Spatial patterns in phototrophic biofilms

The role of physical and biological interactions

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Spatial patterns in phototrophic biofilms

The role of physical and biological interactions

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Chapter 1

General introduction

Background

Spatial scale and structure in ecology

In the last decades, ecological theory has changed focus from the assumption of homogeneity to the recognition of heterogeneity as key for understanding the complexity of nature (Wiens 1989, Levin 1992). Yet the role of organisms in altering this spatial structure at different scales is still a field that requires further attention. Huffaker (1958) was one of the first to demonstrate the importance of spatial scale to ecological processes and patterns by conducting a simple experiment with oranges, mites and mite predators. During these experiments, mites could not co-exist with their predator on a single orange, while on a larger scale a network of multiple connected oranges allowed long-term co-existence of predator and prey. With this simple experiment Huffaker showed that ecological processes are influenced by scale-dependent patterns and that the scale of observation is important to draw valuable conclusions. Since this work, the concept of scale in ecology received increasing attention in various ecosystems (Wiens 1989, Levin 1992, Solan et al. 2003, Sandel and Smith 2009).

Very closely connected to spatial scale is spatial structure ranging from cells to landscapes, e.g. the distribution and abundance of organisms within landscapes (Hutchinson 1953, Turner 2005). Forman and Godron (1981) described landscapes as heterogeneous ecological units, where biotic and abiotic interactions occur in a mosaic of patches that change in structure and functioning through time. Since this work, the interactions between spatial structure of the landscape and ecological processes have been the focus of many studies. Also on a much smaller scale, ecological units can be addressed as landscapes. For example, substrate attached phototrophic biofilms that consist of microorganisms embedded in a slimy matrix of extracellular substances, have been considered as micro-landscapes (Battin et al. 2007). It has been proposed that mechanisms like dispersal create spatial structure in micro-landscapes in a similar way as in larger scale landscapes (Battin et al. 2007). Understanding the mechanisms behind the formation of spatial structure will be essential to understand the functioning of ecosystems from the very small to the very global ecosystem. Along with this, it is difficult to address spatial patterns at a single spatial scale, as small scale interactions can play a key role in forming spatial patterns at larger spatial scales, such as in landscapes (Wiens 1989, Levin 1992, Gustafson 1998, Green and Sadedin 2005, Turner 2005, Battin et al. 2007).

Formation of spatial structure

In the classical view, spatial structure in landscapes primarily results from variation in abiotic factors related to landscape morphology (Turner 2005). However, organisms can also physically alter their environment and create heterogeneity. An example of this can be found in salt marshes where vegetation induces creek formation (Temmerman et al. 2007). Here, feedbacks between vegetation, flow and landform regulates landscape formation in salt marshes. A striking form of spatial structure are the regular spatial patterns that have been described in various ecosystems (Rietkerk and Van de Koppel 2008), including semi-arid ecosystems (Klausmeier 1999), mussel beds (Van de Koppel et al. 2005) and oligotrophic peat lands (Eppinga et al. 2009). In these systems, regular patterns emerge from scale-dependent feedback mechanisms, characterized by a local positive feedback and a longer range negative feedback. Local positive feedback processes improve living conditions through, for instance, the accumulation of nutrients (oligotrophic peat lands) or water (arid-ecosystems), causing small-scale positive feedback (Rietkerk et al. 2002, Eppinga et al. 2009). This positive feedback effect is countered by larger-scale inhibitory processes, for instance, through depletion of resources that cause larger-scale negative feedback (Couteron and Lejeune 2001, Rietkerk et al. 2002, Rietkerk et al. 2004b, Eppinga et al. 2008). These scale-dependent feedback mechanisms form a unifying principle behind the formation of spatial regular patterns in a range of ecosystems (Rietkerk and Van de Koppel 2008). The development of self-organized spatial patterns is predicted to affect ecosystem functioning, altering production rates and ecosystem resilience (Rietkerk and Van de Koppel 2008). Therefore, the occurrence of spatial patterns is not only striking to the eye but also has important emergent effects on ecosystem functioning.

A common characteristic of many self-organized spatial patterns found in ecosystems is that they are generated by organisms that modify their physical environment. These organisms are also referred as ecosystem engineers and are present in many ecosystems covering different trophic levels (Jones et al. 1994). Ecosystem engineers strongly modulate their abiotic conditions which results in strong direct and indirect effects for other species living in the environment. The effect of ecosystem engineers on their environment can reach beyond the spatial scale of their activities, and can persist longer than the life-time of the engineering organism itself (Hastings et al. 2007). A classical example is the engineering effect of beavers that create ponds by damming streams. This damming increases species richness at the scale of the riparian landscape which is much larger than the direct effect of the damming (Wright et al. 2002). Moreover, beavers are active at a site for several years, but after the dam is abandoned the created meadows exist for many more years (Wright et al. 2003). This shows that the temporal and spatial scale of the created physical structures is to some extent independent of the life-time of the engineering organism (Hastings et al. 2007). Moreover, ecosystem engineering can induce habitat heterogeneity by creating patches (Gutierrez and Jones 2006, Wright et al. 2006, Van Wesenbeeck et al. 2007). To what extent patchiness affects ecosystem processes and functioning in a spatial setting has scarcely been studied.

Alternative stable states and discontinuous shifts

A number of studies have put forward that positive feedbacks between organisms and their environment can induce alternative stable states in a wide variety of ecosystems (May 1977, Tilman 1982, Harrison 1986, Chase 1999). Alternative stable states are defined to stably co-exist under the same external environmental conditions when there are no perturbations and have been described mathematically or empirically in marine ecosystems, shallow lakes, woodlands and oceans (Scheffer et al. 1993, Scheffer et al. 2001, Chase 2003, Petraitis and Dudgeon 2004). Gradual changes in external conditions, for instance wave disturbance or grazing, can result in a sudden collapse into an alternative stable state (Scheffer et al 2001). If the ecosystem is close to this shift resilience is low and a very little disturbance can trigger a shift once a critical threshold is reached. The occurrence of alternative stable states has been described in mathematical models of spatially patterned systems such as semi-arid bush land (Rietkerk et al. 2002), peat lands (Rietkerk et al. 2004b) and mussel beds (Van de Koppel et al. 2005). For example, in arid-ecosystems in southern Niger, spatial vegetation patterns are found on hills and slopes, provided that rainfall is sufficient. When rainfall decreases, this vegetated state is predicted to collapse into a bare, degraded state (Rietkerk et al. 2004a). To recover the patterned vegetation requires a significant improvement of conditions (for instance rainfall), far beyond the threshold at which the ecosystem collapsed. In a similar way, model studies point at the potential impact of biotic interactions such as herbivory on pattern formation in semi-arid ecosystems (Rietkerk et al. 2000, HilleRisLambers et al. 2001, Kéfi et al. 2007). These models predict a breakdown of self-organization once a threshold grazing pressure is exceeded, followed by a collapse to a homogeneous state with low vegetation cover (Rietkerk et al. 2000, HilleRisLambers et al. 2001). However, although the potential effects of rainfall has been addressed in empirical studies (Barbier et al. 2006), data showing the influence of top-down control in regulating critical transitions in self-organized ecosystems is scarce.

It is difficult to detect when ecosystems are approaching critical transitions (Clark et al. 2001, Scheffer et al. 2001, Scheffer et al. 2010). Recent work shows that the recovery of ecosystem to small disturbances might be slower when it is close to a discontinuous transition, a phenomenon called critically slowing down (Van Nes and Scheffer 2007, Dakos et al. 2008).

Moreover, increased variance and changing skewness in time series has been associated with rapid changes between ecosystem regimes (Carpenter and Brock 2006, Guttal and Jayaprakash 2009). Nevertheless, a drawback of using these parameters as indicators is that a long time series are needed in order to observe these indicators (Biggs et al. 2009, Scheffer et al. 2009). Therefore changes in spatial patterns might be a more powerful indictor when an ecosystem is approaching a discontinuous shift (Rietkerk et al. 2004a, Scheffer et al. 2009, Dakos et al. 2010). For self-organized spatial ecosystems it is hypothesized that patch size distribution could be a suitable indicator of ongoing transition into a bare state (Rietkerk et al. 2004a, Kéfi et al. 2007). These studies showed that increasing herbivore density changed the spatial configuration of vegetation patterns in dry areas. Despite of the potential value of using the characteristics of spatial patterns as indicator for critical transitions all over the world, this theory has never been tested in other ecosystems than in arid grasslands, limiting the applicability of this theory.

The model system

Phototrophic biofilms

Phototrophic biofilms consist of algae and bacteria embedded in a layer of mucus and are abundant in both fresh as well as marine waters. Phototrophic biofilms are responsible for a significant fraction of total primary production (Underwood and Kromkamp 1999) and are a major food source for macrofauna in marine estuaries (Herman et al. 2000) and freshwater ecosystems (Stevenson 1996). The structure of these biofilms can be rather patchy which has been observed on a different scales, for example the occurrence of seasonal landscape scale patterns (> 1 m, de Brouwer et al. 2000), to smaller scale patterns of < 1 m (Sommer 1999) and < 1 cm (Xavier et al. 2009). Yet, the role of biophysical interactions on the formation of spatial structure at different spatial scales is increasingly studied in ecology. Moreover, the implications for ecosystem functioning and processes of these biological and physical interactions has scarcely been studied.

The cyanobacteria and diatoms that dominate phototrophic biofilms can improve sediment capture and stabilize the sediment on which they live and through this mechanism promote their own living conditions (Stal 2009). Diatoms can excrete extracellular polymeric substances (EPS) (Neumann et al. 1970) which acts as a glue and inhibit erosion by increasing sediment cohesion and decreasing bottom roughness (Paterson 1989, Sutherland et al. 1998a, Sutherland et al. 1998b). This results in increased sedimentation of fine-grained particles (de Brouwer et al. 2000, Montserrat et al. 2009) which subsequently stimulates diatom growth (Van de Koppel et al. 2001) and leads to a state of high algal biomass. Nevertheless, studies on diatom engineering typically lack spatial context, which will be essential for elucidating the mechanisms behind spatial patterns of diatom biofilms, observed in the field (de Brouwer et al. 2000, Rietkerk and Van de Koppel 2008).

Ecosystem engineering of diatoms has been proposed as mechanism behind the formation of a regular, landscape-scale pattern of diatom biofilms on intertidal flats (Rietkerk and Van de Koppel 2008). This landscape is characterized by diatom-covered hummocks alternating with water-filled hollows. Spatial diatom patterns have been observed on intertidal flats during spring (de Brouwer et al. 2000) and disappear again early summer. The consequences of spatial patterns in phototrohic biofilms for ecosystem-level processes have scarcely been studied. There are indications that spatial patterns in benthic algae can affect diversity and stability (Stevenson 1997). Diatom patterns can have important implications for ecosystem functioning at higher trophic levels (Palmer and Poff 1997). Therefore the causes and consequences of spatial patterning in benthic algae are important to elucidate.

Herbivores in phototrophic biofilms

An important part of the biomass that is produced by phototrophic biofilms is consumed by various biota, among which macrobenthos species like polycheates, bivalves and crustaceans or protozoan grazers such as ciliates (Steinman 1996, Herman et al. 1999, Lawrence and Snyder, 1998). These benthic soft bottom herbivores alter many features of benthic phototrophic biofilms such as biomass, primary production, nutrient uptake and taxonomic composition (Gregory 1983, Steinman 1996, Hillebrand and Kahlert 2001, Hillebrand et al. 2002, Kaehler and Froneman 2002). Next to direct effects of grazing, indirect effects such as bioturbation can be an important disturbance for phototrophic biofilms. Bioturbation is the biological reworking of soils or sediment by buried herbivores by for example deposit-feeders, such as the lugworm *Arenicola marina*. Lugworms swallow large amounts of sediment to digest their food (detritus and benthic algae) from this sediment (Meysman et al. 2006) creating a landscape of pits and mound on the intertidal flat which would have been flat in the absence of lugworms (Volkenborn and Reise 2006). Removal of benthic herbivores on intertidal flat resulted in increased sedimentation of small sediment particles and changed microtopograpy of the sediment (Montserrat et al. 2009). Apart from the intertidal flat scale, herbivores also change spatial heterogeneity on a much smaller scale as microscopic observations showed that grazing by e.g. snails changes the biofilm micro-landscape (Lawrence et al. 2002).

Problem definition and objectives

The overall objective of this thesis is to study the role of biological and physical interactions on the formation of spatial structure in phototrophic biofilms at different spatial scales. Spatial pattern formation has been described in many ecosystems The role of bio-physical interactions remains to be elucidated. Therefore I studied spatial pattern formation in phototrophic biofilms emerging from feedback mechanisms between phototrophic biofilms and their a-biotic environment and herbivores.

Hence, I have the following research questions:

- Can interactions between biological and geophysical processes result in formation of spatial patterns in soft bottom communities?
- How do herbivores affect the formation of spatial patterns?
- Do spatial patterns in phototrophic biofilms affect ecosystem-level processes?
- Can spatial self-organized patterns be used for predicting discontinuous shifts in ecosystems?

Outline of this thesis

In this thesis I will examine how spatial patters in phototrophic biofilms can be created by biophysical interactions and how this spatial patterning influences ecosystem processes. Therefore, I studied spatial heterogeneity of benthic algae in the field, in laboratory experiments and in mathematical models, at a variety of scales, from a phototrophic biofilm scale (micrometer) to a landscape scale (> meters).

In Chapter 2, I study how self-organized spatial patterns evolve on intertidal flats (research question 1) and how this can change ecosystem processes (research question 3). I hypothesize that these patterns develop from small-scale positive feedback between diatoms and sediment, interacting with a long range negative feedback by accumulation of water in lower places. I hypothesize that this scale-dependent feedback results in the formation of diatom-covered hummocks and water-filled hollows. This chapter combines field experiments with a mathematical study to elucidate the mechanisms of spatial self-organization from a theoretical viewpoint and to develop an experimental basis for the assumed underlying processes.

In Chapter 3, I focus on the second research question that concerns the implication of herbivory for spatial self-organization on intertidal flats. I hypothesize that herbivores interfere with pattern formation and thereby cause a rapid decline of diatom biomass. To analyze this hypothesis I determined changes in diatom biomass, hummock formation and macrofauna densities during two seasons. I tested the hypothesis that herbivory explains the disappearance of diatom patterning late in the season in a manipulative field experiment where I tested the effect of macrofauna on diatom biofilm formation and landscape dynamics.

The aim of Chapter 4 was to study how the interactions between benthic macrofauna and diatoms interfere with diatom growth on intertidal flats. Therefore I carried out field measurements and laboratory experiments revealing the distribution and preferences of herbivores in relation with resource distribution and landscape position. Moreover, I studied how benthic herbivores affect ecosystem processes such as primary production.

Chapter 5 studies the effect of herbivores on micro-landscapes to answer research question three on how biotic interactions can create spatial patchiness. Therefore I carried out laboratory experiments where I studied the effects of ciliate grazers on the 3D structure of a phototrophic biofilm consisting of several taxa of algae and cyanobacteria on the scale of micrometers.

The last research question on the applicability of spatial patterns for predicting discontinuous transitions, as observed in Chapter 3, is the focus of Chapter 6. Here, I studied changes in spatial configuration in self-organized spatial diatom patterns as diatom biofilms degraded later in season due to increased herbivory. I expect that the spatial configuration of regular diatom patterns changes when herbivore densities increase and thereby broaden the theory on changing spatial configuration of self-organized spatial patterns. So far this has only been described for irregular patterns.

In Chapter 7, I discuss the implications of the results described in this thesis in four themes where I argue for the incorporation of organisms as landscape engineers, the in-cooperation of higher trophic levels in (spatial self-organization) ecology and the importance across ecosystem views for predicting future changes in ecosystems.

Chapter 2

Spatial self-organization on intertidal mudflats through biophysical stress divergence

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Abstract

In this study, we investigated the emergence of spatial self-organized patterns on intertidal flats, resulting from the interaction between biological and geomorphological processes. Autocorrelation analysis of aerial photographs revealed that diatoms occur in regularly spaced patterns consisting of elevated hummocks alternating with water-filled hollows. Hummocks were characterized by high diatom content and a high sediment erosion threshold, while both were low in hollows. These results highlight the interaction between diatom growth and sedimentary processes as a potential mechanism for spatial patterning. Several alternative mechanisms could be excluded as important mechanisms in the formation of spatial patterns. We developed a spatially explicit mathematical model which revealed that scale-dependent interactions between sedimentation, diatom growth, and water redistribution explain the observed patterns. The model predicts that areas exhibiting spatially self-organized patterns have increased sediment accretion and diatom biomass compared with areas lacking spatial patterns, a prediction confirmed by empirical evidence. Our study on intertidal mudflats provides a simple but clear-cut example of how the interaction between biological and sedimentary processes, through the process of self-organization, induces spatial patterns at a landscape level.

Introduction

In the past decade, a large number of studies have reported on regular pattern formation in a wide range of ecosystems (for a review, see Rietkerk and Van de Koppel 2008). Theoretical studies proposed that regular pattern formation is induced by feedback mechanisms acting on different scales Here, facilitation processes locally improve living conditions through, for instance, the accumulation of nutrients (oligotrophic peatlands) or water (arid ecosystems), causing a small-scale positive feedback. This is countered by larger-scale inhibitory processes - for instance, through depletion of resources - that cause a negative feedback (Couteron and Lejeune 2001, Rietkerk et al. 2002, Rietkerk et al. 2004*b*, Eppinga et al. 2009). This scale-dependent feedback mechanism can explain the formation of regular spatial patterns in many ecosystems and is predicted to have important implications for ecosystem functioning in terms of increased production or resilience (Rietkerk and Van de Koppel 2008).

Theoretical studies have mainly focused on concentration of limiting resources when explaining regular pattern formation in ecosystems. However, field studies have pointed to possible alternative mechanisms for explaining regular patterns in ecosystems that are based on divergence of physical stresses, for example, the divergence of water flow or snow (Temmerman et al. 2007, Hiemstra et al. 2002, Larsen et al. 2007, Van Wesenbeeck et al. 2008). On salt marshes, for instance, clumps of vegetation obstruct water flow, which locally improves growth conditions. Divergence of water flow around vegetation clumps increases erosion, which limits plant growth just outside the clump and hence induces a scale-dependent feedback. This mechanism has been suggested to trigger the development of spatial structure in salt marshes and even affect landscape formation in this ecosystem (D'Alpaos et al. 2007, Temmerman et al. 2007, Van Wesenbeeck et al. 2008). Whether it can explain the formation of regular, self-organized spatial patterns has remained unstudied.

In this study, we describe how redistribution of hydrodynamic stress by diatoms can explain the formation of regular patterns on intertidal mudflats. Here, elevated hummocks, covered with diatoms, alternate with water-filled hollows with few diatoms (Fig. 2.1). Spatial patterning on muddy intertidal flats has been observed in a number of studies (Blanchard et al. 2000, de Brouwer et al. 2000, Gouleau et al. 2000, Whitehouse et al. 2000, Lanuru et al. 2007). They range from banded patterns aligned parallel to the flow direction (ridge-runnel systems) in areas where tidal currents are high (Bassoullet et al. 2000, Blanchard et al. 2000, Le Hir et al. 2000, Whitehouse et al. 2000) to round-shaped patterns where water currents are lower (de Brouwer et al. 2000). The linear ridge-runnel patterns are present during all the seasons (Lanuru et al. 2007), while the round-shaped patterns are a seasonal phenomenon (de Brouwer et al. 2000) . Here, a spatial pattern of elevated hummocks alternating

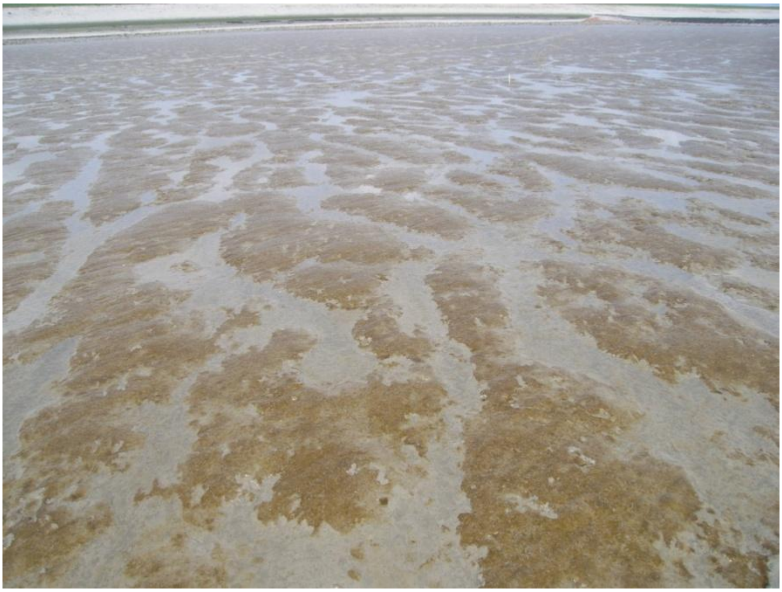
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Figure 2.1: Photograph of a spatial diatom-sediment pattern taken at the intertidal flat the Kapellebank, the Netherlands

with water-filled hollows develops from a homogeneous mudflat in early spring and disappears again in summer (de Brouwer et al. 2000). On top of the hummocks, diatoms accumulate, forming a visible brown biofilm, which in turn is strengthened by the excretion of extracellular polymeric substances (EPS, Neumann et al. 1970). EPS inhibits erosion by increasing sediment cohesion and decreasing bottom roughness (Paterson 1989, Sutherland et al. 1998a, 1998b), resulting in increased sedimentation of fine-grained particles (de Brouwer et al. 2000, Montserrat et al. 2009), which subsequently stimulate growth of diatoms (Van de Koppel et al. 2001). In the hollows, water accumulates during low tide, which will inhibit the buildup of EPS, as EPS dissolves in the overlying water (Blanchard et al. 2000, Paterson et al. 2000). This in turn leads to increased vulnerability to erosion during inundation and subsequently leads to erosion of diatoms and sediment. The interaction between accumulation of sediment on the hummocks and drainage of water toward the hollows is hypothesized to generate a scale-dependent feedback of short-scale facilitation and larger-scale inhibition of diatom growth and was put forward as a possible explanation for the observed spatial patterning (Rietkerk and Van de Koppel 2008). However, this putative stress divergence mechanism has been neither studied mathematically nor tested empirically. Confirmation of stress divergence as a mechanism for the formation of regular self-organized spatial patterns in ecosystems would broaden the conceptual basis of scale-dependent feedback as a structuring process in ecological systems.

Here, we investigated the hypothesis that pattern formation on intertidal mudflats results from spatial self-organization that is caused by scale-dependent feedback between diatom growth, sediment dynamics, and water drainage processes. We developed a mathematical model of this feedback interaction to study stress divergence as a mechanism for the observed spatial pattern and its effect on the functioning of intertidal flats ecosystems. Aerial photographs were analyzed to establish the regularity of spatial patterns observed in the field. Subsequently, we investigated whether diatom biomass and sediment characteristics varied between the hummocks and the hollows to verify model assumptions and to exclude possible alternative mechanisms that could explain the observed spatial patterns. We furthermore tested in the field the model's prediction that patterned tidal flats accumulate more sediment and are more productive than homogeneous flats. This would solidify the concept that self-organized pattern formation affects ecosystem functioning.

A simple model of pattern formation on intertidal mudflats

Description

We developed a simple mathematical model to investigate whether the proposed scale-dependent feedback between diatom growth, sediment dynamics, and water drainage could result in regular spatial patterns on intertidal mud-flats (Fig. 2.1). Observations in the field revealed that the pattern is aligned parallel to the primary drainage direction of the intertidal mudflat. We therefore modelled a one-dimensional cross section of the intertidal mudflat aligned perpendicular to the drainage direction. The patterns in the field were dissected by larger drainage channels (Appendix 2.B). We chose to ignore the large-scale drainage structure, since we were mostly interested in explaining the formation of patches. This simplifying approach allowed us to model small-scale pattern formation without explicitly taking into account large-scale hydrodynamic processes.

We modelled changes in sediment elevation (S; cm), water level (W; cm), and diatom biomass (D; g chlorophyll per m^2) as a function of the interaction between diatom growth, sediment dynamics, and water flow from the hummocks towards the hollows. Diatom growth is described as the balance between growth and losses due to erosion by the overlaying water:

$$\frac{\partial D}{\partial t} = rD\left(1-\frac{D}{k}\right) - ECD\,\frac{W}{q+W} \qquad (1)$$

Here, growth of diatoms is described using the logistic growth equation, where r is the intrinsic growth rate of the diatoms and k is the diatom carrying capacity. Furthermore, E is the maximal sediment erosion rate in the absence of diatoms, C translates sediment erosion to the rate of diatom losses, and q is the water level at which diatom losses are half maximal. Diatom losses by other processes - for example, grazing - are ignored since these are fairly low during development of spatial patterns early in the season.

Changes in sediment level are determined by the balance between deposition and erosion of sediment particles from the overlying water during tidal submergence. Deposition of sediment occurs each high tide as a small amount of sediment settles down from the water column. Erosion depends on diatom biomass since diatoms decrease erosion through the exudation of EPS (Paterson 1989, Sutherland et al. 1998a, 1998b):

$$\frac{\partial S}{\partial t} = S_{in} - E\left(\frac{k - Dp_E}{k}\right)S + A\,\frac{\partial^2 S}{\partial x^2} \tag{2}$$

Sin is the sediment deposition rate and pE is the extent to which sediment erosion is reduced when diatoms are at carrying capacity. In the last term in the equation, A represents the diffusion constant describing slow, gravity-induced dispersion of wet sediment from the hummock to the hollows to avoid the generation of steep hummock edges.

In our model, changes in water level are determined by the residual amount of water left after each tide (Win), the water drainage rate (F) in the direction perpendicular to our cross section, and the lateral flux of water:

$$\frac{\partial W}{\partial t} = W_{in} - WF + \frac{\partial}{\partial x}\left(K(W)W\,\frac{\partial (W+S)}{\partial x}\right) \tag{3}$$

Lateral water flow is modelled as a function of water depth and slope of the water surface (∂(S + W)/∂x), roughly following the Manning formula for open-channel flow, except for assuming a linear relation between water flow and water surface slope. By doing this, we could avoid the complexity of the shallow-water equations and maintain a simple model. Hydraulic conductivity (K(W)) is assumed to be a decreasing function of the water level, reflecting the reduced water flow rate in very thin water layers:

$$K(W) = K_W \frac{TW^4 + p_K}{TW^4 + 1} \tag{4}$$

Here, KW is the conductivity when the water level is high, T translates water level to conductivity, and pK is the proportion of KW to which K(W) reduces when the water level on the sediment approaches zero.

The model provides a strongly simplified representation of biological, hydrological, and sedimentary processes on an intertidal mudflat. It simultaneously considers water drainage from the hummocks to the hollows occurring at low tide and sedimentation processes occurring at high tide. The specific erosion rate is expressed as a monotonic function of diatom biomass, integrating the more complex relation between tidal water flow rate and sediment erosion (Winterwerp and Van Kesteren 2004) over longer time-scales. implifying these processes maintains a tractable model and allows us to explore the effects of scale-dependent feedback mechanisms between diatoms and sedimentary processes on intertidal mudflats without explicitly considering tidal changes in water level. In this way, we aim to follow a principle-seeking approach that provides general understanding of the implications of sediment-diatom-water interactions, rather than a precise description of sedimentary and hydrodynamic processes.

The complexity of the systems (1)–(4) was reduced by adopting a quasi–steady state approach with respect to diatom biomass, assuming that diatom densities equilibrate within each tidal cycle. This allowed us to express diatom biomass algebraically:

$$D = k - \left(1 - \frac{EC}{r}\frac{W}{q+W}\right) \qquad (5)$$

Inserting equation (5) in equations (1) and (2) reduces the model to a system of two partial differential equations. All parameters were derived from data that were collected during this study at reference sites, from the literature, or by estimation if no data were available. Table 2.A1 in Appendix 2.A gives more information on the parameters we have used in the model.

The development of the patterns was simulated numerically with forward Euler integration of the differential equations using Intel Visual Fortran. We simulated a vector of 600 points representing a length of 6 m perpendicular to the flow direction. Starting conditions were given by the homogeneous equilibrium, with a slightly evenly distributed random perturbation with a maximal difference of 0.06 mm. Periodic boundary conditions were adopted since the simulated domain was assumed to be a part of a larger intertidal bed. To avoid numerical instability, we used the harmonic mean of K(W) from two neighbouring points. Simulations were run until stability was reached.

Bifurcation analysis

The occurrence of self-organized patterning is strongly affected by changes in biotic and abiotic conditions, and therefore we investigated how changes in key parameter values affected pattern formation and the predicted buildup of sediment and diatoms. To focus on the parameters that are essential to the model's behavior, we first derived a nondimensional version of the full model (Appendix 2.A). The main factor of variation within and between intertidal flats is erosion, and therefore we investigated how changes in erosion rate affected pattern formation and the predicted buildup of sediment and diatoms. Other parameters from the nondimensional model were also studied and are described in Appendix 2.A. For the bifurcation analysis, we applied the method of spatial dynamics (Champneys 1996), which is an effective method for analyzing spatial steady state solutions (Sherratt and Lord 2007, Knobloch 2008, Wang et al. 2009). The analyses were performed using the bifurcation program AUTO (Doedel et al. 2001).

Results model analysis

Our model analysis shows that the interaction between diatom growth, sediment dynamics, and water drainage induces the development of regularly spaced, diatom-covered hummocks (Fig. 2.2A, solid line). Pattern formation is initialized when a small, random increase in sediment elevation reduces the depth of the water layer and improves diatom growth, which in turn leads to a further increase in elevation. Water that accumulates in slightly lower parts causes the opposite effect as diatom losses increase, leading to more erosion and a further decrease of elevation. In the end, this results in a landscape of regularly placed hummocks with high diatom biomass on the hummocks, where water is drained towards the hollows in which water accumulated (Fig. 2.2C). If the starting conditions are entirely homogeneous, no patterning develops, and both sediment elevation (Fig. 2.2B) and diatoms (Fig. 2.2D) remain low, revealing that spatial interactions are a key mechanism causing the formation of spatial patterns. This suggests that the proposed interaction between diatom growth, sedimentary processes, and water flow toward the hollows can explain the observed patterns on intertidal flats.

Bifurcation analyses revealed that the presence of spatial patterns was strongly dependent on parameter settings. Figure 2.3 presents the result of a bifurcation analysis for changes in the erosion rate E, which is the parameter that most clearly defines differences between intertidal habitats. In the homogeneous equilibrium, sediment level and diatom biomass decrease linearly with increasing erosion rate until diatoms cannot maintain themselves on the intertidal mudflat. This homogeneous equilibrium is unstable to small heterogeneous perturbations when $E > T_2$. Here, small perturbations are inflated and cause the formation of regular spatial patterns (Fig. 2.3, solid dark grey line). The patterned system is globally stable up to erosion rates of $E = T_1$. Within $T_1 < E < LP_1$, the system has two attracting states, where one state is characterized by spatial patterns (solid dark line; represents maximal algal biomass or sediment levels in the patterned solution), while the other is a homogeneous state with few diatoms (dashed black line). Beyond a last threshold, the patterned state becomes unstable, and only a homogeneous state without diatoms is found ($E > LP_1$; solid black line). The bifurcation analyses with respect to other parameters reveal qualitatively similar patterns.

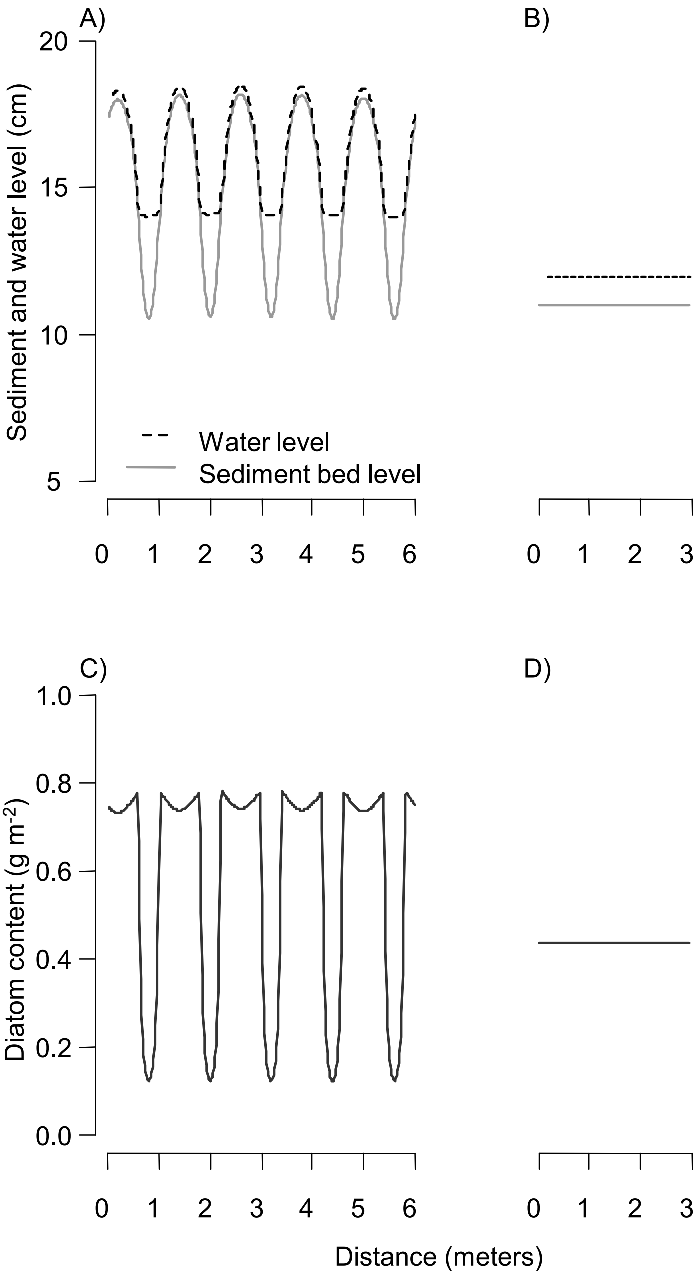


Figure 2.2: Simulated spatial patterns of sediment level, water level (A) and diatom biomass (C) and the simulated homogenous equilibrium for sediment, water level (B) and diatom biomass (D). Light grey lines represent sediment level, black dotted lines represent water level and dark grey lines represent diatom biomass. Parameters used as in online Appendix 2.B.

Pattern formation was found to be most sensitive to changes in the effects of diatoms on sediment erosion (Appendix 2.A).

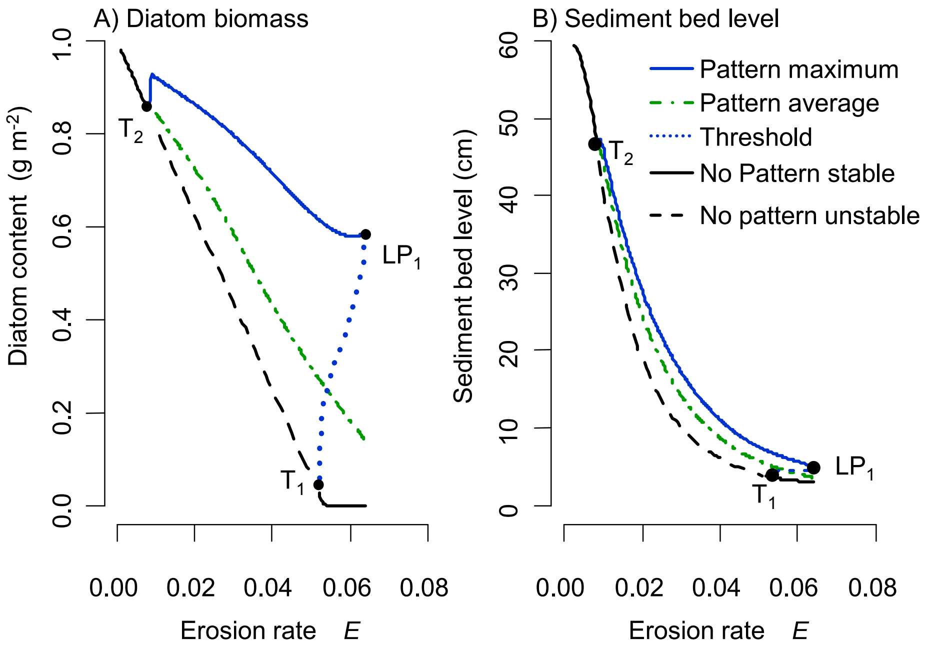


Figure 2.3: Bifurcation diagram of diatom biomass (A) and sediment accumulation (B) based on a single peak solution. The black line represents the homogeneous equilibrium; blue lines represent maximum diatom biomass in the patterned equilibrium, the green line represents average diatom biomass of the whole domain in the patterned equilibrium. Solid lines and dotted lines represent the stable and unstable equilibrium, respectively. T_1 (E=0.039) and T_2 (E=0.012) are Turing instability points and LP_1 (E=0.051) is a limit point.

Our model allows us to investigate the implications of spatial pattern formation on ecosystem functioning. For all parameter values where stable patterns are predicted, average diatom biomass and sediment level are higher in the patterned equilibrium compared with the homogeneous equilibrium (Fig. 2.3, green vs. black lines). Hence, in the patterned state, the intertidal mudflat is predicted to be more productive and accumulate more sediment compared with a homogeneous intertidal mudflat, revealing that spatial patterning has important emergent effects on the functioning of intertidal mudflats that exceed beyond simple generation of heterogeneity and emphasizing the importance of diatom-induced spatial patterns for productivity and sediment capture on intertidal mudflats.

Testing model assumptions and predictions

Description of field site

We conducted a field study to test a number of the assumptions and predictions of the model described in “A Simple Model of Pattern Formation on Intertidal Mud-flats” First, we investigated, using aerial photographs, whether the observed patterns were indeed regular, as predicted by the model. Second, we investigated the differences in chlorophyll-*a* content (a proxy for diatom biomass), sediment erosion thresholds, and sediment characteristics between the hummocks and the hollows. This was done to test the assumptions that underlie the model and to exclude possible alternative mechanisms for the observed spatial patterns. The model analysis identifies two important predictions: (1) self-organized patterns develop even in the absence of underlying environmental heterogeneity and (2) both diatom biomass and sediment accumulation at the scale of the tidal flat ecosystem (e.g., averaged over extensive areas) are higher in the patterned state compared with the homogeneous mudflat state. All experiments and measurements were done on the Kapellebank, an intertidal mudflat situated along the edges of the Westerschelde, the Netherlands (coordinates: 51.458521N, 3.971685E; Fig. 2.1). Spatial patterns are abundant on approximately 75% of the total surface of the intertidal mudflat; they develop each year in spring and disappear at the onset of summer when benthic herbivore abundance increases.

Materials and methods

Pattern observations

We analyzed aerial photographs of spatial patterns to test whether the observed patterns are regular. Aerial photographs were taken with a digital camera (Sony Cybershot DSC-V3) attached to a helium-inflated, blimp-shaped balloon (http://www.floatograph.com), which was attached to a tether line. Photographs were obtained from approximately 50 m height, covering an area of approximately 50 m by 40 m (3,072 pixels by 2,304 pixels; Appendix 2.B). Generally, two types of patterns were detected on the Kapellebank: banded elongated patterns aligned parallel to the flow direction at the sloping sides of the mudflat, and more rounded patterns on the top of the intertidal flat where the slope is less pronounced. From both pattern types, two subsections of 300 pixels by 300 pixels (5 m by 5 m) were selected randomly for analysis, avoiding the larger drainage channels. In the extracted images, dark areas correspond to high diatom concentrations, and light areas correspond to bare sediment (Fig. 2.4A and 2.4D). Visual inspection showed that the intensity of the blue channel within the RGB images reflected diatom biomass most closely, and therefore these pixel values were used for subsequent analysis. From the images, 3,000 pixels were sampled randomly, since the 300 by 300-pixel images were too large to be analyzed entirely. We tested for the spatial autocorrelation by calculating Moran's I using R (http://www.R-project.org). High positive autocorrelation corresponds to more similar diatom biomass at a specific distance, while dissimilarity results in negative autocorrelation (Legendre and Legendre 1998). Since on the sloping sides patterns seemed aligned along the general direction of water drainage, we analyzed the images in two directions: parallel and perpendicular to the average direction of the flow, using categories of 90°.

Underlying heterogeneity

Our model predicts that self-organized patterns could develop in the near absence of underlying heterogeneity. We tested this hypothesis in the field by removing both underlying heterogeneity and benthic diatoms in 2 by 2 m plots, while spatial patterns and diatoms remained untouched in the control plots. We added two additional procedural control treatments. In the first, called diatom removal only, diatoms were scraped from the sediment. In the second, labelled hummock removal only, the sediment was flattened, and the slurry of diatoms that was removed a priori was returned on the experimental plot. This block of four treatments was repeated 10 times following a randomized block design (Sokal and Rohlf 1995). The degree of recovery was determined after 14 days and expressed as the maximal elevation difference on the plot. A detailed description of this experiment is described in Appendix 2.B.

Testing for model assumptions

A crucial assumption for the proposed scale-dependent feedback mechanism is that erosion rates are high in the water-covered hollows compared with the emerged hummocks. In the field, we tested this assumption by determining the differences in diatom biomass, sediment properties, and the sensitivity of the sediment to erosion between the hummocks and the hollows. Therefore, chlorophyll-*a*, silt content, and erosion threshold were measured at hummocks and adjacent hollows. A detailed description of these measurements is described in Appendix 2.A.

The mathematical model predicts higher sediment accretion and diatom biomass for a self-organized patterned intertidal flat compared with a homogeneous intertidal flat. We tested these model predictions in the field were sediment accretion during the season was measured in plots where spatial patterns were present and in plots where no patterning emerged. Sediment bed level measurements were done in April 2009, when patterns were visible, and

June 2009, when patterns had disappeared and the homogeneous bed level was stable, using the sediment erosion bar (SEB) method (Austen et al. 1999). We measured chlorophyll-*a* content in April in both the spatially patterned plots and the homogeneous plots. A detailed description of these measurements is given in Appendix 2.A.

Statistical analysis

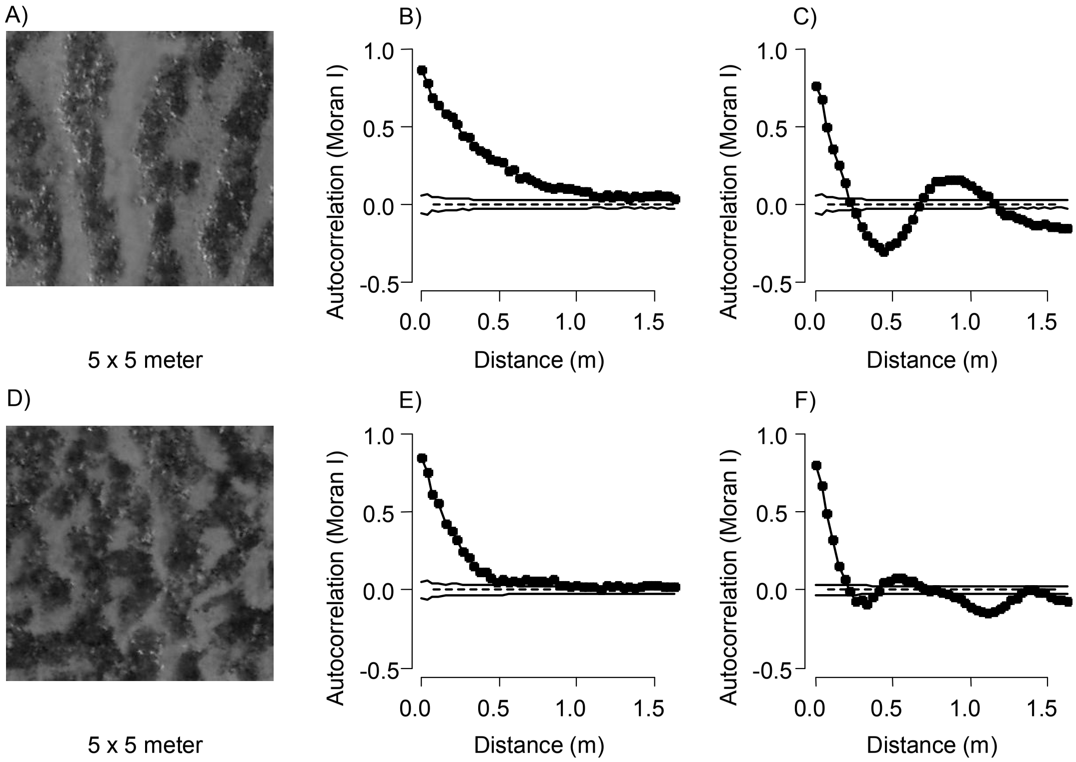
Biotic and abiotic differences between hummocks and adjacent hollows were analyzed using Student's paired t-tests (one-tailed). To further analyze the influence of silt and chlorophyll-*a* content on the variability in the erosion threshold for hummocks and hollows, we used a general linear model (GLM) with landscape position (hummock or hollows) as a fixed factor and silt content and chlorophyll-*a* content as covariates. Using stepwise reduction from the saturated model (Crawley 2005), all non significant factors could be removed, resulting in the best model for explaining differences in erosion threshold. Similarly, a GLM was used to analyze erosion threshold differences as a function of physical characteristics. The influence of spatial patterns on sediment accretion and chlorophyll-*a* content were analyzed using Student's paired t-tests (one-tailed), testing the increase in elevation between April and June. All statistics were computed using R (http://www.Rproject.org).

Results

Pattern observation data and measurements

Spatial analysis of the aerial photographs revealed regular spatial patterning in diatom reflectance that was strongest perpendicular to the tidal flow direction. Patterns closer to the shoreline showed significant positive autocorrelation up to 20-cm lag distance, followed by significant negative autocorrelation from 20 to 80 cm (Fig. 2.4C). This relation was not repeated in the flow direction, where autocorrelation was significantly positive up to 1.5-m distance (Fig. 2.4B), but no negative correlation was found at any distance. This confirms that bands of high diatom biomass were regularly distributed in the direction perpendicular to the flow direction with a wavelength of roughly 1 m. The round-shaped patterns on the top of the intertidal mudflat revealed a similar relation (Fig. 2.4E and 2.4F). These patterns were found to be regular in the direction perpendicular to the tidal flow direction, but no significant negative autocorrelation was observed in the opposite direction (0°), indicating the absence of regularity in this direction. Hence, we found regular patterning on both the sloping sides and the more flat top of the intertidal mudflat, mainly orientated perpendicular to the general direction of water drainage.

Figure 2.4: Autocorrelation (Moran's I) for benthic diatom patterns with their 95% confidence intervals (grey lines). Pictures represent a 5 by 5 meter surface generated by 2 directional analyses that is indicated by the arrows above the graphs. Two different patterns were chosen for this analysis. A banded pattern (A), and a more round shaped pattern (D). Two directions correspond to 0° (B,E) which are parallel with the water line and 90° (C,F), perpendicular to the water line.



Analysis of the field measurements revealed that, in agreement with our hypothesis, higher chlorophyll-*a* content ($P < 0.01$; Fig. 2.5A) and higher erosion thresholds ($P < 0.001$; Fig. 2.5B) were found on the hummocks compared with in the hollows. No significant differences in silt content were found (mean ± SEM: hummocks, 59.4% ± 2.3%, hollows, 57.5% ± 2.9%, $P > 0.05$), indicating that variation in silt content was not the most important factor in determining the patchiness. Differences in chlorophyll-*a* concentration and erosion threshold were to a large part explained by position on either hummocks or hollows. The GLM analysis revealed a model in which the factor landscape (e.g., in a hollow or on a hummock) gave the best fit (on the basis of Akaike information criteria) to measured erosion thresholds compared with models that included chlorophyll-*a* and silt content and their interaction. Stepwise reduction from the saturated model (Crawley 2005), removing any non significant factors, resulted in a model with only landscape position as the explanatory variable ($P < 0.001$). This is probably caused by a strong correlation between landscape position and chlorophyll-*a* content, combined with limited variability of chlorophyll-*a* content within either the hollows or the hummocks.

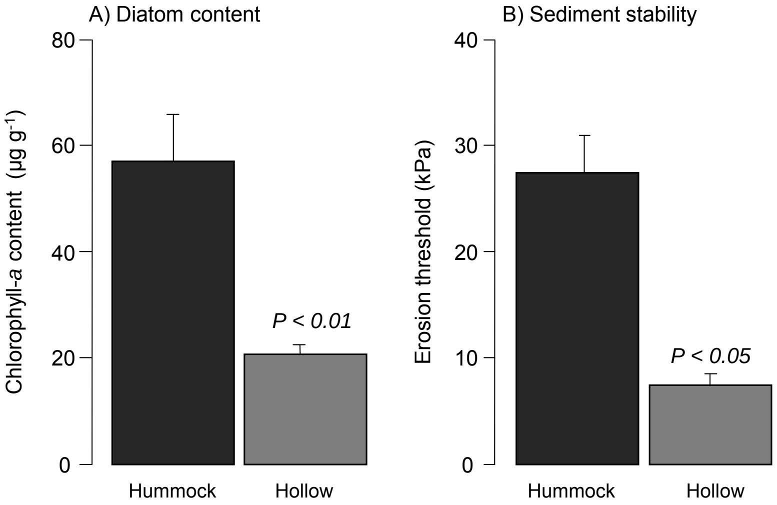


Figure 2.5: Differences in diatom biomass (A) and sediment stability (B) on the hummocks (dark grey bars) and hollows (light grey bars). n=7, error bars denote ± 1 SEM.

We found a strong effect of spatial patterns on sediment accretion during the spring season. Sediment bed level in patterned plots increased by 2.02 ± 0.31 cm (mean ± SEM), which is significantly higher than in homogeneous plots, which increased by 0.71 ± 0.76 cm ($P < 0.05$; Fig. 2.6). Likewise, chlorophyll-*a* was significantly higher in spatially patterned plots compared with homogeneous plots (135.61 ± 2.04 and 81.38 ± 10.74 μg g^{-1}; $P < 0.01$; Fig. 2.6). Hence, these results support the prediction of our mathematical model that self-organized spatial patterns increase the overall sedimentation rate.

Testing for spatial self-organization

Experimental removal of heterogeneity did not affect the formation of hummock and hollows in the experimental plots. Two weeks after treatment application, no differences were found in bed level between control plots (3.12 ± 0.48 cm), treatment plots with hummock and diatom removal (2.50 ± 0.29 cm), or procedural controls (diatom removal only: 2.82 ± 0.31 cm; hummock removal only: 2.58 ± 0.38 cm; Fig. 2.A1A in Appendix 2.A; $P > 0.05$). Similarly, no differences in chlorophyll-*a* content were measured on hummocks between the control plots (130.8 ± 10.8 μg g^{-1}), treatment plots with hummock and diatom removal (131.4 ± 13.81 μg g^{-1}), and procedural controls (diatom removal only: 127.26 ± 11.3 μg g^{-1}; hummock removal only: 136.3 ± 13.8 μg g^{-1}). Similarly, no differences in chlorophyll-*a* content were found in the hollows (control plots: 51.7 ± 9.7 μg g^{-1}; treatment plots: 40.8 ± 8. μg g^{-1}) and procedural controls (diatom removal only: 44.3 ± 4.32 μg g^{-1}; hummock removal only: 48.2 ± 7.29 μg g^{-1}, Fig. 2.A1B; $P > 0.05$). This, in combination with the seasonal disappearance of patterning that was observed each year, makes it unlikely that underlying heterogeneity is a possible alternative mechanism for the formation of self-organized spatial patterns on intertidal flats.

Our results indicate that sediment consolidation, a possible alternative abiotic mechanism for pattern formation, did not differ between the hummocks and hollows. No differences were found for bulk density (1.21 ± 0.10 g cm^3 in hummocks vs. 1.13 ± 0.07 g cm^3 in hollows; $P > 0.05$). Similarly, no differences in water content were found between hummocks and hollows, as both showed 50% ± 3% water content. A GLM analysis, which included sediment consolidation variables as independent factors to explain differences in erosion threshold, identified that landscape position (e.g., hummock or hollow) was the main explanatory variable for the variation in erosion rate. Adding other factors such as bulk density and water content did not improve the explanatory power of the model.

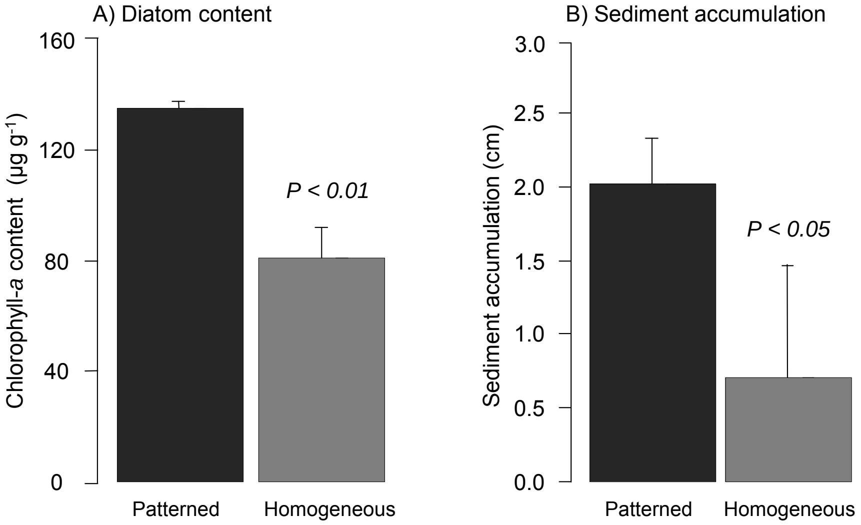


Figure 2.6: Diatom biomass (A) and sediment accumulation (B) for the patterned (dark grey bars) and homogeneous stations (light grey bars). Diatom biomass is expressed as average chlorophyll-*a* content and sediment accretion is expressed as the difference in bed level between April and June. n=3, error bars denote ± 1 SEM.

Discussion

The formation of regular, self-organized spatial patterns has mainly been described in communities driven by resource limitation, where spatial redistribution of resources such as water or phosphate is the driving factor behind spatial self-organization (Rietkerk and Van de Koppel 2008). This study presents an alternative mechanism for the formation of regular patterns: divergence of physical stress by organisms, creating local positive feedback but negative feedback at larger spatial scales. On intertidal mudflats, interactions between diatom growth and geomorphological processes locally improve living conditions for diatoms by increasing the elevation of diatom-covered hummocks. Consequently, increased elevation causes the hollows between hummocks to remain inundated at low water, decreasing diatom cover due to higher vulnerability to erosion and generating a scale-dependent feedback. Model analysis showed that this divergence of hydrodynamic stress induces a regular, self-organized spatial pattern on intertidal mudflats, resulting in a landscape of regularly spaced hummocks and hollows. Field measurements revealed a clear difference in both diatom biomass and erosion threshold between the hollows and the hummocks. This is in line with studies on other intertidal mudflats where regular patterning occurs (de Brouwer et al. 2000, Paterson et al. 2000, Lanuru et al. 2007). Furthermore, our model analyses as well as our experimental results reveal that self-organized patterns increased diatom densities and sediment capture, thereby increasing average elevation by about 2 cm. Hence, spatial pattern formation induced by stress divergence significantly affects the functioning of intertidal mudflat ecosystems.

Stress divergence mechanisms have been proposed in other studies to induce spatial structure in ecosystems (Hiemstra et al. 2002, Larsen et al. 2007, Saco et al. 2007, Temmerman et al. 2007, Van Wesenbeeck et al. 2008). In salt marsh ecosystems, tussocks of *Spartina anglica* improve growth conditions within the tussocks as a result of increased sedimentation but strongly decrease growth potential outside of the tussock. This mechanism induces the formation of creeks during salt marsh development (Temmerman et al. 2007). Stress divergence can be inferred from other studies in patterned ecosystems, such as during the formation of parallel tree lines in the Rocky Mountains (Hiemstra et al. 2002) or ridge and sloughs landscapes in the Everglades (Larsen et al. 2007). These studies, combined with ours, show that stress divergence might be a widely occurring mechanism causing spatial self-organization in ecosystems, broadening the application of spatial self-organization to a wider range of ecosystems.

Testing for alternative mechanisms

Our hypothesis that diatom-sediment interactions could explain the formation of the regular landscape of hummocks and hollows was tested against potential alternative mechanisms that could lead to spatial pattern formation. Possible alternative mechanisms include (1) underlying spatial heterogeneity, (2) increased sediment consolidation reducing sediment erosion on the hummocks, and (3) mechanical disturbance by water rushing through the hollows before submergence of the hummocks; these are all possible physical causes of patterning. We tested the effects of underlying heterogeneity by removing both diatoms and elevation differences in experimental plots. After 2 weeks, both diatom patterning and hummock development reappeared in these plots and were statistically indistinguishable from the control plots. This suggests that the patterns, at least in our experimental plots, are not the result of underlying heterogeneity. We did not observe differences in sediment bulk density or water content between the hummocks and the hollows, typical characteristics of consolidation. These results indicate that consolidation could not explain the observed differences in the sediment erosion threshold, and the diatom-sediment interactions are a more likely cause of the observed spatial patterns. Finally, two lines of reasoning point out that increased mechanical disturbance by water flow alone cannot explain the observed patterns. First, mechanical disturbance would lead to increased erosion in any low location and hence cannot explain the observed regularity. Second, water velocity has been found to be higher on hummocks compared with hollows (Williams et al. 2008), which would result in higher erosion on hummocks, which is opposite of what we found. This leads us to conclude that mechanical disturbance alone is an unlikely mechanism for the observed spatial patterns.

The importance of diatoms in stabilizing sediment has been well established in the literature (Stal 2009), which also includes studies on spatially patterned intertidal flats (Paterson et al. 2000, Lanuru et al. 2007). Removal of benthic algae using biocide can result in extensive sediment erosion, affecting landscape formation (de Boer 1981, Underwood and Paterson 1993). These studies, in combination with other studies, point at the importance of diatom-sediment feedbacks in generating landscape heterogeneity (Van de Koppel et al. 2001, Montserrat et al. 2008). Our own experiments suggest that stress divergence, caused by a local positive feedback between diatom growth and sediment accumulation, is the most likely cause of the observed regular patterns.

Biogeomorphological feedback and ecosystem functioning

Our study reveals a clear effect of self-organized patterns on the functioning of intertidal mudflat ecosystems, since both the model and field data showed that patterned intertidal mudflats have significantly higher diatom densities and accumulation of sediment (Fig. 2.6). The effect of this increase in diatom densities on overall intertidal mudflat productivity has hardly been investigated. Intertidal mud-flats account for up to 50% of the primary production in estuaries (Underwood et al. 1999) and are therefore an important component of estuarine ecosystems. Increased benthic diatom biomass will significantly influence food availability, since diatoms are a major food source for benthic macrofauna (Herman et al. 2000, van Oevelen et al. 2006) and for the planktonic food web (de Jonge and van Beusekom 1992). Therefore, both benthic and planktonic faunal biomass is expected to increase with the presence of patterned intertidal mudflats within estuaries.

Spatial patterning on intertidal flats

Our model analysis predicts increased sediment accumulation in self-organized intertidal mudflats. Although current models describing the morphology of intertidal mudflats have incorporated the effects of seasonal changes in diatom abundance on sediment surface texture (Paarlberg et al. 2005, Le Hir et al. 2007, Borsje et al. 2008), they lack the effects of self-organization induced by diatom-sediment feedbacks. Moreover, the increase in bed level can have important effects on water turbidity in the estuary on scales that far exceed that of a single intertidal mudflat. A back-of-the-envelope calculation reveals that 2 cm elevation of 1 m^2 surface of diatom-induced accumulation of silt on a intertidal mudflat is roughly equivalent to the silt content of about 40 m^2 of open water (assuming an average depth of 10 m in the estuary; Herman et al. 2001). Similarly, benthic diatoms capture, although to a lesser extent, organic particulates from the water column, which can have a substantial effect on the particulate organic matter concentration within estuarine waters (Herman et al. 2001, Staats et al. 2001). In this way, self-organized spatial patterns not only locally affect the functioning of intertidal mudflats but also affect pelagic processes at the estuarine scale.

The bifurcation analysis uncovers two interesting possible implications of self-organization on patterned intertidal mudflats: the occurrence of alternative stable states and the vulnerability to catastrophic shifts. These implications limit our ability to predict how intertidal flats will respond to changes in forcing factors, such as increased flow rates, with respect to both sedimentary dynamics and their biological communities (Van de Koppel et al 2001). It is known that self-organized patterns may indicate the presence of alternative stable states and, as a consequence, a potential for catastrophic shifts, since both phenomena are, in part, the result of similar positive feedbacks (Scheffer and Carpenter 2003, Rietkerk et al. 2004a). On intertidal mudflats, this may result in sudden changes in diatom biomass and subsequent release of fine-grained sediment in response to gradually changing hydrodynamic conditions, which often are difficult to reverse. Addressing such nonlinear dynamics in estuarine systems will be an important future challenge in the study of biogeomorphology to provide more accurate predictions for management and conservation policies.

Our study emphasizes the need to integrate the fields of ecology and geomorphology to come to a general theory of the processes that underlie the formation of natural landscapes. So far, both fields have developed separate bodies of theory (Stallins 2006). Ecology has focused on the concept of spatial self-organization to explain pattern formation of vegetation and other organisms in homogeneous, flat landscapes (Rietkerk and Van de Koppel 2008). Geomorphologists often use a static or linear description of the relation between organisms and physical processes to understand landscape formation, although the effects of vegetation on hydrodynamic and morphological processes are increasingly considered (Le Hir et al. 2000, Paarlberg et al. 2005, Hiemstra et al. 2002, Larsen et al. 2007, Saco et al. 2007, Temmerman et al. 2007, Borsje et al. 2008, Van Wesenbeeck et al. 2008). Mudflat ecosystems provide a unique window on how integration of both fields can lead to detailed understanding of the importance of biogeomorphological interactions and the resulting spatial self-organization on the formation of natural landscapes.

Acknowledgements

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Appendix 2.A

Model parameters, analysis, and testing model assumptions

We present a table of the symbols, interpretation, units, values, and sources used in the model (Table 2.A1). Furthermore, we present the results of a detailed bifurcation analysis of equations x-y ("Model Analysis") to focus on the parameters that are essential to the model's behavior. We also present the detailed methods of the field measurements that were used for testing the model assumptions ("Testing for Model Assumptions").

Table 2.A1: The symbols, interpretation, units, values and sources used in the model.

Symbol	Interpretation	Unit	Value
S_{in}	Sediment input	cm $tide^{-1}$	0.2[a]
E	Maximal erosion rate	$tide^{-1}$	0.03[d]
p_E	Proportion of algae that erode	-	0.90 [d]
K	Carrying capacity of diatoms	g m^{-2}	1 [c]
C	Maximum algal erosion	$tide^{-1}$	15 [d]
r	Intrinsic growth rate diatoms	$tide^{-1}$	0.4 [b]
A	Diffusion factor sediment transport	cm^2 t^{-1}	5 [d]
Q	Half saturation constant diatoms	cm	0.6 [d]
F	Drainage factor	$tide^{-1}$	0.2 [d]
H_{in}	Water level left each tide	cm $tide^{-1}$	0.2 [d]
K_w	Permeability Water	cm t^{-1}	10 [d]
p_K	Porportional of pK	-	0.1
T	Translating water level to permeability	-	200 [d]

[a]Woods and Widdows (2002), [b]Morris (2005), [c]Weerman, unpublished data,

Model analysis

Here, we present the results of a detailed bifurcation analysis of equations x-y. To focus on the parameters that are essential to the model's behavior, we first derived a non-dimensional model from the full model described in equations (1)–(3):

$$\frac{d\delta}{d\tau} = \delta(1-\delta) - \varepsilon\delta C\frac{\omega}{\gamma+\omega} \tag{A1}$$

$$\frac{\partial\sigma}{\partial\tau} = 1 - \varepsilon(1 - p_E\delta)\sigma + \frac{\partial^2\sigma}{\partial\psi^2} \tag{A2}$$

$$\frac{\partial\omega}{\partial\tau} = 1 - \omega\phi + \frac{\delta}{\delta\psi}\left(\kappa(\omega)\omega\frac{\partial}{\partial\psi}\left(\omega + \frac{S_{in}}{H_{in}}\sigma\right)\right) \tag{A3}$$

Here ∂ = Dk^{-1}, σ = rSSin^{-1}, ω = rWHin^{-1}. The spatial dimension is rescaled to ψ = r$^{1/2}$ A$^{-1/2}$ X$^{-1/2}$ and time is rescaled scale to the intrinsic growth rate of the diatoms (t = r). The model has seven rescaled parameters ε = E r^{-1}, γ = q r Hin^{-1}, ϕ = F r^{-1}, κ = K(W) Hin A^{-1} r^{-1}, η=Sin Hin^{-1}, Λ=C, ρ=pE. From this non-dimensional model, we have selected four parameters that were most directly related to habitat characteristics; for example, they would vary in space on tidal flats. These parameters were erosion rate (8), the water level at which diatom losses is half maximal (γ), drainage factor (ϕ), and ρ which describes to what extent sediment erosion is reduced when diatoms are in carrying capacity. For these four parameters we conducted a bifurcation analysis using the bifurcation program AUTO (Doedel et al. 2001). We applied the method of spatial dynamics (Champneys 1996), which is an effective method in analyzing spatial steady state solutions (Sherratt and Lord 2007, Knobloch 2008, Wang et al. 2009). The bifurcation analysis shows that spatial patterns occur for a wide range of parameter values of the erosion rate E and drainage rate F (Fig. 2.A1, gray area). Pattern formation was found to be particularly sensitive to changes in the effects of diatoms on sediment erosion; pattern formation was observed only when pE was larger than 0.5.

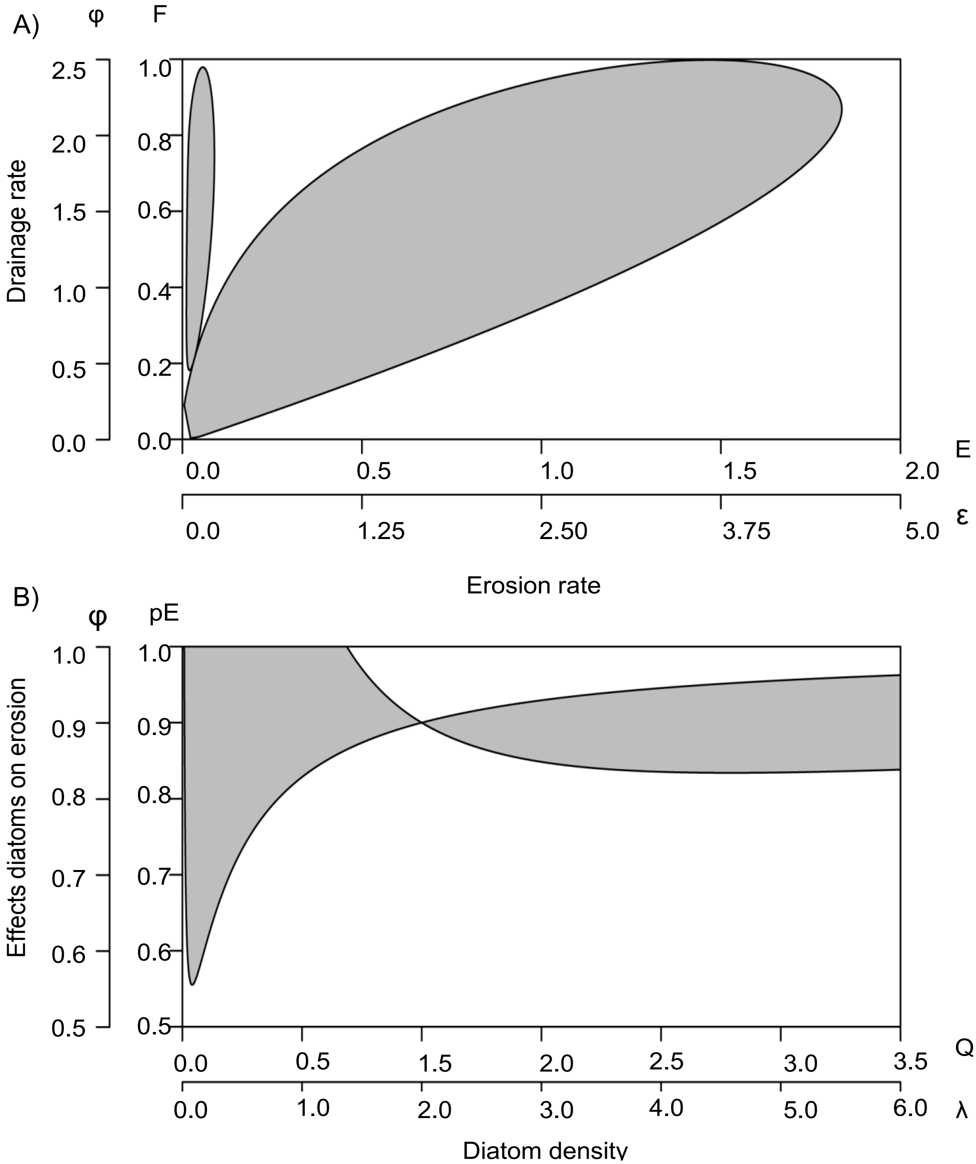

Figure 2.A1: Bifurcation diagram of the sensitivity for changes in erosion rate and drainage factor on spatial patterns (A) and diatom sensitivity and diatom erosion on spatial patterns (B). Grey areas correspond to parameter values where spatial patterns are present and white areas correspond to parameter values where spatial patterns are absent.

Testing for model assumptions

Here, we present the detailed methods of the field measurements that were used for testing the model assumptions. A crucial assumption for the model is that erosion rates are high in the water-covered hollows compared with the emerged hummocks. We tested this assumption by determining the differences in diatom biomass, sediment properties, and the sensitivity of the sediment to erosion between the hummocks and the hollows.

Material and Methods Chlorophyll a content in the top layer of the sediment was used as a measure of diatom biomass, which is the main determinant of the EPS content in the sediment (Paterson et al. 2000) and was measured according to Jeffrey and Humphrey (1975). In April 2007, samples were taken in hollows and on adjacent hummocks during low tide. Surface sediment samples (top 2 mm, inner diameter 36 mm) were taken for chlorophyll-*a* content using a cut-off syringe, and the samples were kept on ice during the fieldwork. Pigment extra ction was done by adding 10 mL 90% acetone to extract the chlorophyll-*a* content, after which this was analyzed using high performance liquid chromatography of the supernatant. Next to the chlorophyll-*a* samples, sediment samples (top 10 mm, diameter 36 mm) for grain size and bulk density analysis were taken using a cut-off syringe. All samples were repeated sevenfold and were kept on ice during the fieldwork. In the laboratory, samples were weighed wet, freeze-dried, and weighed again. After this, the silt and clay content was determined as the fraction of the sediment that is smaller than 63 μm using laser diffraction (Malvern Particle Sizer 2000). We refer to this fraction as silt content in the rest of the article. Differences in sediment shear strength were quantified by using a cohesive strength meter, which provides a measure of the surface normal shear stress threshold beyond which sediment starts to erode, which we refer to as eroding threshold (Tolhurst et al. 1999, 2000). The eroding pressure values measured in this study should not be confused with horizontal bottom shear stress values reported in other studies. The methods that are in use express the eroding threshold in the same units (kPa) but differ in the way the pressure is imposed on the sediment and hence can lead to an order of magnitude difference in threshold values (Tolhurst et al. 1999).

The mathematical model predicts higher sediment accretion and diatom biomass for a self-organized patterned intertidal flat compared with a homogeneous intertidal flat. We tested these model predictions in the field where sediment accumulation was determined as the difference in bed level between April 2009, when patterns were visible, and June 2009, when patterns were at their highest. The sediment bed level differences were compared between plots exhibiting spatial patterns (n=3) and plots lacking clear spatial patterning (n=3); both were present on the Kapellebank, The Netherlands. Bed level measurements were carried out using the sediment erosion bar method, adapted from Austin et al. (1999), where two metal poles of 200 cm length were pushed in the sediment at a distance of 1.5 m away from each other. During the measurements, a portable aluminium frame was placed on top, and the distance from this frame to the sediment was measured in between the poles at 11 points spaced 10 cm from each other with an accuracy of about 1 mm (van Wijnen and Bakker 2001). At each plot, chlorophyll-*a* samples of the top 1 cm of the sediment were taken using a cut-off syringe (diameter 1 cm). In the laboratory, chlorophyll-*a* samples were freeze-dried and analyzed spectrophotometrically after a 48-h extraction in 90% acetone (Jeffrey and Humphrey 1975). Sediment characteristics of these samples were determined as described above.

Statistical analysis. Biotic and abiotic differences between hummocks and adjacent hollows were analyzed using Student's paired t-test (one-tailed). To further analyze the influence of silt and chlorophyll-*a* content on the variability of erosion threshold of hummocks and hollows, we used a GLM with landscape position (hummock or hollows) as a fixed factor and silt content and chlorophyll-*a* content as covariates. Using stepwise reduction from the saturated model (Crawley 2005), all non-significant factors could be removed, resulting in the best model explaining differences in erosion threshold. Similarly, a GLM was used to analyze erosion threshold differences by physical characteristics. The influence of spatial patterns on sediment accretion and chlorophyll-*a* content was analyzed using Student's paired t-tests (one-tailed), testing the increase in elevation between April and June. All statistics were computed using R (http://www.R-project.org).

Appendix 2.B

Spatial patterns and the effects of the removal of underlying physical heterogeneity on the formation of spatial patterns

Here, we present the detailed methods and results of a manipulative field experiment in which we tested the effects of removal of underlying heterogeneity on spatial pattern development.

Figure B1 is an example of an aerial photograph that was used for the regularity analysis (Fig. 2.2). The photograph was taken from a blimp at about 50 m height; dark brown areas correspond to high diatom biomass patches, and light grey areas correspond to almost bare sediment patches.

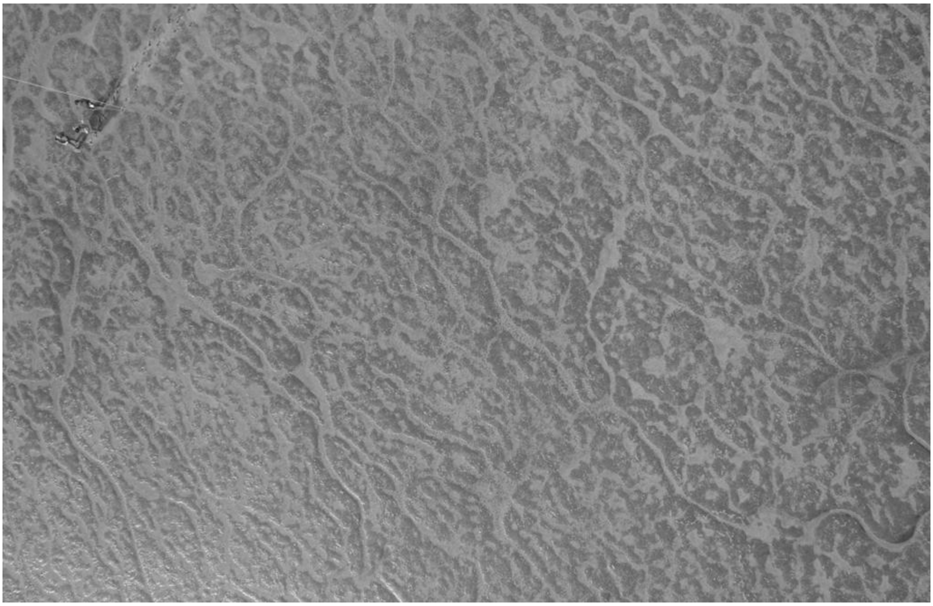

Figure 2.B1: Aerial photograph taken for regularity analysis. Dark grey areas correspond to high diatom biomass, whereas light grey areas correspond to almost bare sediment.

To test the prediction that self-organized patterns could develop in the absence of underlying heterogeneity -for example, even on flat sediment - we removed variation in sediment elevation and diatom biomass.

Materials and methods. On the Kapellebank tidal flat (coordinates), we located 20 plots of 2 m by 2 m in sets of four. We collected the diatoms from the sediment surface using floor sweepers. The top layer of the sediment was then leveled, again using floor sweepers. The recovery of diatom biomass and patchiness within a 2-week period was compared with a control plot, where natural patterning was kept intact. To account for differential effects of diatom removal and sediment leveling, we added two procedural control treatments. In the first procedural control treatment, we removed only the diatoms. In the second, we initially removed diatoms, after which the sediment was leveled and diatoms were smeared back on top of the leveled sediment. All treatments were grouped and replicated five times over the entire intertidal flat following a randomized block design (Sokal and Rohlf 1995). We measured average diatom biomass and bed level differences after 14 days of treatment application. To account for changes in time, we replicated this whole experiment two times within a month. Repeated measurements during the experiment were not possible since entering the plots would disturb the buildup of the spatial structure. At each plot, chlorophyll-*a* samples of the top 1 cm of the sediment were taken using a cut-off syringe (diameter 1 cm). In the laboratory, chlorophyll-*a* samples were freeze-dried and analyzed spectrophotometrically after 48-h extraction in 90% acetone (Jeffrey and Humphrey 1975). Bed level differences were measured using a white board (0.85 m by 0.50 m) on which a grid was drawn that was placed vertically into the sediment in such a way that a hummock and a hollow were included in the pictures. Digital photographs of the grid board (about 2,560 pixels by 1,920 pixels) were geocorrected using Leica image processing software, following the procedure by van der Wal et al. (2005). Hummock development at each location was expressed as the difference between maximum and minimum bed level.

Statistical analysis. The effect of the removal of underlying heterogeneity was determined using a univariate analysis with treatment and sample date as fixed factors and block as a covariate. Normal distribution of the data was checked by visual inspection of Q-Q plots, and Levene's test was used for testing homogeneity of variances.

Results. Treatment application was successful, since after 1 day patterns were still lacking on plots where patterns were removed (Fig. 2.B2A). Time and block did not have a significant effect on chlorophyll-*a* content or bed level differences (ANOVA, $P > 0.05$). Therefore, all the data from different dates and blocks were pooled and analyzed for treatments effects 14 days after the treatment application.

We found that for all treatments, the patterns had fully recovered. No differences were found in bed level between the control plots (3.12 ± 0.34 cm), treatment plots (2.50 ± 0.26 cm), and procedural controls (diatom only: 2.82 ± 0.28 cm; hummock removal only: 2.58 ± 0.34 cm; Fig. 2.B2A, $P > 0.05$). Similarly, no differences in chlorophyll-a content were measured on hummocks between the control plots (133.1 ± 37.5 µg g^{-1}), treatment plots (133.1 ± 37.5 µg g^{-1}) and procedural controls (diatom removal: 124.6 ± 40.5 µg g^{-1}; hummock removal 133.1 ± 37.5 µg g^{-1} or nor in the hollows (control plots: 55.8 ± 19.3 µg g^{-1} and treatment plots: 38.0 ± 17.1 µg g^{-1}) and procedural controls (diatom removal: 46.6 ± 18.6 µg g^{-1}; hummock removal 45.9 ± 11.6 µg g^{-1}cm; Fig. 2.B2A, $P > 0.05$)

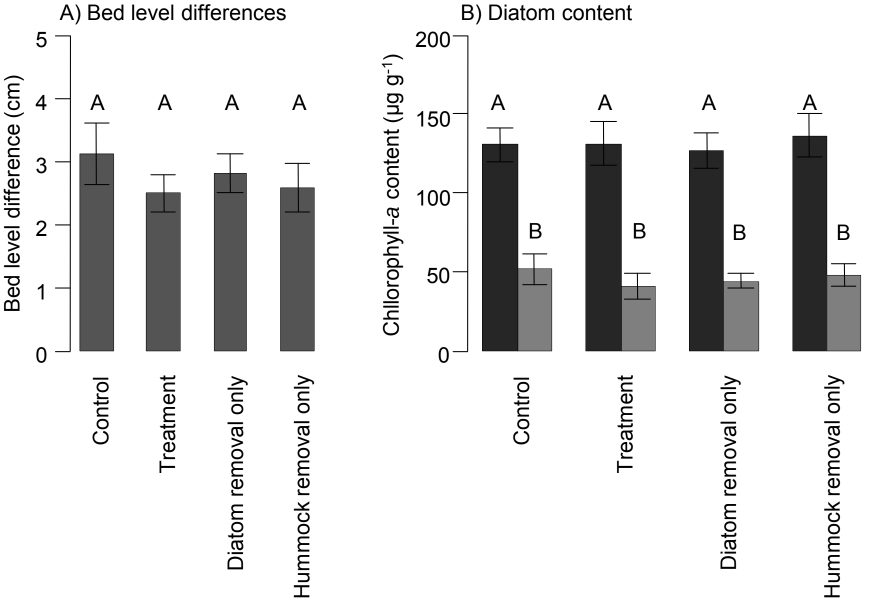

Figure 2.B2: Bed level differences (A) and chlorophyll-a content (B) of the different treatments after 14 days of treatment application. Grey bars in figure D4B represent hummocks and white bars represent hollows (n=10, standard bars denote SEM).

Chapter 3

Top-down control inhibits spatial self-organization of a patterned landscape

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Abstract

Regular, self-organized spatial patterns in primary producers have been described in a wide range of ecosystems, and are predicted to affect community production and resilience. Although consumers are abundant in most systems, the effect of trophic interactions on pattern formation in primary producers remains unstudied. We studied the effects of top-down control by herbivores on a self-organized landscape of regularly-spaced, diatom-covered hummocks alternating with water-filled hollows on an intertidal mudflat. Spatial patterns developed during spring, but were followed by a rapid collapse in summer, leading to a flat landscape with low diatom densities and little variation in sediment bed level. This dramatic decline co-occurred with a gradual increase of benthic herbivores. A manipulative field experiment, where benthic herbivores were removed from the sediment, revealed that both diatom growth and hummock formation were inhibited by the activity of benthic herbivores. Our study provides clear evidence of top-down control of spatial self-organized patterns by benthic herbivores within a biological-geomorphic landscape.

Introduction

Spatial self-organization has been observed in ecosystems all over the world, from tropical arid lands to boreal peat lands (Klausmeier 1999, Eppinga et al. 2009). These self-organized spatial patterns result from strong, local feedback mechanisms, whose effects can scale-up to form regular or scale-free patterns (Pascual and Guichard 2005, Rietkerk and Van de Koppel 2008). Regular patterns emerge where positive and negative feedbacks occur at different spatial scales (Rietkerk and Van de Koppel 2008). In semi-arid ecosystems, for example, vegetation-induced infiltration of rain water into the sediment causes local facilitation between plants, reduces water availability at greater distances, resulting in regularly spaced vegetation patches (Klausmeier 1999, Couteron and Lejeune 2001, Von Hardenberg et al. 2001). Spatial self-organization has important implications for the functioning of ecosystems, as it can increase primary production and enhance the stability of ecological communities (Rietkerk et al. 2002, Van de Koppel and Rietkerk 2004, Van de Koppel et al. 2005).

Top-down control by herbivores on spatially patterned vegetation can have non-linear effects on community production and resilience in self-organized systems (HilleRisLambers et al. 2001, Rietkerk et al. 2004a). Despite their potential impact, little is known about the effect of top-down control on the process of self-organization within ecosystems, or about the potential for herbivores to cause catastrophic shifts in spatially self-organized vegetation (Kéfi et al. 2007). Previous model studies have prediced a breakdown of self-organization once a threshold of grazing pressure is exceeded, followed by a collapse to an homogeneous state with low vegetation cover (Rietkerk et al. 2004a, HilleRisLambers et al. 2001). This collapse affects food web structure and ecosystem function, since an important food source is lost. While it is well-known that predator-prey interactions can cause spatial patterns in ecological communities (e.g. Huffaker 1958, Hassell et al. 1991, Maron and Harrison 1997), there is little empirical evidence for the influence of top-down control on landscape formation in a spatially self-organized ecosystem.

In this study, we test the influence of top-down control on spatial self-organization on an intertidal mudflat. In this ecosystem, a spatial pattern of elevated hummocks alternating with water filled hollows develops each spring (Blanchard et al. 2000, de Brouwer et al. 2000, Gouleau et al. 2000, Whitehouse et al. 2000, Lanuru et al. 2007), due to a strong interaction between diatom growth and geomorphological processes (Fig. 3.1A and Chapter 2). Patterning on tidal flats can be a seasonal phenomenon, in particular on tidal flats where bed level differences between hummocks and hollows are small. In these systems, spatial patterns form in March-April (Fig. 3.1A) and disappear late May-June, returning to a homogenous bare landscape (Fig. 3.1B). Observations from this system suggest that the decline is potentially caused by increased grazing pressure by benthic animals (de Brouwer et al. 2000). The effects that macrobenthic grazers have on the interaction between diatoms and sedimentation have been investigated in a number of field and model studies (Wood and Widdows 2002, Paarlberg et al. 2005, Le Hir et al. 2007), revealing that herbivore grazing can influence sediment dynamics both directly by feeding and indirectly through bioturbation. Hence, intertidal mudflats provide a unique opportunity to investigate the effects of increasing top-down control in spatially self-organized ecosystems, and to test the effects of grazing on self-organization and landscape formation in manipulative experiments.

Here, we present a study on the effect of seasonal changes in top-down control on spatial self-organization and landscape formation on an intertidal flat. We first determined seasonal changes in diatom biomass, elevation differences in the landscape, and herbivore abundance in two consecutive years. We then hypothesized that benthic herbivores cause a decline in diatom biomass and bed level differences leading to a change from a spatial self-organized landscape into a system lacking landscape patterns. To test the causality of this decline, we next performed a manipulative field experiment with defaunated sediment where we applied patches of sediment including and excluding herbivores. We hypothesize that the patches where herbivores are removed reveal increased diatom content and will maintain their bed level relative to the surrounding sediment, while the grazed plots will show a decreased diatom content and bed level. Using this combination of observational and manipulative methods, we found clear evidence for increased top-down control on spatial self-organization, changing the ecosystem from a diatom-covered, patterned mudflat into an erosive mudflat lacking spatial self-organization.

Figure 3.1: Photographs of the intertidal flat the Kapellebank, the Netherlands. In April, a spatially patterned intertidal flat with hummocks and hollows is visible (A). These patterns have disappeared in June and the landscape has changed into a homogenous intertidal flat with low diatom biomass (B).

Materials and methods

Study system

A field study was performed at the Kapellebank, a tidal flat along the edges of the Westerschelde estuary, in the Netherlands (51º27’ N and 3º58’ E). From early spring until the onset of summer, spatial diatom patterns were observed on 80% of the surface of this intertidal flat. Pattern formation is initialized when a small, random increase in sediment elevation improves diatom growth. On top of these elevations, diatoms accumulate, forming a visible brown biofilm, which in turn is strengthened by the excretion of extracellular polymeric substances (EPS) (Neumann et al. 1970). EPS inhibits erosion by increasing sediment cohesion and decreasing bottom roughness (Paterson 1989, Sutherland et al. 1998a, Sutherland et al. 1998b). This results in an increase of sedimentation of fine-grained particles (de Brouwer et al. 2000, Montserrat et al. 2009), which subsequently stimulates diatom growth (Van de Koppel et al. 2001). In the hollows, water accumulates during low tide, inhibiting the build-up of EPS as it dissolves in the overlaying water (Blanchard et al. 2000, Paterson et al. 2000). This in turn leads to increased erosion of diatoms and sediment in the hollows during inundation. The interaction between accumulation of sediment on the hummocks and drainage of water towards the hollows generates a scale-dependent feedback of small-scale facilitation and larger-scale inhibition of diatom growth. This scale-dependent feedback provides a possible explanation for spatial pattern formation when herbivore densities are low (Chapter 2). In the Westerschelde estuary, a peak in microphytobenthos occurs in Apil-June (Herman et al. 2001, Montserrat et al. 2008). During this period, spatial patterns are clearly present at several locations in the estuary, but disappear from the end of May onwards, not returning until spring next year (de Brouwer et al. 2000).

Field observations

To investigate whether increased herbivore numbers are coincident with observed collapse of self-organized diatom patterns and the co-occuring physical landscape on intertidal flats, we monitored macrobenthic animal densities from February until the patterns had disappeared in June in both 2007 and 2008. In 1-2 week intervals during each year, we sampled diatom biomass, macrofauna density, sediment grain size profiles, and elevation differences between hummocks and hollows. Sediment depth profiles were determined monthly April-June in 2007. In 2008, the temporal sampling frequency was lower compared to 2007, as the sampling in 2008 was done to determine consistency of the pattern found in 2007 for patchiness of diatoms and influence of macrofauna.

Difference in relative bed level elevation between hummocks and their adjacent hollows, was measured using a plastic white grid board (0.85 by 0.50 m). The board was pushed in and placed level to the sediment, set perpendicular to the patches in such a way that the grid board included the lowest and highest point of a hollow and hummock, respectively. A digital photo (2560 by 1920 pixels) was taken of the white grid board and the photographs where geo-referenced and geo-corrected using Erdas image processing software (Erdas Imagine Professional v.9.3). To distinguish between the grid board and sediment, an unsupervised classification was made using the image processing software. After classification the topographic profiles were extracted and a value of relative bed level height of every 0.25 cm along the board was calculated (for the detailed procedure of these methods see van der Wal et al. 2005). Bed level differences at each location were determined from this image as the difference between maximum and minimum bed level over the entire horizontal range.

To measure diatom biomass on the intertidal flat, chlorophyll-*a* content in the top layer of the sediment was determined as a measure for diatom biomass on hummocks. Samples were taken of the upper 2 mm of the sediment with a 100 ml syringe, where the end was cut off (inner diam. 36 mm). Samples (n=5) were placed on ice during fieldwork and stored at -80°C upon arrival in the laboratory. Pigment extraction was done by adding 10 ml 90% acetone to lyophilized sediment followed by chlorophyll-*a* quantification by HPLC of the supernatant (Jeffrey and Humphrey 1975). Chlorophyll-*a* concentrations are expressed as µg chlorophyll-*a* per g dry sediment (µg g^{-1}).

Macrofauna density was measured adjacent to the sediment samples taken to determine diatom content. Twenty centimeter deep macrofauna samples (n=3) were extracted using a core (inner diam. 11 cm). The cores were sieved through a 0.5 mm mesh size and fixed with a neutralized 8% formalin solution. Prior to analysis, samples were colored using 0.01 % Rose Bengal. The identification of macrofauna in the cores was done up to genus level to determine the most abundant taxa and calculate total macrofauna abundance.

We monitored changes in sediment grain size along depth profiles, as changes in grain size distribution could reflect accumulation of fine-grained sediment during pattern formation. Samples for sediment grain size analysis (n=5) were taken using a syringe, with the end cut off (inner diam. 36 mm), at the beginning of April, May and June in 2007. Samples were kept on ice during the fieldwork before they were sliced at depths of 0 to 0.5, 0.5 to 1.0, 1.0 to 1.5, 1.5 to 2.0, 2.0 to 3.5 and 3.5 to 5 cm in the laboratory. These subsamples were freeze-dried, after which the silt and clay content was determined as the fraction of the sediment that is smaller than 63 µm using a Malvern Mastersize 2000. This fraction included both clay (< 2µm) and silt particles (2-63 µm), therefore we refer to this fraction as mud content in the rest of this chapter.

Testing the effects of benthic herbivores

A manipulative field experiment was carried out to test the influence of benthic herbivores on the disappearance of spatial patterns. We compared differences in diatom growth and sediment elevation change between natural sediment and sediment where herbivores were removed. The experiment was carried out in May 2007 when diatom patterns had disappeared. Prior to the experiment, sediment obtained from the intertidal flat was frozen (-20 °C) for 48 hours, removing all living macrofauna from the sediment. After defrosting, the sediment was taken back to the intertidal flat where it was applied on the ambient sediment surface. Either natural or defaunated sediment was applied in a round cut-off tube (inner diam. 32 cm), where 4 L of sediment was added (Fig. 3.2A), resulting in hummocks which are slightly smaller compared to the natural hummocks. The cut-off tube was removed after application which resulted in a patch of sediment with macrofauna and without macrofauna (Fig. 3.2B). The defaunated sediment was applied on a plastic sheet to prevent animals from below to enter, taking care that this sheet was not exposed. Each treatment was replicated 5 times. Sediment bed level and chlorophyll-*a* content were measured as described above, at the start of the experiment and after 9 days. The sediment bed level difference between the top of the hummock with the surrounding sediment was taken as measure for hummock bed level. Hummock formation was calculated as the difference in hummock bed level at the start and end of the experiment. Similarly the difference between chlorophyll-*a* concentration at the start and end of the experiment was taken as measure for relative changes in diatom content. We tested the effectiveness of our treatment by sampling macrofauna density at the end of the experiment.

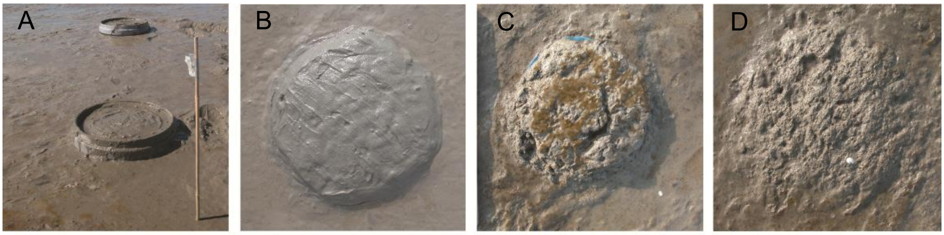

Figure 3.2: Experimental set-up of the manipulative field measurement with tubes filled with 4 L of sediment (A), artificial hummock after removal of the tube (B), patches after nine days of experiments where herbivores were removed (C) and herbivores were present (D). Picture size of B, C and D are 50 cm width and height.

Statistical analysis

One-way ANOVA was used to identify changes in diatom content, macrofauna density and patchiness for the separate sampling dates for both years of the observational study, followed by Tukey HSD post hoc comparison. Normal distribution of the data was ensured by visual inspection of Q-Q plots and Levene's test was used to check for homogeneity of variances. In cases where the data were not normally distributed, log-transformation was performed. An ANOVA was performed with sampling date and vertical distribution as treatments to identify a possible interactive effect of vertical distribution and month on mud content. The experimental data was analysed using Welch's two sample t-test (one tailed). All statistics were computed using R (http://www.R-project.org).

Results

During both years, we observed a gradual increase in benthic diatom biomass early in the season, until chlorophyll-*a* concentrations in the upper 2 mm reached values of 150-180 $\mu g\ g^{-1}$. Then, within about a month, chlorophyll-*a* concentrations dropped to levels below 50 $\mu g\ g^{-1}$ (Fig. 3.3A and B, $P < 0.001$). This decrease was particularly strong in 2007 and appeared to be more gradual in 2008. Bed level differences also decreased significantly during this period, although the decrease was more gradual (Fig. 3.3C and 3.3D, $P < 0.001$). During the season, this process seemed to be irreversible. Once the diatom mats had disappeared, bed level variation decreased and did not come back during the remainder of the year (de Brouwer et al. 2000).

Macrofaunal densities did increase slightly during the period of diatom disappearance in both 2007 and 2008 (Fig 3.3). After the rapid disappearance of benthic diatoms, bed level differences declined, and macrofauna, mostly animals that feed on benthic diatoms and bioturbate the sediment like *Aphelochaeta, Corophium* and *Macoma*, increased very rapidly (Fig. 3.4). These bioturbating taxa dominated >80% of the total benthic herbivore abundances we found, while a minor fraction (<5%) were omnivores. Moreover, mud content in the sediment was reduced and homogenized in depth when macrofauna became more abundant (Fig. 3.5). Analysis of mud content revealed that month explained a significant part of the variance ($P < 0.001$), indicating that the mud content was significantly higher in April compared to May and June. There was also a vertical distribution in mud content where the mud content was significantly higher in the top layer (0-1 cm) in April and May (Fig. 3.5, $P < 0.001$) while in June the mud content in the top layer was equal to lower sediment layers (Fig. 3.5, $P > 0.05$).

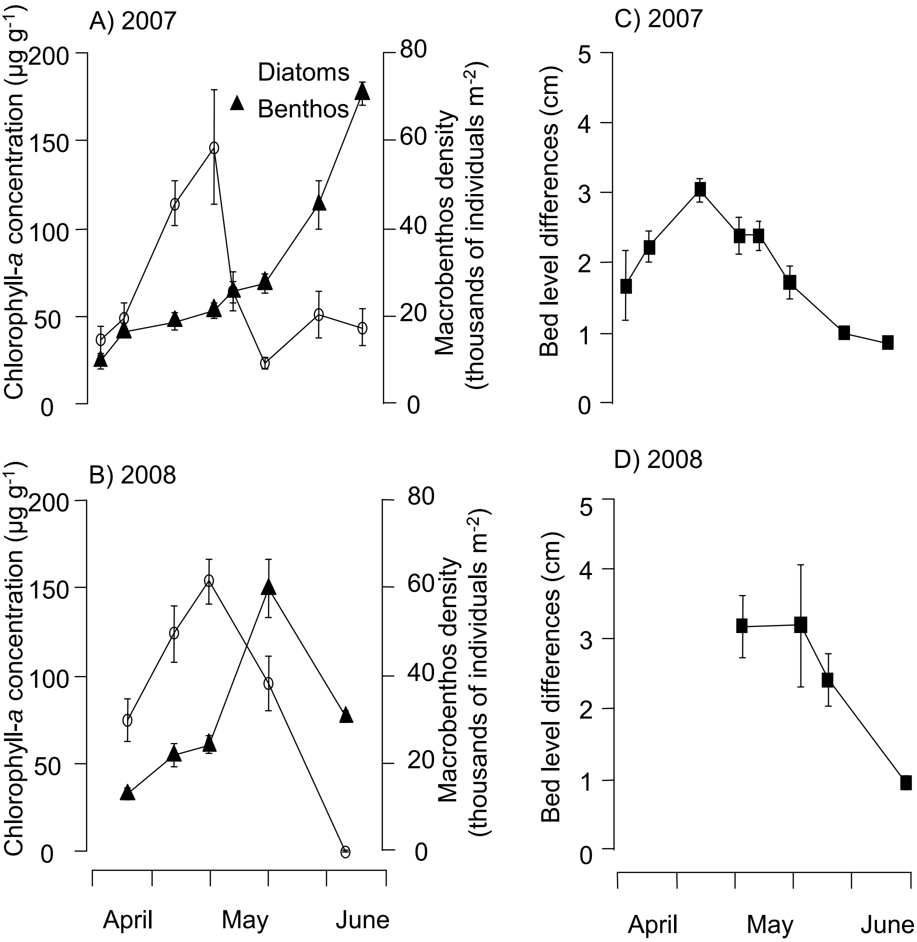

Figure 3.3: Average diatom density ± SEM (round symbols, n=5), macrobenthos density (triangle symbol, n=3; A and C) and bed level differences (square symbol, n=5;) for 2007 and 2008.

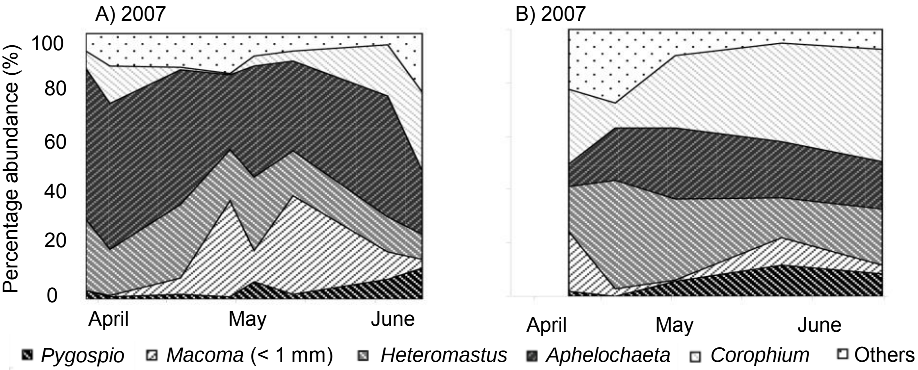

Figure 3.4: Cumulative abundances of most abundant macrofauna taxes that we found during the season in 2007 (A) and 2008 (B)

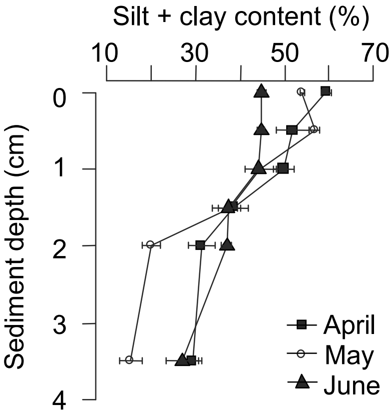

Figure 3.5: The average mud percentage ± SEM (% <63 μm) at different sediment depths at the beginning of April (squares), May (rounds) and June (triangle) 2007.

Experimental test of herbivore influence

The manipulative experiment carried out to test the effect of herbivores on pattern formation and diatom biomass revealed a striking effect of herbivory on both hummock formation and diatom growth. Diatom biomass was significantly higher in defaunated sediments (Fig. 3.6A, $P < 0.05$), being an order of magnitude greater compared to untreated sediment. In addition, nine days after herbivore removal, hummock bed level increased by nearly two centimeters in the defaunated treatments, most likely due to the combined effects of sedimentation within the experimental patches and erosion around the patches. In contrast, hummock bed level decreased in the treatment where herbivores were present (Fig. 3.6B, $P < 0.001$), resulting from erosion on top of the patches. Nine days after treatment application, macrofauna densities were ten times lower in the defaunated treatments (2.8 ± 0.7*1000 ind m-2, mean ±

SEM) compared to the controls (24.3 ± 6.3 *1000 ind. m-2), showing that the effect of our treatment lasted for the entire experimental period.

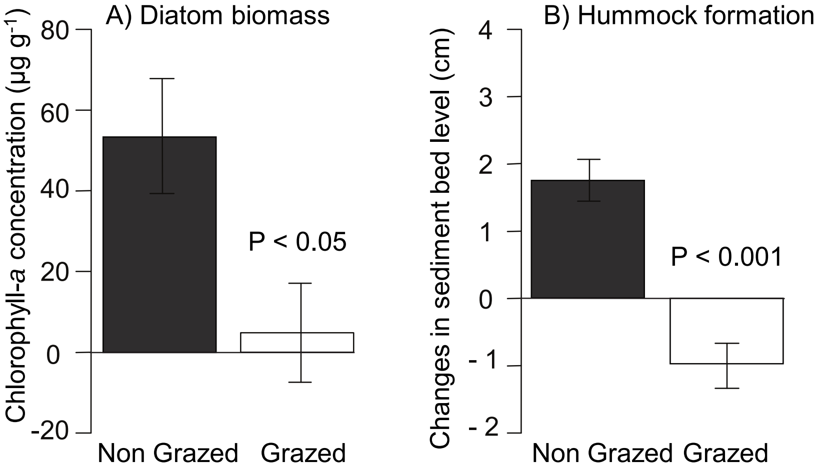

Figure 3.6: Relative differences in bed level ± SEM (A) and chlorophyll-a concentration ± SEM (B) of the field experiment with two treatments: non grazed (black bar, n=5) and grazed (white bar, n=5).

Discussion

Our results reveal a clear top-down effect of herbivores causing the disappearance of self-organized spatial patterns of diatoms and the co-occurring physical landscape. In each of the two consecutive years, a gradual build-up of diatom biomass in the top layer of the sediment was followed by a rapid decline of benthic diatoms to very low biomass, co-occurring with a gradual build-up of benthic animal numbers. During this transition, the landscape changed from a regular pattern of hummocks and hollows into a flat homogeneous landscape with no visible diatom biofilms. The on-set of the diatom population collapse begins after several macrobenthos species, particular *Macoma* larvae, have settled on the intertidal flat, in which was particularly evident in 2007. Benthic invertebrates predominantly prefer primary settlement at sites with high diatom biomass and EPS content (Keough and Raimondi 1995, Dahms et al. 2004, Patil and Anil 2005, Van Colen et al. 2009). Thus the importance of benthic diatoms and associated EPS content on top of the hummocks for settlement of larvae from the water column is evident. A manipulative field experiment revealed that benthic herbivores prevent diatoms from stabilizing the sediment, and thereby may block the mechanism of spatial self-organization, as the absence of herbivores resulted in the re-occurrence of hummocks on the intertidal flat. Our study highlights the effect of top-down control can extend beyond the direct effect of consumption of the prey community in self-organized ecosystems.

Our results are in close agreement with the predictions of theoretical studies on self-organized patterns generated in other systems including arid ecosystems. Mathematical models have predicted that, along a gradient of herbivore impact, arid vegetation undergoes a transition from self-organized spatial patterns to a homogeneous degraded state without vegetation (HilleRisLambers et al. 2001). Increasing density of benthic herbivores, possibly co-occurring with physical disturbance (e.g. wind induced waves), disabled the formation of self-organized patterns on intertidal flats. This resulted in a collapse of the spatially patterned system which was seen in both years of observation. Although the general predictions are consistent with observational studies on the effects of increased grazing on patch formation in arid-grasslands (Rietkerk et al. 2000, Nash et al. 2003, Stavi et al. 2009), experimental evidence for the effects of grazing on the formation of spatial vegetation patterns, and on the interaction between plants and abiotic processes, is limited. Our experimental results confirm the hypothesis that grazing affects the functioning (e.g. sediment accretion and diatom biomass) of self-organized landscapes by interfering with feedbacks between organisms and sedimentary processes. Mechanisms resulting in spatial patterns that act on intertidal flats and semi-arid ecosystems are different and the temporal scale of the effect is much shorter on intertidal flats compared to arid ecosystems. Hence, increased top-down control appears to have similar effects in both ecosystems, emphasizing the generality of our results.

The importance of predator-prey relations and top-down control for spatial self-organization has mostly been studied in systems where predator-prey interactions are the cause of spatial patterns (Hassell et al. 1991, Huffaker 1958). The occurrence of spatial patterns in predator prey systems are for example observed in insect parasitoid-host interactions (Maron and Harrison 1997). In our system, the benthic grazers are important, yet they are not part of the process that causes spatial pattern formation. These grazers rather form a higher trophic level that influences pattern formation by reducing the density of one of the components of the pattern-forming feedback, the diatoms. Herbivores reduce diatom biomass and associated EPS (de Deckere et al. 2001) this can cause a failure of the diatom-sedimentation feedback. Herbivores may block the spatial self-organization as a landscape forming process and in the end induce a flat degraded landscape that lacks significant diatom cover. Prior research has shown that benthic herbivores themselves are under top-down control by predators e.g. migratory birds (Daborn et al. 1993), emphasizing the importance of top-down control in influencing the spatial structure and functioning of intertidal ecosystems.

It is likely that in our system, the decline of diatom biomass cannot be solely explained by direct grazing, but is partly due to the indirect effects of benthic herbivores due to bioturbation and its physical effect on diatom biofilm integrity. Most species we found (>80%) are deposit feeders which feed from a funnel and crawl through or over the sediment while feeding on the diatoms (Ysebaert et al. 2003). While feeding, these herbivores mix sediment and the attached benthic algae (Solan et al. 2003), and may inhibit the build-up of mud in the upper layers of the sediment (Mermillod-Blondin et al. 2004). These indirect effects of benthic grazing in part explain the dramatic decline in benthic algae. Moreover, a number of studies emphasize that, in general, only a small fraction of benthic primary production on tidal flats is directly grazed by macrobenthos (Herman et al. 2000, Van Colen et al. 2010). Nevertheless, the taxa we find just before and during the collapse, e.g. *Corophium, Macoma* and *Aphelochaeta*, feed mostly on benthic diatoms, as is evidenced by their stable isotope ratio (Herman et al. 2000). Hence, their biomass is mostly supported by local diatom primary production, which implies that the intensity of bioturbation is an indirect effect of grazing. Similar effects of herbivore bioturbation affecting sediment texture and soil erosion have been observed on intertidal flats (e.g. Widdows et al. 2000), salt marshes (e.g. Van Wesenbeeck et al. 2007) and in terrestrial ecosystems (e.g. Stavi et al. 2009). For example in arid-ecosystems trampling by herbivores results in water loss and erosion (Stavi et al. 2009). In arctic tundra ecosystems, grubbing of vegetation by geese has a devastating effect plants changing large areas of marsh to bare mud (Jefferies 1988, Iacobelli and Jefferies 1991). This shows that both in terrestrial as well as estuarine ecosystems, herbivores can impose strong indirect effects on biotic components of the ecosystem as a consequence of their grazing, thereby changing landscape morphology.

Ecosystems that exhibit strong feedback mechanisms are associated with catastrophic shifts and bistability, where sudden, irreversible switches to alternative states may occur in response to gradually changing environmental conditions (Scheffer et al. 2001). Spatial patterns can indicate the presence of, and proximity to, a switch point (Rietkerk et al. 2004a, Kéfi et al. 2007). The co-occurrence of both self-organized spatial patterns and a dramatic decline of diatom biomass during spring, in particular in 2007, suggest that two stable states may occur on intertidal flats (Van de Koppel et al. 2001). However, a field experiment where diatom patterns and landscape structure was removed using floor sweepers, showed complete recovery of both diatoms and hummocks within two weeks, indicating the absence of alternative stable states (Chapter 2). Although this experiment was replicated three times during the season until just before the transition into the flat sediment state, no differences between treatments in recovery time were found (Chapter 2). Hence, we have no conclusive evidence for considering them as a catastrophic shift between alternate stable states, even though the decline of diatom biomass was dramatic. Nevertheless, the collapse of diatom densities was striking and remarkably occurs at a density of about 20,000 individuals m^{-2} in both years. This could indicate that there is a macrobenthos density threshold at which the collapse occurs. The precise position of this threshold is likely to vary from system to system, as sedimentary characteristics (e.g. water content) and physical forcing (e.g. winds) also play role in determining the erodability of sediments (de Brouwer et al. 2000, Tolhurst et al. 2008). Diatom biomass can decrease rapidly when exposed to offshore winds, even at relatively low wind speeds (de Jonge & van Beusekom 1995; de Brouwer *et al.* 2000). In 2007, stronger winds were present when diatoms were declining, while conditions in 2008 were less severe, providing a possible explanation for the stronger collapse in 2007 relative to 2008 (Data: Dutch Ministry of Transport, Public Works and Water Management, http://www.hmcz.nl). Hence, it is possible that multiple factors are responsible for the dramatic decline that is observed in 2007. Nevertheless our experiment clearly identifies top-down control as an important factor in determining diatom densities.

Our study highlights that the effect of top-down control can extend beyond the direct effect of consumption of the prey community in self-organized ecosystems. By lowering diatom stocks, benthic herbivores inhibit the diatom-sediment feedback, which is responsible for the spatial self-organization, resulting in the disappearance of self-organized spatial patterns of diatoms and the co-occurring physical landscape. Loss of self-organization has far-reaching implications for intertidal ecosystems. During this diatom collapse, the sediment bed level becomes more erosive and as a consequence the sediment stored in the hummocks is lost to the overlying water within a few weeks. This loss can affect the entire benthic community (Montserrat et al. 2008), as sediment is an important source of nutrients (Van de Koppel et al. 2001) and organic matter (Herman et al. 2000). Moreover, the eroded sediment can increase water turbidity and can thereby affect planktonic as well as benthic primary production (Herman et al. 2001, Staats et al. 2001). Hence, by disabling self-organization processes, top-down control can dramatically influence both community structure and ecosystem functioning.

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Chapter 4

Macrofauna distribution and habitat modification on a spatially patterned intertidal flat

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Abstract

Natural ecosystems can show regular spatial vegetation patterns, which develop from small-scale ecological interactions. Even though studies suggest that grazers can play a major role in controlling vegetation distribution in ecosystems with regular vegetation patterns, the distribution of grazers and the effects of grazing on vegetation in spatially patterned ecosystems remains poorly understood. Here, we study how macrofauna grazers are distributed on a spatially patterned intertidal flat and how they interact with benthic microalgae. The study was carried out on an intertidal flat where each year a regular spatial pattern of diatom-covered hummocks and water-filled hollows develops. Overall benthic macrofauna was equally abundant on hummocks and hollows, while benthic algal biomass was 5-fold lower in the hollows. Nevertheless, some species like *Corophium volutator*, preferred the diatom-covered hummocks. This was confirmed in laboratory experiments where we tested the interactive effect of landscape morphology and benthic algae abundance. Laboratory experiments where we analyzed the grazing effects of *Hydrobia ulvae* and *C. volutator* on benthic algae, revealed that both species increased biomass-specific primary production. This indicates that benthic grazers stimulate algal growth, which explained the high grazer abundances in the nearly bare hollows. Increased production of benthic algae may be a key factor uncoupling herbivore density from diatom biomass both in space and in time, and affecting primary production and spatial patterns on intertidal flats.

Introduction

Natural ecosystems can exhibit strikingly regular spatial patterns which develop from local feedback processes between organisms and the physical environment. Regular landscape patterns have been described in various ecosystems, among which tiger bush in arid ecosystems (Klausmeier 1999), striped oligotrophic peat lands in boreal regions (Eppinga et al. 2009), and mussel beds in intertidal systems (Van de Koppel et al. 2005) are well-known examples (see review: Rietkerk & Van de Koppel 2008). In cases where pattern formation depends on interactions involving primary producers (e.g. benthic algae or vegetation), theoretical predictions suggest that grazers can have a profound effect on spatial patterns (Kéfi et al. 2007). This may result in irreversible shifts from a spatially patterned to a homogeneous state (Rietkerk et al. 2000, HilleRisLambers et al. 2001, Chapter 3). Such a shift can be expected to feed back on the grazers, but despite the assumed role of herbivores in regularly patterned ecosystems, very little is known about the dynamics of grazers in these interactions.

Grazers can affect plant biomass directly by reducing plant standing crop, but they also interact with plants indirectly by releasing nutrients or reducing light limitation (McNaughton 1979). This can have an important effect on plant production (Dyer 1975, McNaughton 1979). The concept of herbivore compensation suggests that at low grazing intensities, primary production increases with increased grazing intensity, while at high grazing intensities the consumption of plant biomass outweighs increased per capita production, which results in a net negative effect of grazing (McNaughton 1979). This phenomenon was first described in terrestrial ecosystems (McNaughton 1979), and in stream ecosystems (Gregory 1983). Several mechanisms have been highlighted as mechanisms likely to explain the increased primary production, such as changes in species composition (Kaehler & Froneman 2002) or increased nutrient regeneration (Steinman 1996). Whether and how this affects pattern formation remains unstudied thus far.

In this chapter, we present a field and laboratory study that investigated the mechanisms influencing the spatial distribution of macrofauna on a patterned intertidal flat. In early spring, regular spatial pattern develops on due to an interaction between benthic algae and sedimentary processes (Chapter 2). This pattern is characterized by regularly spaced, diatom-covered hummocks alternating with almost bare, water-filled hollows (de Brouwer et al. 2000, Chapter 2). Increased herbivore density, as the season progresses, results in a rapid degradation, where the ecosystem shifts into a homogenous tidal flat by both the direct (the removal of benthic algae), and indirect (e.g. bioturbation) effects of grazing (Chapter 3). Two abundant grazers in this system are the amphipod *Corophium volutator* and the gastropod *Hydrobia ulvae.* Both are deposit-feeders and feed predominantly on benthic diatoms.

They have significantly different feeding mechanisms and therefore affect their environment in different ways. *H. ulvae* mixes the sediment horizontally (Orvain et al. 2006) while browsing the sediment for diatoms (Jensen & Siegismund 1980, Levinton & Dewitt 1989). Feeding by *C. volutator* mixes the sediment vertically, as it feeds from semi-permanent burrows in the sediment and gathers sediment particles with its enlarged second antennae (Gerdol & Hughes 1994a, b, Hagerthey et al. 2002, Riisgard & Schotge 2007). These two feeding strategies might result in a different spatial distribution of these grazers within this patchy landscape and hence affect pattern formation and primary productivity.

This study focuses on grazer distribution on a spatially patterned intertidal flat and the effects grazers can have on benthic microalgae. We hypothesized that macrofauna species prefer the diatom-covered hummocks in the field. To test this hypothesis, we measured the distribution of macrofaunal species in the presence or absence of spatial patterns. Moreover, we analyzed the effect of food abundance and landscape morphology on these distributions in the laboratory. We then investigated, in a laboratory experiment, the hypothesis that herbivory stimulated microalgal production, explaining the observation of high macrobenthic biomass at locations with low benthic microalgae biomass. Here, *C. volutator* and *H. ulvae* were added to benthic microalgae biofilms and their grazing effect on these biofilms were measured after several days of grazing. We hypothesized that these macrofauna species are able to change their habitat by increasing biomass-specific production.

Materials and methods

Field sampling: Spatial distribution of benthic microalgae and macrofauna

We measured the distribution of macrofauna and benthic algae on the intertidal flat Kapellebank. This is a muddy intertidal flat (51º27’ N and 3º58’ E) in the Westerschelde, the Netherlands. On this tidal flat each spring a spatial pattern of diatom-covered hummocks and alternating water-filled hollows develops (Chapter 2). Samples were taken early May on top of the hummocks and in the adjacent hollows. In June, when patterns had disappeared, we repeated our sampling. Chlorophyll-*a* content was determined by taking sediment samples from the upper 2 mm of the sediment with a 100 ml-cut off syringe (inner ø 36 mm). Samples (n=5) were placed on ice and stored at -80°C upon arrival in the laboratory. Pigment extraction was done by adding 10 ml 90% acetone to lyophilized sediment followed by chlorophyll-*a* quantification by HPLC of the supernatant (Jeffrey & Humphrey 1975). Macrofauna samples were taken (n = 5) using a core (inner Ø 11 cm, 20 cm deep) next to the samples for chlorophyll-*a* content. The cores were sieved through a 0.5 mm mesh size and fixed with a neutralised 8% formalin solution. Prior to analysis, samples were coloured using 0.01 % Rose Bengal. Identification of macrofauna in the cores was done up to genus level.

Material collection and preparation for laboratory experiments

Sediment was collected at the Kapellebank. After collection, the sediment was mixed and defaunated by freezing it at -20 °C for at least 24 hours. Prior to the experiment, sediment was defrosted and placed in the experimental units. Benthic algae were collected at the Kapellebank by scraping of the top layer of the sediment (± 5 mm). Macrofauna and most of the meiofauna was removed by sieving (mesh size 150 µm) the sediment with filtered seawater (0.45 µm). The diatom suspension was sprayed on a defaunated sediment layer of 5 cm thickness. The benthic algae were subsequently cultured for a week to form a benthic microalgae. *C. volutator* was collected at Kapellebank by sieving sediment (up to 10 cm depth, mesh size 1 mm) and *H. ulvae* was collected 20 km East of the Kapellebank on an intertidal flat near Ritthem (51º27' N and 3º39' E) by sieving the top layer (1 cm) of the sediment (mesh size 1 mm). Both grazers were allowed to adapt to the experimental conditions for two weeks. Experimental conditions were equal for all experiments. A tidal regime was applied (6h:6h) using filtered seawater. Light intensity was 120 ± 4 µW cm^{-2} (mean ± SEM), with a light: dark cycle of 12:12 hours and temperature was set at 13 °C in the laboratory.

Experimental design of the laboratory experiments

We carried out laboratory experiments to test if the distribution of the grazers *H. ulvae* and *C. volutator* is affected by landscape morphology or food abundances. We created artificial hummocks, hollows and flat sediment, both in the absence and presence of benthic algae. A tidal tank (30 × 40 × 10 cm) was established in a temperature controlled room and filled with defaunated sediment. In each tank a randomized block design with in total 30 patches (Ø ~5 cm) and 6 treatments were created. These treatments included 3 types of landscape morphology (hummock, hollow or flat) and 2 levels of food presence (absent or present). Hummocks were ~2 cm higher compared to the flat treatments and hollows were ~2 cm lower compared to the surrounding sediment. Either 100 *C. volutator* individuals or 100 *H. ulvae* individuals were randomly added to the experimental tank. Preliminary experiments showed that the settlement of *C. volutator* took longer compared to *H. ulvae*, therefore, *H. ulvae* presence on the patches was counted after 1h and *C. volutator* abundance was counted after 24h. In total 10 different tanks with different landscape configurations were tested for each grazer.

Grazing effects on benthic algae

Laboratory experiments were carried out to measure the effects of macrofauna grazing on benthic algal biomass and production. Experimental units consisted of PVC cores (Ø 12.5 and height of 10 cm) filled with defaunated sediment. Benthic algae were scraped off the sediment in the benthic algae culture tanks and mixed with filtered seawater, to create a mixture of benthic algae, small sediment particles and other organic material. During low tide, this mixture was sprayed on defaunated sediment in the experimental cores as a thin layer. Biofilms were allowed to grow for 7 days, after which grazers were added at different densities (0, 600, 1900, 3800 individuals m^{-2}). After 18 days, the control cores reached a steady state and therefore we measured photosynthetic parameters in all biofilms using the Imaging-PAM (Walz, Effeltrich) after these 18 days. Using the Imaging-PAM benthic algal biomass was measured as maximum fluorescence (Fm) after 15 minutes of dark adaptation of the biofilms (Kromkamp et al. 1998, Barranguet & Kromkamp 2000). There was a good correlation between the Fm values and the chlorophyll-*a* concentration (mg C m^{-2}; Appendix A), therefore we recalculated the Fm values into chlorophyll-*a* concentration. Fluorescence measurements were done during the low-tide cycle. Diatoms in the laboratory did not show their natural migratory rhythm, therefore the time of these measurements during the cycle did not influence the fluorescence signal. Following the Fm measurements, electron transport rate (ETR) measurements were carried out by exposing the biofilms to ten photon flux levels, ranging from 0-702 µmol m^{-2} s^{-1}. These ETR curves were fitted according to Webb et al. (1974), using the least squares method. In this method, the maximum rate of ETR, the initial slope and the light saturation parameter were calculated to estimate the photosynthetic productivity (mg C m^{-2} day^{-1} of these biofilms according to (Barranguet & Kromkamp 2000). Biomass-specific primary production was calculated by dividing the production (mg C m^{-2} day^{-1}) by the chlorophyll-*a* concentration (mg C m^{-2}).

Statistical analysis

Field collection: The field data was analyzed using a one-way ANOVA where we tested if diatom content, *C. Volutator* density, *H. ulvae* density or total macrofauna density were significantly different between the hummocks, or hollows, and on flat sediment. Normal distribution of the data was ensured by visual inspection of Q-Q plots. Levene's test was used to check for homogeneity of variances (Zar 1999). When data did not meet these criteria, we performed a natural log transformation of the data. To indicate where differences occurred, a post hoc comparison test was performed using the Tukey-Kramer procedure at 0.05 significance level.

Laboratory experiments: The influence of diatoms biomass and landscape morphology on *C. volutator* or *H. ulvae* abundances was analyzed using a two-way ANOVA. Here, we tested for interactive effects of the fixed factors of diatom content (present or absent) and landscape morphology (hummock, hollow, flat). To overcome the problem of pseudoreplication we used tank as random categorical predictor. The data were checked for normal distribution and homogeneity of variance, appropriate transformation was applied if these criteria were not met.

Grazing effects: In the laboratory experiment, we analyzed the effects of grazing on benthic algal biomass, carbon production and biomass-specific production using linear regression. For all statistical analyses significance levels were set at $p < 0.05$ and analyses were performed using R (http://www.R-project.org).

Results

Field observations: Diatom biomass was an order of magnitude higher on the hummocks compared to hollows the no patterned landscape (Fig. 4.1A; Table 4.1). We observed little effect of diatom presence on macrofauna distribution, as total macrofauna densities did not differ between algal-covered hummocks or almost bare hollows (Fig. 4.1B). However, there were clear differences between *C. volutator* and *H. ulvae* abundances. We observed higher *C. volutator* abundances on hummocks (Fig. 4.1C), as compared to both hollows and flat sediment while *H. ulvae* is distributed homogeneously in all locations (Fig. 4.1D).

Table 4.1. One-Way ANOVA results for benthic algal biomass, *Corophium volutator*, *Hydrobia ulvae* and total macrofauna abundances tested against landscape morphology. –: no transformation performed

	Landscape morphology			Transformation
	MS	F	p	
Benthic algal biomass	2,002	77.63	***	Logarithmic
Total macrofauna	$7.7 * 10^5$	8.921	**	-
Corophium volutator abundance	$1,1 * 10^8$	11.20	**	-
Hydrobia ulvae abundance	$3.1 * 10^5$	0.214	NS	-

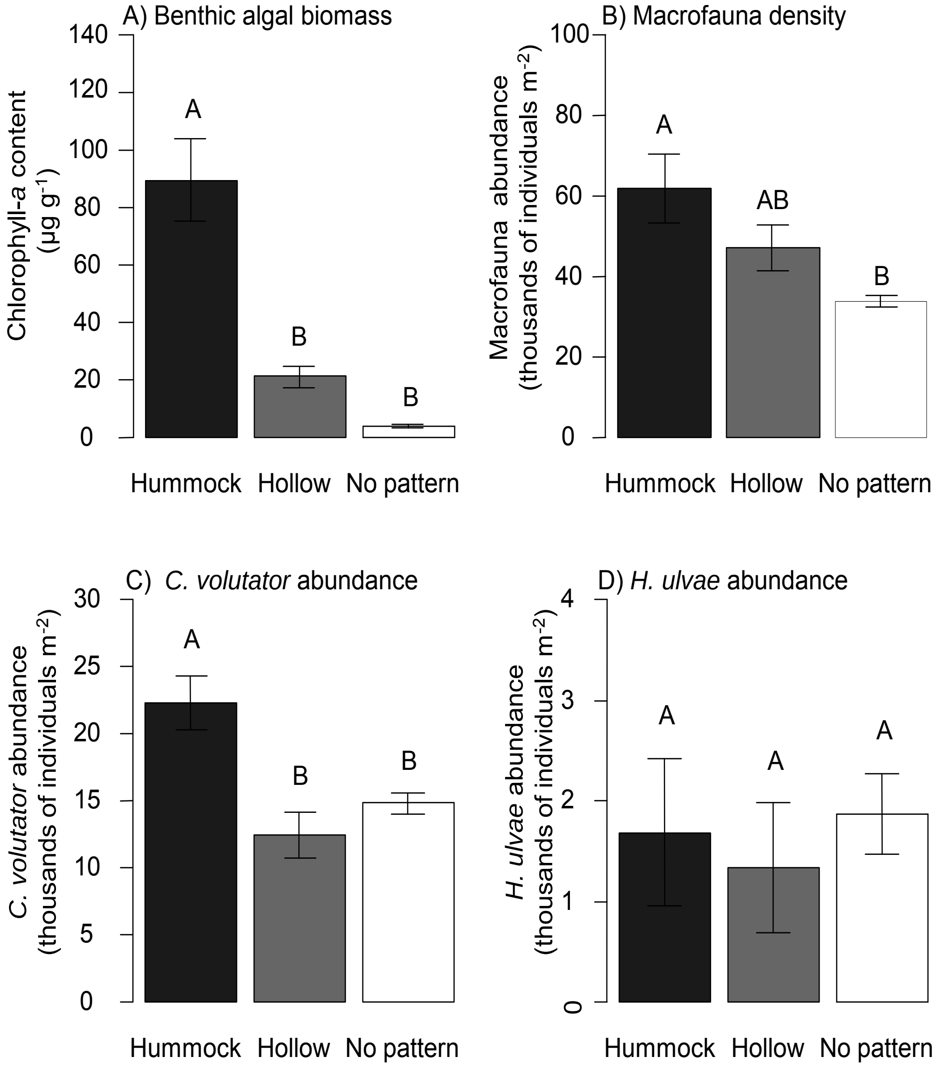


Figure 4.1: Field measurements on the average density of benthic microalgae biomass (A), total macrofauna density (B), *Corophium volutator* density (C) and *Hydrobia ulvae* density (D) expressed as thousands of individuals on a square meter. Black and grey bars indicate abundances on hummocks and in hollows when patterns were visible (May) White bars indicate abundances when patterns had disappeared (June) Error bars ± SEM, n=5.

Laboratory experiments: We tested whether *H. ulvae* or *C. volutator* showed preference for a specific landscape position (hummock, hollow or flat) or if it was generally attracted to food enriched patches. These experiments demonstrated the aggregation of both grazers in enriched food patches where both species showed at least a doubled abundance (Fig. 4.2, Table 4.2). Moreover, landscape morphology showed to have a clear effect on species distribution. Although *H. ulvae* showed to prefer flat enriched algae patches, this effect was just not significant (P = 0.05, Fig. 4.2A). *C. volutator* preferred diatom-covered hummocks over water-filled hollows or flat patches (Fig. 4.2B).

Table 4.2: Two-Way ANOVA results for influence of landscape morphology, food abundance (benthic algae) and landscape morphology × food abundance on *Corophium volutator* and *Hydrobia ulvae* abundances.

Grazer	Source of variation	SS	df	MS	F	p
C. volutator	Food abundance	33.36	1	33.36	71.79	***
	Landscape morphology	1.23	2	0.61	1.32	NS
	Landscape × Food	6.69	2	3.45	7.42	***
H. ulvae	Food abundance	66.69	1	66.69	140.0	***
	Landscape morphology	0.47	2	0.23	0.49	NS
	Landscape × Food	2.88	2	1.44	3.03	NS

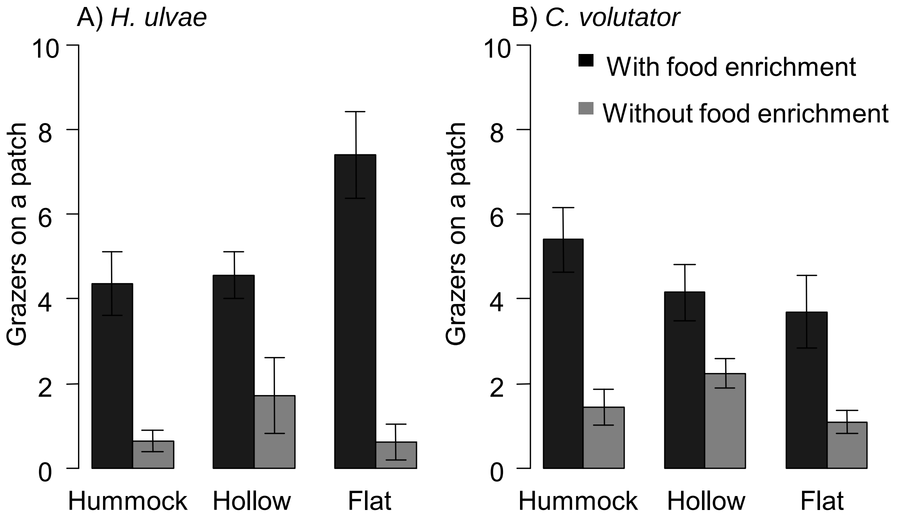


Figure 4.2: Average H. ulvae (A) and C. volutator (B) on the patches with food enriched (black bars) and non food enriched patches (grey bars) with the different landscape morphology treatments (hummock, hollow or flat). Error bars ± SEM, n=5.

Grazing effects: Our laboratory grazing experiments revealed a clear influence of grazers on benthic algal biomass and production. As expected, both grazers reduced benthic algal biomass with increasing grazer density (Fig. 4.3A; Table 4.3). At the highest grazer densities, both grazers reduced algal standing stock by 50%. Although, similar grazer concentrations showed comparable effects on benthic algae removal, primary production was affected differently by species. Carbon production did not change with increasing *C. volutator* density, whereas for *H. ulvae* a slight but significant decrease was found in primary production with increasing grazer densities (Fig. 4.3B). Biomass-specific production increased with both grazers (Fig. 4.3C). Increased grazing by *C. volutator* revealed a stronger increase of biomass-specific production compared to similar densities of *H. ulvae.*

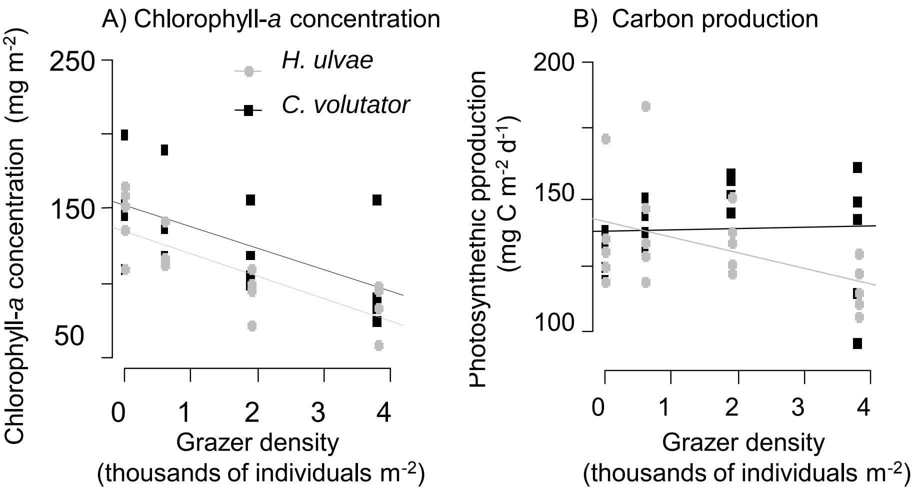
Table 4.3: Test statistics and *P*-values for the linear regression analysis of benthic algal biomass, carbon production and biomass-specific production on grazer density.

C. volutator	a	b	r^2	P
Benthic algal biomass	152.70	-14.41	0.34	**
Carbon production	137.65	0.49	0.05	NS
Biomass-specific production	0.91	0.09	0.40	**
H. ulvae				
Benthic algal biomass	134.51	-15.14	0.61	***
Carbon production	141.26	-5.88	0.17	*
Biomass-specific production	1.07	0.12	0.32	**

. * = P < 0.05, ** = P < 0.01, *** = P < 0.001, NS = Not Significant

Discussion

In this study, we investigated the response and effects of two benthic herbivores on a spatial pattern of benthic diatoms. Our results revealed that the combination of grazer-induced enhancement of biomass-specific production and the interactive effect of landscape morphology and resource heterogeneity provided a putative explanation for the spatial and temporal distribution of macrobenthic herbivores. While clear differences in benthic algal biomass were observed between hummocks and hollows, whereas grazers were relatively insensitive to these landscape morphology differences in the field. There was no difference in abundance between the hollows and hummocks in *H. ulvae* and only a slight difference in *C. volutator* between the hollows and hummocks. Assuming that grazers cannot survive at zero benthic algal biomass, it is surprising to see that so many grazers are found in the hollows, in spite of the



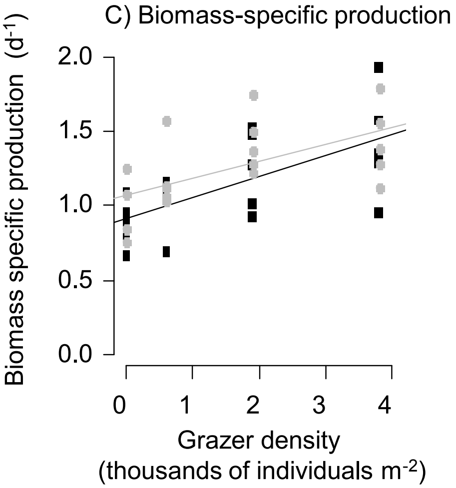


Figure 4.3: Laboratory experiments on the grazing effects of *H. ulvae* (grey lines; dots) and *C. volutator* (black lines; squares) on benthic algal biomass (A, µg chlorophyll-a g^{-1} sediment), carbon production (B, g C $year^{-1}$) and biomass-specific production (C, area specific production/algal biomass. Error bars ± SEM, n=5.

low levels of food. This suggests that there is no, or only a very weak, negative feedback from the loss of benthic algae in June on the grazer standing stock. This observation may be explained by the increased biomass-specific production of benthic algae, induced by both *H. ulvae* and *C. volutator* grazing. Compensated production seems to be essential in the apparent insensitivity of benthic herbivores to spatial and temporal variation in the availability of their resources.

Laboratory experiments revealed a clear preference of both *C. volutator* and *H. ulvae*, for patches of high algal biomass indicating that food availability was the prime determinant of the spatial distribution of these grazers. From other studies it is known that both species accumulate in patches with high food abundance (Morrisey 1988a, Lawrie et al. 2000). Mud snails move slower once they have reached high density food patches (Forbes & Lopez 1986); along with the ability to follow mucus trail of others (Bretz & Dimock 1983) this results in a rapid assembly of large groups on abundant food resources. Previous studies also concluded that *C. volutator* aggregates towards patches with higher food abundance (Lawrie et al. 2000, Hamilton et al. 2006). Nevertheless, in the field the relative concentration on high-diatom hummocks compared to low-diatom hollows was weak to absent. *C. volutator* densities differed by less than a factor two, where diatom biomass differed by a factor of five. Field data from a similar intertidal flat showed that benthic macrofauna can reach highest densities at intermediate food densities (Van der Wal et al. 2008). This suggests that, above a certain minimum diatom biomass, macrofauna density is relatively independent of algal biomass.

Next to food abundance, we also tested the influence of landscape morphology on the distribution of grazers. Responses to landscape morphology were found to be species-specific in our study. This is in line with previous studies, the amphipod *C. volutator* showed preference for diatom-hummocks, while the mud snail *H. ulvae* showed a slight preference for flat and depressed patches (Grant 1981, Hogue & Miller 1981, Barros et al. 2004). However, these previous studies were all conducted in the field, which makes it very difficult to distinguish the effect of landscape morphology and other factors like variations in hydrodynamic forcing, silt content and food availability between crests and troughs (Lanuru et al. 2007). Our controlled experiments showed that landscape morphology did influence *C. volutator* abundances which imply that resource heterogeneity is not the sole explanation for macrofauna distribution.

The feeding mode of grazers is an important aspect of the effects that herbivores have on plant primary production (Carpenter 1986, McClanahan 1994). The browser *H. ulvae* and the central place forager *C. volutator* both have different feeding mechanisms and although the highest grazer density we applied in our laboratory study is still quite low compared to peaks in natural abundances, both showed different effects on the biofilm biomass and production (Fig. 3). *H. ulvae* and *C. volutator* are known to have a strong effect on benthic algal biomass even at low grazer concentrations (Lopez-Figueroa 1987, Morrisey 1988b, Gerdol & Hughes 1994a). Although the effects on biomass were similar in our study, the effect of herbivores on biomass-specific production was much stronger for *C. volutator* compared to *H. ulvae*. *C. volutator* mixes and ventilates the sediment much deeper compared to *H. ulvae* (Meadows et al. 1990, Orvain et al. 2004). This may enhance the flux of nutrients to the sediment surface. Since benthic microalgae are often nitrogen-limited (Hillebrand & Sommer 1997) enhanced bioturbation and subsequent release of nutrients could explain the higher biomass-specific production under *C. volutator* grazing compared to *H. ulvae* grazing. This is in line with studies explaining the increased biomass of macroalgae in relation with increased nitrogen availability (Bracken 2004, Godbold et al. 2009). Also changes in taxonomic composition of the benthic algae are a plausible mechanism. *H. ulvae* is a grazer on sub-dominant species, while *C. volutator* consumes mostly dominant taxa which increase species richness (Hagerthey et al. 2002). This habitat modification by macrofauna clarifies the observation of high macrobenthic biomass in the hollows, where algal biomass is much lower compared to the hummocks.

The results presented in this study show some interesting similarities with the concept of grazing lawns in terrestrial studies. In grazing lawns, herbivory leads to increased plant production because it increases nutrient regeneration and favors grazing-tolerant species. As a consequence, the independence of herbivores from food biomass is enhanced by the increase of biomass-specific production rate as an indirect effect of grazing. This interaction results in a mosaic of heavily grazed patches surrounded by inaccessible bushes (McNaughton 1984). In our intertidal system, grazing was found to stimulate primary production in a similar way. The dense diatom mats on the hummocks in the patterned state could be equivalents of the terrestrial inaccessible bush vegetation. However, the analogy with terrestrial systems breaks down on the long run. The decrease of benthic algal biomass disrupts the biophysical feedbacks between diatom growth and sedimentation of fine-grained materials, leading to a dramatic collapse of both diatom biomass and the resulting landscape morphology on intertidal mudflats (Chapter 2). This suggests that by their insensitivity to food abundance, at least on the short term, herbivores can have a profound impact on the spatial structure of ecosystems, similar to what is found in terrestrial ecosystems (Iacobelli & Jefferies 1991, Jefferies et al. 1994).

Our results, combined with our previous work in this ecosystem (Chapter 2, Chapter 3) revealed that the interactive effects of landscape form, resource heterogeneity, and grazing-induced enhancement of benthic algae production provided a putative explanation for the spatial and temporal distribution of macrobenthic grazers on a patterned intertidal flat. These results will increase the ecological understanding of the distribution and dynamics of grazers in spatially patterned ecosystems.

Acknowledgements

The authors would like to thank Dingeman de Visser and Jos van Soelen for their help in the laboratory experiments. Jacco Kromkamp is thanked for his assistance in analyzing the Imaging PAM data.

Appendix 4.A

The relationship between Fm values and the chlorophyll-*a* concentration (mg C m^{-2}) was estimated by analyzing chlorophyll-*a* content and Fm values of different aged biofilms. These biofilms grown as described in the materials and methods section:”*Grazing effects on benthic algae”* Chlorophyll-*a* samples were taken from these different aged grown biofilms by using the contact core method, where a metal disc was used to take a sediment sample for chlorophyll-*a* concentration analysis of approximately 2 mm deep. A detailed description of this contact core method can be found in Heywill et al. (2002). The sediment samples were freeze dried for 48h. These freeze dried samples were analyzed in the laboratory to determine chlorophyll-a concentration. Chlorophyll-*a* content was determined spectrophotometrically after 48h 90% acetone extraction (Jeffrey & Humphrey 1975). Data was analyzed using linear regression in R (http://www.R-project.org).

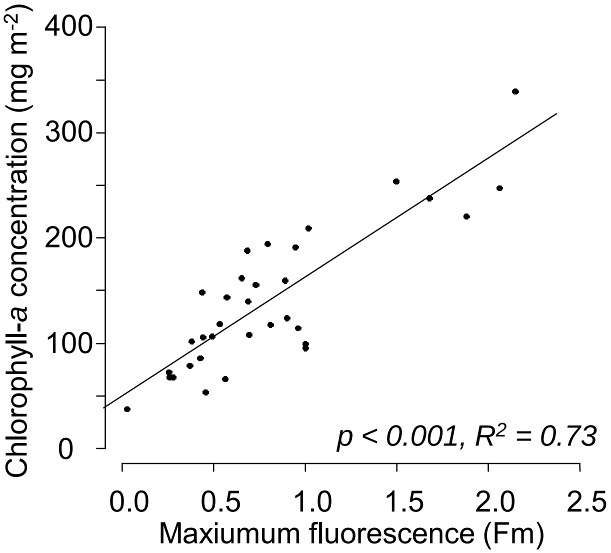
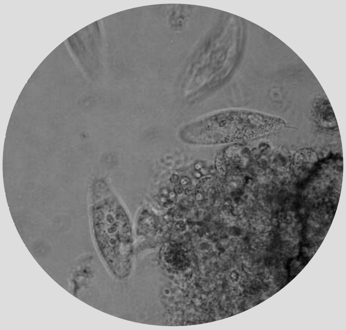


Figure 4.A1: The correlation between the maximal fluorescence (Fm) and chlorophyll -*a* concentration. The inset shows the statistical analysis of the linear regression.

Maximum fluorescence values were positively correlated with chlorophyll-*a* concentration in the top 2 mm of the sediment (Fig. 4.A1, $P < 0.001$). These results are in agreement with similar studies that correlated Fm values measured with a PAM with chlorophyll-*a* content (Honeywill et al. 2002, Kromkamp et al. 2006).

Chapter 5

Ciliates as engineers of phototrophic biofilms



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Abstract

Phototrophic biofilms consist of a matrix of phototrophs, bacteria and extracellular polymeric substances (EPS), which are spatially structured. Despite wide-spread exploitation of algae and bacteria within phototrophic biofilms, for example by protozoans, the ‘engineering’ effects of these ciliates on the spatial heterogeneity of phototrophic biofilms is poorly studied. We studied the potential engineering effects of two ciliates, *Urostyla* sp. and *Paramecium bursaria*, on the spatial heterogeneity of synthetic multi-species biofilms. Biomass of phototrophic organisms, EPS and bacteria was analysed three dimensionally using Confocal Laser Scanning Microscopy. Spatial heterogeneity and cover of the phototrophs, bacteria and EPS were determined at several depths within the biofilm. Ciliate species did not interfere with the overall development of the phototrophic algae as thickness of the biofilms was equal between biofilms with and without the presence of ciliates, even though ciliate abundance did affect spatial heterogeneity of biofilm components. When *Urostyla* was present, it reduced aggregation in EPS and bacteria and increased EPS biovolume. This implies a local facilitating effect of ciliates on photosynthetic activity. Biofilms to which *Paramecium* was added did not differ from controls in phototrophs, EPS cover and biovolume. Nevertheless, ciliates affected the spatial heterogeneity of these components as phototrophs and EPS became more evenly distributed. This study shows that ecosystem engineering by organisms does not only occur at large spatial scales, e.g. grasslands and estuaries, but also plays a role at the microscopic scale of biofilms. This effect on spatial heterogeneity was not driven by substantial exploitation of biofilm components, but via the subtle engineering effects of ciliates.

Introduction

The ability of organisms to cause physical changes in their environment has been studied extensively since Jones et al. (1994) introduced the concept of 'ecosystem engineering' (see reviews Wright and Jones, 2006; Hastings *et al*., 2007). Organisms can have pronounced effects on their environment; for instance, the removal of herbivorous snails from a rocky beach resulted in the formation of a dense algal mat and an associated accumulation of sediment (Bertness, 1984). Ecosystem engineering by organisms can increase habitat heterogeneity by creating patches that differ from their surroundings. These patches can influence ecosystem processing and community structure (Gutierrez and Jones, 2006; Wright and Jones, 2006; Van Wesenbeeck *et al*., 2007; Chapter 2). The engineering effect of macroscopic organisms has mainly been studied at larger spatial scales (metres), but the effects of microscopic organisms, such as ciliates, on the physical structure of biofilms is still poorly studied. Nevertheless, it has been shown that small scale heterogeneity can influence ecosystem processes at larger scales (Battin *et al*., 2003b).

Phototrophic biofilms, consisting of phototrophic organisms and bacteria embedded in a matrix of extracellular polymeric substances (EPS), are an abundant component of the interface between water and the substratum in both freshwater and marine systems (Stevenson et al., 1996; Underwood and Kromkamp, 1999). Phototrophic biofilms exhibit complex heterogeneous structures, formed by individual cells or groups of cells at the micrometre scale, these structures have been referred to as 'micro-landscapes' (Battin *et al*., 2007). Micro-landscapes incorporate a range of forms, including maze-like structures (Xavier et al., 2009), mushroom-like structures (Klausen *et al*., 2003) or regular patterns (Thar and Kuhl, 2005). Despite their small scale, it has been demonstrated that changes in these structures can affect ecosystem processes, such as primary production and respiration (Cardinale *et al*., 2002; Battin *et al*., 2003b; Yang *et al*., 2000; Battin *et al*., 2007). For example, increased areal porosity in biofilms will favour internal mass transport, while clustering of the biofilm components will result in a lower surface to volume ratio causing the opposite effect (Yang *et al*., 2000). Therefore, it has been argued that biofilm spatial heterogeneity can play an important role in the functioning of aquatic ecosystems at a larger scale (Battin *et al*., 2003b).

Despite the fact that phototrophic biofilms are an important food source for many aquatic invertebrates and protozoan grazers (Epstein, 1997; Hillebrand and Kahlert, 2001; Lawrence *et al*., 2002; Parry *et al*., 2004), the effect of protozoans on the spatial heterogeneity of phototrophic biofilms is poorly understood. The exploitation of phototrophic biofilms by a variety of grazing invertebrates has been clearly demonstrated; for instance, grazing by snails, ostracods and mayflies (larvae) has been shown to reduce biofilm thickness and the abundance of algae (Lawrence *et al*., 2002). Unlike the apparently indiscriminate scraping by some macroinvertebrate species, the small size of protozoan grazers suggests that their feeding may be more localized and selective for certain components of the biofilm (either algae, bacteria or mucus), a proposition that has been supported by microscopic observations on many benthic ciliates (Fenchel, 1975). Grazing by protozoans can affect benthic bacterial communities in several ways, e.g. modifying morphology due to size specific feeding (Hahn and Höfle, 2001). Moreover, it has been demonstrated that in both mono- and multi species heterotrophic biofilms, localized grazing by protozoans influences the small-scale spatial structure of biofilms (Bohme et al., 2009; Lawrence and Snyder, 1998; Matz *et al*., 2004; Weitere *et al*., 2005).

Here, we report on the influence of two ciliates, sp. and *Paramecium bursaria* on the two- and three-dimensional spatial heterogeneity of multi-species biofilms grown under laboratory conditions. *Urostyla* is an omnivore and feeds on particles in phototrophic biofilms, including diatoms, algae, cyanobacteria and detritus (Berger, 2006). *Paramecium* feeds mainly on bacteria, and can increase its own growth rate and maximum population density by a symbiotic relationship with the green algae *Chlorella* sp. (Karakashian, 1963). Both species are expected to act as ecosystem engineers by affecting the structure of biofilms as a result of consuming particles and by affecting the motility of these particles (Gray, 1952; Nisbet, 1984; Berger, 2006).

Our aim was to explore the influence of ciliates on the spatial heterogeneity of phototrophic biofilms. We hypothesized that both ciliate species would affect biofilm spatial heterogeneity directly by consumption of phototrophs, bacteria and EPS, or indirectly, as a result of their feeding behaviour. Using confocal microscopy we measured bacterial and algal cell densities and the abundance of extracellular polysaccharides (EPS) at different depths in the biofilm. We analysed possible effects of ciliate grazing and abundance on the spatial heterogeneity of these three biofilm components.

Materials and methods

Algae and ciliate cultures

The non-axenic phototrophs used in this study were obtained either from the Culture Collection Yerseke) or isolated from floodplain sediment (Van der Grinten *et al*., 2005). For the experiments, seven different photosynthetic microbes were used, including a diatom, single celled and filamentous cyanobacteria and a green alga (Table 5.1). These phototrophs were kept in Erlenmeyer flasks containing glass beads (Ø 490–700 ml) as an artificial substratum and 100 ml of modified WoodsHole (WC) medium (Guillard and Lorenzen, 1972; Van der Grinten *et al*., 2005). The flasks were closed with a cellulose plug and illuminated from above with fluorescent, cool-white tubes (45 µmol s^{-1} m^{-2}) under 12h light/12h dark regime at 15°C. To keep cultures in the exponential growth phase, 50% of the volume (50 mL) was replaced weekly with 50 mL new WC-medium (Van der Grinten *et al*., 2005).

Two ciliate taxa were used in this study, *Paramecium bursaria* (100 µm) and *Urostyla* sp. (150 µm). Both species were isolated from sediment just below the water line in the river Rhine flood plain near Wageningen, the Netherlands (51° 57´ 12.39´´ N, 5° 39´ 49.37´´ E). After isolation the ciliates were cultured in the same medium under similar light and temperature conditions as the phototrophs. The ciliate cultures were fed twice a week with mixed algae. Ciliates were starved for three days before the experiment began.

Table 5.1: Photosynthetic microbes used in this study, the group of phototrophs to which they belong, the growth form they usually occur in and their origin.

Algae	Group	Growth form	Source
Nitzschia perminuta	Diatom	Single cells	Floodplain in the river Rhine, the Netherlands
Synechococcus	Cyanobacterium	Single cells	CCY0621, pond in Leiden, the Netherlands,
Pseudanabaena	Cyanobacterium	Filaments	CCY0619, pond in Leiden, the Netherlands
Synechocystis	Cyanobacterium	Single cells	CCY0622, pond in Leiden, the Netherlands
Limnothrix	Cyanobacterium	Filaments	CCY0637, Friesland, the Netherlands
Leptolyngbya	Cyanobacterium	Filaments	CCY9627, source unknown
Chlorococcum	Green alga	Single cells	CCY0626, pond in Leiden, the Netherlands

Experimental set-up

A mixture of the cultured phototrophs was inoculated in several six-welled plates and allowed to grow in 10 mL of WC medium on glass discs (Ø 24 mm). Experimental conditions were similar to culture conditions. After 4 days the discs were covered with a thin phototroohic biofilm layer. The discs were transferred to new six-welled plates containing 6 mL of fresh WC-medium. For each ciliate species, six wells were inoculated with 150 ciliates and three wells, without ciliates were established as controls. Ciliates were counted under a binocular microscope before use and were selected individually with a pipette. For each ciliate taxon this resulted in a ciliate abundance treatment of 33 cm^{-2} (n=6) and control treatments without ciliates (n=3). After 7 days in the presence of ciliates, the experiment was terminated and ciliates were counted to determine if they had reproduced during the experiment. Subsequent confocal microscopy measurements were performed using confocal laser scanning microscopy (CLSM) on the biofilms.

Photosynthetic biomass during the experiment

We used a non-destructive method to follow biofilm development during the experiment. Each day phototrophic biomass was measured using Pulse Amplitude Modulated Fluorometry (PAM, Water-PAM/F, Heinz Walz GmbH). The minimal fluorescence (F0; determined after 30 minutes of dark adaptation) was used as a measure of photosynthetic biomass. To account for spatial heterogeneity of the biofilm the F0 was measured at three random points on the glass disc.

Three- dimensional imaging of the biofilms

We analysed phototrophic, EPS and bacteria abundances in the biofilms in three dimensions using Confocal Laser Scanning Microscopy (CLSM; Zeiss LSM 510). Prior to confocal imaging the ciliates were carefully removed from the biofilms by rinsing with WC medium. Preliminary experiments showed that this method was efficient and did not damage the structure of the biofilm. Following the removal of ciliates, biofilms were stained according to Lawrence et al. (1998). EPS biomass was quantified by staining with a fluorescently labelled lectin of *Canavalia ensiformis* (L.) (Con-A, tetramethylrhodamine conjugated: Molecular Probe). One mL of a 0.1 g L^{-1} Con-A solution was added to the biofilm on each disc, incubated for 30 minutes at 20° C and subsequently rinsed carefully four times with filtered WC-medium. Syto 9 (Molecular Probe) was used to stain bacterial DNA, 100 µL of 0.835 µM Syto-9 solution was applied to each disc and incubated for 5 minutes at 20° C. After staining, biofilms were kept in filtered WC medium until confocal imaging was performed. Phototrophic biomass (diatoms, green algae and cyanobacteria) was quantified through chlorophyll auto-fluorescence. A detailed description of the use of fluorescent dyes and autofluorescence in biofilms can be found in Lawrence et al. (1998).

The 0.17 mm thick glass disc was fixed in a sample holder, allowing the measurement of fully hydrated biofilms. For each biofilm, three 3D images were measured at three random positions on each glass disc using a 40x/0.75 NA (Zeiss, PlanAprochromat®). These CLSM scans represented an area of 230.4 x 230.4 μm^2 (X-Y) and 40 µm depth (Z). A series of optical sections was made at 1 µm intervals, starting at the glass surface towards the top of the biofilm. Autofluorescence of the phototrophs was excited at 633 nm and detected using a long-pass 650 filter. The Con-A stain was exited at 543 nm and the signal emitted was detected using a 560–615 nm band-pass filter. The Syto-9 stain was excited at 488 nm and detected using a 505–550 nm band-pass filter. LSM

Image Browser 4.0 was used to convert the CLSM files into uncompressed TIFF format for each section, after which image analysis could be performed. These TIF-images had the resolution of 512 x 512 pixels at eight bit colour depth (256 grey values).

Image analysis

We analysed the TIFF files using PHLIP version 0.7, a freely available Matlab-based image analysis program (Mueller *et al*., 2006; http://phlip.sourceforge.net). The converted TIFF files were imported using PHLIP-ML (Merod *et al*., 2007) followed by a three-dimensional extension of the Otsu algorithm, applied for calculating a threshold value for each subsample. This algorithm is based on an analysis of a histogram of the image, after which the grey image was converted into a binary image of pixels of either presence of absence of phototrophs, EPS or bacteria (Xavier *et al*., 2001). Following Mueller *et al.* (2006), we calculated the cover of phototrophs, bacteria and EPS, which represents the percentage of pixels where phototrophs, EPS or bacteria are present at different depths in the biofilm. In addition, total biovolume was calculated for the treatments and control.

Biofilm morphology was described using three parameters; mean biofilm thickness, roughness coefficient and spatial variance of the biofilm at different depths. For the main biofilm thickness and the roughness coefficient PHLIP 0.7 was used, in which the maximal height (Z) of each pixel in the xy plane was recorded and averaged to represent the mean thickness. The roughness coefficient (R) was obtained by the standard deviation of these Z values in the xy plane. Spatial variance (SV) was calculated to describe spatial heterogeneity in the biofilms at different depths. Spatial variance (SV), which indicates the spatial dispersion of the data in the CLSM slices, is either uniform (SV<1), random (SV=1) or aggregated (SV>1). The binary images were converted into grains of 50x 50 pixels (22 x 22 µm), this grain size was the average grain of the three components, where SV did not change with increasing grain size. This binning resulted in 100 50 x 50 pixel bins, in which the value was assigned of the mean from the 50 x 50 pixels (Fig. 5.1). From these 100 values the spatial variance (SV) was calculated as the standard deviation of 100 grains divided by the overall mean. SV was corrected for the binomial distribution of our data (Zar, 1999).

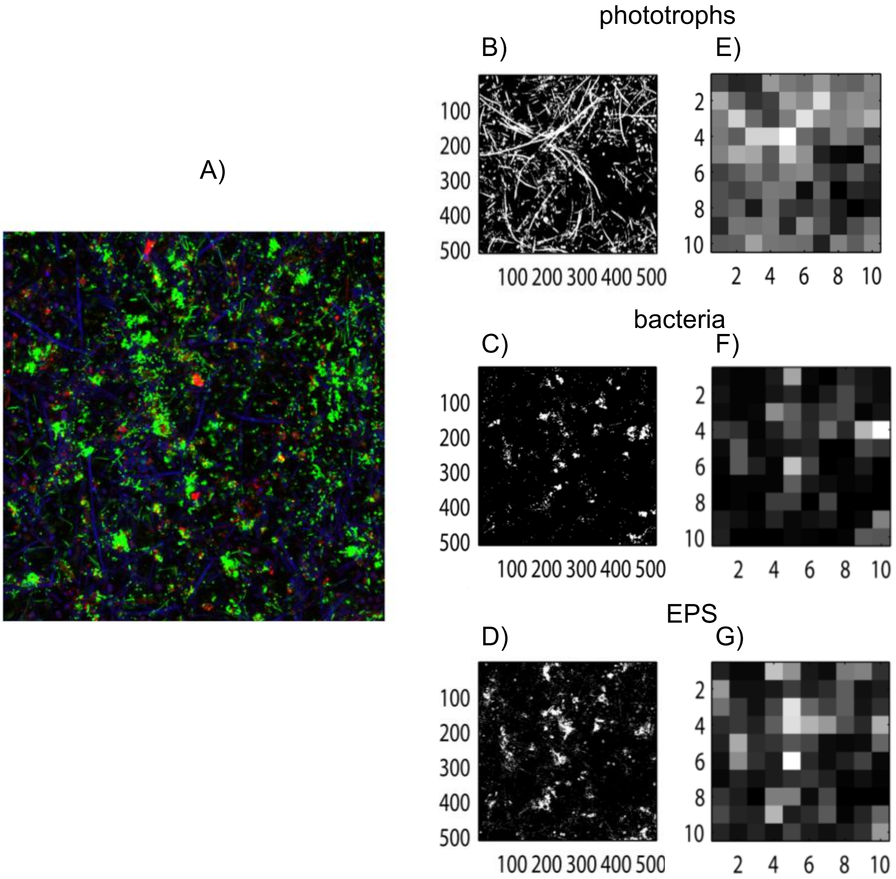

Figure 5.1: Confocal Laser Scanning Microscopy image (A) with autofluorescence of the phototrophs (blue), EPS (red) and bacteria (green) components. Binary images after thresholding are shown of respectively phototrophs (B), bacteria (C) and EPS (D). Here white indicates that the component (phototrophs, bacteria or EPS) is present and black indicates that the component is absent. The images created from the spatial variance analysis are shown in E, F and G. Here the CLSM signal is averaged in the bins (50 x 50 pixels), with white areas corresponding to high coverage and black areas corresponding to low average coverage. From these averages the coefficient of variation was calculated with a SV of 131 for phototrophs, 127 for EPS and 87 for bacteria.

Spatial cross-correlations (Pearson's correlation coefficient) between the different components were calculated to indicate if grazers changed the spatial distribution of components within the biofilms. For this the 512x512 binary-pixel images were used, from which cross-correlation was calculated using the xcorr function in Imaging Processing Toolbox of Matlab (2007b). We measured the cross-correlation at the depth with the highest cover (4 μm).

Data analysis

Measurements of the CLSM microscopy and PAM fluorometry were done by analysing three random samples on each glass disc. The influence of ciliates on algal biomass measured with the PAM was analysed using a repeated measure ANOVA design, with time and grazing treatment (ciliates present and control) as fixed factors. To account for the repeated measures, disc was nested as a random factor within grazing treatment. The effects of grazing were tested against the error variability of "discs within grazing"; the effects of time and time x grazing were tested against the error variance ('within discs', Neter et al., 1985). Differences between control and grazed treatments on cover and spatial variance were analysed in a similar way using a nested ANOVA, with depth and treatment (ciliates present and control) as factors in a full factorial design. Biofilm biovolume, biofilm thickness, roughness coefficient and cross-correlations between biofilm components were analysed similarly with glass disc nested within treatment. Normal distribution of the data was ensured by visual inspection of Q-Q plots and Levene's test was used to check for homogeneity of variances. In cases where the data were not normally distributed, a double square root transformation was performed. All significance levels were at $P < 0.05$ and statistics were computed using R (http://www.Rproject.org).

Results

Phototroph and ciliate abundance

During the experiments both ciliates reproduced, the density of *Urostyla* increasing from 33 to 61 ± 16 cm^{-2} (mean ± SEM) and *Paramecium* from 33 cm^{-2} to 47 ± 4 cm^{-2}. Phototrophic biomass in both experiments, measured by PAM, increased significantly during the experiment (both species $P < 0.05$; Fig. 5.5.2). Biofilms where *Urostyla* was present had a slightly higher algal biomass ($P < 0.05$; Fig. 5.2A) compared to control biofilms, while this was not observed for biofilms where *Paramecium* was introduced ($P > 0.05$; Fig. 5.2B).

Exploitation of phototrophic biofilm components

CLSM measurements revealed that phototrophs and EPS dominated in terms of biovolume and cover in all biofilms, while the contribution of bacteria was low (Fig. 5.3B and E). Biofilms with *Urostyla* showed slightly greater cover of phototrophs close to the substratum, as the interaction treatment x depth was significant (*P* < 0.05) although overall algal biovolume was not affected (*P* > 0.001; Fig. 5.3C) and total biovolume of EPS in the biofilm was greater than in the control (*P* < 0.05; Fig. 5.3C inset). 0.05; Fig. 5.3A inset). EPS cover in biofilms with *Urostyla* was higher close to the glass disc, as the interaction term treatment × depth was significant (*P* < *Paramecium* reduced and bacterial abundance close to the substratum (*P* < 0.01; Fig. 5.3E), and total bacterial biovolume was also lower in biofilms where *Paramecium* was present compared to the control (*P* < 0.01; Fig. 5.3E inset).

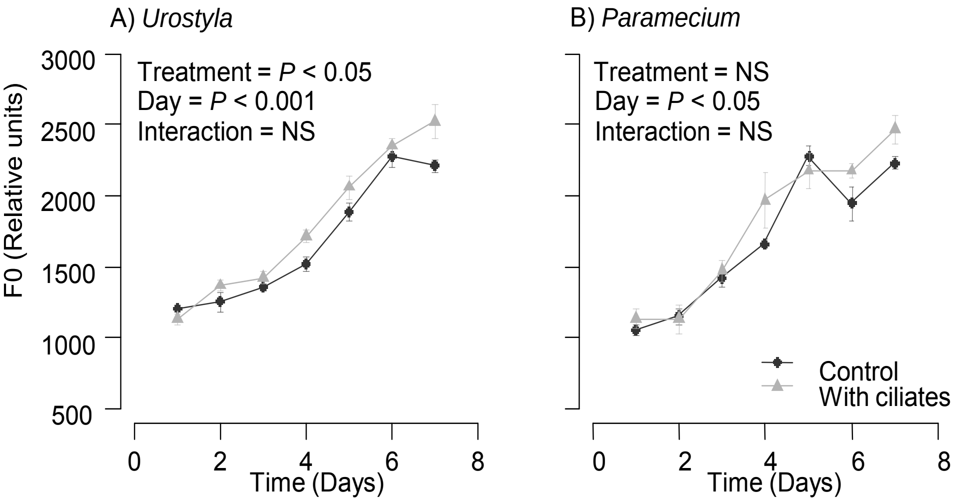

Figure 5.2: Biofilm development expressed as minimal fluorescence (F0) in time (days) for A) biofilms with *Urostyla* present, and B) biofilms with *Paramecium* present (means ± SE).

Biofilm morphology

No differences in biofilm thickness and the roughness coefficient were observed when ciliates were present compared to the controls (*P* > 0.05; Table 5.2). The analysis of spatial heterogeneity, using spatial variance (SV), revealed that all biofilm components were aggregated (Fig. 5.4; SV >> 1). Ciliates had an effect on overall spatial variance at all depths. The aggregation of bacteria close to the glass substratum was lower in the presence of *Urostyla* as the interaction term depth x treatment was significant (*P* < 0.001; Fig. 5.4C). The aggregation of phototrophs (*P* < 0.05; Fig. 5.4D) and EPS (*P* < 0.01; Fig. 5.4F) was also lower in presence of *Paramecium*. *Paramecium* also affected algal spatial variance in the z-dimension, as interaction terms between treatment and depth were significant (*P* < 0.001; Fig. 5.4D). These results show that aggregation in phototrophic biofilms was generally lower in the presence of ciliates.

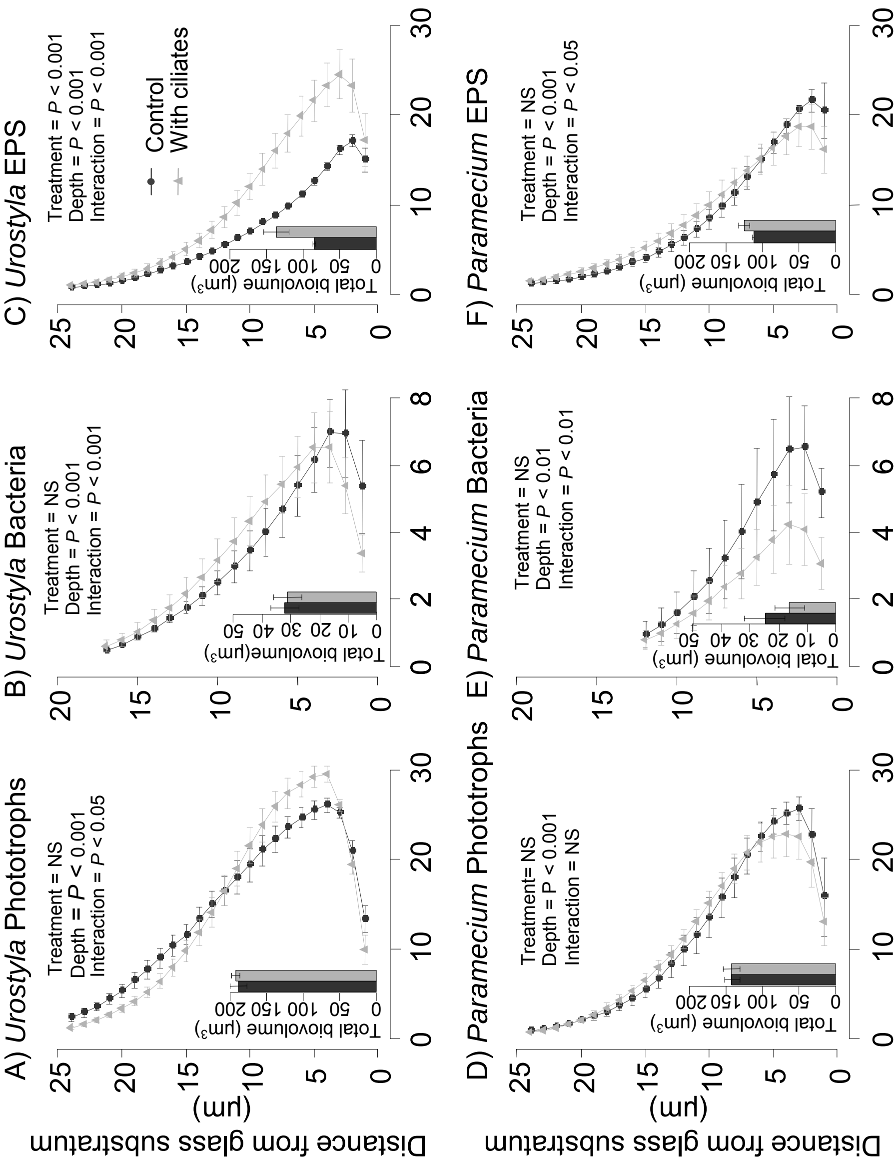

Figure 5.3: Cover (%) of the different biofilm components; phototrophs (A and D), bacteria (B and E) and EPS (C and F) for the two ciliates *Urostyla* (A-C) and *Paramecium* (D-F) at different distances from the glass substratum (µm) (means ± SE). Bar graphs show the overall biovolume (µm^3) for the different components. Grey bars represent the treatments with ciliates present and black bars represent control treatments (means ± SE). Note: the bacterial component in the biofilms was very thin and did not occur in the layers further from the glass substratum.

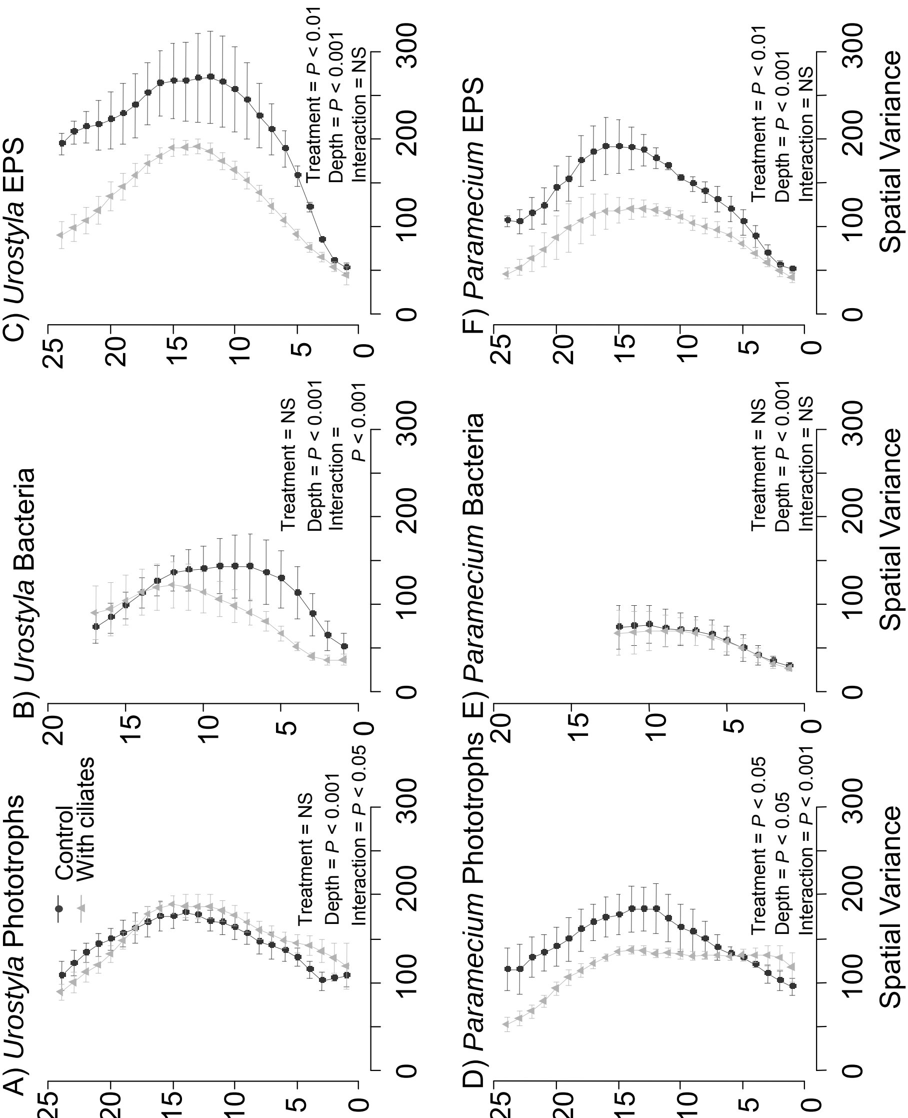

Figure 5.4: Spatial variance of the different biofilm components; phototrophs (A and D), bacteria (B and E) and EPS (C and F) for the two ciliates *Urostyla* (A-C) and *Paramecium* (D-F) at different distances from the glass substratum (µm) (means ± SE. Grey line represent the treatments with ciliates present and black bars represent control treatments (means ± SE). Note: the bacterial component in the biofilms was very thin and did not occur in the layers further from the glass substratum.

Table 5.2: Biofilm morphology parameters (thickness and roughness coefficient) of the biofilms with ciliates (*Paramecium* or *Urostyla*) and the controls

Parameter	Channel	Ciliate	Control	Ciliates
Thickness (µm)	Phototrophs	*Paramecium*	23.14 ± 0.74	21.78 ± 0.62
		Urostyla	23.67 ± 0.14	24.43 ± 0.19
	Bacteria	*Paramecium*	12.61 ± 1.34	11.48 ± 0.77
		Urostyla	17.01 ± 0.81	16.04 ± 0.82
	EPS	*Paramecium*	24.78 ± 0.67	23.23 ± 0.46
		Urostyla	23.88 ± 0.50	25.13 ± 0.51
Roughness coefficient	Phototrophs	*Paramecium*	0.47 ± 0.01	0.44 ± 0.02
		Urostyla	0.47 ± 0.02	0.45 ± 0.01
	Bacteria	*Paramecium*	0.46 ± 0.01	0.46 ± 0.01
		Urostyla	0.39 ± 0.01	0.45 ± 0.02
	EPS	*Paramecium*	0.45 ± 0.01	0.44 ± 0.01
		Urostyla	0.43 ± 0.01	0.42 ± 0.01

Cross-correlation of the three biofilm components

The degree of spatial correlation between phototrophs, bacteria and EPS was calculated to determine if ciliates affected spatial interactions between biofilm components. Significantly higher cross-correlation was found between phototrophs and EPS for the biofilms where *Urostyla* was present compared to the control biofilms ($P < 0.05$; Table 5.3), while other cross-correlations were not affected by *Urostyla*. *Paramecium* significantly lowered the cross-correlation between bacteria and EPS ($P < 0.01$; Table 5.3).

Discussion

Our results suggest that two ciliates *Urostyla* sp. and *Paramecium bursaria* can affect the spatial heterogeneity of synthetic multispecies phototrophic biofilms. The impact of ciliates was evident, despite their moderate densities. The densities of protozoans at the end of the experiment in this study (61 ± 16 cm^{-2} and 47 ± 4 cm^{-2} of *Urostyla* and *Paramecium*, respectively) were rather low compared to ciliate densities in natural biofilms, which can reach 100 cm^{-2} (Reiss and Schmid-Araya, 2008). However, in nature biofilms are inhibited by many different species of ciliates (Dopheide *et al*., 2009), which may have more complex effects on spatial heterogeneity compared to our single grazer experiments. Hence, this study suggests that effects on spatial heterogeneity

Table 5.3: Cross-correlations (Pearson's correlation coefficient) of the biofilms of the controls and treatments (presence of *Paramecium* or *Urostyla*) and their effects.("–", no effect; "↓", decreased cross-correlation between components; "↑", increased cross-correlation between the components).

Cross-correlation	Control	Ciliates	Effect	
Paramecium				
Phototrophs-EPS	0.195 ± 0.010	0.192 ± 0.010	-	
Phototrophs –Bacteria	0.043 ± 0.005	0.038 ± 0.002	-	
Bacteria-EPS	0.191 ± 0.022	0.097 ± 0.009	↓	$P < 0 .01$
Urostyla				
Phototrophs -EPS	0.172 ± 0.012	0.212 ± 0.010	↑	$P < 0.05$
Phototrophs -Bacteria	0.049 ± 0.003	0.003 ± 0.002	-	
Bacteria-EPS	0.170 ± 0.017	0.150 ± 0.010	-	

were probably not driven by substantial exploitation of biofilm components but via subtle engineering effects. The ecosystem engineering capacities of ciliates have been previously addressed in studies on bacterial biofilms (Bohme *et al*., 2009, Lawrence and Snyder, 1998). Bohme et al. (2009) showed that the formation of microcolonies of bacteria was both suppressed and stimulated in bacterial biofilms grazed by ciliates (Bohme *et al*., 2009). In the present study with phototrophic biofilms, similar changes in spatial heterogeneity were observed in the presence of ciliates. These changes were probably not caused by direct feeding, since there were no effects of bacteria ingestion by *Paramecium* on the spatial heterogeneity of bacteria. For example, phototrophs in biofilms with *Paramecium* were not affected in terms of cover or biovolume, while spatial heterogeneity was affected. Therefore, we postulate that changes in spatial heterogeneity were the result of indirect effects, caused by ciliates crawling through and over the biofilm (Berger, 2006). Similarly, at a larger scale in both terrestrial and wetland ecosystems, organisms can impose strong indirect effects on the spatial heterogeneity and landscape morphology as a consequence of their foraging behaviour, e.g. by trampling (Stavi *et al*., 2009; Jefferies, 1988; Iacobelli and Jefferies, 1991; Chapter 3). Here we show that that ecosystem engineering by organisms does not only occur at such large scales (Hastings *et al*., 2007) but also plays an important role in generating the micro-landscape of phototrophic biofilms, caused indirectly by the activity of ciliates.

In contrast to effects on spatial heterogeneity, algal biomass was unaffected (though based on CLSM data from PAM measurements, a small increase in phototrophic biomass was found). This is surprising since *Urostyla* can feed on algae, although ciliate concentrations were still rather low, which might have made it difficult to detect the exploitation of algae. High *Paramecium* abundance resulted in lower bacterial densities and EPS coverage in the biofilms at depths close to the glass disc. Since *Paramecium* feeds primarily on bacteria (Karakashian, 1963) this also decreased cross-correlation between phototrophs and bacteria. While most studies addressing the grazing of protozoans on phototrophic biofilms report negative effects of exploitation on resources (Epstein, 1997; Parry, 2004), our results show that ciliates can have a stimulating effect on resources, as grazing by *Urostyla* increased EPS biovolume. It is most likely that this increased EPS originates from EPS excretions of phototrophs, as the cross-correlation between phototrophs and EPS was the strongest of all correlations and this increased in biofilms in the presence of *Urostyla*. The increased EPS content where *Urostyla* was present might have affected our biofilms in different ways, as EPS has several functions in biofilms. For example, EPS increases the nutrient uptake from the overlaying water (Flemming and Wingender, 2001) and EPS can also serve as a protection against protozoan grazing (Pajdak-Stos, et al., 2001). The latter mechanism has also been observed in protozoan-grazed bacterial biofilms, where microcolony formation has been identified as a major protective mechanism in bacterial biofilms (Matz *et al*., 2004; Weitere *et al*., 2005). This suggests that *Urostyla* abundance stimulated EPS formation in algae, as a possible defence mechanism against ciliate grazing.

In addition to the lack of effects of ciliate presence on algal biomass, no effects on the average thickness and roughness of the biofilms were recorded. One explanation could be due to the low thickness of our biofilms, in which no complex 3D structures could be formed. In general, biofilms are composed of different morphotypes of various algae species that are important for the three-dimensional spatial structure of the biofilm. Ciliates can affect these three dimensional structures. For example, the cyanobacterium *Leptolyngbya* can grow in typical vertical clusters in the absence of copepods, while it forms a compact biofilm with a network of filaments in the presence of copepods (Caramujo *et al*., 2005). Our biofilms were in the early stage of development and reached a thickness of about 25 µm, whereas mature natural biofilms are typically up to 2 mm thick (Battin *et al*., 2003a). However, our observations do indicate that ciliates affected the micro-landscape (as discussed above) even in the early stages of development. This initial effect can have an impact during biofilm maturation, as ciliates can strongly influence the succession of benthic microalgae by selectively feeding on vulnerable species (Admiraal, 1977). Algal biofilms in their early developmental stage grow on a bare substratum, where attachment strength might be important for grazing resistance (Sommer, 1999). Later, this primary biofilm can become overgrown by filamentous algae (e.g. *Amphipleura rutilans* (*Trentepohl)* and *Haslea crucigera* (Wm. Smith)), which are resistant to grazing e.g. by periwhinkels (*Litorina litorea* (L.) when the algae are mature (Sommer, 1999). If ciliates affect spatial architecture in the stages of biofilm development, it is likely that, by interfering in the competition between algal species or morphotypes, ciliate engineering also affects spatial structure in mature biofilms. However, mature biofilms are often heavily exploited by larger herbivores such as gastropods and mayflies (Lawrence *et al*., 1998), and structuring of the biofilm by micro-fauna may not be detected.

In interpreting the effects of ciliates on spatial patterning observed in this study, it is important to take note of the scale of observation. Spatial variance changed in a similar way over all our replicates, suggesting that the effect of ciliate species on spatial variance is consistent at the scale addressed. In our study the CLSM images covered an area of 230.3 x 230.3 µm, which was slightly larger than ciliate length (ca. 150 µm in our cultures). At the selected grain scale (22 x 22 µm), which is an order of magnitude smaller than the size of our ciliates, spatial heterogeneity changes, i.e. the biofilm became more homogenous. This does not rule out that at larger scales (larger than an image of 230.3 x 230.3 µm) heterogeneity is affected differently, similar to what has been observed in other studies, where the effect on heterogeneity depended on the scale of observation (Adler et al, 2001; Sandel and Smith, 2009). Moreover, previous studies showed that biofilm complexity and community can change ecosystem processes at a larger scale (Newman and Banfield, 2002; Hoellein *et al*., 2010; Battin *et al*., 2003b). Therefore, it would be interesting to predict how small-scale ciliate grazing influences larger scale patterns and processes. However, before predicting how grazing at small spatial scales affects larger-scale spatial patterns and ecosystem processes, the scale-dependent feedbacks that underlie this spatial pattern formation should be elucidated. Moreover, mathematical descriptions of ecological patterns and their parameterization by observations are needed to predict the effects on larger scales (Battin *et al*., 2007).

In conclusion, this study showed that selective feeding on several components of young phototrophic biofilms by two different ciliate species alters biofilm morphology. Obviously this effect on spatial heterogeneity was probably not driven by massive exploitation of biofilm components, but by the subtle engineering effects of just a limited number of individual ciliate. The spatial architecture was distinctly affected by the two ciliate species, with individual effects on bacteria, algae and EPS; hence, we speculate that the spatial effect can cascade to mature biofilms. Nevertheless, to predict and understand the effects of ciliates on spatial heterogeneity of phototrophic biofilms the mechanisms should be verified at larger spatial and temporal scales.

Acknowledgments

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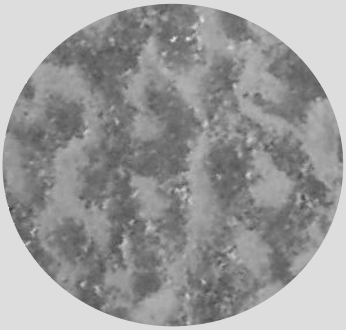
Chapter 6

Changes in patch-size distribution precede degradation in spatial self-organized mudflat ecosystems



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Abstract

Self-organized spatial patterns, regular and irregular, have been proposed as possible indicators for regime shifts in ecosystems. Until now, this hypothesis has only been tested in dryland ecosystems. Here, we focus on intertidal mudflats where we study the spatial patterns along a temporal change towards degradation. On intertidal mudflats, spatial patterns develop in early spring from the interaction between diatom growth and sedimentation but disappear rapidly when benthic herbivory increases in early summer, leading to a nearly bare mudflat. We followed the patch-size distributions of diatom biofilms during the season until all biofilms had degraded. We found a consistent change in the size distribution of diatom patches; from a truncated patch size distribution that is characteristic of regular patterns towards a power-law patch-size distribution, before all diatom biofilms were lost. The changing spatial configuration of patches co-occurred with the loss of diatom-sediment feedback processes that generate the regular patterns. This indicates a failure of the self-organization process that underlies regular patterning when this system degraded. In a part of the tidal flat area that was covered by irregular patterns, the underlying positive feedback was weak or inexistent, suggesting a lack of self-organization. For both types of patterns, the path to degradation co-occurred with the loss of the larger patches in the system, which is in agreement with dryland studies. Nevertheless, the direction of change in the shape of the patch size distribution was opposite to that found in dryland systems, because of the different mechanisms underlying the spatial patterns. Our results emphasize the necessity to understand the characteristics of the undisturbed patterns and their underlying ecological mechanisms before spatial patterns can be used as indicator of ecosystem degradation, and confirm the need for the development of ecosystem-specific indicators.

Introduction

Self-organized spatial patterns have been described in a range of terrestrial and aquatic ecosystems (Pascual and Guichard 2005; Rietkerk and Van de Koppel 2008). These patterns develop from strong feedback mechanisms that can generate coherent spatial patterns at a large spatial scale, ranging from regular to irregular patterns depending on the underlying mechanisms (Pascual and Guichard 2005; Rietkerk and Van de Koppel 2008). Recently, self-organized spatial patterns have been put forward as potential indicators for ecosystem degradation in response to changing levels of environmental or biotic stress in arid ecosystems (Rietkerk et al. 2004). In sub-Saharan drylands, a combination of low rainfall and scale-dependent feedback between vegetation and its limiting resource, water, can induce regular, self-organized spatial vegetation patterns (Barbier et al. 2008). Decreases in rainfall are predicted to induce a sequence of changes in the shape of these patterns before the system collapses to a degraded, desert state (Rietkerk et al. 2004). In Mediterranean ecosystems, a local facilitation mechanism due to the ameliorating effect of the vegetation on its local abiotic environment generates self-organized but irregular spatial vegetation patterns. These patterns lack a dominant spatial scale and are characterized by a power-law patch size distribution with many small and a few larger patches (Kéfi et al. 2007). When the vegetation degrades under overgrazing, the patch size distribution progressively loses its larger patches and becomes truncated. These combined studies spurred the hypothesis that consistent and predictable changes in the statistical characteristics of spatial vegetation patterns could be used as indicators for ongoing degradation to a desert state. Since desertification might involve tipping points at unknown thresholds and is often difficult to reverse, the development of indicators based on spatial patterns is important for ecosystem management to predict when catastrophic change is looming.

The search for predictable changes in characteristics of self-organized spatial patterns has focused on vegetation patchiness in drylands (Guttal and Jayaprakash 2009; Kéfi et al. 2007; Lin et al. 2010; Maestre and Escudero 2009; Rietkerk et al. 2004; von Hardenberg et al. 2010), restricting the application of this theory to a single ecosystem type. Moreover, most studies tested changes in patch-size distributions by comparing spatially distinct systems along a gradient of decreasing rainfall or increasing grazing intensity (Kéfi et al. 2007; Lin et al. 2010; Maestre and Escudero 2009). When comparing spatially distinct ecosystems, the environment may vary in various aspects, introducing possible confounding factors. Whether these patterns also show consistent changes preceding a temporal shift to a degraded state remains unstudied. Hence, the hypothesis that self-organized patterns show a coherent, predictable change in spatial structure when evolving towards a degraded state would benefit from further testing in other ecosystems, including the explicit analysis of time series of how spatial patterns change in time.

In this Chapter, we described temporal changes in the spatial configuration of benthic diatom patterns on the Kapellebank intertidal mudflat in the Netherlands. On 80% of the study area, regular spatial patterns consisting of diatom-covered hummocks alternating with water-filled hollows develop each spring (Blanchard et al. 2000; de Brouwer et al. 2000; Lanuru et al. 2007), due to interactions between diatom growth and geomorphological processes (Weerman et al. 2010). On the remaining 20% of the study area, an irregular patchy structure of benthic diatoms is visible, but bed level differences are absent, suggesting the lack of strong feedback-mechanisms in these areas. On the whole study area, as time progresses, a gradual and seasonal increase in benthic herbivory causes a sudden shift from a spatially patterned intertidal flat with high diatom biomass into homogeneous, almost bare sediment with constant bed level at around early June each year (Weerman et al. 2011). This intertidal mudflat ecosystem provides unique characteristics that make it an extremely interesting case study: i) the presence of spatial patterns, both regular and irregular, and ii) the build-up and collapse of the system which can be observed at short time scales and is repeated every year. This system thus offers an excellent opportunity to investigate changes in spatial pattern characteristics along a degradation gradient.

Our aim was to test the hypothesis that changes in patch size distributions along a temporal gradient preceded degradation in spatially-organized mudflat ecosystems. Therefore, we took aerial photographs and determined the bed level elevation profiles of spatial diatom patterns during the season when spatial patterns were just visible (April) until the period when spatial patterns were degraded due to high benthic herbivore numbers (June). We first applied autocorrelation analysis on the diatom patches to test for regularity. We then tested for the presence of diatom-sedimentation feedbacks by determining the relation between bed level elevation and diatom biomass. Finally, we analyzed the patch size distributions from classified aerial photographs. We then studied whether and how spatial patterns varied along degradation in intertidal mudflats, and we put these results in the broader context of the literature on spatial indicators, in particular those developed in drylands.

Materials and methods

Study site and experimental set-up

A field study was performed at the Kapellebank, a tidal flat along the edges of the Westerschelde estuary, in the Netherlands (51º27’ N and 3º58’ E). From early spring until the onset of summer, regular spatial diatom patterns were observed on 80% of the surface of the intertidal flat. Regular spatial patterns develop from small initial differences in diatom densities forming a visible brown biofilm, which in turn is strengthened by the excretion of extracellular polymeric substances (EPS) (Neumann et al. 1970). EPS inhibits erosion by increasing sediment cohesion and decreasing bottom roughness (Paterson 1990; Sutherland et al. 1998a; Sutherland et al. 1998b). This results in an increase of net sedimentation of fine-grained particles and the local formation of slightly elevated hummocks (de Brouwer et al. 2000; Montserrat et al. 2009), which subsequently stimulates diatom growth on the hummocks (Van De Koppel et al. 2001). In the hollows, in between the hummocks, water accumulates during low tide, inhibiting the build-up of EPS since EPS dissolves in the overlaying water (Blanchard et al. 2000; Paterson et al. 2000). This in turn leads to increased erosion of sediment and diatoms in the hollows during submergence. The interaction between accumulation of sediment on the hummocks and drainage of water towards the hollows generates a scale-dependent feedback of local scale facilitation and longer range inhibition of diatom growth, providing an explanation for regular spatial pattern formation when herbivore densities are low (Weerman et al. 2010). The remaining 20% of the intertidal flat was characterized by irregular patterns.

Six 12 × 12 meter plots were marked on the Kapellebank in which sediment bed level and diatom cover was determined in April, May and June 2008. Three plots were chosen in an area with regular hummock-hollow structure (hereafter referred to as the “regular plots”); three control plots were chosen in an area on flat sediment lacking regular spatial patterns (hereafter referred to as the “irregular plots”). We analyzed the six plots for spatial patterns of benthic algae and sediment bed level during these months.

Spatial patterns of benthic algae

Aerial photographs for pattern analysis were taken with a digital camera (Sony Cybershot DSC-V3) attached to a helium-inflated blimp-shaped balloon (www.floatograph.com), which was attached to a tether line. Photographs were obtained from approximately 50 meters height covering an area of approximately 50 × 40 meters (3072 × 2304 pixels). The corners of the plots were marked with a colored marker which was used to locate the plots during the image processing.

Landscape morphology

To collect morphological data of the landscape in the six plots a Riegl LSM Z-210 3D laser scanner (Riegl Instruments) was used and controlled by the software RiScan Pro. This laser scanner collects data points of x,y,z coordinates of the sediment surface, using an infrared laser beam emitted in precisely defined angular directions controlled by a spinning mirror arrangement. Prior to the scans, five reflectors were placed around the plot, the position of these reflectors was measured using a DGPS (Thales, the Netherlands) and these were automatically located by the RiScan Pro software which used the DGPS data for georeferencing the data points measured by the laser.

Data analysis

Indicators for spatial self-organization: First, we analyzed the regularity of the patterns using the autocorrelation (Moran I) of the diatom patches (Legendre and Legendre 1998). Since the entire photographs were too large to analyze, we selected a square of 75 × 75 pixels (3 × 3 meters) in the middle of each plot for autocorrelation analysis. Autocorrelation analysis was performed perpendicular to the flow direction because regularity is most prominent in this direction (Weerman et al. 2010). Second, we investigated the underlying mechanisms of these patterns by checking for the presence of positive feedback. Therefore, we generated a 300 × 300 digital elevation matrix for each plot from the landscape morphology data measured with the laser scanner. After fixing the corners of these plots by using their DGPS co-ordinates, the data points from the Riegl laser scanner were interpolated into 300 × 300 matrix using a nearest-neighbour interpolation. Similarly, the aerial photographs were rescaled in the same resolution of the laser scanner data, therefore the photographs were rescaled to 300 × 300 pixels using a sliding neighborhood filter in Matlab 2007b. We then analyzed the correlation between diatoms and sediment bed level of 3000 randomly selected points of the aerial photographs and landscape morphology data images using Pearson’s correlation coefficient.

When scale-dependent feedbacks are intact we expect high diatom numbers on top of the hummocks and low diatom numbers in the hollows. Therefore, a positive correlation between diatoms and sediment bed indicates a functional scale-dependent feedback between diatoms and sediment (Weerman et al. 2010).

Patch size distribution. From the raw aerial photographs, the 12 by 12 meter plots were selected and a binary image was created from each plot by discriminant analysis. This discriminant analysis was used to classify the pixels for each photograph into two classes: “diatoms” or “bare sediment”. The classes were based on manually selected pixels (n=15) in each class from which the brightness in the red, green and blue band was recorded and extrapolated to the other pixels in the image. For each picture, the classification method was checked by comparing the binary images with the raw photographs. From this binary image the cover fraction was calculated and patches were assigned using a Moore neighborhood of 8 adjacent pixels. A minimal patch size was set at 4 pixels to avoid artifacts at patch edges affecting patch-size distributions. For each unique patch size, the number of occurrences was calculated. From this vector of patch sizes and their occurrences, the inverse cumulative distribution was calculated and an upper-truncated power-law was fitted to the inverse cumulative distribution according to Burroughs and Tebbens (2001). Note that it differs from the fitting procedure typically used in previous studies for non-cumulative patch size distributions, e.g. by Kéfi et al. (2007) or Maestre and Escudero (2009). These authors used a binning-based method but this takes away the information about the patch-area distribution within each bin (White et al. 2008). Therefore we used a cumulative distribution in which no binning is used. The patch size distributions were fitted using the following equation describing an upper-truncated cumulative number-size distribution based on a power-law (Burroughs and Tebbens 2001).

$$M(r) = C(r^{-\gamma} - r_T^{-\gamma}) \qquad (1)$$

Where M(*r*) is the cumulative fitting function of patches of size *r* (in cm^2), *C* is a constant, γ is the scaling exponent, r_T is the patch size at which the truncation occurs. For the fitting procedure we used a pseudo-random search algorithm (pricefit module) which is provided in the R-package ecolMod (Soetaert and Herman 2009). Appendix 6.A shows that although this method is sensitive to the size of the plots, the truncation is determined by the size of the observed patches, rather than an artifact of plot size.

To give an indication of the degree of the truncation of the curves, we defined ‘observed truncation’ as the ratio between intercept of M(*r*) of the full truncated power-law model and the power-law model without truncation, but with similar scaling exponent (Appendix 6.A, Fig.6.A2). This gives information about the shape of a given distribution by showing how much it is bent compared to the case where the same distribution is fitted with a pure power law (i.e. a straight line).

Differences in cover, average patch size, the scaling exponent (*γ*), the patch size at which the truncation occurs (*rT*) and the observed truncation between different sampling times were analyzed using Students' paired t-test (two-tailed).

Results

In both regular and irregular plots, the cover fraction of diatoms decreased significantly between April and May (Fig 6.1; Fig.6.2A, regular: $P < 0.01$, irregular: $P < 0.01$). This degradation co-occurred with a decrease in average patch size (Fig. 6.2B, regular: $P < 0.01$, irregular: $P < 0.05$). In June, no diatom patches could be detected, and therefore we argue that the ecosystem can be considered as completely degraded. In the rest of this chapter, we thus focus on the analysis of the patterns in April and May.

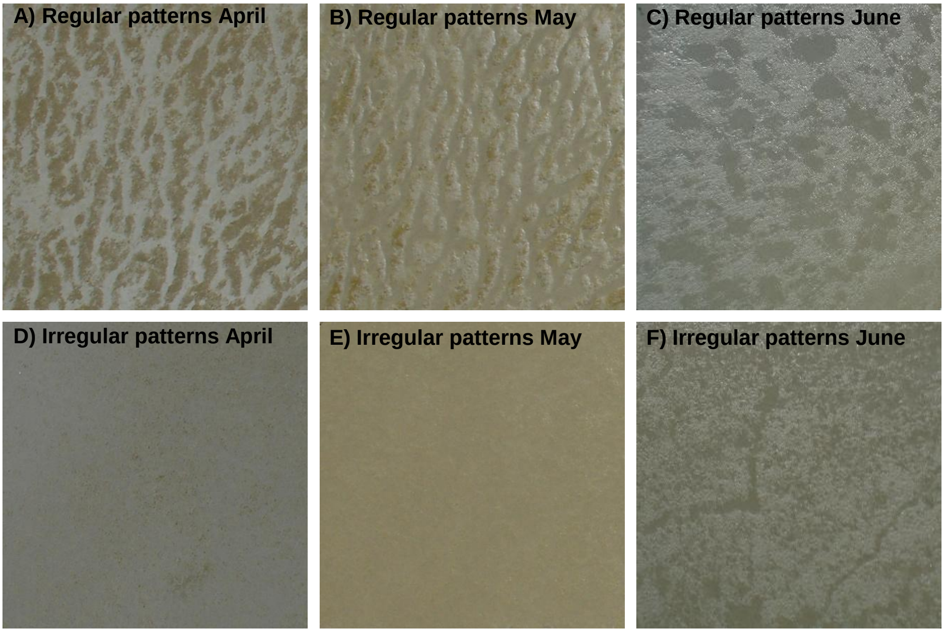

Figure 6.1: Aerial photographs of plots in April, May and June for the location with regular spatial patterns (A-C) and the location with irregular spatial patterns (D-F). Dark brown areas correspond to diatom abundance while grey areas correspond to bare sediment. Plot size is 12 by 12 meters.

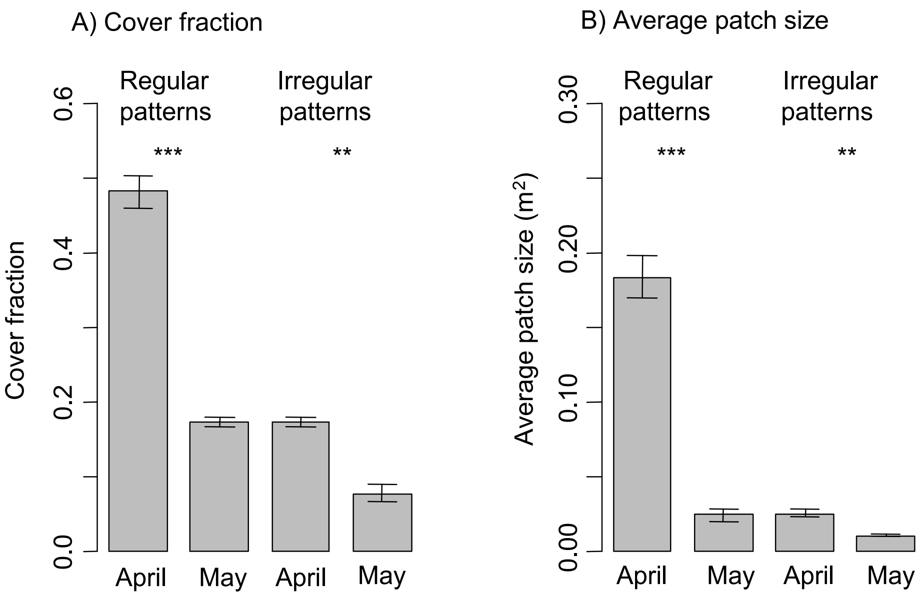

Figure 6.2: Cover fraction (A) and average patch size (B) of the irregular and regular plots in April and May. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Aerial photographs of the plots revealed clear visual differences between the regular and irregular plots (Fig. 6.1), which were confirmed by autocorrelation analysis. The regular plots showed positive autocorrelation up to 20 cm lag distance, followed by significant negative autocorrelation from 20 to 80 cm (Fig. 6.3A). This confirms that in these plots bands of high diatom biomass were regularly distributed in the direction perpendicular to the flow direction with a wavelength of roughly 1 m. In May, this regularity was lost since only positive Moran I values were found (Fig 6.3). The irregular plots showed only positive Moran I values in both April and May, indicating a lack of regularity in these plots (Fig. 6.3C and D).

To investigate possible feedbacks underlying the observed patterns, we looked at the correlation between diatom abundance (as estimated from the aerial photographs) and sediment bed level elevation in April and May (Fig. 6.4). In the regular plots, a strong positive correlation was found in April (Fig. 6.4A), while the correlation was absent or very weak in May (Fig. 6.4B). This suggests that the change in spatial configuration observed in the regular plots co-occurred with the disappearance of the scale-dependent feedback between diatom growth and sedimentation processes. In the irregular plots, no or very weak correlation was found in both April and May (Fig. 6.4C and D), which indicates that no feedbacks were underlying the observed irregular spatial patterns.

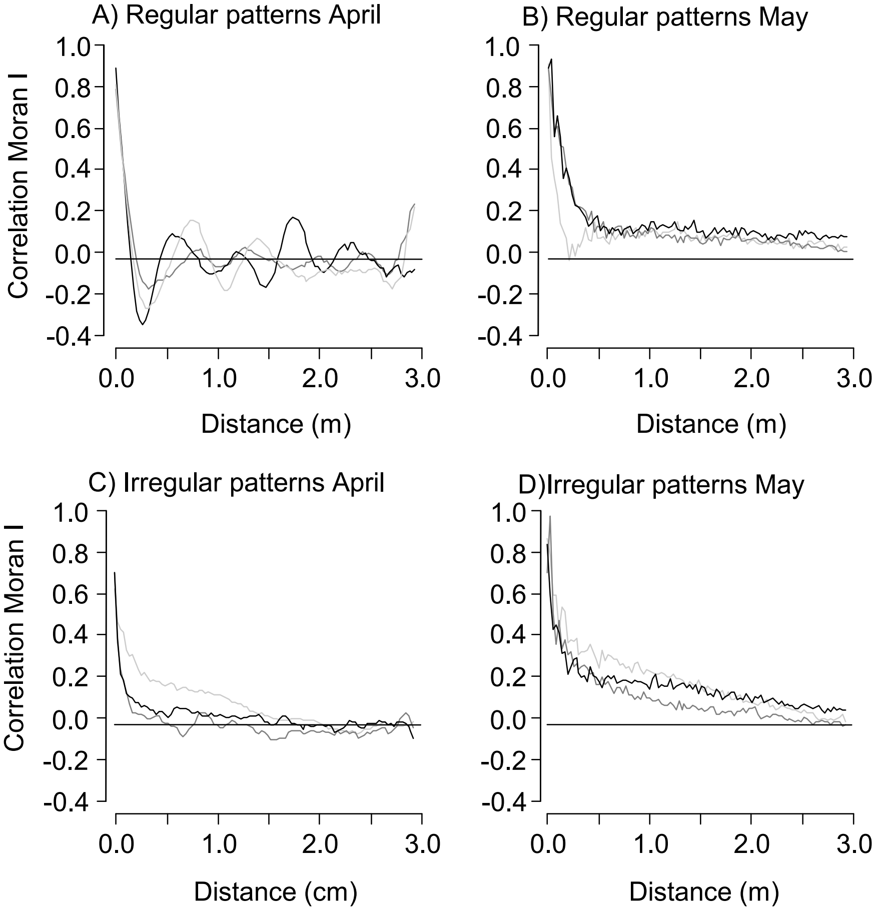

Figure 6.3: Autocorrelation analysis of the diatom patterns with the Moran I values for the regular patterns in April (A) and May (B), irregular plots in April (C) and May (D). The three different colors indicate the three different plots.

Consistently with the regularity of the patterns revealed by autocorrelation analysis, the patch size distributions of the regular plots in April were characterized by an excess of intermediate patches compared to a pure power law. This suggests the presence of a dominant spatial scale which is a characteristic of regular patterns (Fig. 6.5A). The other distributions (regular plots in May and irregular plots in April and May) approached a power-law distribution (Fig. 6.5 B-D). For the regular plots, the scaling exponent (*γ*) was found to be higher in May compared to April (Fig. 6.6A, regular: *P* = 0.12). The same trend was observed in the irregular plots but was not significant (Fig. 6.6A). At the same time, we observed a clear decrease of the truncation

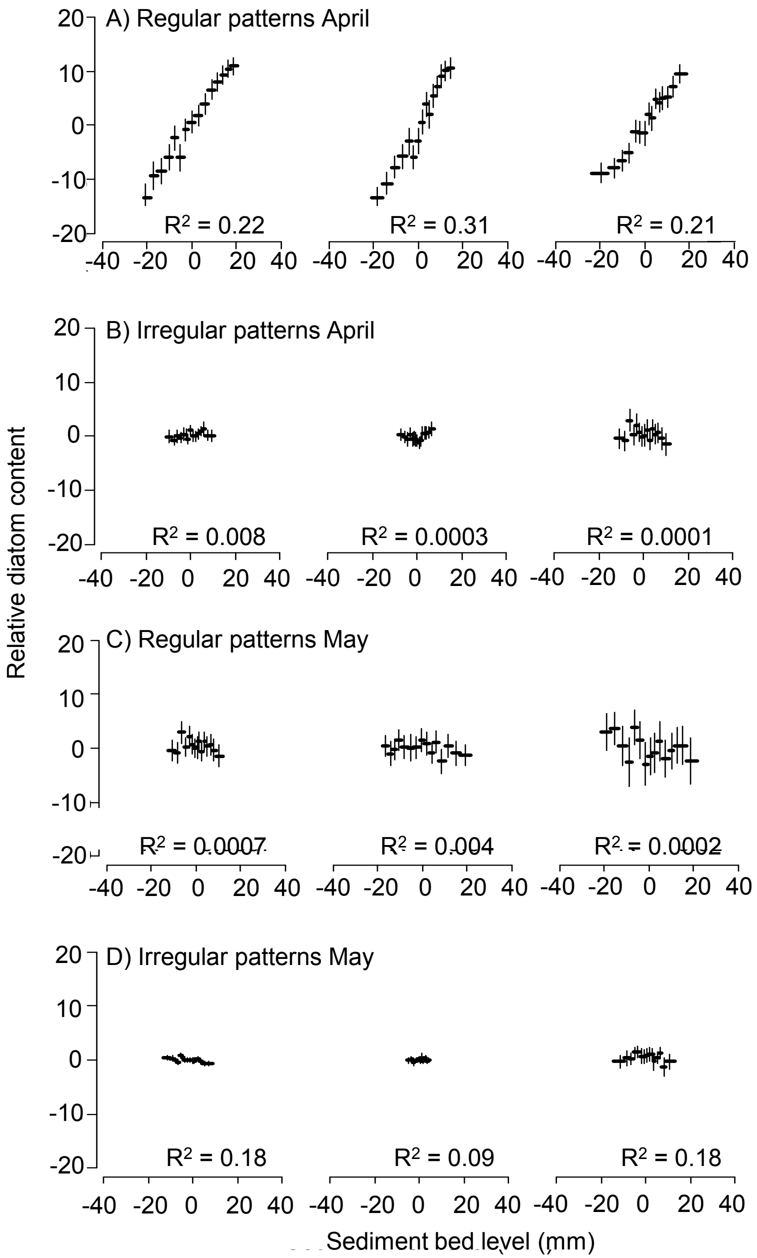

Figure 6.4: The response of relative diatom abundance as a function of bed level. The data are split into 15 classes of the variables, with an equal number of observations in each class with the mean value of each class and the 95% confidence intervals.

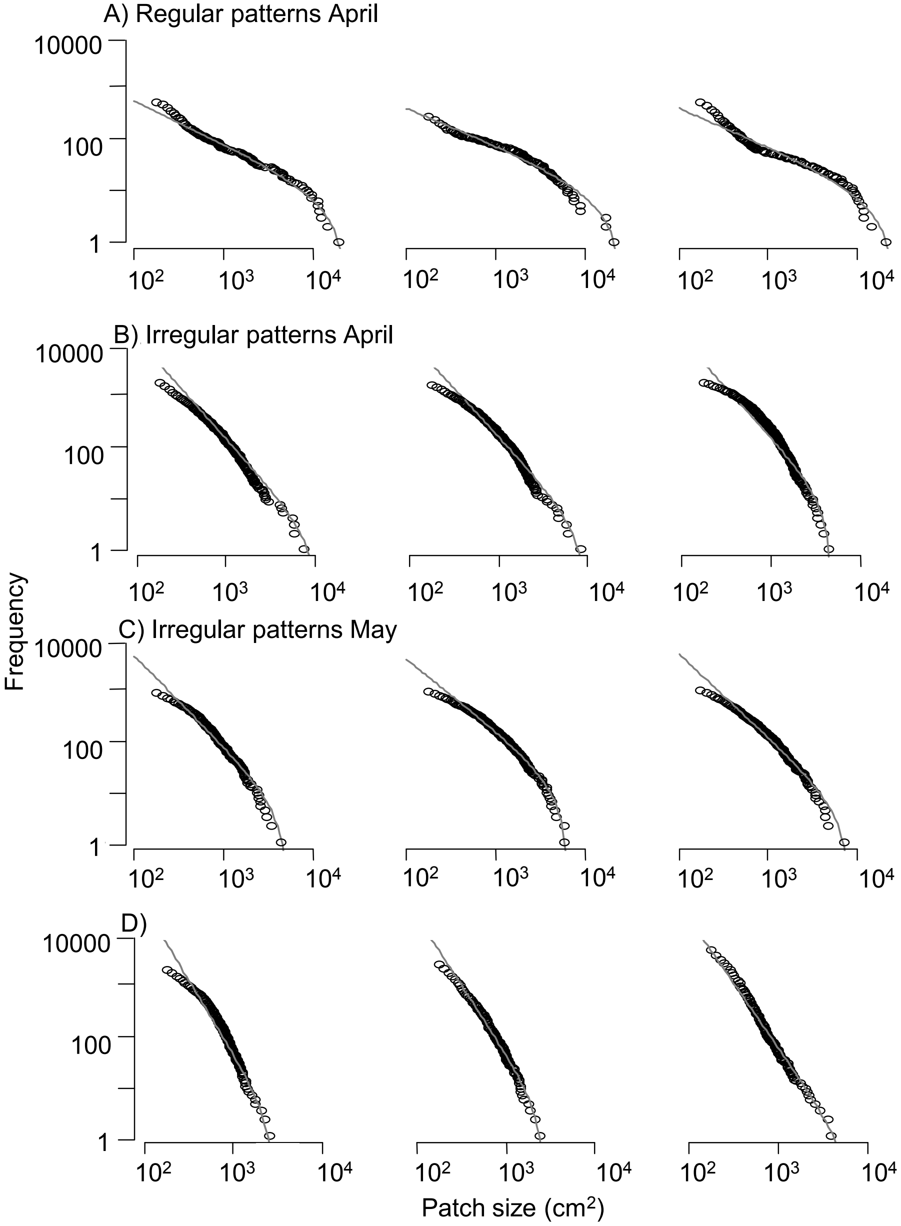

Figure 6.5: Patch size distribution of diatom patches from three replicas of each location and month. The x-axis represents the patch size (m2) and the y-axis represents frequency of patch size. Circles are observed values and grey line is the fitted model.

parameter in the regular plots between April and May (Fig. 6.6, r_T: $P < 0.01$), while this was not significant (but also decreasing) in the irregular plots. Looking more into the details of the shape of the distributions, the observed truncation was significantly different for regular plots between April and May (observed truncation: $P < 0.01$), which indicates that the distributions become closer to a pure power law distribution as degradation increases. The irregular plots did not show any significant change between these months ($P > 0.05$).

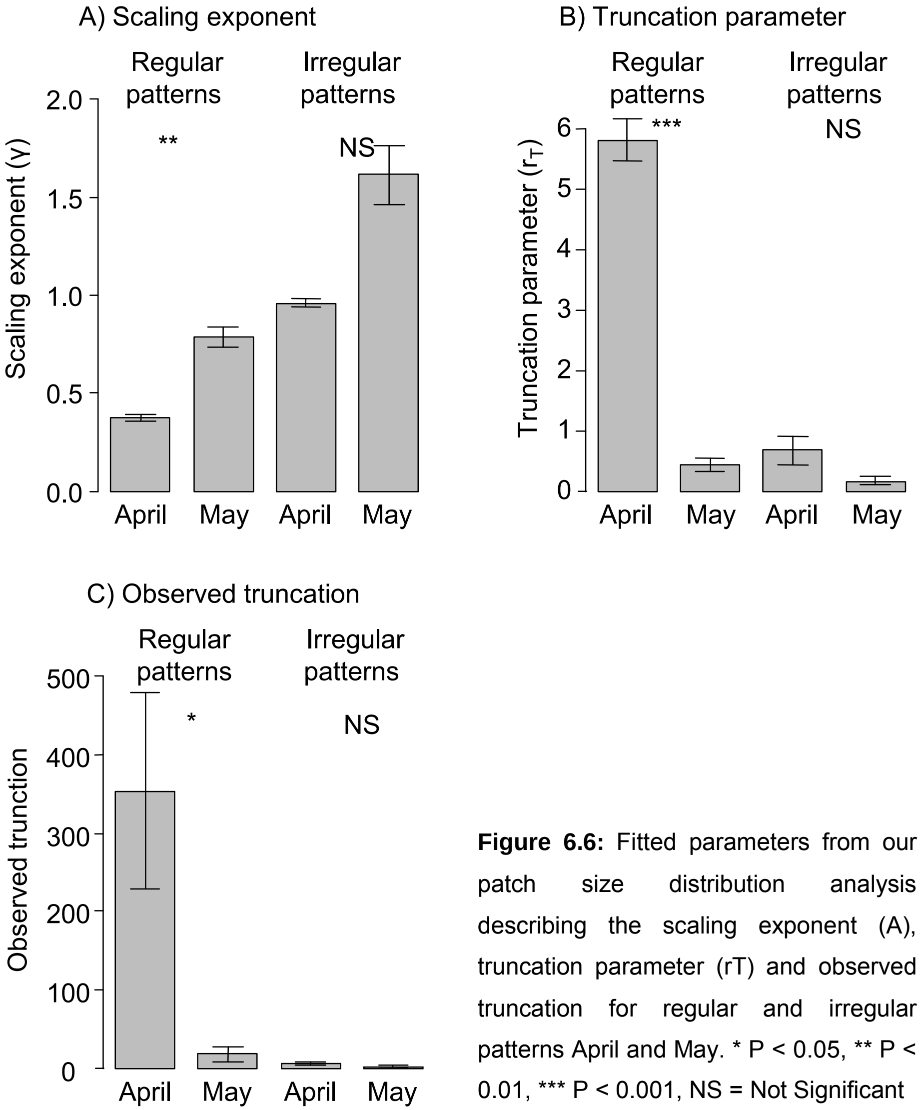

Figure 6.6: Fitted parameters from our patch size distribution analysis describing the scaling exponent (A), truncation parameter (rT) and observed truncation for regular and irregular patterns April and May. * P < 0.05, ** P < 0.01, *** P < 0.001, NS = Not Significant

Discussion

Recent studies have proposed that changes in the spatial configuration of self-organized vegetation patterns in drylands can serve as possible indicators of ecosystem degradation (Kéfi et al. 2010; Rietkerk et al. 2004). To investigate universality of this theory to other ecosystems, we studied the changes in spatial patterns of diatoms during the process of degradation in intertidal flats (Weerman et al. 2011). Our results showed consistent changes in size distribution of the self-organized regular patterns as the disturbance, caused by herbivores, increased. This is generally in agreement with the idea that the spatial organization of ecosystems might provide information regarding the level of ecosystem degradation. However the specific types of changes observed in this study were not all in agreement with the trends observed in Mediterranean ecosystems.

Most of our study area was covered by regular patterns of diatom biofilms due to a scale-dependent feedback between the diatoms and sedimentation (Weerman et al. 2010). With ongoing degradation, the scale-dependent diatom-sediment feedback was lost. This co-occurred with the loss of the regularity of the spatial patterns and associated changes in the shape of the distribution towards a power-law distribution. Similar to dryland ecosystems (Kéfi et al. 2007; Kéfi et al. 2011; Lin et al. 2010), we observed an increase in the scaling exponent and decrease of the truncation parameter; this clearly indicates a loss of the larger patches of the distribution as the system became more degraded. However, the changes in the shape of the distribution differed greatly from those observed in drylands, suggesting even an opposite trend (Kéfi et al. 2007; Kéfi et al. 2011; Lin et al. 2010). In drylands, undisturbed vegetation experiencing low grazing pressure was characterized by a power-law patch size distribution. An increasing upper-truncation of this power-law was found when grazing pressure increased (Kéfi et al. 2007, Lin et al. 2010). We observed an opposite sequence, where undisturbed regular patterns showed fitted a truncated patch size distribution. This truncation was lost with increasing grazing pressure and developed towards a power-law. This last result can be explained by the differences in the type of patterns studied. On the one hand, the Mediterranean ecosystems studied were presumably self-organized systems (although the mechanisms have not been systematically measured in the field). The dominant underlying ecological mechanism was thought to be local facilitation (not a scale-dependent feedback) and there was no indication that the strength of this mechanism decreases along a degradation gradient. Thus the patterns remained self-organized and irregular along the whole transition to degradation. On the other hand, in the intertidal mudflat studied here, the transition to degradation was associated with a shift in the type of patterns, from regular patterns, characterized by a dominant spatial scale, to irregular patterns characterized by a wider range of patch sizes. Part of the study area was covered by irregular patterns which were not associated to a scale-dependent feedback mechanism, and thus probably not self-organized. Interestingly, these patterns showed similar general trends as the regular patterns along degradation, although none were significant.

Although several studies have argued that spatial patterns could be used to predict the onset of degradation and catastrophic collapse (Kéfi et al. 2007; Rietkerk et al. 2004), the use of power-laws as a monitoring tool to gauge ecosystem degradation levels is still in its infancy, as shown by the recent discussions in the literature (Kéfi et al. 2010; Kéfi et al. 2007; Maestre and Escudero 2009; Maestre and Escudero 2010). Our results contribute to this discussion and suggest that the changes in spatial patterns that can be expected with ongoing degradation are determined by the type of patterns that characterizes the undisturbed system, and by the ecological mechanisms underlying these patterns. Understanding the “pattern attractor”, i.e. the type of pattern that can be expected under undisturbed conditions (e.g. regular or irregular, self-organized or not), is essential before predictions on how degradation can affect the patch size distribution can be safely made. Therefore we argue that, at the very least, the presence of self-organization processes in any spatially heterogeneous ecosystem needs to be empirically verified, before spatial patterns can be used as indicator for ongoing or imminent degradation.

When studying degradation in patchy ecosystems, loss of cover fraction of vegetation or of other organisms is the most straightforward way to assess the degree of deterioration of the ecosystem (Maestre and Escudero 2009; Maestre and Escudero 2010; Reynolds et al. 2007). Nevertheless, several field observations have showed that there is no causal relationship between spatial patterns and cover (Bautista et al. 2007; Lin et al. 2010). Moreover, ecosystems with strong feedback mechanisms can respond non-linearly, involving tipping points, to gradually increased stress (Rietkerk et al. 2004; Scheffer et al. 2009). Because the cover fraction at which a tipping point occurs is unknown, cover fraction alone is not sufficient to indicate the proximity of a tipping point in the ecosystem (Kéfi et al. 2010). However, our study demonstrates that the change of the scaling exponent reflected increasing fragmentation by grazing activity, as the scaling exponent γ increases with level of degradation. Nevertheless, increase in the scaling exponent provides no indication of the breakdown of key feedback processes that could initiate a catastrophic response. This is underlined by an equally strong change in the scaling exponent found in those parts that only contain irregular diatom patches as in the regular plots of our mudflat ecosystems with clearly developed diatom biofilms. Rather, we observed that a clear change in truncation of the patch size distribution and loss of spatial autocorrelation accompanied the breakdown of diatom-sedimentation feedbacks in the regularly patterned plots.

Other indicators for possible non-linear responses, like increased spatial autocorrelation and variance in proximity of a tipping point have recently been proposed (Dakos et al. 2010; Guttal and Jayaprakash 2009). This is not in agreement with our study, where we found decreasing autocorrelation along a degradation gradient. This was due to the loss of the scale-dependent feedback which occurred simultaneously as the degradation. Moreover, it is likely that a set of indicators might be more powerful in predicting regime shifts than a single indicator (Kéfi et al. 2011). In this study, we suggest that for regular self-organized patterns a combination of the observed truncation and autocorrelation, both signatures of regularity, are the most reliable assessment of ecosystem health.

Concluding, our results show that changes in the structure of spatial patterns can provide important clues about the level of degradation in self-organized ecosystems. However, the sequence in which patterns change with ongoing degradation, as well as the type of changes, depends on the underling structuring mechanisms that generate these patterns, e.g. whether they result from feedback leading to self-organization, and what type of self-organization. Therefore, we propose that before spatial patterns can be used to predict degradation in ecosystems, it is important to 1) resolve the mechanisms that underlie both the formation and degradation of spatial patterns, 2) develop a description of the pattern attractors that describe the expected patterns in undisturbed conditions, and 3) to develop indicators of both the structure of the spatial patterns and of the strength of the underlying feedback mechanisms, before a reliable assessment of ecosystem status can be made. Only when fulfilling above three criteria, spatial structure can become a promising indicator to address the proximity of a nonlinear response towards complete degradation.

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Appendix 6.A: Detailed description of the methods

Validation truncation

In this appendix we explain the method and results of the analysis which we preformed to test if the limited plot size could have caused the ‘observed truncation’ in Fig 6.4. We doubled the size of the patches at the edge of our plots and we compared the patch size distribution of this doubled patch size data to our original data. The doubled truncated patch size data resulted in similar patch size distributions compared to our original data (Fig. 6.A1). The AIC values from both analysis showed that most of the graphs fitted the original data better, expect of a few graphs of patterned plots in both April and May (Table 6.A1). The differences in the parameters that we found in our original data are still observed on our doubled patch size data. The truncation parameter (rT) and the observed truncation were significant for regular plots between April and May ($P < 0.001$ and observed truncation $P < 0.05$), while similar to the original data the truncation parameter and observed truncation did not change in the irregular plots ($P > 0.05$). The scaling exponent (γ) was significant for both pattern types between the months (regular: $P < 0.001$ and irregular $P < 0.05$). These results indicate that mainly on the patterned plots, where patch sizes are bigger, the truncation parameter is partly influenced by our limited plot size. However, the significance of the fitted parameters reveal that most of the truncation can be explained by biological processes as doubling the size of the truncated plots resulted in a minor change in parameters.

Table 6.A1: The differences in parameters for both models, on the original data and the doubled patch size data. The model selection is based on lowest AIC with minimal difference of 2.

Pattern	Month	Original Data					Doubled edge patches					Best Fit
		C	γ	rT	Obs.Trunc	AIC	C	γ	rT	Obs.Trunc	AIC	
Unpatterned	April	1.36	0.96	0.93	0.54	297.66	1.59	0.93	2.23	1.65	338.32	Original
Unpatterned	April	2.02	0.93	0.89	0.60	530.61	2.45	0.90	1.26	2.71	527.33	Doubled
Unpatterned	April	1.74	1.00	0.22	0.19	635.96	1.74	1.00	0.37	1.74	648.75	Original
Patterned	April	12.63	0.40	5.17	3.63	104.07	13.21	0.40	11.37	655.29	95.94	Doubled
Patterned	April	15.30	0.35	5.96	4.29	147.94	16.50	0.34	12.90	4135.00	128.43	Doubled
Patterned	April	13.75	0.36	6.34	4.39	180.45	14.74	0.35	13.31	1989.63	188.17	Original
Unpatterned	May	0.01	1.75	0.13	0.06	381.86	0.01	1.77	0.16	0.07	429.28	Original
Unpatterned	May	0.09	1.32	0.07	0.05	120.49	0.10	1.31	0.12	0.17	131.52	Original
Unpatterned	May	0.16	1.25	0.30	0.15	175.17	0.01	1.79	0.30	0.08	442.12	Original
Patterned	May	1.08	0.90	0.25	0.19	225.88	0.91	0.94	0.56	0.91	310.99	Original
Patterned	May	5.78	0.69	0.38	0.34	293.60	5.69	0.69	0.50	12.33	345.54	Original
Patterned	May	2.59	0.80	0.64	0.48	335.70	3.15	0.77	0.66	4.45	270.21	Doubled

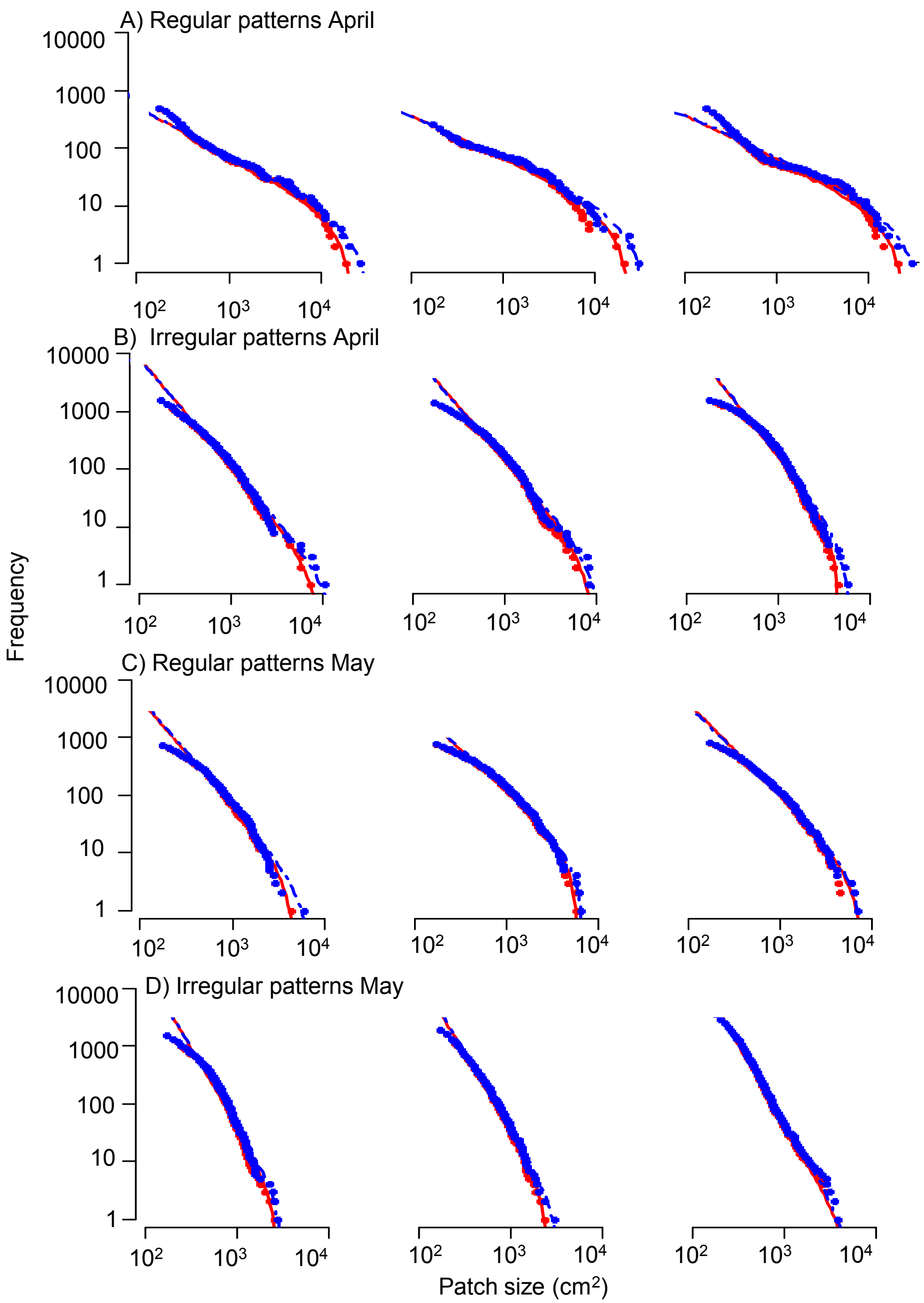


Figure 6.A1: Patch size distribution of diatom patches with patch sizes on the x-axis and frequency on the y-axis. Two data sets and model fits are plotted: 1) Original data in red and 2) double patch sizes of the truncated data in blue.

Observed truncation

The ‘observed truncation’ is defined as the difference between intercept of M(r) of the full truncated power-law model and the power-law model without truncation, but with similar scaling exponent. This observed truncation parameter is calculated as the ratio between the intersection point with the x-axis of the truncated model and a power-law model with the same scaling parameter, but where truncation is absent (Fig. 6.A2).

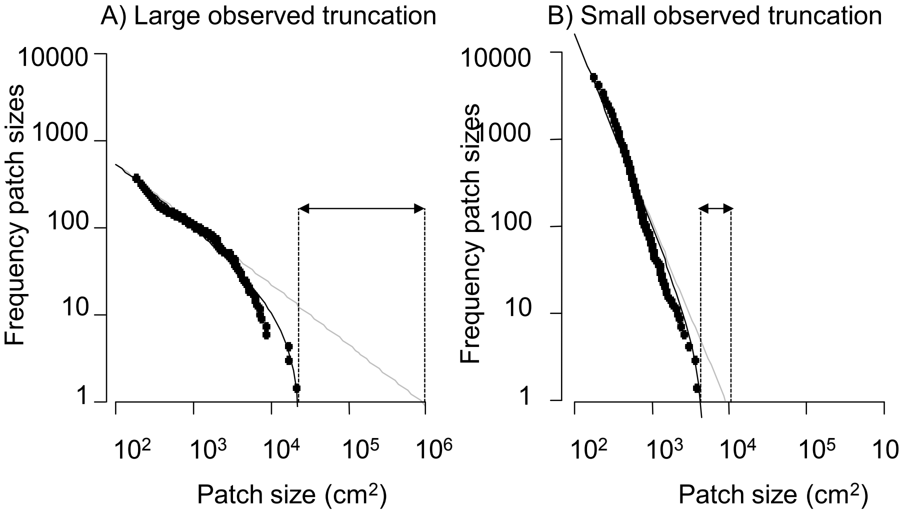
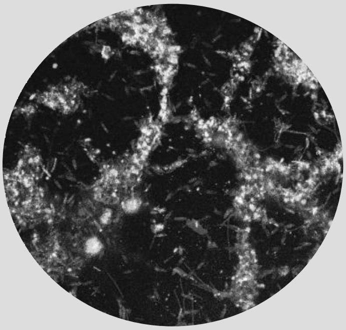


Figure 6.A2: Patch size distribution of diatom patches with patch sizes on the x-axis and frequency on the y-axis. Two models are fitted through the data (dotted points), 1) truncation model (black line) and 2) the truncation model where the truncation is set to zero (grey line). The observed truncation is calculated as the ratio of the intersection point of the truncated model and the same model where the truncation is set to zero. The observed truncation is indicated with the dashed lines and arrows in between. Figure A shows a plot with a high observed truncation (583) and figure B shows a plot with low observed truncation (1.32).

Chapter 7

General discussion



Introduction

In this thesis, I have presented a study on the interactions between biological and physical processes that determine the formation of spatial structure of intertidal mudflats. The interaction between diatoms, water drainage and sedimentation processes was found to be a key determinant of the formation of a regular landscape of diatom-covered hummocks and water-filled hollows. These spatial patterns were found to affect ecosystem functioning by increasing total diatom content and net sedimentation on the scale of the entire mudflat (Chapter 2). Subsequently, I showed that top-down control by benthic herbivores has a major influence on landscape formation, affecting benthic phototrophic biofilms at microbial scale (µm) as well as on intertidal flat scale (m). Herbivores controlled pattern formation following the seasonal collapse of the diatom biofilm patterns (Chapter 3) and in the laboratory ciliate grazers were found to decrease clustering in the micro-scale landscape of photrophic biofilms (Chapter 5). Top-down control did not only affect landscape formation, but also increased net primary production (but not biomass), which was validated by laboratory experiments (Chapter 4). Analysis of changes in the spatial characteristics of spatial patterns as they degraded due to increased grazing intensity revealed predictable changes in their spatial configuration which point out that there is a potential use of spatial patterns as an indicator for rapid shifts to a degraded state (Chapter 6).

In this Chapter I will discuss the implications of the results presented in this thesis, addressing them according to the following points:

- Organisms as landscape engineers
- Scaling up from organisms to landscapes
- Top-down control on landscape formation
- Implications for spatial self-organization theory

Organisms as landscape engineers

Spatial heterogeneity of landscapes has mainly been studied in terrestrial ecosystems where wetland ecosystems such as rivers, streams and estuaries have been considered as elements of a landscape mosaic. A framework for integrating wetland ecology with landscape ecology was developed by Wiens (2002), which is based on several themes explaining how landscapes are structured, how they function and how they affect ecological processes. Organisms are considered as passive entities, responding to landscape patterns. However, the work in this thesis shows that, to the contrary, organisms can themselves shape their own habitat, the community in which they live, and even the geomorphic landscape by interacting with physical and chemical processes.

The integration of the disciplines of ecology and geomorphology, also referred as biogeomorphology, has been an increasing focus of study in the last decade (Murray et al. 2002, Murray et al. 2008). The study of the interaction between biological and geomorphological processes revealed that organisms can have pronounced effects on landscape formation (Stallins 2006). In this thesis I have shown that microphytes, when forming concentrated phototrophic biofilms, structure the intertidal mudflat landscape by inducing spatial patterns (Chapter 2), similar to what was found for the well known engineering effects of macrophytes like sea grasses (Van der Heide et al. 2010). This landscape formation process can be disturbed by herbivores, which reduce the impact of engineers on the physical or chemical landscape by reducing biomass. This will affect ecosystem functioning at scales that exceed the direct effect of these organisms. Moreover, organisms can change ecosystem functioning by generating spatial feedbacks, like in Chapter 2 where benthic diatoms increased diatom density and sedimentation. Despite of these clear-cut effects on ecosystem functioning caused by spatially explicit interactions, spatial heterogeneity has only scarcely been addressed in the field of biogeomorphology. This line of evidence, extending upon Wiens (2002), highlights that organism traits are a major factor in physical-ecological interactions.

The role of structuring organisms, mainly ecosystem engineers, has been studied broadly in various ecosystems since Jones et al. (1994) launched the term ecosystem engineers. However, cross-system comparisons have scarcely been made. This is potentially an important tool to provide insight into the importance of engineering effects for landscape formation. The process may differ between terrestrial and wetland systems, because of the dominant role of water flow as a structuring factor in the latter. Water exerts a strong and variable physical force on wetlands ecosystems. As a consequence, water-flow can generate a dynamic patch structure in wetland ecosystems, e.g. by trapping of sediment by vegetation and affecting the redistribution of water flow as a result of this (Temmerman et al. 2007, Van Wesenbeeck et al. 2008). Although patch dynamics are considered more important in wetland ecosystems than in terrestrial ecosystems (Palmer et al. 2000), processes that cause regular patterns were found to be similar across wet and dry biomes. Hence, despite of critical differences between terrestrial and aquatic landscapes, cross-system studies also point to the universal nature of ecological pattern formation.

Scaling up from organisms to landscapes

The previous chapters showed that scale is fundamental in ecology, since it determines how we study and interpret patterns and processes which can act at different temporal and spatial scales (Wiens 1989). Landscape formation can be studied at different scales, e.g. from landscape scale (Chapter 3) up to micro-landscape scale (Chapter 5). Moreover, spatial scale might be strongly coupled to temporal scale. In this study, large-scale patterns typically developed on a longer time scale, e.g. landscape scale patterns in diatom biofilms take weeks to develop (Chapter 2), while micro-scale patterns develops within days (Chapter 5). Moreover, the temporal and spatial scale at which biogeomorphological interactions affect the environment depend on the strength and the rate of dissipation of the remaining physical structure once the engineering by organisms has stopped (Hastings et al. 2007).

Time scales addressed in this thesis range from days in micro-landscape biofilms (Chapter 5) up to seasons in landscape scale patterns (Chapter 3). Hence, it would be interesting to predict how these effects would cascade through to longer time scales. For example, the effects ciliates have on micro-landscape biofilms could have significant effects on mature biofilms. A modeling study revealed that disturbing biofilms on a relative short time scale (<10 days) still show in significant changes in biofilm composition at longer time scales (>100 days) (Van der Grinten 2004). Therefore, it is plausible that the ciliates also affect biofilm spatial structure at longer timescales. An intriguing question would be if this short-time scale effect can have a snowball effect on the larger temporal and spatial scale like on the spatial patterns described in Chapter 2. To answer this question we first must understand how information is transferred from small to large scale and vice versa including the time scale in these transitions (Levin 1992). Empirical evidence for these temporal and spatial cascading effects is very scarce and would be a challenge for future research.

Trophic control on landscape formation

Research in this thesis showed that organisms of higher trophic levels not only followed landscape structure, (Chapter 4), but also created and affected it (Chapter 3 and 5). I studied the interactions between benthic herbivores and landscape formation, where organisms such as *Hydrobia ulvae* and *Corophium volutator* graze upon diatoms, the dominant inhabitants of phototrophic biofilms. On both a landscape scale of meters (Chapter 3) and on a within-biofilm scale of micrometers (Chapter 5), herbivores affect the landscape by their direct and indirect feeding effects. Similar indirect effects of feeding herbivores on landscape patterns have been observed in a wide range of ecosystems. In arid ecosystems trampling by herbivores results in water-loss and erosion of sediment, creating micro-elevations and depressions (Stavi et al. 2009). Similarly, in tundra ecosystems, the grubbing of salt-marsh vegetation by geese has a devastating effect on salt-marsh plants by changing large areas to bare mud (Jefferies 1988, Iacobelli and Jefferies 1991). These studies combined with the results of Chapter 3 and 5 confirm that in both terrestrial and wetland ecosystems, herbivores can have a major impact on landscape formation

Trophic interactions can have a profound effect on the effectiveness of ecosystem engineers in modifying their environment. In marine ecosystems, one of the first examples explaining how the seasonal changes in sediment stability on intertidal flats are a result of trophic control has been described by Daborn et al. in 1993. Here diatoms stabilized the sediment in early spring, until *Corophium volutator* numbers started to increase and bioturbated the sediment while feeding on diatoms. From there the sediment destabilized until the arrival of the sandpipers. The birds caused a decrease of the *Corophium* population and hence indirectly increased sediment stability again (Daborn et al. 1993). Jones et al. (1997) referred to this as ‘coupled engineering and trophic cascades’ where the sandpiper only can have such a big effect because the diatoms and *Corophium* are ecosystem engineers. The coupling between ecosystem engineers and trophic control has been observed in several ecosystems, for instance in kelp forest (Estes and Palmisano 1974) and is predicted to have strong community wide effects (Hacker and Bertness 1996).

Interaction between organisms and their environment can integrate with other types of interactions to form complex networks that are typical of most natural communities (Olff et al. 2009). These networks involve trophic interactions, like the broadly studied predator-prey interactions (Hutchinson 1959, Paine 1966, Chapter 4) or non-trophic interactions such as plant-pollinator interactions or other interactions were the involved organisms not directly eat one another, but influencing each other by other means (Bascompte 2007). Both trophic and non-trophic interactions were found to play a major role in the formation and collapse of spatial structure in phototrophic biofilms. Direct feeding of benthic herbivores on benthic algae was observed in Chapter 4, where benthic herbivores depleted the algae. However, I found evidence for indirect positive effects of grazing on diatom growth, as grazing increased biomass-specific primary production, possibly caused by increased nutrient availability. This phenomenon has also been described in terrestrial ecosystems (McNaughton 1984), were grazing increases grass production in so-called grazing lawns. This increased production can cascade towards higher trophic levels and increases productivity of the entire foodweb (Ulanowicz 1997). Increased production as a result of indirect effects of grazing is a possible explanation for the continued increase in abundance in benthic herbivores on our tidal flat late in the season (Chapter 3). By reducing benthic algae stock, and therefore reducing the ecosystem engineering capacity of the diatoms, herbivores indirectly release the sediment which is stored in the hummocks (Chapter 2), affecting sedimentary processes in both benthic as well as pelagic ecosystem (Chapter 2).

Implications for self-organization theory

The process of spatial self-organization has been put forward as a unifying explanation for spatial patterns observed in ecosystems that lack conspicuous underlying heterogeneity. Self-organized patterns have been observed in ecosystems all over the world, ranging from arid bush lands (Rietkerk et al. 2002), peat lands (Eppinga et al. 2009), mussel beds (Van der Koppel et al. 2005), and, with the results presented in this thesis, intertidal mudflats. Theoretical studies emphasize that in many cases these patterns emerge from a scale-dependent feedback mechanism (Rietkerk and Van de Koppel 2008). Here, facilitation processes locally improve living conditions through, for instance, the accumulation of nutrients (oligotrophic peat lands) or water (arid-ecosystems), causing a small-scale positive feedback. This is countered by larger-scale inhibitory processes, for instance, through depletion of resources that cause a negative feedback (Couteron and Lejeune 2001, Rietkerk et al. 2002, Rietkerk et al. 2004b, Eppinga et al. 2008). This scale-dependent feedback mechanism was shown to be a unifying explanation for regular spatial patterns in various ecosystems (Rietkerk and Van de Koppel 2008).

So far studies focused on the redistribution of resources as the prime process behind scale-dependent feedback. In this thesis, I show that diatom-sediment interactions also induce a scale-dependent feedback, but via an entirely different mechanism. On intertidal flats, a close interaction between diatom growth and increased sedimentation induces a locally increased drainage of water. By locally increasing elevation, water, which reduces the effectiveness of EPS and hence biofilm integrity, is diverted to places that do not have increased elevation. Although resource concentration and stress-divergence involve different feedback processes, both mechanisms have spatial accumulation of stress as a common feature of their scale-dependent mechanism. Organisms accumulate resources like nutrients (Eppinga et al. 2009) or water (Rietkerk et al. 2002) in ecosystems where the resource concentration mechanism is the underlying mechanisms behind spatial pattern formation. In ecosystems where the stress-divergence mechanism is the underlying mechanism, the accumulation of hydrodynamic stress results in the formation of spatial patterns. On tidal flats, water is accumulated in lower places which dissolves the EPS and results in a negative feedback that underlies the scale-dependent feedback (Chapter 2). This accumulation of hydrodynamic stress might be the main driver for regular diatom patterns on intertidal flats. Since the positive feedback between diatoms and sediment is a wide spread phenomenon on intertidal flats (for review see Stal 2009), it is likely that the negative feedback mechanism is limiting on most tidal flats in the formation of regular diatom patterns. Water needs to accumulate at lower places in order to cause the EPS to dissolve and to erode the sediment with incoming or outgoing tide. Similar hydrodynamic stress divergence has been described for *Spartina anglica* patches, where *Spartina* patches locally reduce water flow rate, promoting plant growth, but divert flow energy around the patches where it causes the formation of a gully by increased erosion (Van Wesenbeeck et al. 2008). Hence, my study in intertidal mudflats, in combination with the results from other intertidal studies, points out that stress divergence is an alternative mechanism for pattern formation, and might be a common principle in systems that experience energetic stress caused by flowing water of wind.

Mathematical studies predict that spatial self-organized patterns can have pronounced effects on ecosystem functioning, for instance by increasing resilience or primary production (Rietkerk et al. 2004a, Van de Koppel et al. 2005). Field-based experiments exploring the effects of self-organized spatial patterns on ecosystem functioning are still very scarce (Schmitz, 2010). Few field studies on spatial patterns showed that evenly spaced distribution of organisms like termites or mussels increased biomass of these organisms (Van de Koppel et al. 2008, Pringle et al. 2010), and that this effect can even cascade to higher trophic levels (Pringle et al. 2010). In this study, we found clear evidence that patterned parts of intertidal flats accumulate more diatom biomass and sediment during the season as compared to parts that had no patterns, further solidifying the concept that self-organization has emergent consequences for ecosystem functioning.

Seasonal monitoring revealed that diatom biomass accumulates gradually early in the season, but decreases rapidly in early summer (Chapter 3). The combined grazing and bioturbation of benthic fauna causes a release of the sediment that is stored in the hummocks in early summer, which is likely to increase the suspended sediment load in the water column, and hence reduce pelagic production (Chapter 3). Similar seasonal phenomena have been described for marine intertidal systems (Van der Wal et al, 2010, Herman et al. 2000, Staats et al. 2001b, Montserrat et al 2008, Anderson et al. 2001), yet the decrease in benthic algae during these seasonal patterns was more gradual compared to the decrease of benthic algae described in Chapter 3). This implies that intertidal flats with self-organized regular patterns experience a stronger decrease in phototrophic biomass, relative to tidal flats lacking these spatial patterns. To what extent the rise and demise of spatial patterns on mudflats affects estuarine sediment budgets in both natural and human-disturbed mudflats systems remains an important topic for future research.

Our field measurements showed a sudden collapse from a patterned towards a degraded, mudflat lacking dense phototrophic biofilms. Sudden regime shifts have been observed in a wide range of ecosystems (Scheffer 2001), and impose a major challenge to both conservation and restoration policies. Predicting these rapid non-linear shifts is therefore a major goal in conservation ecology (Scheffer et al 2009). Ecosystems where rapid shifts occur are characterized by strong feedback mechanisms. In these systems, regime shifts might therefore be difficult to reverse (Scheffer et al. 2001, Rietkerk et al. 2004, Scheffer et al. 2009). It has been proposed that the changes in spatial patch size configuration of self-organized patches follows a predictable sequence towards degradation (Rietkerk et al 2004a, Kéfi et. al 2007). This puts forward the hypothesis that changes in the characteristics of spatial patterns can be used as an indicator for imminent degradation, as has been observed along spatial gradients towards degradation in arid grasslands. This hypothesis was tested in this thesis, where we found indeed a consistent change in spatial configuration of diatom patches as diatom biofilms are degrading due to increased grazing activity.

Most studies have analyzed spatial self-organized patterns using mathematical models. Despite the impact of spatial self-organized patterned ecosystems on ecosystem functioning very little empirical verification has been addressed. Hence, this thesis shows that predicting the behavior of spatial self-organized patterns it is important to resolve the underlying feedback mechanisms that result in spatial patterns (Chapter 6). Combining theoretical with empirical verification can generate new ideas for spatial self-organized ecosystems which are valuable for management and conservation policies.

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Summary

Ecological theory acknowledges the importance of spatial heterogeneity in ecosystems. Yet, the ecological mechanisms that generate persistent spatial heterogeneity in ecosystems, and their implications for ecosystem functioning, are still scarcely studied. In the past decades, a large number of studies have emerged describing the formation of coherent spatial patterns in systems all over the world. Strikingly, in many systems, there was little underlying heterogeneity that could explain the patterns. This has lead to the hypothesis that the patterns were self-organized, suggesting that they resulted from ecological interactions, of from interactions between organisms and their physical environment. Mathematical studies indicate that self-organized spatial patterns affect ecosystem functioning, for instance by increasing production. Moreover, it can make these systems vulnerable to sudden and dramatic shifts between ecosystem states. However, the development of spatial patterns and their impact on ecosystem functioning have scarcely been studied in a real-world ecosystem. Therefore, I have studied pattern formation in soft bottom communities in benthic ecosystems and I focus on the following research questions:

- Can interactions between biological and geophysical processes result in formation of spatial patterns in soft bottom communities
- How do herbivores affect the formation of spatial patterns?
- Do spatial patterns in phototrophic biofilms affect ecosystem-level processes?
- Can spatial self-organized patterns be used for predicting discontinuous shifts in ecosystems?

The first research question was addressed by studying the formation of spatial patterns in phototrophic biofilms on a landscape scale (meters, Chapter 2 and 3), and patterns in phototrophic biofilm at a microscopic scale (micrometers, Chapter 5). On intertidal flats, landscape patterns of diatom-covered hummocks and water-filled hollows develop each year in early spring, and disappear very rapidly again in early summer (Chapter 2). These spatial patterns developed from internal feedbacks between diatom growth and sedimentation processes. Field measurements and a manipulative experiment confirmed that increasing densities of benthic herbivores inhibited spatial patterns to develop after spatial patterns had disappeared. This indicates that within a timeframe of days, the intertidal flat changes from a spatial patterned system into a homogeneous system where herbivores regulate landscape formation by inhibiting spatial patterns to re-establish.

Remarkably, despite of low algal benthic biomass on the tidal flat, herbivore abundances continue to increase, after the collapse of the diatom biofilms. Laboratory experiments with two commonly occurring benthic herbivores, the mudsnail *Hydrobia ulvae* and the amphipod *Corophium volutator*, showed that these species increase diatom production. This would allow herbivores to survive low food availability, hence maintaining high herbivore density and control of diatom biomass (Chapter 3). Similar effects were observed on a smaller scale where I experimentally investigated the influence of herbivores on phototrophic biofilms on a micrometer scale. These results show that ciliates influence the 3D landscape of thin phototropic biofilms, by homogenizing patches of algae, EPS and bacteria without substantial removal of biomass of these components. Therefore, I argue that this influence of ciliates on landscape formation in young biofilms can have pronounced effects on ecosystem functioning at longer timescales, like when biofilms are mature (Chapter 5). These explorations indicate that interactions between ecological and geomorphological processes play a key role in structuring ecological systems at different spatial and temporal scales.

The second research question, on the influence of pattern formation by the ecosystem engineering activity of diatoms on ecosystem functioning, was studied on the hummock-hollow patterns observed on tidal flats (Chapter 2). Here the model output predicted that spatially patterned intertidal flats contained more diatoms and had increased sediment accretion compared to homogenous intertidal flats. This model prediction was confirmed by field measurements, where higher sedimentation and benthic algae content was measured in spatially patterned plots relative to plots that lacked patterns. This shows that spatial self-organization in benthic biofilms changes ecosystem functioning, which is expected to affect benthic ecosystems.

In Chapter 6, I analyzed whether spatial patterns provided an a-priori indication of the rapid shifts from spatially patterned ecosystem dominated by diatom biofilms to degraded sediment lacking any diatom cover, as was observed in Chapter 3. Therefore, I analyzed a sequence of aerial photographs of diatom biofilm patterns as increased herbivory degraded the diatom patterns, until diatom biofilms patterns had altogether disappeared in early summer. Patch size analysis revealed that the distribution of diatom patches changed in a systematic way with increased degradation, supporting the hypothesis that a coherent change in patterning precedes ecosystem degradation. However, I argue that before self-organized spatial patterns can be used as indicator of imminent degradation in ecosystems, it is important to resolve the mechanisms that underlie the formation of spatial patterns, and develop ecosystem-specific indicators of imminent degradation.

Concluding, this thesis provides an empirical basis for both the processes that generate and change (self-organized) spatial patterns in phototrophic biofilms and the effects that biofilms have on ecosystem functioning. By inducing spatial self-organization, interactions between ecological en geomorphologic processes can change ecosystem structure and functioning at scales that exceed the scale of their direct effects. Trophic interactions were shown to be of particular importance as they, by reducing diatom biomass and changing landscape formation. Despite of their prevalence in wetland ecosystems, these themes are hardly considered in other studies on biota-sediment interactions. Including both trophic as biophysical interactions in future research on landscape formation of wetlands will increase understanding of patterns and processes in spatially patterned ecosystems, where effects can cascade through trophic levels and through spatial scales. Combining theoretical with empirical research can generate new perspectives on the effects of biophysical interactions on landscape formation, ecosystem functioning and the potential for regime shifts which is valuable for management and conservation policies.

Samenvatting

Ruimtelijke heterogeniteit wordt in de ecologie erkend als een belangrijke factor in het functioneren van ecosystemen. Er is echter weinig bekend over de ecologische mechanismen die deze ruimtelijke heterogeniteit in landschappen veroorzaken. De afgelopen jaren zijn veel studies gedaan naar de mechanismen achter het spontaan ontstaan van ruimtelijke patronen in omgevingen zonder onderliggende abiotische heterogeniteit. Uit deze studies is de hypothese naar voren gekomen dat deze patronen gevormd worden door zelfregulerende processen in de ecologische interacties tussen organismen en hun fysische omgeving. Wiskundige modellen suggereren bovendien dat de patronen het functioneren van een ecosysteem beïnvloeden. Zo kunnen dergelijke patroonrijke ecosystemen een hogere productie hebben vergeleken met ecosystemen zonder zelfregulerende patronen. De organisatie in patronen draagt ook bij aan de weerstand tegen veranderingen waardoor er minder snel omslagpunten in het ecosysteem kunnen voorkomen. Begrip over het functioneren van zelforganiserende ecosystemen wordt gehinderd door het ontbreken van studies over het effect van hogere trofische niveaus zoals herbivoren. Om dit gat in de kennis op te vullen, beschrijf ik in dit proefschrift het onstaan van ruimtelijke patronen in diverse aquatische systemen, met de volgende onderzoeksvragen als uitgangspunt:

- Kunnen bio-fysische interacties ruimtelijke patronen op waterbodems veroorzaken?
- Kunnen ruimtelijke patronen in bentische fototrofe biofilms ecosysteemfuncties veranderen?
- Kunnen herbivoren het onstaande ruimtelijke patroon veranderen?
- Kunnen ruimtelijke patronen voor het voorspellen van omslagpunten in ecosystemen gebruikt worden ?

Voor het beantwoorden van de eerste onderzoeksvraag heb ik onderzoek uitgevoerd op verschillende ruimtelijke schalen. In Hoofdstukken 2 en 3 bestudeer ik ruimtelijke patronen op de schaal van meters en hun ontwikkeling door het seizoen. In Hoofdstuk 5 bestudeer ik patronen op de schaal van bentische fototrofe biofilms (microscopisch) over korte tijdsperiodes (dagen).

Op getijdenplaten kan een jaarlijks terugkerend fenomeen waargenomen worden, waarbij een kenmerkend landschap van heuvels en dalletjes ontstaat in de vroege lente, dat weer verdwijnt rond mei. De heuveltjes zijn bedekt met een dikke diatomeeënmat, terwijl de dalletjes met water zijn gevuld. Ik heb in een wiskundig model het ontstaan van deze patronen gesimuleerd. De heuvels en dalletjes ontstaan door schaalafhankelijke terugkoppelingen tussen bentische diatomeëen en sediment (Hoofdstuk 2). Observaties als experimenten in het veld bevestigden het bestaan van de terugkoppelingsmechanismen en toonden aan dat het model correcte voorspellingen maakt. Ze hebben bovendien aangetoond dat herbivore bodemdieren verantwoordelijk zijn voor de verdwijning van de patronen binnen een tijdsbestek van enkele dagen. Na de omslag blijft de getijdeplaat voor de rest van het jaar vlak, met een lage biomassa van diatomeeën. Herbivoren hebben een direct effect doordat zij de diatomeeën begrazen. Indirect veranderen zij de stabiliteit van het sediment door bioturbatie. Deze twee processen leiden tot de omslag in de ruimtelijke structuur van het ecosysteem. Opmerkelijk is dat de aantallen bentische herbivoren op deze kale, vlakke plaat door blijven groeien ondanks de vermindering van de diatomeeënbiomassa. Om het mechanisme hierachter te onderzoeken, heb ik laboratoriumexperimenten uitgevoerd waarin dit fenomeen gesimuleerd kon worden. Twee veel voorkomende bentische herbivoren, de slijkgarnaal Corophium volutator en het wadslakje Hydrobia ulvae, bleken in staat te zijn om door begrazing de productiviteit (productie per eenheid biomassa) van diatomeeën te verhogen. Dit verklaart waarom de herbivoren door kunnen groeien op een sediment met weinig diatomeeën.

Op een microscopische schaal zijn herbivoren ook in staat om het microlandschap te veranderen (Hoofdstuk 6). Dit is gebleken uit laboratorium experimenten waar ciliaten waren toegevoegd aan fototrofe biofilms. Binnen enkele dagen was waar te nemen dat begrazing door ciliaten de 3-dimensionale structuur van de biofilm homogeniseerde (Hoofdstuk 6). Uit deze verschillende studies heb ik kunnen concluderen dat bio-fysische interacties een sleutelrol spelen bij het vormen van landschappen op verschillende temporele en ruimtelijke schalen.

Voor de tweede onderzoeksvraag heb ik onderzocht hoe ruimtelijke patronen op getijdenplaten het functioneren van een ecosysteem kunnen beïnvloeden. In een model werd een getijdenplaat met patronen vergeleken met een getijdenplaat zonder patronen. Het model voorspelde dat bij ruimtelijke patronen een hogere biomassa van diatomeeën en een hogere accumulatie van sediment verwacht kunnen worden. In het veld zijn deze voorspellingen getoetst op enerzijds een getijdenplaat met het heuvel- en dalenpatroon en anderzijds een controle plek waar geen ruimtelijk patroon zichtbaar was (Hoofdstuk 2). De veldmetingen bevestigden de voorspellingen van het model en hebben tot de conclusie geleid dat belangrijke functies in het ecosysteem zoals productie en vastlegging van sediment door patroonvorming worden veranderd.

In Hoofdstuk 6 heb ik bestudeerd of omslagpunten in ecosystemen, zoals waargenomen in Hoofdstuk 3, voorspeld kunnen worden door veranderingen in ruimtelijke patronen te bestuderen. Hiervoor heb ik luchtfoto's van patronen in benthische diatomeëen op getijdenplaten die van begin april tot het verdwijnen van de patronen in juni genomen werden geanalyseerd. Deze analyse liet een consistente verandering zien van de structuur van deze ruimtelijke patronen, nadat er een omslagpunt bereikt was van een getijdenplaat met een heuvel- en dalstructuur naar een kale, vlakke plaat. Hieruit kon ik concluderen dat de statistische kenmerken van zelfregulerende patronen gebruikt kunnen worden als voorspeller van omslagpunten. Hierbij moet benadrukt worden dat deze eigenschappen geen globale voorspellers zijn van omslagpunten, omdat ze afhankelijk zijn van de mechanismen die leiden tot het ontstaan van de ruimtelijke patronen. Per ecosysteem dienen specifieke indicatoren geselecteerd te worden voor het voorspellen van omslagpunten.

Dit proefschrift bevat een solide empirische basis om aan te tonen dat bio-fysische interacties ruimtelijke structuren veranderen in (zelfregulerende) fototrofe biofilms. Daarnaast laat dit proefschrift zien dat deze interacties een effect hebben op het functioneren van het ecosysteem. Trofische interacties waren van belang in het homogeniseren van ruimtelijke patronen in het landschap op verschillende ruimtelijke en temporele schalen. Ondanks dat deze processen erg belangrijk zijn is er tot op heden nauwelijks onderzoek aan het cascade effect van biota-sediment interacties op hogere trofische niveaus. Het meenemen van trofische en bio-fysische interacties in toekomstig onderzoek naar ontwikkeling van waterbodems zou kunnen leiden tot een beter begrip van ecosystemen in hun volledige ruimtelijke en temporele dynamiek. Bovendien zou het combineren van theoretische en toegepast onderzoek nieuwe inzichten geven naar de effecten van bio-fysische interacties op vorming van landschappen, het functioneren van ecosystemen en de potentie tot omslagpunten in ecosystemen. Deze nieuwe inzichten kunnen waardevol zijn voor het ontwikkelen van natuurbeleid in estuaria waar sediment dynamiek een belangijke rol speelt.

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Uiteraard zijn er buiten mijn werk om ook vele mensen betrokken geweest met wie ik mijn euforische momenten maar ook klaagzangen kon delen. Ten eerste met mijn huisgenoot Ina, ik vond het gezellig om samen een huis te delen in Yerseke. Door de week zijn er ook vele lekkere maaltijden genuttigd gevolgd door fanatieke potjes Kolonisten van Katan; eerst met Thomas en Nicole en later met Henk, Nienke en Paul. Super bedankt voor deze gezellige avonden, waar ik tot 10 heb leren tellen....of was het toch 13? Tetsuro and Sayuri, arigato for bringing Japanese culture and food into my life! Dedmer, sinds onze HBO tijd bewandelen we hetzelfde pad, het is altijd erg fijn om even met je bij te kletsen over wetenschappelijke avonturen, maar ook over koetjes en kalfjes. Ik hoop dat we dat nog vele jaren kunnen doen!

De weekenden bracht ik meestal in Wageningen door waar vele mensen zorgden voor de nodige afleiding. Alsof ik door de week nog niet genoeg modder had gezien zorgde het “Bugs in the Picture”-fiets clubje ervoor dat ik (af en toe) zondagochtend vroeg in het bos te vinden was ploeterend op de MTB door...de modder. Fietsmaatjes en buurtjes Hans en Joke zorgden voor de spontane bezoekjes, middagborrels en gebakken eieren. Ik hoop ondanks dat we geen buren meer zijn dat we elkaar blijven zien! De rustige weekenden werden af en toe bruut verstoord door afspraakjes met Tessa en Sophie. Bedankt voor de vele onvergetelijke herinneringen van tapijt knuffelen, pompoenen tot de kabouterkrukjes. Jan Jelle en André, ik heb jullie tijdens mijn tijd in Yerseke niet heel veel gezien, maar de keren dat we elkaar zagen was het altijd ouderwets gezellig en nu ik weer in het hoge Noorden werk is kunnen we elkaar wat makkelijker zien, waar ik erg naar uitkijk! De Meindersveen-clan zorgde voor de nodige afleiding in de vorm van kamperen en lekker wandelen.

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EJWeerma

Ellen J. Weerman

Curriculum vitae

Ellen Jolien Weerman was born in Roden, the Netherlands, in 1980. She grew up in Roden and later in Nieuw-Roden in the province of Drenthe. After finishing secondary school, she went to the Van Hall Inistitute in Leeuwarden to study Environmental Sciences. During the last years of her studies she was introduced into marine ecological research during an internship on the spawning of bivalve larvae at the Royal NIOZ at the Isle of Texel, the Netherlands. Here she got the enthusiasm of ecological research which was followed by internships at the University of Napier in Edinburgh by a project on intersex in Gammarus species and a Bachelor thesis at the University of Amsterdam in a project on copper toxicity in phototrohpic biofilms. These projects motivated her to continue in research and followed her studies at Wageningen University where she studied Aquatic Ecology and Hydrology. Here she graduated in 2005 by finishing her thesis on the influence of pharmaceutical residues in surface water on the competition between two algae species and on the cascading effects of this competition to higher trophic levels. This work was presented during the 2005 ASLO conference in Santiago de Compestella in Spain.

In October 2005, she started a PhD project under the umbrella of Centre for Wetland Ecology which was collaboration between the department Spatial Ecology of the Netherlands Institute of Ecology and the department Aquatic Ecology and Ecotoxicology at the University of Amsterdam. During her research she focused on the role of physical and biological interactions in the formation of spatial patterns of phototrophic biofilms. She carried out field surveys, field and laboratory experiments and modeling work. In this period she supervised several Master and Bachelor students during their thesis and internships. She also presented her research at conferences in the Netherlands, France, Belgium, Germany and the USA. Furthermore, during her PhD research she was PhD representative of the central works council of the Netherlands Institute of Ecology.

At present, she is employed as a post-doctoral researcher in the Community and Conservation group of Groningen University. Here she focuses on the influence of ecosystem engineers on food-web dynamics in the Wadden Sea.

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