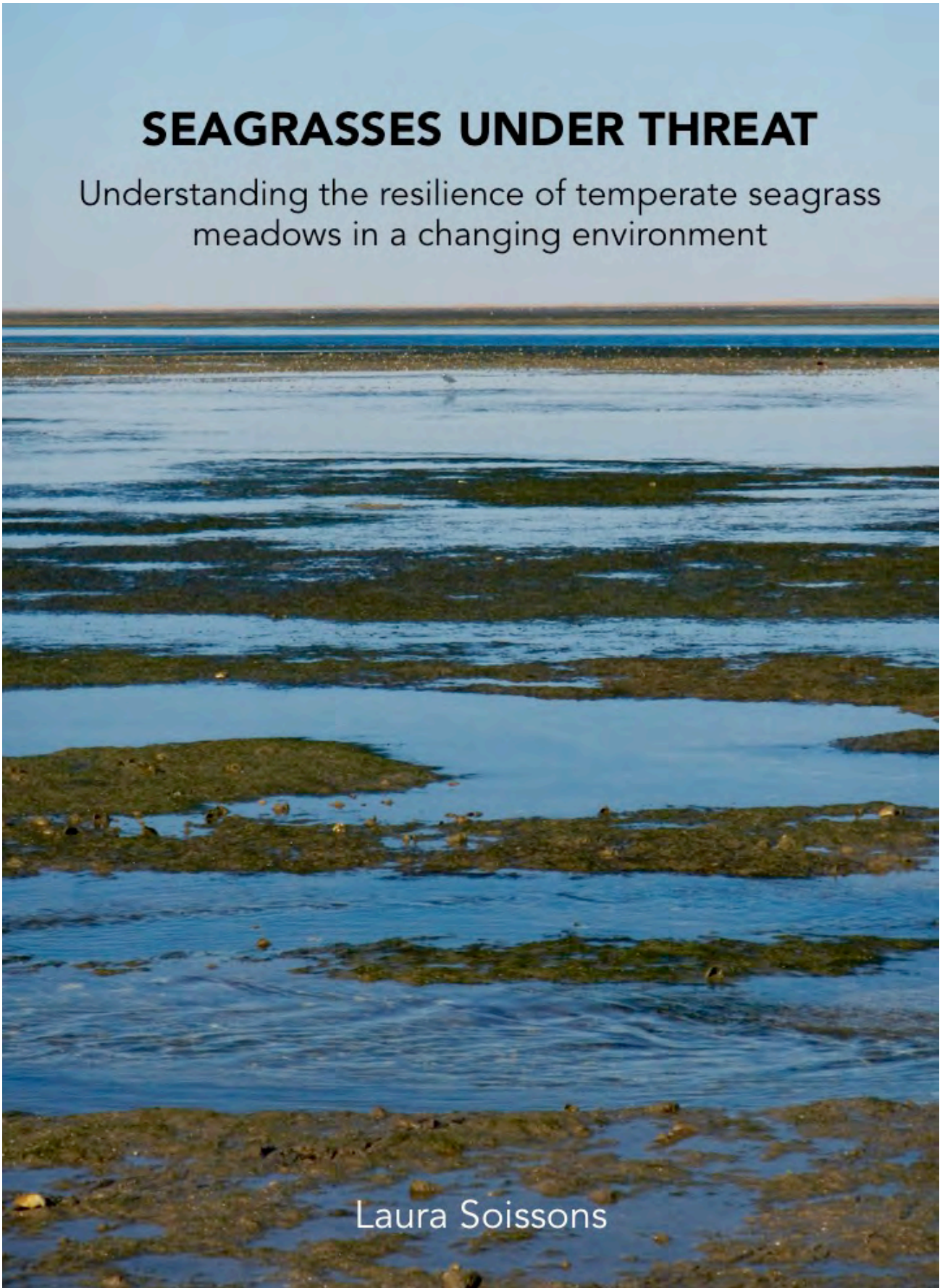


# **SEAGRASSES UNDER THREAT**

Understanding the resilience of temperate seagrass meadows in a changing environment

Laura Soissons









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## CHAPTER 1

### Introduction: Seagrass meadows under threat

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Coastal areas are well known for the numerous services they provide. They form preferential areas for many fisheries, allow the development of complex and diversified ecosystems, which promote the expansion of various human activities from transport to industry development and tourism. But it has now been strongly acknowledged worldwide that coastal ecosystems are threatened and particularly by human activities (Halpern et al. 2008). Anthropogenic impacts range from oil platforms, to agricultural runoff and intensive fisheries (and many others). Their effect on coastal areas and ecosystems are nowadays subject to many investigations (Orth et al. 2006, Halpern et al. 2008, Eriksson et al. 2010). By changing environmental conditions such as hydrodynamics that induce sediment disturbance, change in soil chemistry, nutrient enrichment and so on, mankind has contributed to drastic changes in ecosystem functioning (Eriksson et al. 2010). These changes threaten key-stone ecosystems such as coral reefs (Kleypas et al. 1999, Hughes et al. 2003); mangroves (Valiela et al. 2001); saltmarshes (Bromberg Gedan et al. 2009) and seagrass meadows (Orth et al. 2006, Waycott et al. 2009), the focus of the present thesis.

As a consequence of direct (e.g. trampling) or indirect (e.g. dredging, eutrophication, overfishing) anthropogenic impacts (Duarte et al. 2004b, Unsworth et al. 2014), a decline of seagrass meadows has been evidenced in an increasing number of coastal areas (Orth et al. 2006, Waycott et al. 2009). Seagrasses worldwide have been disappearing at an alarming and accelerating ( $7\% \text{ yr}^{-1}$  in the 1990s) rate of  $110 \text{ km}^2 \cdot \text{yr}^{-1}$  since 1980 (Waycott et al. 2009) (Figure 1.1). In the Mediterranean sea, the increasing number of declining meadows over the past century has been ascribed to the rising and diversification of human activities (Marbà et al. 2014). In Arcachon Bay along the French Atlantic coast, 33% of the *Zostera noltii* coverage was lost between 1989 and 2007, with and even greater loss of 78% of the *Zostera marina* coverage in the adjacent channels between 1988 and 2008 (Plus et al. 2010).

In the Oosterschelde (the Netherlands), land reclamation and artificial coastal protection methods such as the construction of a storm-surge barrier to protect the people living along the estuary might have caused seagrass decline and a surface coverage reduction of about 80% (Suykerbuyk et al. 2012). Furthermore, studies showed that mussel harvest might have directly affected and reduced the coverage of *Zostera marina* and *Zostera noltei* meadows in the Dutch Wadden sea; or that mussel culture had indirectly affected *Zostera marina* beds in France (Duarte et al. 2004b).

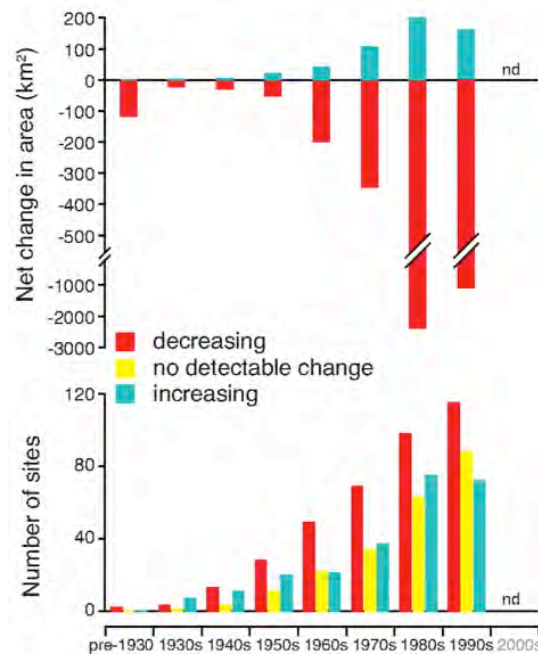


Figure 1.1: Figure from Waycott et al. (2009) showing the decadal trend in seagrass areal extent with: top graph: the measured net change in seagrass area, calculated as the net change across each decade; and bottom graph: the number of sites in each category (decreasing, increasing, or no change) by decade.

Dredging has been responsible for a loss of more than 21 thousands hectares of seagrass vegetation during the last 50 years (Erftemeijer and Lewis 2006). In the Yellow River Delta in China, a rapid loss

and collapse of seagrass has been observed, potentially linked to the intensive land reclamation and industrialisation for oil drilling, high pollutant loads and excess nutrient inputs (see Box 1.1).

### **But what are seagrasses and why do we care?**

#### **Box 1.1** Lessons learned from a rapid seagrass decline: The Yellow River Delta

The Yellow river delta (YRD) is the second largest river in the world in terms of sediment load over the last thousand years (Li et al. 2009) (Figure 1.2). It experiences a wide range of human-induced disturbances via land reclamation or dredging activities for oil or salt industries as well as intensive agriculture and aquaculture activities. Such activities have a significant impact on flow directions, sediment and nutrient load at the mouth of the yellow river. Added to natural seasonal changes like high hydrodynamics and erosion due to stronger winds in winter and low hydrodynamics leading to sediment deposition in summer (Yang et al. 2011), human activities modified dramatically the environment and caused a serious decline in tidal flat ecosystems.

In 2011, there was only one site in the YRD that suggested the presence of ecosystem engineers like seagrasses (Figure 1.2). The site was located southward of the YRD mouth on a tidal flat recently modified by the construction of a dyke along the coast. It was an intertidal flat structured in separated areas alternating mudflats dominated by snails (*Bullacta exarata*), with macroalgae flats and then *Zostera japonica* meadows. In 2011, the seagrass meadow was already poor and declining with highly dynamic conditions revealed by the presence of well-defined sand ripples (personal observations). In spring 2012, the patches of seagrasses previously found were getting sparse and hard to find (Figure 1.2). At the peak of growth, patches became sparser to completely absent over the summer. When seagrass cover was supposed to be at its maximum, only dead rhizomes and black leaves were found. By the end of summer 2012 the seagrass meadow completely disappeared. In spring 2013, no seagrasses grew back in that area, where the sediment became coarser, exposed to increasing hydrodynamic stressors and drought due to the dike construction.



Figure 1.2: Google image of the Yellow river Delta area in the Bohai sea, China. The black box represents the location of the seagrass meadow described with a picture of sparse seagrass shoots taken in May 2012 before the meadow completely disappeared.

There are many reasons that could explain the sudden collapse of this *Zostera japonica* meadow, described as very dense by local fishermen (pers. com.). It could be the anthropogenic influence but also a combination of changing environmental variables and pollution from the Yellow River and its surroundings. So far, no historical data reporting the existence of this meadow or anything related to the status of this particular tidal flat before the dike construction on 2009 have been found (from what we know). Stakeholders in that area will not give up expanding their activities but also want to consider their impact and the need to preserve their environment. It is thus important to emphasize this sudden collapse and its consequences and try to understand the reasons of their decline and how we made it happen.

## Seagrasses in temperate systems

Seagrasses are marine flowering plants (Figure 1.3) widely distributed worldwide (Short et al. 2007) and represent one of the most valuable resources in the coastal landscape for the ecosystem services they provide (Orth et al. 2006). Seagrasses can be found along the coast from intertidal to subtidal areas, forming extensive and highly dynamic monospecific or multispecific meadows, in both tropical and temperate regions (Short et al. 2007). Their development and distribution depend however on various conditions such as light and nutrients availability (Duarte 1991, Grice et al. 1996, Wicks et al. 2009), sufficiently sheltered hydrodynamic conditions (varies per species), and sediment characteristics (Koch 2001, De Boer 2007, Eriksson et al. 2010). With a total of 12 genera, there are over 50 referenced seagrass species of different shapes and sizes found worldwide (Figure 1.3). These various seagrass species differ in their growth strategies, from slow to fast growers, presenting different traits that potentially determine their capacity to resist and to recover from stresses and disturbances.

In temperate systems, seagrasses are known to have distinct seasonal growth and rhizome proliferation (Duarte 1989; Hemminga and Duarte 2000; Larkum et al. 2006) (Figure 1.3). The main mechanisms controlling the seasonal growth of seagrasses are changes in light intensity and temperature (Dennison 1987, Duarte 1991, Olesen and Sand-jensen 1993, Ochieng et al. 2010, Marbà et al. 2012). Generally, plants start to grow in spring, until mid-summer when they reach their maximum shoot density and cover to build up their carbon reserves (Madsen 1991). Then senescence starts in late summer with the release of seeds; leaves get thinner and shorter, and shoot density becomes lower (Hemminga and Duarte 2000, Larkum et al. 2006). In winter, the vegetation is very sparse (Orth et al. 2012). For dwarf eelgrasses in northern areas, only the below ground biomass – with limited leaves cover – and seed banks remain until spring, when new shoots grow again.

To overcome the winter months, seagrasses exploit the non-structural carbohydrate reserves (i.e. starch and sucrose) gained during the summer period (Madsen 1991, Alcoverro et al. 1999, Olivé et al. 2007, Lee et al. 2007, Govers et al. 2015). The amount of carbohydrates needed for seagrass survival over the winter periods depends on abiotic factors, i.e. temperature and light availability; but also on internal factors, such as respiration and growth (Madsen 1991, Alcoverro et al. 2001, Govers et al.

2015). Chances of survival during winter depend on the plant's capacity to build up its carbohydrate reserves during summer (Govers et al. 2015).

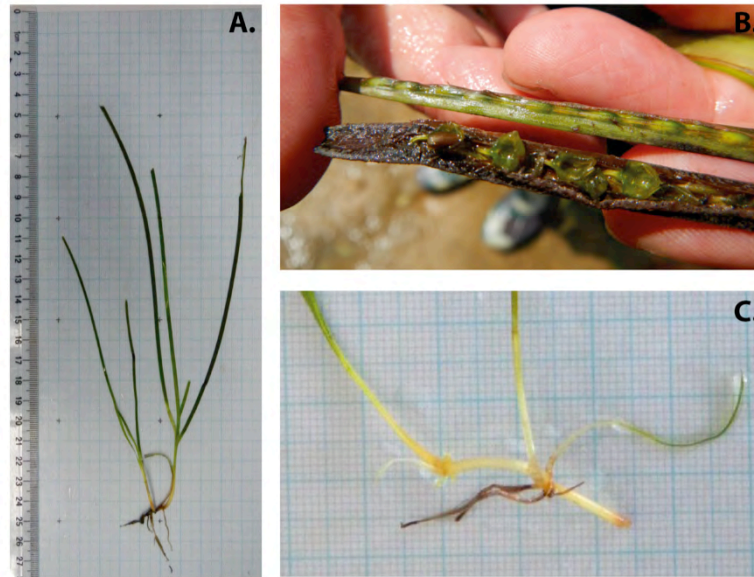


Figure 1.3: Pictures of seagrass illustrating their morphological structure (A) and their sexual (B) and asexual (C) reproduction strategies. A. *Zostera noltei* at its peak of growth (max. leaves length and width) showing an apical shoot and the 1<sup>st</sup> following shoot attached to their roots and rhizomes. B. Sexual reproduction represented by seed-bearing leaves of the seagrass *Zostera marina*. C. Zoom into the root (brown) and rhizome systems (yellow) of *Zostera noltei* to represent their asexual growth through rhizome elongation (this picture: growth towards the left direction).

### Seagrasses matter

The presence of seagrasses and their biophysical interactions lead to the existence of feedback loops, fundamental for the stability of coastal ecosystems (Suding et al. 2004, van der Heide et al. 2007, 2011, Carr et al. 2010, Suykerbuyk et al. 2012). Indeed, seagrasses are defined as ecosystem engineers (Jones et al. 1994, Bos et al. 2007) as their presence modifies their environment and allows the development of many organisms (Figure 1.4).

For instance, they play a major role in stabilizing intertidal flats under various hydrodynamic conditions (Christianen et al. 2013). Their seasonal presence reduces bed erosion thresholds (Fonseca and Fisher 1986) as a result of waves and bed shear stress reduction by leaves. Hence, sediment resuspension is reduced (Widdows et al. 2008) which leads together with direct sediment trapping (Hendriks et al. 2008) to bed elevation (Bos et al. 2007, Ganthy et al. 2013), and improved water clarity. The effect on water clarity and sediment trapping varies as a function of growth and shoot height and density (Koch 2001, van der Heide et al. 2011, Ganthy et al. 2015) (Figure 1.4).

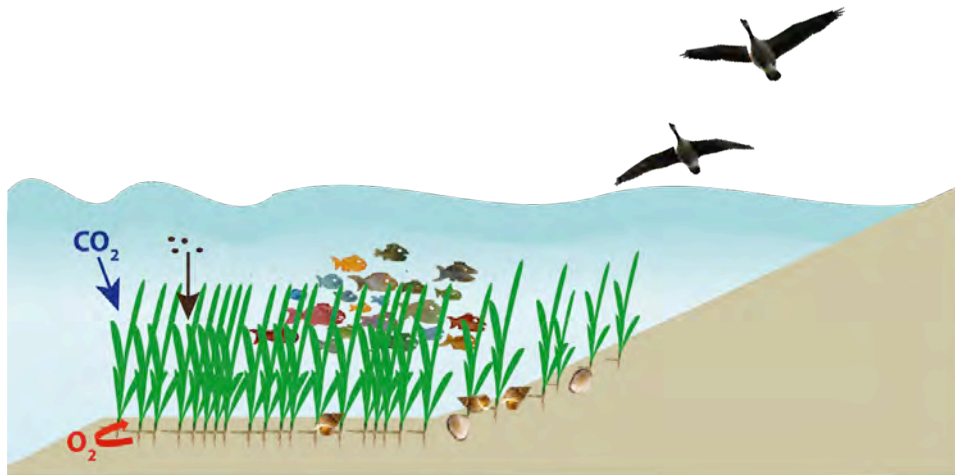


Figure 1.4: Illustration of seagrass ecosystem engineering capacity: Wave and current attenuation, sediment trapping (brown arrow), improved water quality, carbon sequestration (blue arrow),  $O_2$  release for detoxification (red arrow), nursery – shelter and food, increased biodiversity.

Seagrasses form very productive ecosystems (Costanza et al. 1997, Duarte and Chiscano 1999). They greatly contribute to carbon sequestration (Fourqurean et al. 2012), nutrient cycling (Hemminga et al. 1991) and interact with other key ecosystems such as mangrove forest or coral reefs in tropical systems (Lai et al. 2013, Gillis et al. 2014a, 2014b, 2015).

Because of their capacity to adapt to their environment, seagrass meadows can form good indicators for ecosystem health (Orth et al. 2006).

They are nowadays subjected to various monitoring programs and listed as biological quality elements in international or national directives (Borum et al. 2004, Duarte et al. 2004a, 2004b, Borja et al. 2011, 2013; see Box 1.2) for the evaluation of coastal ecosystem health status.

**Box 1.2** European and International programs for the conservation of seagrasses

The so-called “global crisis” for seagrasses initiated the development of various monitoring and conservation programs worldwide in the past decades (Duarte et al. 2004a) involving scientists, stakeholders and local populations. Organisations such as SeagrassWatch or SeagrassNet were created to form “worldwide ecological monitoring programs that investigate and document the status of seagrass resources and the threats to this important and imperilled marine ecosystem” (from: [www.seagrassnet.org](http://www.seagrassnet.org)). These programs thus both intend to monitor seagrass health status and increase awareness worldwide on seagrasses, their role and the threats they undergo.

Moreover and in order to further protect coastal areas and ecosystems, directives such as the European Water Framework directive (WFD, 2000/60/EC) and later on the Marine Strategy Framework Directive (MSFD, 2008/56/EC) have been implemented (Borja et al. 2013). In accordance with the WFD, “good ecological status” in transitional and coastal waters was to be achieved by 2015 by using biological quality elements, or bio indicators. Bio indicators were defined according to 3 main criteria: wide distribution, sedentary habitat and susceptibility to environmental conditions (Foden and Brazier 2007, Montefalcone 2009). Relevant for each criterion, seagrasses became good candidates for the evaluation of coastal waters ecological status and listed as part of the annex V of the WFD as biological quality elements. As a consequence, an increasing amount of studies and the development of various multivariate indexes reporting their distribution and health status were developed in Europe, such as the Bipo (Lopez y Royo et al. 2010, 2011), PREI (Gobert et al. 2009) or POMI (Romero et al. 2007) for *Posidonia oceanica*, the CYMOX (Oliva et al. 2012) for *Cymodocea nodosa*, the ZoNI (García-Marín et al. 2013) for *Zostera noltei*, or depth-light distribution studies for *Zostera marina* (Krause-Jensen et al. 2005, Foden and Brazier 2007).

## Main threats to seagrasses

One of the main threats to seagrasses comes from eutrophication, or nutrient enrichment, indirectly or directly affecting them. Indirect effects are observed through the proliferation of macroalgae in the water column, leading to an increase in organic matter fluxes and to anoxia (Short and Burdick 1996, Brun et al. 2003c, Nelson et al. 2008). Eutrophication can also directly damage the morphological, physiological and biomechanical traits of seagrasses (Lee et al. 2004, Armitage et al. 2011, La Nafie et al. 2012, 2013) due to nutrient excess and ammonium (van Katwijk et al. 1997) or nitrate (Burkholder et al. 1992) toxicity.

The other major threat to seagrasses is associated with plant removal through various factors such as: anchoring, local human activities like collection of fauna (Alexandre et al. 2005, Cabaço et al. 2005), increased (local) sediment dynamics (burial and erosion) resulting from large scale human interventions in the coastal zone (infrastructure, coastal protection, dredging) (Ruiz and Romero 2003, Erftemeijer and Lewis 2006, Cabaço and Santos 2007, Cabaço et al. 2008, Manzanera et al. 2011, Han et al. 2012), increased bioturbation (Valdemarsen et al. 2011, Delefosse and Kristensen 2012) or over-grazing (de longh et al. 1995, Eklöf et al. 2008a). Such factors can lead to the creation of gaps of different sizes in seagrass beds. Seagrasses can recover from such disturbances through regrowth from rhizomes (Marbà and Duarte 1998), by growing in gaps from the edges (Rasheed 1999), or by growing rhizomes upward or downward in the sediment (Han et al. 2012). Seagrass recovery after small-scale disturbance, can differ depending on the seasonal growth of plants (Bell et al. 1999), the size and type of the disturbance (Rasheed 1999, 2004), the rate of seagrass vegetative growth (i.e. rhizome elongation), abiotic conditions, burial, reproduction (Valdemarsen et al. 2010) and resistance to stress/disturbances.

With the recent increase of human activities and the pressure of global environmental trends on seagrass meadows (Orth et al. 2006), both stressors (eutrophication and plant total or partial removal) can occur simultaneously and synergistically affect their survival, growth and properties. The synergistic effect of stressors has already been observed for the combined influence of light and hydrodynamics (de los Santos et al. 2010) or waves and nutrients (La Nafie et al. 2012) for instance. The individual and synergistic effect of stressors, such as nutrient enrichment (eutrophication) and small-scale disturbances (gap creation) can reduce the

resistance and recovery potential of seagrasses. Hence the system resilience can also be affected (van Nes and Scheffer 2007; Scheffer et al. 2001, 2009; Chisholm and Filotas 2009), leading to direct changes in the local seagrass meadows properties or to collapse.

### Seagrass resilience and the alternative stable state theory

Because of their diversity and complexity, coastal ecosystems, such as seagrasses, may respond in different ways to changes and the increasing amount of stresses and disturbances they undergo (Scheffer et al. 2001). It has been shown theoretically that many coastal ecosystems, including seagrasses (van der Heide et al. 2007, 2010a, Carr et al. 2010, 2012, van Katwijk et al. 2011), may follow the alternative stable state theory. This theory implies hysteresis between two stable states and the risk of unexpected fast collapse (Scheffer et al. 2001, 2009, Scheffer and Carpenter 2003, van Nes and Scheffer 2007) (Figure 1.5). Indeed, the recent increase in seagrass loss, mainly due to changes in soil chemistry, nutrient loading, hydrodynamics and sediment dynamics conditions (Orth et al. 2006, Waycott et al. 2009) may induce catastrophic shifts, switching the system from one (vegetated) to an alternative (unvegetated) stable state (Scheffer et al. 2001, van der Heide et al. 2007).

Switches from one state to the other are complex and occur when the system reaches a tipping point (or fold bifurcation point). When ecosystems are at a first equilibrium defined as first state (Scheffer et al. 2001, 2009), they can adapt to gradually varying external conditions. But over time, the continuous increase in external perturbation reduces the system's resilience (van Nes and Scheffer 2007) (Figure 1.5) and, consequently, even a small perturbation over the critical threshold can make the system switch to another stable state (Scheffer et al. 2001, 2009, Chisholm and Filotas 2009) which may imply collapse of the system. Measurements of such critical thresholds and the identification of tipping points are hard but some indicators can be used. Early warning signals of external conditions can be used to identify critical thresholds by measuring variance and dynamics of external conditions (Scheffer et al. 2009). Furthermore, and as resilience remains a difficult parameter to measure directly, studies refer to the Critical Slowing Down (CSD) as a good indicator of whether a system is getting close to a bifurcation/tipping point

(Scheffer et al. 2009). Critical slowing down happens when a monitored variable returns more slowly to equilibrium after a small perturbation (Dakos et al. 2011) and means that the resilience of the system is dramatically reduced. Transitions in ecosystems have been observed and described broadly and it appeared that the alternative stable state theory could relate to a wide variety of ecosystems ranging from rangelands to marine systems like mussel beds, oyster reefs, saltmarshes and seagrasses. Nevertheless, it still remains unknown whether the indicators or early warning signals described can be applied easily to all ecosystems in practice.

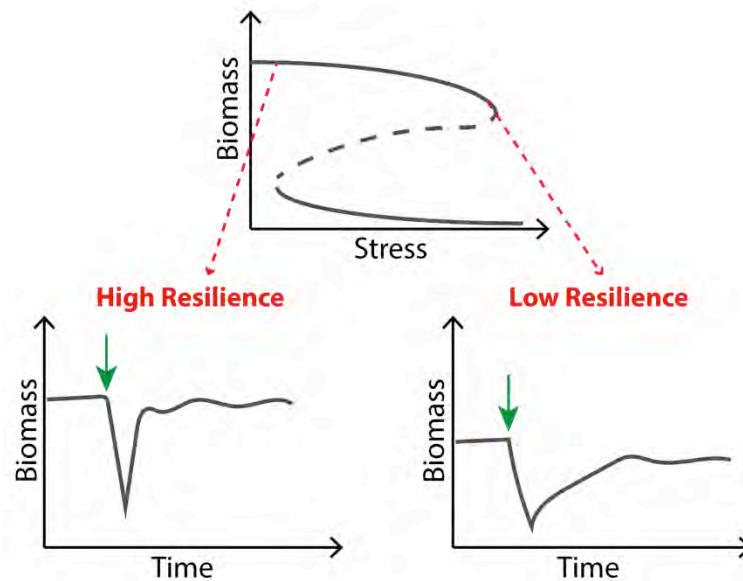


Figure 1.5: Representation of the two stable states (full lines on the top graph) of a system represented by its biomass along a stress gradient. At high resilience, if a small disturbance occurs (green arrow) the system will recover fast to its original biomass. At low resilience, and close to a bifurcation point, if a small disturbance occurs it will take longer for the system to recover and maybe not back to its original biomass. This is the phenomenon of critical slowing down. At the bifurcation point the system might switch to a second stable state. The dotted line on the top figure represents the unstable state between two states (full lines). Figure adapted from van Nes and Scheffer (2007).

Ecosystem engineers such as seagrasses have the capacity to react to smooth changes in external conditions and keep the system to a stable state (Scheffer et al. 2001). Being organisms capable to adapt to their environment, seagrasses can acclimate to changing conditions and adapt their morphological, physiological and mechanical traits (Peralta et al. 2005, 2006, Cabaço et al. 2009, de los Santos et al. 2010, 2013, La Nafie et al. 2013), making them more resilient under threats. There are, however, possibilities that external conditions push the system towards their critical threshold or fold bifurcation point (Scheffer et al. 2009). When even a small disturbance occurs, the dramatic response of the system could lead to a potential collapse of seagrass ecosystems (van der Heide et al. 2007, van Wesenbeeck et al. 2008), by affecting their resistance to stress and recovery potential after a disturbance and thus the resilience of the ecosystem in both the short and the long-term (see Box 1.3).

### **Box 1.3** Terminology relative to ecological concepts used in this thesis

- **Stress:** condition that restricts production such as light, temperature, nutrients, water, or pollutants (Grime, 1977).
- **Disturbance:** partial or total destruction of the plant biomass, by e.g. animal activities: grazing; human activities: trampling, dredging; erosion, storms, fire, desiccation (Grime, 1977).
- **Resilience:** Following Holling (1973), the term 'resilience' refers to the size of the valley, or basin of attraction, around a state, which corresponds to the maximum perturbation that can be taken without causing a shift to an alternative stable state. In the present thesis, we consider the resilience of the system as the combination between resistance to stress and recovery from disturbance.
- **Resistance:** In this thesis, and sensu Grime (1977) and Wissel (1984) resistance represents the property of a system to remain essentially unchanged, and to persist/tolerate a stress through time.
- **Recovery:** In this thesis we consider recovery as the capacity of the system to return to its original stable state after a disturbance. Recovery is used as a measure of a potential Critical Slowing Down of the system when reduced.

## Aim of the thesis

In a stressed environment, the recovery dynamics of seagrasses can be slowed down or cease completely when the resilience of the system is lowered. As seagrasses are a valuable resource for fisheries or coastal protection, it is thus important to preserve them. To do so, there is urgent need to better understand the mechanisms and dynamics of seagrass resilience under threats and their consequences for the long-term survival of seagrass meadows, locally and on larger-scales (i.e. along large climatic gradients). This, in order to prevent dramatic declines similar to the one that occurred in the Yellow river Delta (see Box 1.1) and the subsequent loss of all the ecosystem services they provide.

Through a series of manipulative field experiments, the present thesis hence focused on gaining a **better understanding of the resilience of seagrass meadows against threats**, such as eutrophication in temperate systems. This can be done by evaluating (i) how to better prevent the collapse of seagrass meadows before it happens; and (ii) how changes in environmental conditions (natural or human-induced) might impact on seagrass meadows' health and (reduced) resilience. To answer these questions (see Box 1.4 for research questions and hypotheses) the thesis has been divided into three parts being: (1) Indicators of resilience, (2) strategies for resilience and (3) the influence of global gradients on resilience. Firstly, we focused on the evaluation and comparison of indicators for seagrass resilience (Chapter 2); as well as the effect of timing of the disturbance on seagrass resilience and indicators (Chapter 3). Secondly, we looked at the importance of species-specific growth rates vs. ecosystem engineering as strategies for seagrass resilience to a combination of stresses and disturbances (Chapter 4). Thirdly, a special focus on the large-scale evaluation of seagrass resilience mechanisms along a latitudinal – global - gradient was made in order to: evaluate the influence of short-term vs. long-term stress events on their seasonal carbon storage and resilience (Chapter 5); to assess the seasonal and latitudinal variation in seagrass mechanical resistance to stress (Chapter 6); and to evaluate the consequences of pollen-limitation on seagrass reproductive effort and conservation (Chapter 7).

**Box 1.4** Questions and hypothesis

		Questions	Hypothesis
INDICATORS	Chapter 2	How does the response of two seagrass indicators differ under threat (nutrient enrichment)?	Nutrient enrichment will reduce the response of both traditional (cover) and theoretical (Critical slowing down) indicators.
	Chapter 3	What is the effect of timing of a disturbance on seagrass resilience and indicators of resilience?	The response of indicators is dependent on the timing of the disturbance, which in turns affect the overall resilience to disturbance of the two studied seagrass meadows.
STRATEGIES	Chapter 4	What is the relative importance of seagrass ecosystem engineering vs. growth rate as strategies for their resilience?	<p><u>Hyp 1.</u> Sensu Grime: Fast-growers are expected to be better recolonisers but less resistant to stressors than slow-growers.</p> <p><u>Hyp 2. (alternative)</u> Due to their ecosystem engineering capacity, fast-growers can improve their resistance to stress and become more resilient.</p>

## THE INFLUENCE OF GLOBAL GRADIENTS

Chapter 5	<ul style="list-style-type: none"> <li>• How do unpredictable short-term stress events affect the seagrass capacity to withstand seasonal changes?</li> <li>• How can this affect seagrass long-term resilience and survival in a globally changing environment?</li> </ul>	<p><u>Hyp 1.</u> Carbohydrate reserves in seagrass rhizomes vary along their seasonal growth and across a latitudinal gradient.</p> <p><u>Hyp 2.</u> Short-term stress events might hamper the seagrass capacity to store their carbohydrate reserves, affecting their survival overwinter.</p>
Chapter 6	<ul style="list-style-type: none"> <li>• How do seagrass mechanical traits vary along a latitudinal and seasonal gradient?</li> <li>• What is the influence of seagrass morphological and physiological traits on their mechanical response to eutrophication?</li> </ul>	<p>Hyp 1. There are disparities in seagrass mechanical traits related to their distribution along a climatic gradient.</p> <p>Hyp 2. Seagrass mechanical traits are negatively affected by nutrient enrichment stress and this response is related to their morphological and physiological response to eutrophication (C:N ratio).</p>
Chapter 7	Is there an Allee effect in hydrophilous plant, i.e. seagrass populations along the Atlantic European coast?	Pollen limitation may be a common Allee effect for seagrasses, particularly in declining or fragmented and evergreen populations.

**Box 1.5** Species studied in this thesis

▪ ***Zostera marina* (L.)**

Commonly referred to as eelgrass (Figure 1.6a), it is the most widespread seagrass species in temperate systems in the Northern hemisphere (Short et al. 2007, 2010a). It can be found in lower intertidal flats as an annual plant as well as in subtidal areas as a perennial plant. Its leaves can reach a length of 1 m and a width of 8 mm. Its rhizome elongation rate is 26 cm.year<sup>-1</sup>, making it a relatively slower grower as compared to *Zostera noltei* or *Zostera japonica*.

▪ ***Zostera noltei* (Hornem)**

Commonly named dwarf eelgrass (Figure 1.6b), it is also and mostly referred to in the literature as *Zostera noltii*. It is a temperate seagrass species found in intertidal areas along the North-eastern Atlantic coast (Short et al. 2007, 2010b). Its leaves can reach a length of 20 cm and a width of 0.5-1.5 mm. It is considered as a fast-growing species with a rhizome elongation rate of 68 cm.year<sup>-1</sup>. In Southern areas, this species is leaf-bearing throughout the year (Buia and Mazella 1991; Peralta et al. 2000, 2005; Cabaço et al. 2009, Cabaço and Santos 2012), but in north Atlantic Europe the plants overwinter as small rhizome fragments with few or no leaves (Vermaat and Verhagen 1996).

▪ ***Zostera japonica* (Asch. & Graebn.)**

Commonly referred to as Japanese or dwarf eelgrass, and native from the Eastern Pacific, *Zostera japonica* is also invasive in the Western United States (Short et al. 2007, 2010c). It is found in intertidal areas in the uppermost part of the flat in temperate systems and is commonly distributed close to *Zostera marina* meadows in Asia (Figure 1.6c). *Z. japonica* shares the same biological traits than the European dwarf eelgrass *Zostera noltei*, as both species differ from each other on only two genetic markers, their main difference being in their geographical distribution.

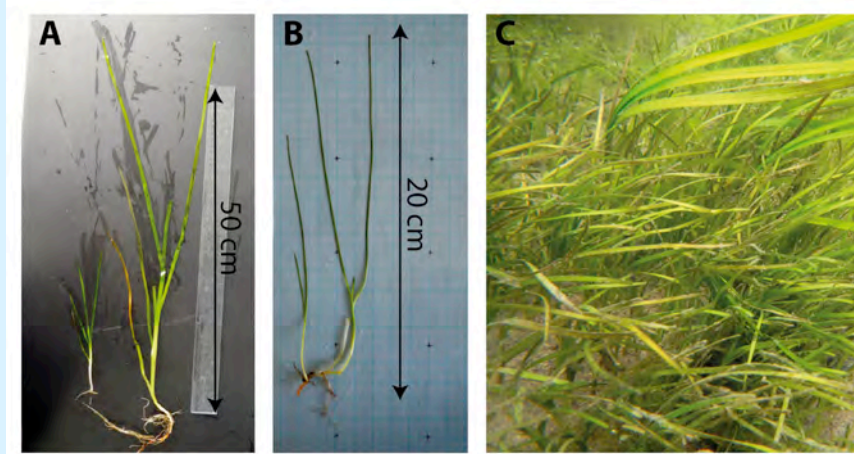
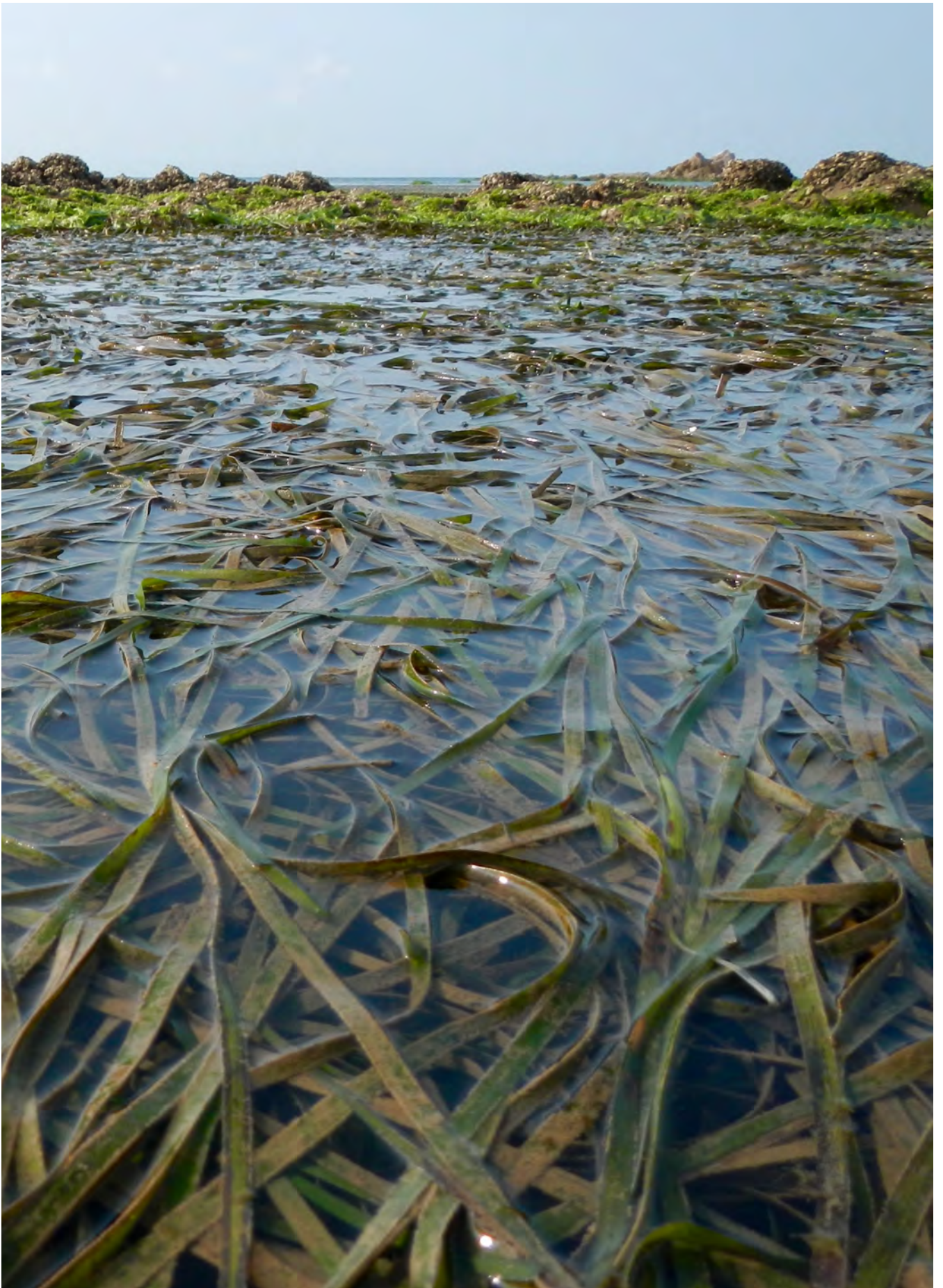


Figure 1.6: Photos of A. a *Zostera marina* adult shoot (right) and newly developed shoot from seedling (left); B. a *Zostera noltei* shoot at its peak of growth; and C. *Zostera japonica* (front) and *Zostera marina* (back) co-occurring in a lagoon in temperate Eastern China.



## CHAPTER 2

### Cover vs. recovery: contrasting response of two indicators in seagrass beds

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#### Abstract

Despite being a highly valuable key-stone ecosystem, seagrass meadows are threatened and declining worldwide, creating urgent need for indicators of their health status. We compared two indicators for seagrass health: standing leaf area index versus relative recovery from local disturbance. Disturbance was created by removing the aboveground biomass and by recording the rate of regrowth of *Zostera marina* meadows exposed to contrasting wave regimes and nutrient stress levels.

Within the experimental period, relative regrowth in gaps was around 50 % in most plots, except for the ambient nutrient treatment at the sheltered site, where it exceeded 100 %. The two indicators showed an opposite response to disturbance: the higher the standing Leaf area index, the lower the relative recovery from disturbance. This conflicting response raises the question on the proper interpretation of such indicators to estimate seagrass health and resilience, and how to ideally monitor seagrass ecosystems in order to predict collapse.

## Introduction

Seagrasses represent one of the most valuable resources in the coastal landscape for the ecosystem services they provide. Seagrass meadows can be found in coastal areas worldwide, are defined as keystone species (Zieman et al. 1999) and are known to be highly sensitive to environmental status (Orth et al. 2006). Their development and distribution depend on various conditions such as light and nutrient availability (Duarte 1991; Grice et al. 1996; Wicks et al. 2009), sufficiently sheltered hydrodynamic conditions and low sediment dynamics (Koch 2001; Eriksson et al. 2010). Despite their capacity to adapt and to cope to some extent with environmental changes, seagrasses suffer rapid and large-scale losses worldwide, their distribution is declining and their survival threatened (Orth et al. 2006). Anthropogenic influences, causing changes in soil chemistry, nutrient loading, hydrodynamics and sediment dynamics are responsible for the seagrass disappearance over the last 40 years (Orth et al. 2006 and references therein; Waycott et al. 2009).

With the rapid loss of seagrasses, monitoring programs were initiated in the last two decades to better estimate the evolution and status of seagrasses (Duarte et al. 2004a). For most monitoring programs like *Seagrass watch* or *Seagrass Net*, seagrass density or percent ground cover are commonly used indicators to evaluate a meadow status along transects or quadrats (McKenzie et al. 2003, Duarte et al. 2004a, Short et al. 2006). With these measurements, seagrass status can be evaluated by comparing cover maps over defined periods of time and to observe the evolution and status of the meadow (i.e. healthy or in decline). These monitoring programs also use environmental parameters such as water and sediment quality in combination with seagrass measurements to infer the causes of changes in seagrass cover and distribution (Duarte et al. 2004a, Short et al. 2006, Neckles et al. 2012).

Several recent studies have argued that seagrass systems follow the alternative stable state theory, implying hysteresis in the transition between vegetated and unvegetated states (van der Heide et al. 2007, 2010a, Carr et al. 2010, 2012). This has profound effect on the resilience of the system, i.e. the capacity of recovery of the system to its initial state (equilibrium) after a perturbation. According to Holling (1973), resilience refers to the size of the valley, or basin of attraction, around a state, which corresponds to the maximum perturbation that can be taken without causing a shift to an alternative stable state (cf. Scheffer et al. 2001). As resilience is a difficult

parameter to measure directly, recovery rates from disturbance are used as an indicator. This is based on model explorations (e.g. van Nes and Scheffer, 2007) which showed that at higher stress levels, when the system approaches its tipping point, it will exhibit a slower recovery rate from disturbance. This phenomenon is referred to as 'critical slowing down' (Dakos et al. 2011). It still remains largely unknown whether critical slowing down can be used in practice as an indicator or early warning signal across ecosystems (Hastings and Wysham 2010). As a matter of fact, the main support for the existence of critical slowing down originates from theoretical models based on long-term data and on specific systems (Boettiger and Hastings 2013).

As seagrasses are disappearing fast worldwide, there is, in addition to good monitoring programs, need for indicators for their capacity to recover from disturbances. In this study, we aim to find the relationship between (i) a traditionally used *indicator for seagrass health* from global monitoring programs (i.e., seagrass cover) and (ii) a theoretically suggested *indicator for seagrass health in terms of resilience to disturbances* (i.e., critical slowing down). To compare both indicators, we combined vegetation monitoring with a disturbance-recovery experiment by above-ground biomass removal, at two nutrient-stress levels (i.e., ambient vs. nutrient enriched) and at two hydrodynamic contrasting field sites (i.e., relatively sheltered vs. wave exposed). Sediment nutrient enrichment was used to impose contrasting stress levels within each field site, to which both indicators can respond. Stress differences can be due to creation of eutrophic conditions or by alleviating nutrient limitations. For both nutrient levels, at both sites, disturbance was imposed by removing the above-ground cover by mowing the leaves, as typically occurs due to animal grazing or boat anchoring. Overall we aim to test the hypothesis that the *indicator for seagrass health* (i.e., seagrass cover) and the *indicator for seagrass resilience* (i.e., critical slowing down) give similar response to site-specific conditions and nutrient induced stresses, but may vary in the strength of their response. That is, we compare the correlation between the responses of two indicators (Leaf Area Index, as a quantitative proxy for the generally by experts quickly estimated seagrass cover, for *seagrass health* versus critical slowing down of recovery for *seagrass resilience*) under different interacting environmental settings (i.e., wave exposed versus sheltered and ambient nutrient vs. nutrient enriched).

## Material and Methods

### Field sites

Indicators of seagrass health were compared at two sites varying in their exposure to hydrodynamics, located in the Shandong province (China) close to the city of Weihai (Figure 2.1). The sheltered site (SS) is located in "Yuehu lagoon" or "Swan Lake" (N37° 20' 58.2"; E122° 34' 48.4") and has a small tidal inlet (86 meters wide) and shallow waters (< 2 meters) all over the lagoon. In contrast, Dongchu Island (N37° 02' 28.1"; E122° 34' 11.4") is a more exposed site (ES) with strong hydrodynamics and a rocky shore open to the sea. Both sites have a dense and healthy *Zostera marina* (Linnaeus, 1753) meadow, which is also exploited for aquaculture in SS (i.e., mainly for sea cucumber and shellfish).



Figure 2.1: Site localisation in the Shandong province

Hydrodynamics were not measured during the experiment, but the geographical situation and wind fetch of both sites allowed us to define their relative exposure: a shallow lagoon as SS and an open-sea system with

a rocky shore and visible waves on the shore close to the meadow as ES (personal observations). In winter, SS is a refuge for swans migrating from Siberia and eating on the seagrasses but not as their main food (personal communication with local people). The sheltered site is expected to have stronger anthropogenic influences due to its limited water exchange with the open sea and high human population density along the shore.

### Experimental design

A nutrient addition experiment was implemented simultaneously in both ES and SS seagrass meadows at the beginning of the growing season (i.e., 4<sup>th</sup> of June 2012). Each treatment was replicated 5 times, i.e. 10 fertilized plots (5 with a gap and 5 without a gap), and 10 non-fertilised control plots (5 with a gap, 5 without a gap). We measured standing Leaf Area Index ( $LAI_{standing}$ ) as a proxy for the traditional monitoring method (i.e. percent cover, indicator 1) and we derived the relative recovery in mown gaps as a proxy for resilience, related to critical slowing down. Instead of using multi-parametric indexes developed as part of the European Water Framework Directive (i.e. ZoNI, POMI, BIPO; Krause-Jensen et al. 2005; Marbà et al. 2012; García-Marín et al. 2013; Mascaró et al. 2013),  $LAI_{standing}$  was chosen as a proxy to evaluate a simple and common indicator such as cover in comparison with more theoretical concepts by using regrowth/recovery (critical slowing down) in the assessment of seagrass health and resilience (i.e. seagrass watch). Relative recovery (%RC) was obtained by expressing the absolute regrowth in the mown graph ( $LAI_{regrowth}$ ) as percentage of the simultaneously occurring increase in leaf area in the surrounding vegetation (plots without gap,  $DLAI_{standing}$ ). The experiment ran from the 4<sup>th</sup> of June until the 19<sup>th</sup> of July 2012, giving a total of 46 days at both sites.

Nutrient enrichment was applied by adding slow release inorganic fertilizer (N:P:K = 26:11:11) directly into the sediment, thus creating two nutrient levels: ambient nutrient (no addition) and high nutrient treatment (1500 kg N/ha added). Concentrations for the high treatment were calculated as a function of nitrogen addition since nitrogen is documented as the limiting nutrient for seagrasses in many temperate systems (Zimmerman et al. 1987, Touchette and Burkholder 2000a, 2000b). Nutrient additions were chosen to be really high, comparable to levels used in agriculture in the Shandong province (Ju et al. 2006). The desired quantity of fertilizer was evenly injected with syringes into the sediment for each circular plot of 1 m diameter (0.78 m<sup>2</sup>). High nutrient ('nutrient') and

ambient nutrient ('control') treatments were randomly distributed in the study area at both sites and in seagrass areas of similar cover.

Circular gaps of 0.5 m diameter in the centre of each plot were created by mowing the leaves, leaving the below ground and sheaths in place. Rhizomes around the gaps were cut to limit lateral carbon transfer and to limit shoots recovery on the basis of their reserves outside the gap, in order to measure regrowth independently from the surrounding meadow. The leaves cut from each gap treatment were used to measure tissue content (C:N ratio) and total removed biomass.

### Sampling and analysis

Seagrass, water, sediment and porewater samples were collected at the start and the end of the experiment to evaluate the status of the meadows. Water temperature and depth, as well as light available to the seagrasses, were monitored over the experimental period. Light and water temperature were measured using two HOBO Pendant Temperature/Light loggers (64k – UA-002-64, ONSET) at a frequency of 1 measurement every 15 minutes during the whole experiment. Water depth was monitored by using Sensus Ultra pressure sensors (SU-R-B, Reefnet Inc.), set up with the standard configuration provided by the constructor (Reefnet Inc.). Two of each logger were placed in couples (i.e., 1 Hobo and 1 Sensus ultra) within the study area for each site. Salinity was measured every two weeks by using a YSI Pro multimeter.

**Seagrass samples:** Seagrasses were sampled in the experimental area at the start of the experiment in a surface of 0.09 m<sup>2</sup> for biomass and morphological measurements and replicated three times. The leaves mown in the 0.5 m gaps were kept for biomass measures. At the end of the experiment, gap-control plots were sampled in 0.09 m<sup>2</sup> cores and all plants in the 0.5 m diameter gaps were collected. After mowing/sampling, seagrasses were directly cleaned a first time in seawater before being transported to the laboratory for measurements. The plants were carefully rinsed and cleaned in fresh water to remove epiphytes and any sediment left. The total number of shoots per sample, and their total wet biomass were directly noted. Subsamples of 10 shoots per sample were randomly selected for morphological measurements. Leaf length and width, root length, rhizome length and thickness, number of leaves per shoot, and single shoot wet biomass were measured for all replicates. Then, for the whole sample, leaves, rhizomes and roots were carefully separated, frozen

and subsequently freeze-dried for dry biomass measurements. Leaf surface per shoot, above ground (AG) and below ground (BG) biomass, AG/BG ratio were calculated from the averaged values of dry biomass and morphological measurements. As a proxy for estimated cover, the Leaf Area index of the standing seagrass meadow ( $LAI_{standing}$ ;  $m^2 \cdot m^{-2}$ , ie.  $m^2$  of leaf.  $m^{-2}$  of surface) was calculated as the product of leaf surface per shoot ( $m^2$ ) times the shoot density ( $m^{-2}$ ).  $LAI$  was measured by using the total leaf width and leaf length to compute the surface of each leaf and then multiply by the shoot density per  $m^2$ .  $\Delta LAI_{standing}$  is calculated as the difference between  $LAI_{standing}(t_{end})$  and  $LAI_{standing}(t_0)$ . The  $LAI_{regrowth}$  in the mown gaps, i.e. the  $LAI$  recorded after 46 days of regrowth, was used as a proxy for absolute recovery. To enable comparison between sites, we calculated the relative recovery (%RC) by dividing  $LAI_{regrowth}$  by  $\Delta LAI_{standing}$  and multiplying by 100%. We assumed that a lower relative recovery is indicative for a slower return of the system to its initial state and hence is representative of a critical slowing down in the system response to disturbance.

**Sediment samples:** Sediment samples were collected at the start and the end of the experiment by inserting a clean and single use plastic 20 ml syringe into the sediment to collect the top 5 cm of sediment. Once sampled, sediment was placed into individual plastic bottles and conserved into iceboxes for transportation to the laboratory where they were stored in a freezer before analysis. Sediment grain size measurements were done on freeze-dried samples using a Mastersizer 2000 Laser particle Sizer (Malvern Instruments Limited, UK). TN% and %TOC in sediment were analysed by a CNS Analyzer (Vario MACRO CN) on dry and ground samples.

**Porewater and water samples:** 20 ml syringes connected to Rhizon MOM 5 cm female luer (19.21.22F) (Rhizosphere research product, Wageningen, The Netherlands) were used to sample porewater directly in the field. The Rhizon was placed into the surface sediment and the syringe left at the top of the sediment while the Rhizon progressively extracted porewater from the first 5 cm of sediment. Water samples were collected in the experimental area at the start and at the end of the experiment with 50 ml single use plastic syringes. Collected porewater and water samples were transferred into a plastic bottle after being filtered with pinhole filters of 25 mm diameter and 0.45  $\mu m$  pore size connected to the syringe. Samples were transported into iceboxes before being frozen at the laboratory. Porewater and water total nitrogen,  $NO_3^-$ ,  $NO_2^-$ ,  $NH_4^+$ , and total phosphorus were measured using a nutrient auto-analyzer utilizing gas-segmented continuous flow analysis (AutoAnalyzer 3, Branluebbe, Germany).

### Statistical analysis

Differences between sites at the start of the experiment, independent of treatments, were analysed using the non-parametric Kruskal-Wallis test, as the dataset could not meet with the homogeneity of variances and normality assumptions. Differences in seagrass morphology at the start and end of the experiment, and between sites at the end of the experiment for control treatments were then tested with independent T-tests on SPSS (IBM® SPSS® Statistics Version 19). The influence of the two factors 'Site' (SS vs. ES) and 'Nutrients' (enriched vs. control) and their interaction effect on leaf surface per shoot, shoot density and LAI ( $LAI_{standing(t_{end})}$  and  $\Delta LAI_{standing}$ ), absolute ( $LAI_{regrowth}$ ) and relative recovery (%RC) from gaps after 46 days were checked with 2-way ANOVA. Statistical differences between means were measured using independent samples T-tests with only one factor treatment (a combination of site and nutrient enrichment treatment) and by grouping variables according to each factor. Normality and homogeneity of the data were previously checked, along with interactions between factors. When necessary, data were transformed to meet with ANOVA assumptions. Data are presented as means ( $\pm SE$ ), and three significance level of 1%, 5% and 10% are used in all analyses to create three levels of statistical significance, respectively: high significance (\*\*\*,  $p < 0.01$ ), intermediate significance (\*\*,  $p < 0.05$ ) and low significance (\*,  $p < 0.1$ ).

(next page)

Table 2.1: Water porewater, sediment and seagrass characteristics of both sites at start and for the two nutrient treatments at the end of the experiment (only for undisturbed plots). The p-values following Kruskal-wallis and Independent T-tests are included for differences between <sup>1</sup> control and nutrient enriched plots at the end of the experiment; and <sup>2</sup> between start control values and end values in control and nutrient enriched plots.

Exposed site										Sheltered site			
		Start		End		p-value <sup>‡</sup> (start vs. End)		Start		End		p-value <sup>‡</sup> (start vs. End)	
		control	nutrient	p-value <sup>†</sup>	control	nutrient	p-value <sup>†</sup>	control	nutrient	p-value <sup>†</sup>	control	nutrient	p-value <sup>†</sup>
Water	NO3- (μM)	1.3 ± 0.2	1.3 ± 0.2		0.852			0.8 ± 0.2	0.13 ± 0.05		0.021**		
	NH4+ (μM)	5.8 ± 0.7	0.6 ± 0.002		0.001***			10.6 ± 3.9	0.7 ± 0.03		0.036**		
	NO2 (μM)	0.15 ± 0.004	0.04 ± 0.01		<0.001***			0.09 ± 0.02	0.04 ± 0.02		<0.001***		
	PO4 (μM)	0.04 ± 0.006	0.04 ± 0.002		0.933			0.9 ± 0.3	0.03 ± 0.001		0.021**		
Porewater	NO3- (μM)	3 ± 1.1	0.9 ± 0.4	1.7 ± 0.5	0.276	0.073*		9 ± 0.8	1.4 ± 0.6	1 ± 0.6	0.688	0.009***	0.006***
	NH4+ (μM)	14.3 ± 4.5	2 ± 1	4.9 ± 2.5	0.311	0.013**		10.9 ± 2.2	2.6 ± 1.4	4.7 ± 2.9	0.5	0.016**	0.096*
Seagrass	C:N ratio in leaves	21 ± 0.7	16 ± 0.6	16 ± 0.7	0.914	<0.001***		24 ± 0.3	27 ± 5	33 ± 2	0.153	0.562	<0.001***
	C:N ratio in rhizomes		35 ± 2	32 ± 1	0.226				24 ± 2	24 ± 0.3	0.009***		
	Leaf length (mm)	157 ± 22	543 ± 87	542 ± 20	0.991	0.013***		176 ± 8	716 ± 33	826 ± 88	0.358	<0.001***	0.002***
	Leaf width (mm)	3.7 ± 0.03	5.6 ± 0.4	5.6 ± 0.3	0.98	0.053*		4.8 ± 0.2	7.2 ± 0.3	7.5 ± 0.2	0.537	0.002***	<0.001***
	Leaf surface per shoot (mm <sup>2</sup> )	3295 ± 456	10763 ± 2317	12956 ± 894	0.452	0.034**		449 ± 365	23088 ± 3068	24555 ± 2392	0.725	0.025**	0.003***
	Shoot density (m <sup>-2</sup> )	444 ± 40	878 ± 113	947 ± 138	0.707	0.03**		433 ± 50	298 ± 31	375 ± 23	0.084*	0.05*	0.265
	Leaf Area Index	1.5 ± 0.3	9.8 ± 3.03	12.6 ± 1.9	0.492	0.052*		1.9 ± 0.2	6.2 ± 0.4	9.3 ± 0.6	0.008***	0.001***	<0.001***
	Internode length (mm)		13.4 ± 2.2	12.6 ± 2.1	0.814	0.006***			19.9 ± 0.5	18.5 ± 1.7	0.486	<0.001***	0.002***
	AG:BG ratio	3 ± 1	2.4 ± 0.2	3.6 ± 0.7	0.138	0.335		3 ± 0.3	4.6 ± 1.3	4.6 ± 0.7	0.997	0.001***	0.127
	AG biomass (g)	6 ± 1	52 ± 8	55 ± 3	0.762	0.04**		7.3 ± 0.5	41 ± 4	38 ± 8	0.788	0.008***	0.018**
	BG biomass (g)	1.7 ± 0.2	22 ± 3	17 ± 3	0.244	0.002**		2.6 ± 0.2	10.4 ± 1.5	8 ± 2	0.409	0.347	0.047**

## Results

### Seagrass meadows characteristics and environmental status

At the start, no significant differences for most morphological measurements were observed between the two *Zostera marina* seagrass meadows (Table 2.1). Both sites presented a similar Leaf Area Index (LAI). Inorganic nitrogen in the porewater at the non-enriched plots decreased strongly during the experimental period (Table 2.1). At the exposed site (ES), the ammonium concentrations decreased 8-fold ( $p=0.053^*$ ), whereas nitrate only decreased 3-fold ( $p=0.053^*$ ). At the sheltered site (SS), porewater ammonium concentrations decreased 4-fold ( $p=0.016^{**}$ ) and nitrate concentrations 7-fold ( $p=0.009^{***}$ ). In general, porewater nitrogen concentrations were lower at ES as compared to SS at the start of the experiment ( $p=0.025^{**}$ ; Table 2.1), but not at the end (Table 2.1). Following nutrient addition, at the end of the experiment higher ammonium porewater concentrations were measured at ES ( $p=0.028^{**}$ ) and they tended to be increased at SS ( $p=0.096^*$ ), whereas nitrate porewater was lower at nutrient enriched sites of SS ( $p=0.006^{***}$ ) and not significantly affected at those of ES (Table 2.1).

Nitrate and phosphate concentrations in the seawater were generally higher in ES than in SS ( $p=0.076^*$  and  $p=0.025^{**}$  for Nitrate;  $p=0.009^{***}$  and  $p=0.024^{**}$  for Phosphate, at start and end respectively), and decreased in both sites over the experimental period (Table 2.1). Ammonium concentrations were higher in SS than in ES ( $p=0.024^{**}$ ; Table 2.1).

Both sites presented a contrasting granulometry with a D50 of  $119.11 \pm 26.03 \mu\text{m}$  (fine sand) at the Sheltered site and of  $478.08 \pm 121.47 \mu\text{m}$  (medium sand) at the Exposed site. The grain size distribution is in accordance with our observation on site exposure.

On average over the experimental period, temperatures were higher ( $p=0.001^{***}$ ) in ES ( $19.5 \pm 0.07^\circ\text{C}$ ) than in SS ( $18.8 \pm 0.05^\circ\text{C}$ ). Water temperature showed a similar increase at both sites of  $2.6^\circ\text{C}$  during the experimental period. Salinity was also higher in ES ( $p=0.004^{***}$ ) with values on average of  $31.79 \pm 0.07\text{‰}$  in ES and of  $31.45 \pm 0.03\text{‰}$  in SS. Light available for the plants was higher ( $p<0.001^{***}$ ) in ES ( $121.36 \pm 3.8 \mu\text{E.s}^{-1}.\text{m}^{-2}$ ) than in SS ( $40.45 \pm 2.06 \mu\text{E.s}^{-1}.\text{m}^{-2}$ ).

### Nutrient enrichment and site effects on $LAI_{standing}$ (indicator 1) and other static meadow characteristics

Mostly no main effect of nutrient addition was detected on  $LAI_{standing}$  (indicator 1), shoot density and leaf surface per shoot (Table 2.2). Nevertheless, at the sheltered site nutrient enrichment resulted in an increased  $LAI_{standing}$  (1.5 times,  $p=0.008^{***}$ ; Figure 2.2c and Table 2.3) and tended to have an increased shoot density (1.2 times,  $p=0.008^*$ , Figure 2b and Table 2.3). Leaf surface per shoot (Figure 2.2a) was approximately 175% higher at the sheltered site (SS) than at the exposed site (ES) whereas shoot density was approximately 225% higher at ES than at SS (Figure 2.2a, 2b and Table 2.3).  $LAI_{standing}$  was 1.5 times higher in ES than in SS in the nutrient treated plots ( $p=0.095^*$ ), but there was no difference between sites in the control plots (Table 2.3, Figure 2.2c). There were no interaction effects of site and nutrient addition on  $LAI_{standing}$ , shoot density and leaf surface per shoot (Table 2.2). No significant amounts of epiphytes were noticed at both sites among treatments over the experiment.

### Nutrient enrichment and site effects on the relative recovery (%RC; indicator 2) and the absolute recovery ( $LAI_{regrowth}$ )

The above ground – below ground ratio was similar at the start and the end of the experiment. In addition the above-ground biomass removed at the start of the experiment was not significantly different between the two sites, which allowed us to compare regrowth and relative recovery over the experimental period.

Relative recovery (%RC; indicator 2) was higher at the sheltered site than at the exposed site, and was negatively affected by the nutrient enrichment (Table 2.3, Figure 2.2e). However, relative recovery also showed an interactive effect, indicating a stronger negative nutrient effect at the sheltered site (Table 2.3, Figure 2.2e).

In the control treatment, relative recovery was 3 times higher in SS than in ES (Figure 2.2e), resulting in a relative recovery in SS that even exceeded the  $LAI_{standing}$  in the undisturbed meadow (Figure 2.2c and 2.2e) and for most replicates. On the other hand, the nutrient treatments at both sites and the control site of ES showed a much lower relative recovery below 50% in all instances (Figure 2.2e; Table 2.4). This shows a clear difference between sites where SS has a higher recovery than ES only in the control treatment, whereas it has a lower  $LAI_{standing}$  compared to the three others showing a regrowth of half or less as compared to the surrounding

meadow. Absolute recovery did not significantly differ between sites and nutrient treatments or their interaction (Figure 2.2d; Table 2.3).

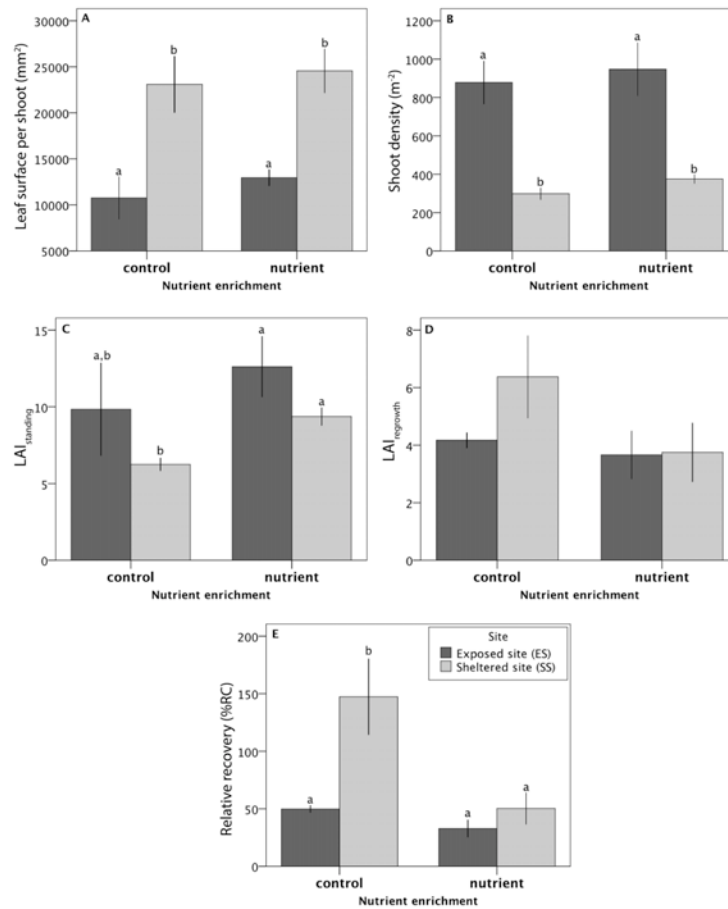


Figure 2.2: Seagrass morphological parameters (mean  $\pm$  SE) at the end of the experiment ( $t=46$  days) and group statistics (a/b). Leaf surface per shoot (a) refers to the leaf length times leaf width per shoot ( $\text{mm}^2$ ), shoot density (b) represents the number of shoots per  $\text{m}^2$  ( $\text{m}^{-2}$ ).  $\text{LAI}_{\text{standing}}$  (c) is the product of shoot density and leaf surface per shoot (dimensionless) and represents the indicator 1 for 'control' and 'nutrient' treatments.  $\text{LAI}_{\text{regrowth}}$  (d) or Absolute recovery represents the LAI, so indicator 1 for gap treatments ('control' and 'nutrient'). Relative recovery (e) represents indicator 2 and is the recovery from gaps as a function of  $\Delta\text{LAI}_{\text{standing}}$  expressed in percent.

Table 2.2: Two-way ANOVA p-values of the effect of site, nutrient addition and their interaction on shoot density, Leaf area parameters and recovery of *Zostera marina*

	Shoot density [m <sup>-2</sup> ]	Leaf surface per shoot [mm <sup>2</sup> ]	LAI <sub>standing</sub>	ΔLAI <sub>standing</sub>	LAI <sub>regrowth</sub>	Relative recovery [%RC]
Site	<0.01***	0.048**	0.076*	0.05*	0.277	0.014**
Nutrient	0.435	0.442	0.118	0.118	0.15	0.015**
Site*Nutrient	0.969	0.626	0.922	0.921	0.313	0.061*

Table 2.3: p-values of the 2 indicators LAI<sub>standing</sub> (as a proxy for cover) and relative recovery (as a proxy for resilience), as well as LAI<sub>regrowth</sub>, ΔLAI<sub>standing</sub>, Leaf surface per shoot and Shoot density following independent samples t-tests for main effects site and nutrient enrichment.

	Leaf surface per shoot [mm <sup>2</sup> ]	Shoot density [m <sup>-2</sup> ]	LAI <sub>standing</sub>	ΔLAI <sub>standing</sub>	LAI <sub>regrowth</sub>	Relative recovery [%RC]
Exposed Vs Sheltered						
Control	0.036**	0.005***	0.305	0.258	0.261	0.097*
+N	0.011**	0.003***	0.13	0.095*	0.95	0.327
Ambient Vs High Nutrient						
Exposed site	0.452	0.707	0.492	0.492	0.613	0.139
Sheltered site	0.725	0.084	0.008***	0.008***	0.217	0.083*

### Correlation cover – recovery

The two indicators,  $LAI_{standing}$  (i.e. quantitative proxy for cover) as *indicator 1 for seagrass health* and the relative recovery (%RC) as *indicator 2 for seagrass resilience*, showed an inverse relationship with a  $R^2=0.82$  and correlation coefficient  $r=0.9$  (Figure 2.3a). This means that the two indicators gave an opposite prediction on ecosystem health, indicating that the relative recovery is lower in meadows with a higher cover. Nutrient enrichment clearly affected the response at both sites by increasing the  $LAI_{standing}$  whereas relative recovery tended to decrease. This effect is most clear at the sheltered location. Indicators in ES responded similar but with more variability (i.e., large SE). The correlation between absolute recovery and  $LAI_{standing}$  also gives a negative relationship with coefficients  $R^2=0.76$  and  $r=0.87$  (Figure 2.3b), demonstrating that the inverse correlation between LAI and recovery is not the result of an autocorrelation caused by comparing  $LAI_{standing}$  and relative recovery (= absolute recovery divided by  $LAI_{standing}$ ).

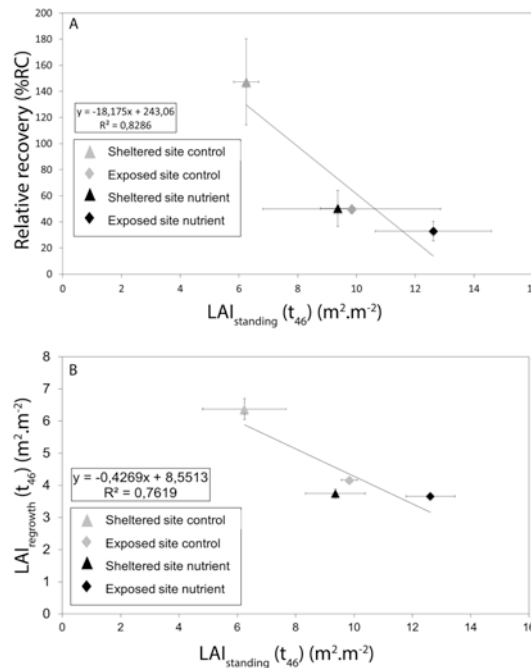


Figure 2.3: (A) Correlation between relative recovery from gaps (%RC) and  $LAI_{standing}$  at the end of the experimental period ( $t=46$  days) and (B) Correlation between Absolute recovery ( $LAI_{regrowth}$ ) and  $LAI_{standing}$  at the end of the experiment.

## Discussion

Seagrasses are threatened worldwide (Orth et al. 2006), emphasizing the need for better indicators of their health status and their resilience to disturbance (Duarte et al. 2004a). We compared the response of two indicators of seagrass health (i.e., leaf area index as a direct indicator of health versus relative recovery as an indirect indicator of resilience) in different stress levels from hydrodynamic exposure and nutrient availability. Our experiment showed that the two indicators were inversely related with a lower recovery when cover was high (Figure 2.3). The sheltered site had much larger plants and lower shoot densities than the exposed site (Figure 2.2), resulting in a  $LAI_{standing}$  that was comparable among sites (being a product of plant dimensions and shoot density). The first indicator, LAI used as a quantitative proxy for plant cover, tended to increase with nutrient addition (Figure 2.2c), whereas the second indicator, relative recovery, tended to decrease in response to nutrient addition (see Figure 2.2e). This opposite response may explain the inverse relation between both indicators.

### Best estimate for seagrass health and resilience?

The two indicators imply a negative relationship (see Figure 2.3): when  $LAI_{standing}$  increases, relative recovery decreases. This observation seems contradictory with the concept behind the basic monitoring approaches looking at biomass or cover to estimate a seagrass meadow resilience and health status (Orth and Moore 1988, McKenzie et al. 2003, Short et al. 2006) and the concept of seagrass conservation. Seagrass meadows with a high cover, relating to a healthy status, are supposed to have higher tolerance to stress. Our measurements indicate that the more biomass a vegetation has, the harder it is to recover to the pre-disturbance level, as this requires re-growing a lot of biomass. If the cover is lower, this may provide a better chance to fully recover as it requires less regrowth. The control treatment at the sheltered site had even more growth than the undisturbed plots by a factor 1.5 (Figure 2.3), which indicates that, at this site, space and/or light may have been limiting in spite of the relatively low standing biomass, as was for example also observed in heavily grazed meadows (e.g. Christianen et al. 2012). Carbon reserves (data not available) could have played an additional role in the evaluation of recovery, as they might differ between sites as a function of local abiotic parameters and meadow conditions over winter. Under higher wave-induced disturbance in the exposed site, we observed the highest cover but lower relative recovery

(Figure 2.3). Overall, our results suggest that a high  $LAI_{standing}$  may not always indicate high resilience against disturbance.

Relative recovery in our experiment was used to emphasize the effect of Critical Slowing Down, an indicator developed after Wissel (1984). In their review of early warning signals to predict critical transitions, Scheffer et al. (2009) refer to Critical Slowing Down when a system becomes increasingly slow in recovering from small perturbations. This indicator can be detected in systems via early warning signals such as an increase in variance of the monitored variable, an increase in auto-correlation and a slower recovery from disturbances (Scheffer et al. 2009, Dakos et al. 2011). Such early warning signals have been shown to be accurate in some model simulations (van Nes and Scheffer 2007, Scheffer et al. 2009, Dakos et al. 2011), but not in others (Hastings and Wysham 2010, Boettiger and Hastings 2013). Nonetheless, in practice it remains difficult to verify and use them. Measuring relative recovery or even recovery rates seems the most appropriate method to relate to experimental and easily collectable data. The reduction in recovery rates in the sheltered site in the presence of nutrient addition, suggests that nutrient addition would reduce the resilience of the system, putting the system closer to its bifurcation point (Wissel 1984, Scheffer and Carpenter 2003, van Nes and Scheffer 2007, Scheffer et al. 2009, Downing et al. 2012). The same is observed at the exposed site, be it to a less strong extent. Henceforth, our recovery measurements, used as proxy for critical slowing down, suggest that nutrient addition reduces the resilience of the seagrass meadows (Figure 2.3). This is in line with general theory, but our results also show that the anthropogenic pressure, assumed to be very high in the sheltered site as compared to the exposed site (in terms of eutrophication), does not form yet a strong stress regime to the seagrasses in our experimental area, as  $LAI_{standing}$  generally increases when nutrients are added.

The inverse relationship between cover and recovery (as indicator for approaching collapse) may well be a common plant biological phenomenon in eutrophication gradients. The mechanisms underlying this phenomenon may result from the unimodal response of plants to nutrient enrichment. Plant dimensions generally increase following fertilisation, leading to longer and wider leaves and thus increased cover and above-ground biomass (Short 1983, Marschner 1995). However when nutrients, particularly nitrogen, are in excess, the plant physiological and growth response are negatively affected. This is due to nitrogen toxicity, and in

aquatic or marine systems additional algal overgrowth, which both lead to a higher demand of carbon skeletons, thus causing physiological imbalance, ultimately leading to mortality and collapse (Pearson and Stewart 1993; Marschner 1995; for seagrasses reviewed in: Touchette and Burkholder 2000a; Burkholder et al. 2007). Along this eutrophication trajectory, prior to collapse, increasing plant cover thus may coincide with increasing physiological imbalance, which likely diminishes plant recovery potential.

### **Seagrass health indicators: a timing effect?**

Our results show that two basic indicators can give an opposite indication on seagrass health status and on their resilience to environmental stresses. Monitoring tools developed for several years by using cover or some other static morphological parameters are relevant when applied in time over specific locations to understand the status of a seagrass meadow and its evolution in time. However, our results show that a single measurement may be misleading as an assessor of the health status of a seagrass bed. According to our results, an increased cover may be accompanied by reduced recovery potential showing reduced resilience and indicating nearness of collapse, which is contradictory with most seagrass conservation and monitoring goals. Nevertheless, in order to interpret cover data, monitoring and studies on species resilience need a system-specific approach, depending on several external variables in the surrounding environment but also need to include information on the timing and spatial organisation (i.e. patchiness, depth distribution, meadow edge, etc.) of the system studied (Neckles et al. 2012). The choice of indicators, as suggested by the European Water Framework Directive, should include several parameters at the individual, the population and the landscape scale (i.e. multi-parametric indexes for seagrasses such as POMI, BIPO, ZoNI, etc; Mascaró et al. 2013). The aim of our study was to investigate how a basic and simple indicator (as used in Seagrass watch but also in multi-parametric studies) could alone compare to a more theoretical approach (critical slowing down) in estimating seagrass health status and resilience.

Our experiment was run between June and mid-July, which corresponds to the first phase of the growing season for these plants. Because seagrasses are seasonal plants, timing represents an important parameter to consider when measuring recovery. The seasonal development of LAI may affect both standing LAI and regrowth rate and the outcome of such experiment, requiring further study. In addition, the

length of the time period used for measuring regrowth could have an effect on the indicators' response. Further research on LAI and recovery over time is required to better evaluate seagrass health and resilience to disturbance over the growing season. Furthermore, our study showed that the response and resilience to disturbances is affected by site-specific environmental variables. A site-specific approach involving a higher resolution in space and time would further elucidate resilience dynamics.





## CHAPTER 3

### Understanding seagrass resilience in temperate systems: the importance of timing of the disturbance

*Ecological Indicators (2016)*

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#### Abstract

Temperate seagrass meadows form valuable ecosystems in coastal environments and present a distinct seasonal growth. They are threatened by an increasing amount of stressors, potentially affecting their capacity to recover from disturbances. We hypothesized that their resilience to disturbances is affected by seasonal dynamics. Hence, we investigated the effect of the timing of the disturbance on seagrass Leaf Area Index (as a proxy for presence, or 'visible' status), recovery from disturbance (as a proxy for meadow resilience), and rhizome carbohydrates (as a proxy for longer term resilience) by a series of four disturbance-recovery field experiments spread over the growing season at two sites in Shandong Province, China. During the course of the growing season, we found the highest recovery at the start of the growing season, lowest recovery when Leaf Area Index peaked around mid-growing season, and intermediate recovery when Leaf Area Index decreased at the end of the growing season. Rhizome carbohydrates were not affected by disturbances during any of the four experimental periods and could not explain the low recovery during mid-growing season. The two sites differed in exposure and in the occurrence of incidents like a green tide and storms, which affected recovery. However, general patterns were similar; timing strongly influenced the indicator of meadow resilience and its correlation with presence during the two main seagrass growth phases. Our results emphasize the importance of carefully considering timing in the evaluation of seagrass resilience in temperate systems. Furthermore, our study implies that, to effectively protect seagrass beds, conservation management should aim at avoiding disturbances particularly during the peak of the growing season, when resilience is lowest.

## Introduction

Seagrasses are key marine angiosperms capable of creating extensive meadows through clonal growth (Hemminga and Duarte 2000; Larkum et al. 2006). Their presence in intertidal as well as subtidal areas worldwide modifies the environment and creates suitable habitats for many benthic organisms and juvenile fish (Heck et al. 2003, Hughes et al. 2009). But seagrasses are globally increasingly threatened. Threats come mainly from anthropogenic activities and the subsequent environmental changes they induce (Short and Wyllie-Echeverria 1996, Orth et al. 2006, Waycott et al. 2009, Hughes et al. 2009). It has been widely acknowledged that, over the last two decades, the combination of increasing disturbances and stresses has led to seagrass decline and occasionally sudden collapse. Recovery of such decline can be cumbersome, as seagrass meadows may act as bi-stable ecosystems, with the bare state being the alternative attractor (van der Heide et al. 2007, Carr et al. 2010, 2012). To be able to manage and to protect remaining seagrass meadows, it is important to gain fundamental insight in their resilience to disturbance.

Temperate seagrass ecosystems are known to have distinct seasonal growth and rhizome proliferation (Duarte 1989; Hemminga and Duarte 2000; Larkum et al., 2006). The main mechanisms controlling the seasonal growth of seagrasses are changes in light intensity and temperature (Dennison 1987, Duarte 1991, Olesen and Sand-jensen 1993, Ochieng et al. 2010, Marbà et al. 2012). Generally, plants start to grow in spring, until mid-summer when they reach their maximum shoot density and cover to build up their carbon reserve that allows them to overwinter and regrow the next year (Madsen 1991). Then senescence starts in late summer with the release of seeds, leaves get thinner and shorter, and shoot density becomes lower (Hemminga and Duarte 2000, Larkum et al. 2006). In winter, the vegetation is very sparse (Orth et al. 2012), only the below ground biomass – with limited leave presence – and seed banks remain until spring when new shoots grow again. To overcome the winter months, seagrass plants exploit the non-structural carbohydrate reserves gained during the summer period (Madsen 1991, Alcoverro et al. 1999, Olivé et al. 2007, Lee et al. 2007, Govers et al. 2015). The amount of carbohydrates needed for seagrass survival over the winter periods depends on abiotic factors, i.e. temperature and light availability; but also on internal factors, such as respiration and growth (Madsen 1991, Alcoverro et al. 2001, Govers et al. 2015). Chances of survival during winter thus depend on the plant's capacity to build up its carbon reserves during summer (Govers et al. 2015).

Given these strong seasonal dynamics, it may be expected that seagrass resilience in temperate systems will be strongly affected by its growing season, making the recovery to a disturbance strongly sensitive to the period the disturbance occurs. It becomes essential to clearly pinpoint the specific time in their growth cycle when seagrass resilience would be either the most or least severely affected.

The resilience of a system is its ability to return to its initial form or state after a disturbance (van Nes and Scheffer 2007, Scheffer et al. 2009). Resilience can be evaluated by looking at indicators of an ecosystem health status (for seagrasses e.g. cover, or multi parametric indexes such as ZoNI, POMI, BIPO; Krause-Jensen et al. 2005, Marbà et al. 2012, García-Marín et al. 2013, Mascaró et al. 2013) or more dynamic early warning signals that indicate a potential critical transition of the system to another stable state, such as “critical slowing down” (Scheffer et al. 2009, Dakos et al. 2011). Critical slowing down is defined as “a phenomenon that happens when a monitored stable variable of the system returns more slowly to equilibrium after a small perturbation” (Dakos et al. 2011). It is evidenced, for instance, by a decrease in recovery rate when the system gets close to a transition point, and its resilience gets lower (van Nes and Scheffer 2007, Chisholm and Filotas 2009). Recent work shows that the choice of indicators (cover vs. critical slowing down) is important to consider, as they may provide an opposed response in their evaluation of seagrass health and resilience (Soissons et al. 2014). If resilience of temperate seagrass meadows would be affected by the different stages of the growing season, the indicators cover and critical slowing down may be expected to give different outcomes for seagrass resilience over the season.

In this study, we aim to assess the importance of timing over the growing season on seagrass resilience to disturbances. More specifically, we question whether seagrass recovery, as a measure for resilience and as a proxy for nearness of collapse (‘critically slowing down’, cf. Scheffer et al. 2009) and carbohydrates in the rhizomes, potentially representing a longer term resilience (cf. Govers et al. 2015) are dependent on the timing of a disturbance over the growing season. If the resilience differs along the growing season, our study will allow identifying the timing in the seagrass growth cycle that lead for better recovery and the timing when the beds are particularly vulnerable to disturbances. A manipulative experiment was implemented and repeated at different stages of the growing season on *Zostera marina* intertidal, seasonal stands at two sites in Shandong

province, China, differing in their exposure to hydrodynamics and geographical setting (Figure 3.1). We measured Leaf Area Index (as a proxy for cover, representing seagrass presence or 'visible' status), relative recovery and carbohydrate content in rhizomes at each of the sequential, identical experiments.

### Material and methods

#### Study sites and experimental design

In order to encompass the entire growing season, a series of 4 strictly identical resilience experiments were implemented at two sites within dense *Zostera marina* meadows (average densities ranging from 450 to 1080 shoots.m<sup>-2</sup> at sites) but contrasting in their hydrodynamic conditions: Swan Lake, hereafter referred as Sheltered site (SS) and Dongchu Island, hereafter referred as Exposed site (ES) (Figure 3.1). Both sites are located in the Shandong province in China and are submitted to different pressures and stochastic events such as green tides during the summer season (at SS), storms (at ES) and human activities (at both sites). Hydrodynamics were not measured during the experiment, but the geographical situation (Figure 3.1) and wind fetch of both sites allowed us to define their relative exposure as sheltered versus exposed.

The shallow lagoon was a relatively sheltered site (SS) compared to the open-sea system with a rocky shore and visible waves on the shore close to the meadow (i.e., exposed site; ES). This relative difference in exposure was confirmed by the difference in granulometry with a D50 of  $119 \pm 26 \mu\text{m}$  (fine sand) at the SS and a D50 of  $478 \pm 121 \mu\text{m}$  (medium sand) at the ES (see Soissons et al., 2014 for more information on both field sites).

In temperate systems, like for these two sites, the seagrass *Zostera marina* has a seasonal growth with shoots and seeds developing in spring until summer when, usually in July, they reach a maximum presence in terms of shoot density, size and cover (Zhang et al. 2014; and personal observations). In order to cover the growing season and to ascertain to capture the peak of seagrass presence at both sites, 4 sequential, independent and identical resilience experiments, with a slight overlap in timing for the 2 experiments during the expected peak seagrass cover (Zhang et al. 2014; personal observation), were implemented. The temporal resolution of the four resilience experiments was based on previous studies

in the area, aiming to cover the entire growing season (Zhang et al. 2014; personal observation during 2012): *i*) experiment “Exp 1” at the beginning, when cover increases (9 May - 7 June 2013); *ii*) experiments “Exp 2” and “Exp 3” around the peak of the seagrass cover (7 June – 8 July and 26 June – 25 July 2013, respectively); and *iii*) experiment “Exp 4” at the end of the growing season presenting a decrease in cover (25 July – 24 August 2013) (for schematic representation see Figure 3.2). In each experiment, to assess seagrass resilience we measured recovery rates over a four week period; within this period we harvested four times: Harvest 1 (H1), at the start of the experiment for control and initial plant parameters; Harvest H2 and H3, respectively two and three weeks after the beginning of the experiment; and Harvest H4, at the end of the experiment after four weeks from the start (Figure 3.2).

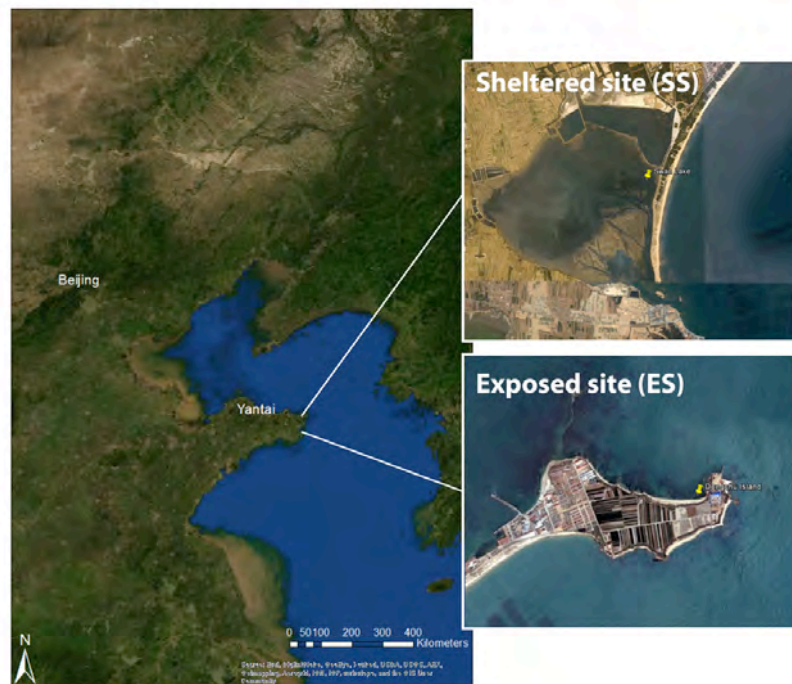


Figure 3.1: Satellite images of the two study sites in the Shandong province, China.

At each site, for each resilience experiment of four weeks, the procedure was identical: 30 plots (15 disturbed and 15 controls) were randomly allocated on a small area of apparent homogeneous seagrass

density (average density of 450 to 1080 shoot.m<sup>-2</sup> at SS and ES respectively) at start; different areas were selected for each of the four experiments (located at approx. 10 m distance from each other) to avoid working with an already sampled plot. From the 30 plots, 15 were randomly defined as controls and the other 15 as disturbed plots, leaving 5 replicates for control and 5 replicates for disturbed plots for each harvest time (i.e. Harvest H2, H3 and H4, after 2, 3 and 4 weeks, respectively) (Figure 3.2). As disturbance, circular gaps of 0.3 m diameter in the centre of each plot were created by clipping the leaves at the start of each resilience experiment, leaving the below ground and sheaths in place. Clipping was chosen to mimic grazing from birds (swans migrating in this region), or the effect of boat anchoring and shell collection by local fishermen, removing parts of the seagrass. It represents one type of disturbance experienced by seagrass meadows and was used, for this study, in order to get a general conceptual understanding of how timing of disturbances affect resilience. It also allowed us to investigate the short-term regrowth of disturbed plants. The size of a disturbance can affect the rate of recovery (Rasheed 1999, 2004, Macreadie et al. 2014).

For our experiments, 0.3 m diameter gaps were chosen from our experience with previous experiments in the same area as an optimal size for direct recovery measurements in a short-time period (4 weeks long experiments), to match the scale of the disturbance we mimic, and to minimize the overall impact of the experiment on the meadow (Soissons et al. 2014). Rhizomes around the disturbed plots were cut to limit recovery by colonization from the edges. Control plots were positioned similarly but remained untouched until harvest time (Figure 3.2).

Note that each resilience experiment (i.e. Exp's 1 to 4, representing timing) was independent from the others: each starting with a disturbance (i.e. leaves clipping and subsequent clipped leaves biomass measures) with an additional harvest (n=5) of full plant material (i.e. above and below-ground material, sampled within the experimental area) that served as an initial measurement of Leaf Area Index (H1), then followed by 3 other harvest time (H2 to H4). Each resilience experiment lasted 4 weeks in total.

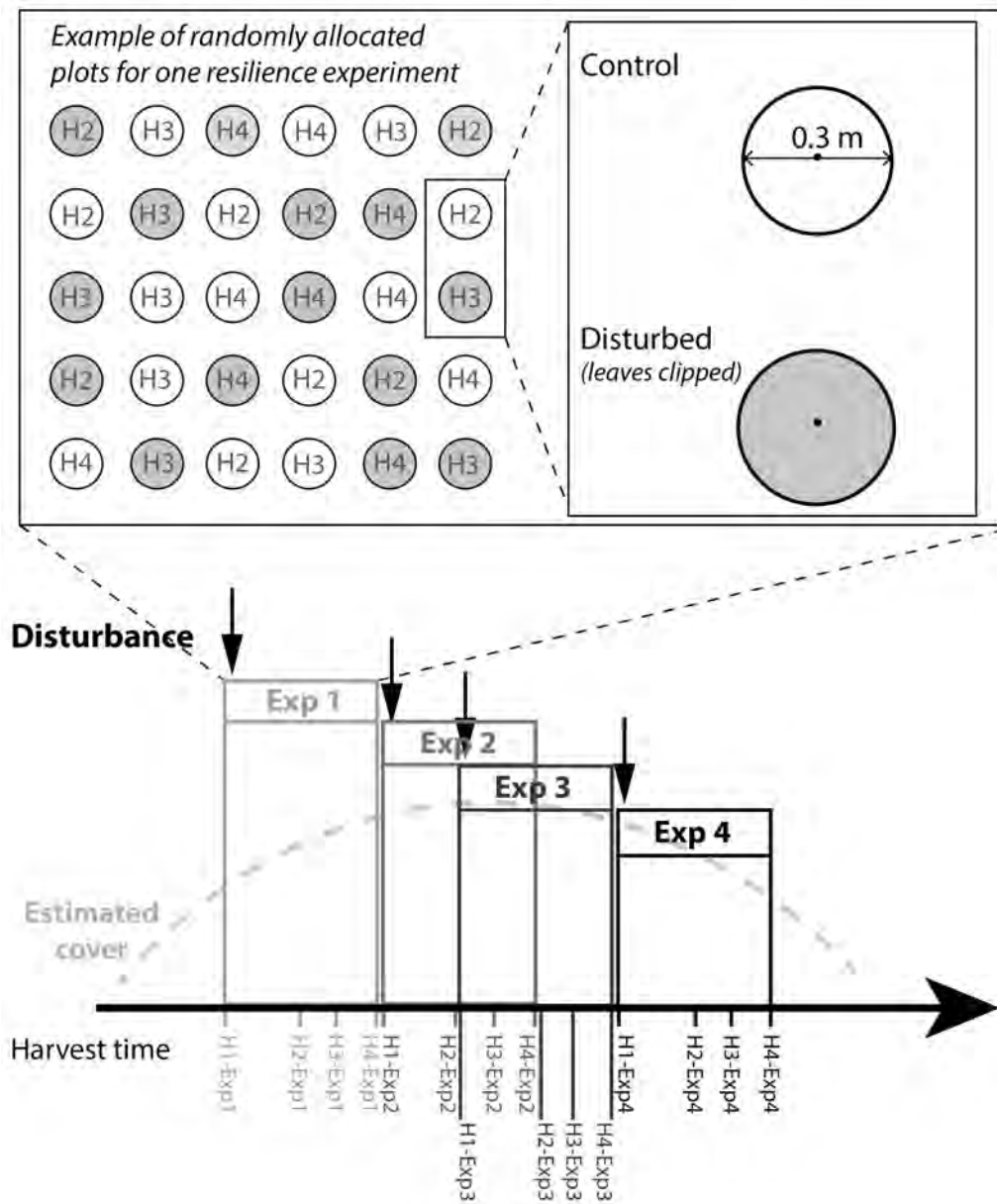


Figure 3.2: Diagram of the experimental design and time frame for all resilience experiments. The upper diagram shows the random distribution of the plots according to their treatments (empty circles represent the control plots, grey circles represent the disturbed plots) and the different

harvest times. In the lower diagram, the four independent, sequential, identical resilience experiments to study timing are represented along the time line and referred to as Exp 1 to 4. The slight overlap between Exp 2 and Exp 3 was designed to better capture the peak of the growing season. The downward black arrows at the start of each resilience experiment represent the disturbance created by clipping the leaves. The four harvest times (H1 to H4) are represented per resilience experiment along the timeline. H1 was done at the start of each resilience experiment within the experimental area for control samples only, and thus is not represented in the upper diagram.

### Harvesting and analysis

**Seagrass harvesting:** At the start of each resilience experiment, whole plant samples (i.e. including above and below-ground material) were harvested within the experimental area for initial morphological measurements (H1,  $n=5$ ) in a surface of  $0.07 \text{ m}^2$ . The leaves clipped in the  $0.3 \text{ m}$  diameter gaps to create the disturbance at the start of each resilience experiment ( $n=15$ ) were not used for morphological measures. Then, for each following harvest time (H2, H3 and H4 after 2, 3 and 4 weeks, respectively), whole plants in 5 control and 5 disturbed plots were sampled in  $0.3 \text{ m}$  cores ( $0.07 \text{ m}^2$ ) so that all plants in the  $0.3 \text{ m}$  diameter gaps were collected. After harvest, seagrasses were directly cleaned a first time in seawater in the field before being transported to the laboratory for measurements. All plants were carefully rinsed and cleaned in fresh water to remove epiphytes and any sediment left. The total number of shoots per sample was directly noted. Subsamples of 5 representative shoots per sample were randomly selected for morphodynamic measurements. Leaf length, width and the number of leaves per shoot were measured for all replicates. Then, for the whole sample, leaves, rhizomes and roots were carefully separated, and subsequently freeze-dried for dry biomass measurements and carbohydrates analysis.

Leaf surface per shoot was calculated from the averaged values of morphodynamic measurements. The Leaf Area index of the standing seagrass meadow ( $\text{LAI}_{\text{standing}}$ ;  $\text{m}^2 \cdot \text{m}^{-2}$ ) was calculated as the product of leaf surface per shoot ( $\text{m}^2$ ) times the shoot density ( $\text{m}^{-2}$ ) from control plots. LAI was chosen as a proxy for seagrass cover, representing seagrass presence or 'visible' status, as quantifying stem density and leaf area provided us with a more precise and reproducible method than by making cover estimates.

The  $LAI_{regrowth}$  ( $m^2.m^{-2}$ ) in the clipped gaps (i.e. disturbed plots) collected at each harvest time was used as a proxy for absolute recovery during the corresponding time period (i.e. harvest times: H2, after 2 weeks; H3, 3 weeks and H4, after 4 weeks). To enable comparison between sites for each harvest time, a relative recovery in terms of LAI was defined. We calculated the relative recovery (RC; %) by dividing  $LAI_{regrowth(t)}$  by  $LAI_{standing(t)}$  (Equation 3.1).

$$(3.1) \quad \%RC_{(t)} = \frac{LAI_{regrowth(t)}}{LAI_{standing(t)}} \times 100$$

This relative recovery also provides us with a proxy for the evaluation of a potential critical slowing down (i.e. a slower recovery rate of a monitored variable after a disturbance), which is a measure of nearness to collapse (van Nes and Scheffer 2007, Chisholm and Filotas 2009, Dakos et al. 2011). Relative recovery was preferred over absolute recovery to estimate the recovery as a function of the growing season and because the experimental design already took recovery as a function of the growing season into account by being spread over the seasonal growth of plants. Indeed, we expected LAI to vary with time and thus to affect the relative recovery. We assumed that a lower relative recovery was indicative for a slower return of the system to its initial state and hence was representative of a critical slowing down in the system response to disturbance.

**Carbohydrate measurements:** Freeze-dried and grinded rhizome samples were carefully shipped from China for carbohydrate measurements. Analyses of carbohydrates were completed at the Netherland Institute for Sea Research (NIOZ) in Yerseke; following a method developed after Yemm and Willis (1954). First, soluble sugars -glucose, fructose and sucrose- were extracted out of the freeze-dried plant material by using an 80% ethanol solution. The residue was hydrolyzed with diluted hydrochloric acid (3%HCL) to convert all the sugar compounds into carbohydrates. Subsequently the fraction was boiled at 100°C for 30 minutes. An Anthrone coloring reagent was added and the color intensity was measured at 625 nm. By calibration with a d-glucose standard series, carbohydrate content in rhizomes was measured in mg carbohydrates per gram dry plant material (Yemm and Willis 1954). All samples were measured in duplicate and a new calibration curve was prepared for every series of measurements.

**Statistical analysis:** The influence of three factors being ‘timing’ (i.e. the sequential, identical resilience experiments Exps 1 to 4), ‘harvest time’ and ‘site’ and their interactive effect on leaf surface per shoot, shoot density in controls and LAI ( $LAI_{standing}$ ), absolute ( $LAI_{regrowth}$ ), relative recovery (%RC) in disturbed plots were checked with a 3-way ANOVA (univariate) on SPSS (IBM® SPSS® Statistics Version 21). For carbohydrate content in rhizome (only measured for the last harvest time, H4), the 3-way ANOVA included the effect of ‘timing’ (i.e. the sequential, identical resilience experiments Exps 1 to 4), ‘site’ and ‘disturbance’ and their interactive effect. Statistical differences between means were measured using the Tukey post-hoc tests. The correlation between indicators was tested with a linear regression model. Assumptions for regression as well as normality and homogeneity of the data were previously checked, along with interactions between factors. Data are presented as means ( $\pm$ SE).

Table 3.1: Three-factors ANOVA p-values of the effect of resilience experiments representing timing (Exps’ 1 to 4), harvest time (within each experiment), site and their interactive effect for the dependent variables  $LAI_{standing}$ ,  $LAI_{regrowth}$  (absolute recovery) and Relative recovery (%RC)

	$LAI_{standing}$	$LAI_{regrowth}$	%RC
Timing	<0.001***	<0.001***	<0.001***
Harvest time	0.273	0.995	0.394
Site	<0.001***	<0.001***	0.015***
Timing* Harvest time	0.004***	0.742	0.321
Timing* Site	0.003***	<0.001***	0.018***
Harvest time* Site	0.667	0.462	0.283
Timing* Harvest time* Site	0.002***	0.261	0.714

## Results

During the experimental period, two storms occurred at the exposed site, and two green tides at the sheltered site. During the green tide, algal cover (mainly *Chaetomorpha linum*) was up to 265 g DW.m<sup>-2</sup>. Such events are common in the region (personal communication, Qiuying Han and local managers). No exceptional variations in the water temperature were noticed either in the winter before we implemented the experiment or during the growing season, although air temperatures in August were 2°C higher than normal (data obtained from the meteorological station in Rongcheng, Weihai, China).

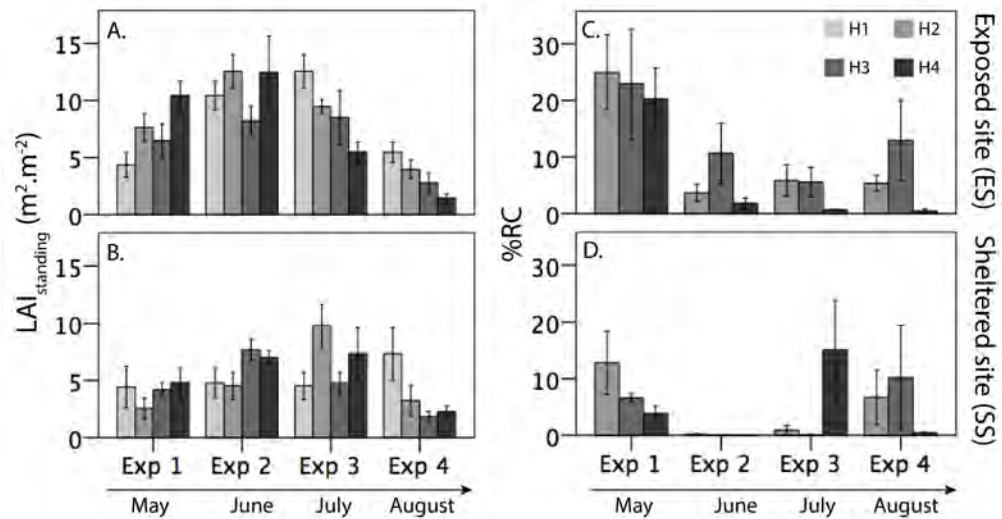


Figure 3.3: Leaf Area Index of the standing stock (LAI<sub>standing</sub>) in m<sup>2</sup>.m<sup>-2</sup> (A. and B.; data based on control plots) and Relative recovery (Critical slowing down) in %RC (C. and D.; data based on disturbed plots) at both sites (Exposed site (ES) for A. and C., and Sheltered site (SS) for B. and D.) at the different resilience experiment (Exp) and harvest times (H).

### Leaf Area Index in relation to the growing season and across sites

Leaf Area Index (LAI<sub>standing</sub>) measurements at both sites show that the standing stock develops as expected and in line with the growing season: LAI progressively increases from May until the beginning of July before decreasing in July-August (Figure 3.3a and 3.3b). LAI<sub>standing</sub> was

similar to the  $LAI_{standing}$  in the preceding year at the same sites (values in 2012 between 6 and 10  $m^2.m^{-2}$ ; Soissons et al. 2014). Differences between sites and timing (i.e. differences between the sequential, identical resilience experiments Exp's 1 to 4) are strong (Table 3.1 and 3.2), as well as their interactive effect (Table 3.1):  $LAI_{standing}$  values are higher at site ES with values up to  $12.6 \pm 1.5 m^2.m^{-2}$  at the top of the growing season (H1-Exp 3, Figure 3.3a) whereas the highest standing stock values at SS only reached  $9.8 \pm 1.9 m^2.m^{-2}$  (H2-Exp 3, Figure 3.3b). At neither site, no significant differences were found between the two resilience experiments implemented at the peak of growth season (Exp 2 and Exp 3; Table 3.2). Other post-hoc tests indicated for the exposed site (Tukey:  $p < 0.01$ ) that  $LAI$  values increased from experiments Exp 1 to Exp 2 and decreased at Exp 4 as compared to Exp 3. Similarly, at the sheltered site, Exp 3 presented significantly higher  $LAI_{standing}$  values than Exp 1 (Tukey:  $p = 0.03$ ) and Exp 4 (Tukey:  $p = 0.013$ ).

Table 3.2: p-values, following post-hoc Tukey HSD test (from 3-factor ANOVA) on the effect of timing (i.e. differences between the resilience experiments, Exp's 1 to 4) for the dependent variables  $LAI_{standing}$ ,  $LAI_{regrowth}$  (absolute recovery) and %RC (Relative recovery). Exp1-Exp4 correspond to the four sequential, identical resilience experiments and represent timing in the growing season

		$LAI_{standing}$	$LAI_{regrowth}$	%RC
		ng	th	
Exp 1	Exp 2	0.001***	<0.001***	<0.001***
	Exp 3	0.017**	0.005***	0.003***
	Exp 4	0.01***	<0.001***	0.005***
Exp 2	Exp 3	0.839	0.801	0.847
	Exp 4	<0.001***	0.972	0.637
Exp 3	Exp 4	<0.001***	0.552	0.988

Harvest time did not show any statistical differences as such (Table 3.1), but a significant interactive effect between harvest time and resilience experiments is seen for  $LAI_{standing}$  (Table 3.1). This interactive effect indicates an increasing  $LAI_{standing}$  between harvest times in the first experiment and decreasing  $LAI_{standing}$  between harvest times in the last experiment, as is expected.

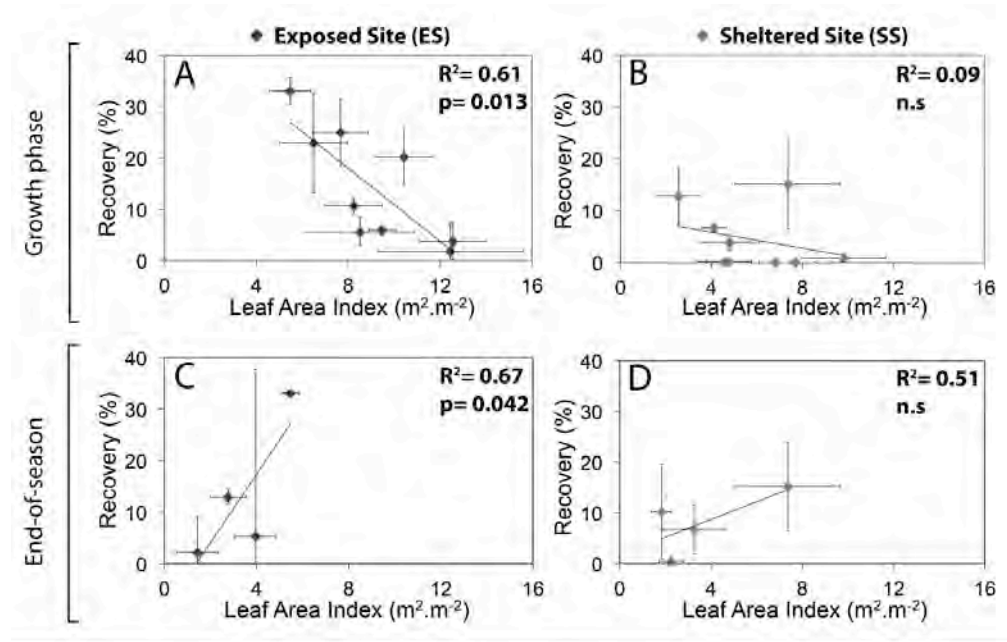


Figure 3.4: Correlation between indicators: Relative recovery (%RC) as a function of Leaf Area Index ( $LAI_{standing}$ ;  $m^2.m^{-2}$ ) in control plots. A and B: Correlation during the growth phase at the Exposed site ES (dark markers) and Sheltered site SS (grey markers) respectively; C and D: Correlation during the End-of-season phase at ES and SS respectively. The growth phase corresponds to the period from May until mid-July; the end-of-season phase corresponds to the period from end of July till end of August. Bars represent standard errors for both indicators.  $R^2$  and p-values are displayed on each graph (n.s for when  $p > 0.05$ ).

### Recovery from disturbances along the growing season and across sites

Both absolute recovery ( $LAI_{\text{regrowth}}$ ; recovered LAI after leaves clipping) and relative recovery (%RC) show an opposite trend to the standing stock with high values at the beginning (Exp 1), a decrease over the growing season, and a small increase towards the end of the growing season (Figure 3.3c and 3.3d; Table 3.2). Differences between sites and timing (i.e. differences between the sequential, identical resilience experiments Exp's 1 to 4) were significant on  $LAI_{\text{regrowth}}$  and %RC values as well as their interactive effect (Table 3.1 and 3.2). However, in all instances %RC did not increase any further after the first harvest time (after 2 weeks), never reaching values higher than 25%RC, meaning that the gaps might 'never' completely recover over the growing season (Figure 3.3c and 3.3d). Post-hoc tests show that regrowth differed over time at the exposed site, with the first experiment being higher than the subsequent experiments (Tukey:  $p < 0.001$ ).

### Correlation between indicators (LAI vs. Relative recovery)

In order to evaluate the correlation between the two indicators, data were compared according to two phases: a growth phase, where  $LAI_{\text{standing}}$  is increasing at both sites, Exp 1 until the end of Exp 3 (from May until mid-July); and an end-of-season phase, evidenced by a decrease in  $LAI_{\text{standing}}$  from the end of Exp 3 until the end of the last resilience experiment, Exp 4 (mid July-August). No significant regression was found at SS. At ES, during the growth phase, a negative relationship was found between %RC and  $LAI_{\text{standing}}$  ( $R^2 = 0.61$ ;  $p = 0.013$ ; Figure 3.4a). During the end-of-season phase, this correlation was positive for ES ( $R^2 = 0.67$ ;  $p = 0.042$ ; Figure 3.4c).

### Carbohydrate reserves

Carbohydrate reserves were evaluated by measuring non-structural carbohydrates in rhizomes at the end of each experiment. The evolution of rhizome carbohydrate content differed between the two sites and along the entire experimental period but was not affected by disturbance (Table 3.3; Figure 3.5).

Table 3.3: Three-factors ANOVA p-values of the effect of site, timing (i.e. resilience experiments (Exps'1 to 4), disturbance and their interactive effect for the dependent variables carbohydrate content in rhizomes at the end of each resilience experiment.

	Carbohydrate content in rhizomes
Site	<0.001***
Timing	0.003***
Disturbance	0.433
Site*Timing	<0.001***
Site*Disturbance	0.549
Timing*Disturbance	0.049**
Site*Timing*Disturbance	0.407

Timing (i.e. comparing sequential, identical resilience experiments Exps'1 to 4) had an interactive effect with site, as well as with the disturbance treatment (Table 3.3; Figure 3.5). At ES, rhizome carbohydrate content showed an increase during the growth phase (between Exp 1 and Exp 2, Tukey:  $p < 0.001$ ) and a decrease during the end-of-season phase (between Exp 3 and Exp 4, Tukey:  $p = 0.014$ ; Figure 3.5). The maximum carbohydrate content at ES was  $56.3 \pm 0.97 \text{ mg.gDW}^{-1}$  at the peak of the growing season in control plots (Exp 2). At SS, rhizome carbohydrate content decreased over the growing season, with values declining almost 2-fold from  $80.3 \pm 12.7 \text{ mg.gDW}^{-1}$  for Exp 1 to  $43.8 \pm 1.6 \text{ mg.gDW}^{-1}$  at Exp 4 in controls ( $p = 0.001$ ; Figure 3.5). No linear relationship was found between carbohydrate content (as an explanatory variable) and recovery.

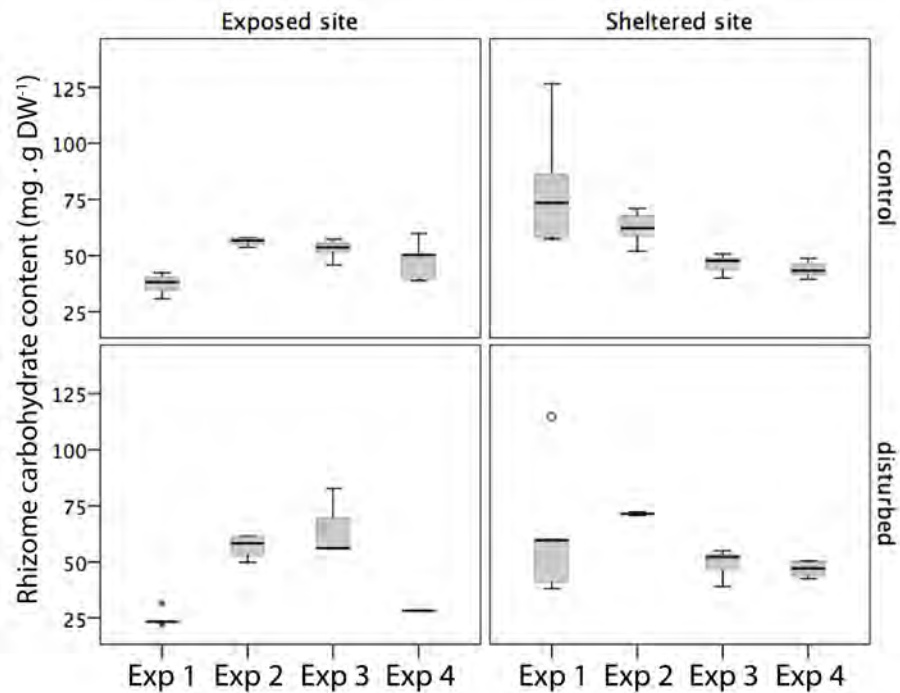


Figure 3.5: Boxplots representing the rhizome carbohydrate content in  $\text{mg.gDW}^{-1}$  at the end of each resilience experiments (X-axis). Columns represents the sites (Exposed site ES and Sheltered site SS), rows represent the treatment (control and disturbed). Symbols (° and \*) represent outliers.

## Discussion

Seagrass presence and seasonal growth in temperate systems are controlled by abiotic variables such as light availability, hydrodynamics and temperatures (Marsh et al., 1986; Duarte 1991; Hemminga and Duarte 2000) and their seasonal changes (Duarte 1989, 1991, Olesen and Sand-jensen 1993, Zharova et al. 2001, Hansen and Reidenbach 2013). This seasonal growth of seagrasses is determinant for their winter survival and chances for seagrass expansion over time as they build up carbon reserves over the growing season (Madsen 1991, Alcoverro et al. 1999, 2001, Govers et al. 2015). Present results emphasize the influence of different phases of the growing season on the resilience of temperate seagrass meadows against disturbance. In our series of identical experiments over the growing

season at two sites, recovery from small-scale disturbances (i.e. above-ground biomass removal by clipping) was higher in the first experiments (at the beginning of the growing season) than in the experiments during the peak of the growing season, when seagrass cover had increased. This effect was found at both sites, despite their difference in exposure and in stochastic events (one site experiencing, green tides, the other site experiencing storms). Carbohydrates content in rhizomes varied between sites and over the growing season, but were not affected by the experimental small-scale disturbances applied throughout the growing season.

### **The effect of timing on resilience in temperate systems**

According to the theoretical concepts of critical slowing down and resilience (van Nes and Scheffer 2007, Scheffer et al. 2009, Dakos et al. 2011), a decrease in recovery rate indicates that the resilience against disturbances of the system is lowered and potential collapse is nearer. Our study shows that, at both research sites, recovery from a small-scale disturbance (clipping) is lowest during the peak of the growing season. Thus, when Leaf Area Index peaked, resilience of the meadows is indicated to be lowest. The slight increase in recovery at the end of the season would imply a higher resilience again at that stage. In general, this demonstrates the need to include a time dimension into the evaluation of resilience in seasonal systems. More specifically our findings imply for management that disturbances should be avoided during the peak of the growing season. It is however noted that a lower recovery at one specific moment in time may represent a low short-term resilience of the plants, but does not necessarily imply a reduced longer-term resilience. Also, it might be expected that repetitive disturbances would at some point lead to no recovery and drastically affect the plant's capacity to overwinter and regrow for the next season (Di Carlo et al. 2011).

Understanding timing effects on resilience is clearly particularly important in bi-stable seagrasses ecosystems (van der Heide et al. 2010b, Carr et al. 2010, 2012), but may also apply to other bi-stable temperate ecosystems such as e.g. temperate shallow lakes (Scheffer et al., 2001, Scheffer and Van Nes 2007); salt-marshes (van Wesenbeeck et al. 2008); temperate reefs (Baskett and Salomon 2010); and temperate floodplains lakes (Chaparro et al. 2014). A time-scale in the evaluation of resilience and potential transition to another stable state (for instance, unvegetated) is to be considered at two levels: (i) Recovery might slow down in time as an

indicator of a lower resilience (van Nes and Scheffer 2007, Chisholm and Filotas 2009, Dakos et al. 2011) but also (ii) might vary along the growth curve of the system as a function of its growth rate, and seasonal changes.

### **The effect of timing on indicators of seagrass presence and resilience**

Indicators of seagrass presence (LAI) and resilience (recovery from small-scale disturbance, or Critical Slowing Down) showed an opposite response during the growth phase with recovery decreasing when the standing stock increased similar to results of Soissons et al. (2014). In contrast, both indicators were positively related at the end of the growing season at the exposed site, which to our knowledge was not observed before. Present results are opposite to results from tropical seagrasses measured by de Longh et al. (1995), where a full recovery was observed during the wet season (when biomass/cover increases) and no recovery was measured during the dry season after dugong grazing (i.e. mostly above-ground removal). This difference could be explained by this seagrass population being close to collapse, or, more likely, by the stronger role of timing during the growing season and more extreme changes in abiotic variables in temperate systems (Baskett and Salomon 2010, Chaparro et al. 2014).

During an event of green tide at the sheltered site, rather common in that region, with green algae (mainly *Chaetomorpha linum*) covering the seagrasses, the Leaf Area Index in the controls was less affected than the disturbed plants, though lower than at the exposed site. This implies that stress reduces resilience, which is in line with the CSD-theory (van Nes and Scheffer 2007, Scheffer et al. 2009, Dakos et al. 2011). It might be speculated that the plants, under disturbance and additional stress (green tides/algae shading) might experience a trade-off between recovering from the disturbance through re-growth and maintaining their below-ground stock for better chances to survive the winter period. Such trade-off might explain the difference in recovery rates, although small at both sites at the peak of growth, between ES and SS. Most importantly, the timing of the disturbance and the seasonal changes in seagrass presence and biomass played an overruling role on both resilience and capacity to recover, regardless of site and stress level.

### **Seagrass resilience in relation to carbohydrate content in rhizomes**

Surprisingly, in our experiment, rhizome carbohydrate content did not show any change after disturbances, whereas differences between sites were present. At the sheltered site, rhizome carbohydrate content remained high in disturbed plots even though recovery was very low or close to 0. During a green tide with large algae cover at the sheltered site, carbohydrates had the tendency to decrease but only in controls as has been observed in Spain on *Zostera noltei* plants shaded by the macroalgae *Ulva rigida* (Brun et al. 2003a). Our results suggest that the plants do not use the carbohydrates stored in the rhizomes for recovery from small-scale disturbances in summer.

In temperate systems, plants use carbon reserves such as carbohydrates stored in rhizomes to cope with winter stresses and regrow next season (Madsen 1991, Alcoverro et al. 1999, Govers et al. 2015). A higher standing stock –and thus recovered stock– would ensure a better chance for winter survival, and carbon reserves can serve as an indicator for winter survival, as a colder winter might greatly reduce their carbohydrate reserves and thus their resilience (Govers et al. 2015). Our study did not investigate the link between current and past growing season, however no exceptional variations in the water temperature were noticed either in the winter before we implemented the experiment or during the growing season. Overall our results hence suggest that small-scale disturbances during the growing season do not affect winter survival.

### **Conclusion and implications for seagrass conservation and management**

Seagrasses worldwide form essential ecosystems in the coastal landscape. Unfortunately, they are submitted to various and repetitive stresses, mostly due to the increasing development of anthropogenic activities (Orth et al. 2006, Waycott et al. 2009, Unsworth et al. 2014) as well as effects of climate change. A growing number of seagrass meadows are nowadays monitored and submitted to management plans, as part of Marine Protect Area (MPA) schemes for instance, to conserve them (La Manna et al. 2015). In economically strongly developing countries, as e.g. China, land reclamation and the intensive use of coastal areas are still ongoing and increase at high rates (Ma et al. 2014). Our finding that both seagrass sites show a low capacity to recover over their seasonal growth (i.e. lower than 50%) is illustrative of the threats to seagrass in such rapidly

developing areas, particularly considering the almost complete absence of other seagrass beds in a wide area around them (Yellow river mouth, personal observation).

In our study, the seasonal changes in standing stock and growth, as well as recovery capacity, were more likely due to spring and abiotic fluctuations than to carbon reserves. Hence, carbohydrate content in rhizomes, in our experiment, did not form a good predictor for the plant's capacity to recover over their growing season. Our results illustrate the need to pinpoint the time in the growing season when the seagrass response to a disturbance would be the least or the most damaging for their short-term resilience. The knowledge of seasonal changes in presence but also in the resilience of keystone ecosystems such as seagrasses becomes then essential in order to preserve them from future collapse. For instance, our study shows that a disturbance at the peak of growth could result in a lower recovery than at the beginning of the growing season. This might thus reduce their net production and lead to their decline. Henceforth, it is essential to carefully consider the timing at which a potentially disturbing activity such as e.g. dredging or trawling is carried out, as timing was shown to have considerable impacts on recovery rates, implying a reduced resilience.

Overall, these results provide conceptual insights that may help management of seagrass meadows, for conservation purposes, especially with respect to the importance of regulating the timing of human disturbances in coastal areas. Considering the seasonal growth and changes of coastal ecosystems is essential, in order to better preserve them and to prevent their potential collapse. Indicators are needed to evaluate resilience, but the effect of timing must be considered as it forms a fundamental factor to integrate for both the use of indicators and the evaluation of resilience, in seagrass as for any other seasonal systems.





## CHAPTER 4

### Ecosystem engineering may alter the expected response to stress and disturbances as based on Grime's growth strategies

*Submitted*

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#### Abstract

Plant species can be characterised by different growth strategies, related to their inherent growth and recovery rates, shaping their response to stresses and disturbances (Grime 1977). Ecosystem engineering offers however an alternative way to cope with stress: by reducing it. By means of an experimental study on two contrasting seagrass species (i.e. the relative slow-growing *Zostera marina* vs. the fast-growing *Zostera japonica*), we explored how growth strategies versus ecosystem engineering capacity of plants affect their resistance to stress (i.e., organic material additions to the sediment) and recovery from disturbance (i.e., above-ground removal). Ecosystem engineering was assessed by measuring sulphide levels in the sediment porewater, as seagrass plants can keep sulphide levels low by aeration of the rhizosphere.

Non-surprisingly, we observed that the fast-growing species had a relative high capacity to recover from disturbance as compared to the slow-growing species. The fast growing species was able to maintain a high standing stock (Leaf Area Index) at increasing stress levels, because of its ecosystem engineering capacity (i.e., constant low porewater sulphide at all organic material levels). Its capacity to recover from disturbance decreased with increasing stress, as the biomass loss reduced the ecosystem engineering capacity (i.e., increasing sulphide concentrations with increasing organic material levels). In contrast to Grime's growth strategies, the slow-growing species was not able to maintain its standing stock (Leaf Area Index) under organic material stress, which we ascribed to a weak ecosystem engineering ability (i.e., strongly increasing sulphide concentrations with increasing organic material levels). Overall, our study suggests that plant responses to

stress and disturbance may deviate from the response predicted by Grime's growth strategies under the influence of ecosystem engineering. Indeed, as shown for the fast-growing species, the combination of low cost investment in growth while having ecosystem engineering that alleviate stress, hence creates a short cut for resilience by making it both fast in recovery (fast growth rate) and more resistant (ecosystem engineering). We argue that both concepts should be considered together in the evaluation of plant's resilience.

## Introduction

Based on their spatial distribution over habitat types and their ability to respond to disturbances, plants have been broadly divided into three growth strategies: competitive, ruderal and stress-tolerant species (Grime 1977). Competitive species (adapted to low stress and low disturbance) and ruderal species (adapted to low stress, high disturbance) typically exhibit a high capacity to extend in space through vegetative growth and, thus, present fast growth rates to better compete for light and other resources (competitors) or to recover from disturbances and improve chances for survival (ruderals). Unlike competitors, ruderal species also present a rapid seedling production and establishment (Grime 1974). For those two strategies, the energy investments in plant tissues are generally relative low. In contrast, stress-tolerant species (adapted to high stress and low disturbance) typically present a rather slow growing rate with long-lived, relatively 'expensive' leaves and a capacity to store resources for extended growth (Grime 1977), providing better defence and structure in their tissues (higher C:N, lignin and secondary metabolites).

The theory of Grime (1977) has proven to be important in understanding a species' resilience in terms of resistance to stress and recovery from disturbance, and makes an important research topic in current ecology (Mumby and Anthony 2015). A species' resistance to stress can be defined as the ability to maintain an unchanged standing stock under stress. Resistance represents both a species' capacity to tolerate the stress (stress-tolerant strategy) or to avoid the stress (stress avoiding strategy) by physiological or morphological adaptations. A species' recovery capacity from disturbance can be defined as its ability to regrow after a local disturbance back to its original state.

A completely different way to cope with stress may come from an organisms' ability to modify its abiotic environment. The latter is often referred to as ecosystem engineering, a term used to indicate species that have the ability to cause state changes in biotic or abiotic environment (Jones et al. 1994) via their own physical structure (autogenic, for instance trees) or via the transformation of non-living or living material (allogenic, for instance beavers creating dams). Ecosystem engineers often – but not exclusively – occur in relatively stressful environments (Crain and Bertness 2006).

The concept of plant growth strategies (Grime 1977) arose well before the concept of ecosystem engineering was introduced into ecological theory (Jones et al. 1994) and both concepts have to our

knowledge not been thoroughly linked. Growth strategies seem to display a trade-off between resilience mechanisms: the fast-growing ruderals and competitors typically present high recovery rates after disturbances either via vegetative growth or seedling re-establishment, whereas stress-tolerant species are typically slow growing and depend on their ability to resist the stress (Grime, 1977; Kilminster et al. 2008). To our knowledge, it is unknown to which extent this trade-off might be affected by a species' ability to modify its environment by ecosystem engineering. Hence we aim at identifying the relative importance of a species' resistance to stress and recovery from disturbance to both its growth rate and its ecosystem engineering capacity, using a fast and a slow growing seagrass species with contrasting ecosystem engineering strategies as model system.

There are various species of seagrasses worldwide, representing different growth strategies as a function of their size and rhizome elongation rate (i.e. fast growing or slow growing species). It has been shown that this difference in growth rates is related to seagrass allometry (Duarte 1991, Hemminga and Duarte 2000): fast growing seagrasses are generally rather small with relatively high rhizome elongation rates (Marbà and Duarte 1998). Both fast and slow growing seagrass species have been recognised as autogenic ecosystem engineers (Jones et al. 1994) that attenuate hydrodynamic energy and stabilize sediment (Fonseca and Fisher 1986, Bos et al. 2007, Widdows et al. 2008, Ganthy et al. 2013). As photosynthetic organisms, seagrasses have the capacity to engineer the sediment chemistry by the release of oxygen via their root system (Greve et al. 2003), thereby escaping sulphide toxicity in organic-rich sediments (Marbà et al. 2009; Frederiksen et al. 2006; Jovanovic et al. 2015; Pedersen and Kristensen 2015). In case that oxygen leakage is too low, there is the risk of sulphide intrusion into the relatively permeable tissues (Holmer and Kendrick 2013). Alternatively, seagrass may escape sulphide toxicity by making relatively impermeable roots, which are protected from toxin intrusion. This morphology also reduces the Radial Oxygen Loss (ROL), thus resulting in a lower engineering of the sediment (Frederiksen et al. 2006, Hasler-Sheetal and Holmer 2015, Jovanovic et al. 2015, Pedersen and Kristensen 2015).

The present study aimed at evaluating the relative importance of *i)* growth strategies and *ii)* ecosystem engineering capacity, in determining the resilience of two co-occurring contrasting seagrass species in terms of their resistance to stress and recovery from disturbance. The two co-

occurring seagrass species we compared were the slow-growing eelgrass *Zostera marina* with low ROL and the fast-growing dwarf eelgrass *Zostera japonica*. Based on Grime's theory, our null-hypothesis is that the slow-growing *Zostera marina* will be more resistant to stress (i.e., anoxic sediment) but will present a lower recovery from disturbance (i.e. leaf removal) as compared to the fast-growing *Z. japonica*. Based on ecosystem engineering capacity (i.e., detoxification of anoxic sediment by oxygen loss from the roots), our alternative hypothesis is that the ecosystem engineering capacity of the fast-growing species will override Grime's theory, causing the fast-growing species to be both faster in recovery and more resistant to stress due to having a faster growth rate and a stronger ecosystem engineering capacity by oxygen release. To test these hypotheses, we assessed along an organic matter gradient the plants' capacity (i) to maintain standing stock (LAI) under increasing organic matter loading; (ii) to recover from disturbances such as above-ground removal and (iii) to engineer their habitat via sediment detoxification (quantified through porewater sulphide measurements).

## Material and methods

### Study site

The experiment was implemented simultaneously on stands of *Zostera marina* and *Zostera japonica* species co-occurring in the "Yuehu lagoon" or "Moon Lake" (N37° 20' 48.6"; E122° 34'10.9"). This is a sheltered lagoon located in the Shandong province (China) close to the city of Weihai (Figure 4.1). The lagoon has a small tidal inlet (86 meters wide) and shallow waters (< 2 meters). The two seagrass stands are located within the same intertidal area inside the lagoon (Figure 4.1) and are thus submitted to identical environmental conditions. Water temperatures oscillate between 18 and 21°C in summer for a salinity of  $31.45 \pm 0.03\%$ . Hydrodynamics were not measured during the experiment, but the geographical situation (Figure 4.1), wind fetch and average granulometry (D50 of  $119 \pm 26 \mu\text{m}$ , i.e fine sand) of the co-occurring seagrass stands allowed us to define their relative exposure as sheltered (see Soissons et al. 2014 for more information on the field site).

### Experimental design

To apply a stressor, an organic matter (OM) gradient was created in order to obtain a range of increased sulphide levels into the sediment and

porewater within the rhizosphere. For each seagrass stand, four levels of OM were added into the sediment ( $n=10$  per OM addition level). OM was composed of carbon (cellulose) only, to avoid the additional effect of nutrient contained in some other organic matter substrates (Govers et al. 2014b). Hence, a mixture of shredded coffee filters and potato starch in a 1:1 proportion (i.e. 1 gram of coffee filter for 1 gram of potato starch) was used in different quantities to create the four levels: no OM [no OM added]; 1xOM [ $100 \text{ g C} \cdot \text{m}^{-2} = 3.5 \text{ g} + 3.5 \text{ g}$  (starch + filters) per plot]; 4xOM [ $400 \text{ g C} \cdot \text{m}^{-2} = 14 \text{ g} + 14 \text{ g}$  (starch + filters) per plot] and 10xOM [ $1000 \text{ g C} \cdot \text{m}^{-2} = 35 \text{ g} + 35 \text{ g}$  (starch + filters) per plot]. OM was inserted into the sediment in all plots randomly allocated within each seagrass stand in areas of apparent homogeneous density. Mean shoot densities were  $433 \pm 62$  and  $6366 \pm 609 \text{ n.m}^{-2}$  for *Zostera marina* and *Zostera japonica* meadows respectively.

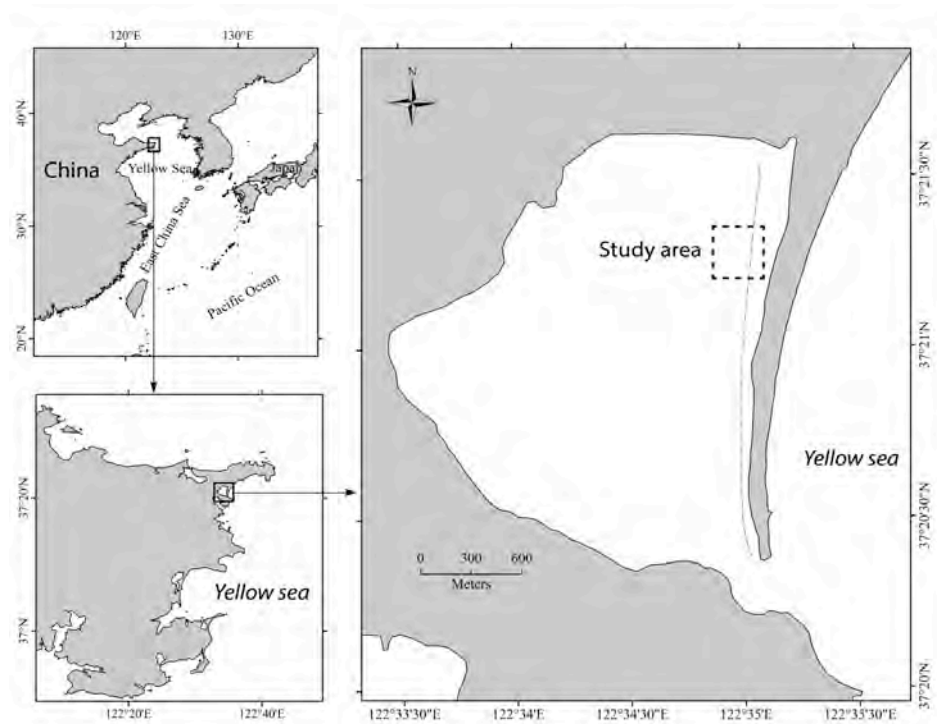


Figure 4.1: Map and localisation of the study area within the 'Moon Lake' lagoon, Shandong province, China.

To measure recovery from disturbances, we removed the above-ground biomass of the plants, by clipping leaves in a circular gap of 0.3 m diameter in the centre of the plots, and leaving below ground parts and sheaths in place to allow regrowth in a short-term. This disturbance was imposed for half of the OM addition plots (i.e., 5 randomly selected out of 10 per treatment), 10 days after OM addition. Leaf clipping was chosen to mimic grazing from birds (swans migrating in this region), or the effect of boat anchoring and shell collection by local fishermen, removing parts of the seagrass. Rhizomes around the disturbed and undisturbed 0.3 diameter plots were cut to limit recovery by colonization from the edges on the basis of their reserves outside the gap, in order to measure regrowth independently from the surrounding meadow. A total of 80 plots were thus created: two seagrass species and 8 treatments (OM addition = 4 levels; disturbance = 2 levels) replicated 5 times, giving a fully-balanced experiment that ran for 30 days after gap creation from the 22<sup>nd</sup> of July until the 20<sup>th</sup> of August 2013.

### Sampling and analysis

**Seagrass samples:** At gap creation (i.e. 10 days after OM addition) seagrasses were randomly sampled in the experimental area, i.e. outside the plots, in a surface of 0.07 m<sup>2</sup> for control biomass and morphological measurements and replicated five times for both *Zostera marina* and *Zostera japonica* meadows. At the end of the experiment, all plots were sampled in 0.07 m<sup>2</sup> cores so that all plants in the gaps were collected. After sampling, seagrasses were directly cleaned a first time in seawater in the field before being transported to the laboratory for measurements. Then, all plants were carefully rinsed and cleaned in fresh water to remove epiphytes and any sediment left. The total number of shoots per sample was directly noted. Then subsamples of 5 shoots per sample were randomly selected for morphological measurements (number of leaves per shoot, leaf length and width). The leaf surface per shoot was calculated from the averaged values of the morphological measurements. The Leaf Area index of the standing seagrass meadow ( $LAI_{standing}$ ; dimensionless, being calculated as m<sup>2</sup> of leaf surface per m<sup>2</sup> of soil surface area) was calculated as the product of leaf surface per shoot (m<sup>2</sup>) times the shoot density (m<sup>-2</sup>) from undisturbed plots. Relative recovery from disturbance (RC%; %) was calculated by dividing  $LAI_{regrowth}$  ( $LAI$  measured in the disturbed plots) by  $LAI_{standing}$  for each OM addition level. In order to assess the relative resistance to stress, a relative  $LAI_{standing}$  (RLAI%; %) was

also computed by dividing the  $LAI_{standing}$  values of the treated plots (1xOM, 4xOM and 10xOM) by the  $LAI_{standing}$  in the no OM plots (only in undisturbed plots).

**Porewater samples:** Porewater was sampled to quantify sulphide concentration as a result of OM addition and disturbance, in relation to plant species. Sampling was done both during gap creation to observe the first short-term effect of OM addition (i.e. 10 days after OM addition) and at the end of the experiment to observe changes after 30 days (i.e. 40 days after OM addition). Pore water samples were obtained using 20 ml syringes connected to rhizon MOM 5 cm female luer (19.21.22F) (Rhizosphere research product, Wageningen, The Netherlands). The rhizon was placed into the surface sediment and the syringe left at the top of the sediment while the rhizon progressively extracted porewater from the first 5 cm of sediment. Sulphide measurements were done directly after sampling in the field for all samples. Collected porewater samples were directly transferred into a plastic bottle after being filtered with pinhole filters of 25 mm diameter and 0.45  $\mu$ m pore size connected to the syringe. An Ion selective electrode Ag-S (AGS15XX Electrode, Consort, Turnhout, Belgium) was used connected to the voltmeter for sulphide measurements in samples fixed with a buffer solution in a 1:1 proportion (4 ml of samples + 4 ml of buffer solution). The electrode was calibrated prior to field measurements; mV measurements were converted into mM for sulphides.

**Statistical analysis:** Differences between species in terms of  $LAI_{standing}$  in untreated (undisturbed-no OM added) plots at the start and the end of the experiment were checked using a 1-way ANOVA. Treatment effects, i.e. 'OM addition' and 'disturbance' were additionally tested on porewater sulphide levels for both seagrass species together by using a 3-way ANOVA; and individually by using a 2-way ANOVA. The influence of two factors being 'seagrass species' and 'OM addition' and their interactive effect on  $LAI_{standing}$ , relative recovery from disturbances (RC%) and relative resistance to stress (RLAI%) were checked with a 2-way ANOVA. The effect of 'OM addition' on  $LAI_{standing}$ , relative recovery from disturbances (RC%) and relative resistance to stress (RLAI%) was also checked for each seagrass species individually with a 1-way ANOVA. Statistical differences between means were estimated per species using the Tukey post-hoc tests for 'OM addition' only. Normality and homogeneity of the data were previously checked, along with interactions between factors. All tests were achieved

on SPSS (IBM® SPSS® Statistics Version 21). Data are presented as means ( $\pm$ SE).

## Results

### Ecosystem engineering capacity: ability to suppress sulphide build-up

No significant increase of sulphide concentration in the porewater of untreated plots was observed over the experimental period (Figure 4.2). However, the addition of organic matter (carbon only) led in most treatments to higher sulphide concentrations within the vegetated sediment as measured from porewater sulphide concentration at the end (i.e., day 40) of the experiment (Table 4.1; Figure 4.2).

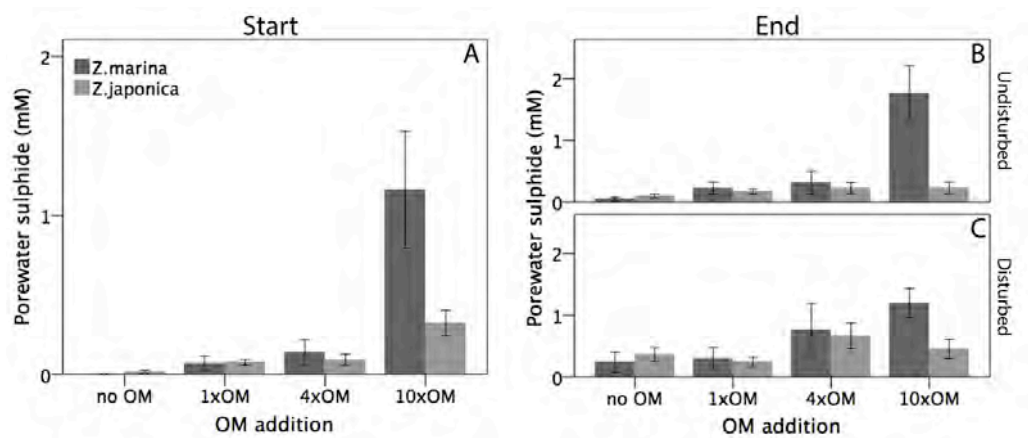


Figure 4.2: Porewater sulphide concentration in plots along the OM addition gradient: A. at the start of the experimental period (i.e. at gap creation, 10 days after OM addition); and at the end of the experimental period: B. in control plots (undisturbed); and C. in disturbed plots for both species. Error bars represent the standard error of the mean.

This increase in sulphide content was the highest and fastest for the high OM level (10xOM), especially in plots of the slow-growing *Zostera marina* (Tukey:  $p < 0.001$  in all instances, Figure 4.2). In general, the fast-growing *Zostera japonica* showed lower sulphide concentration in the sediment/rhizosphere, indicating a better detoxification (= ecosystem engineering) than the slow growing species *Zostera marina*, even at the

high OM level (Table 4.1; Figure 4.2). For the fast-growing species, disturbance strongly decreased the detoxification capacity; at the two higher OM levels, porewater sulphide was 0.5-0.7 mM, which is more than double the value of 0.2-0.3 mM in the undisturbed plots (Table 4.1; Figure 4.2). In contrast, the disturbance had no significant effect on the porewater sulphide levels of the slow-growing species, where sulphide levels were constantly high (Table 4.1).

Table 4.1: Statistical values for the effect of treatments (disturbance and OM addition gradient) on porewater sulphide levels with species as a factor (3-way ANOVA) or for each species individually (2-way ANOVA)

	2-way ANOVA					
	3-way ANOVA		<i>Zostera marina</i>		<i>Zostera japonica</i>	
	F	p	F	p	F	p
Species	8.982	0.004	-	-	-	-
Disturbance	2.059	0.156	0.04	0.843	9.551	0.004
OM addition	10.911	<0.001	10.896	<0.001	1.831	0.161
Species*Disturbance	1.147	0.288	-	-	-	-
Species*OM addition	7.901	<0.001	-	-	-	-
Disturbance*OM addition	1.658	0.185	1.38	0.266	0.838	0.483
Species*Disturbance*OM addition	0.925	0.434	-	-	-	-

Table 4.2: Statistical values for the influence of the OM addition on  $LAI_{standing}$ ,  $LAI_{regrowth}$ , Relative resistance (RLAI%) and Relative Recovery (RC%) with species included (2-way ANOVA) or for each species individually (1-way ANOVA)

	2-way ANOVA		1-way ANOVA			
			<i>Zostera marina</i>		<i>Zostera japonica</i>	
	F	p	F	p	F	p
<b><math>LAI_{standing}</math></b>						
Species	11.45	0.002	-	-	-	-
OM addition	2.59	0.07	3.37	0.045	3.16	0.054
Species*OM addition	3.93	0.017	-	-	-	-
<b><math>LAI_{regrowth}</math></b>						
Species	17.77	<0.001	-	-	-	-
OM addition	2.96	0.048	3.45	0.046	3.17	0.053
Species*OM addition	2.57	0.073	-	-	-	-
<b>RLAI%</b>						
Species	15.89	<0.001	-	-	-	-
OM addition	2.58	0.07	3.37	0.045	3.15	0.054
Species*OM addition	3.92	0.017	-	-	-	-
<b>RC%</b>						
Species	17.46	<0.001	-	-	-	-
OM addition	2.79	0.058	0.32	0.81	3.11	0.056
Species*OM addition	2.57	0.073	-	-	-	-

#### Standing stock: proxy for resistance to stress and recovery

Leaf area index of the standing stock ( $LAI_{standing}$ ) was the same for both species at the start (ANOVA:  $F=1.632$ ;  $p=0.237$ ) as well as at the end of the experimental period when untreated (ANOVA:  $F=0.07$ ;  $p=0.798$ ; Figure 4.3a). OM addition resulted in a reduction of  $LAI_{standing}$  and relative

resistance to stress (RLAI%) for the slow growing species, *Zostera marina*, even when OM additions were low (Table 4.2; Figure 4.3a). For *Zostera japonica*, LAI<sub>standing</sub> and the relative resistance to stress (RLAI%) both significantly only decreased at the highest OM level (10xOM) (Tukey:  $p=0.03$  for both).

Relative recovery from disturbance (RC% for leaves clipped) was significantly higher for the fast-growing than for the slow-growing species (Table 4.2; Figure 4.3b). The fast-growing species presented a lower recovery when OM was added for all three levels of addition as compared to the control treatment (Table 4.2; Figure 4.3b).

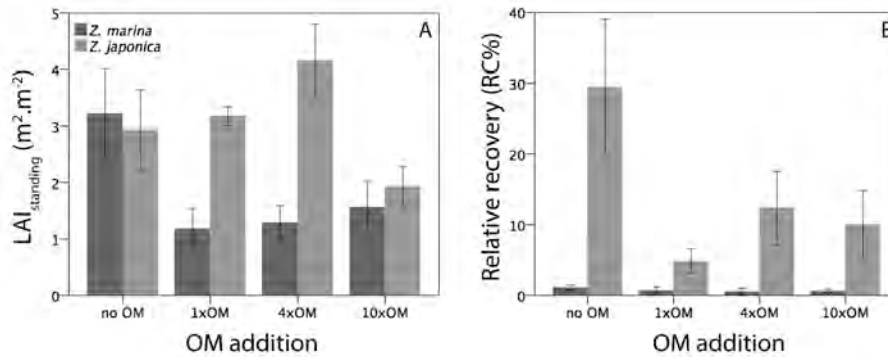


Figure 4.3: LAI<sub>standing</sub> values (A) and relative recovery from disturbances (RC%; B) at the end of the experimental period along the anoxia gradient for both species. Error bars represent the standard error of the mean.

#### The importance of growth rate vs. ecosystem engineering: testing our hypothesis

To evaluate the relative importance of i) growth strategies and ii) ecosystem engineering capacity in determining the resilience of two co-occurring contrasting seagrass species, we compared the expected (schematised line in Figure 4.4) and observed (data points in Figure 4.4) plant response for relative recovery from disturbances (i.e., RC% representing regrowth into gap; Y-axis Figure 4.4) and the relative resistance to stress (i.e., RLAI% expressed as the LAI<sub>standing</sub> of OM-stressed plots relative to control plots; X-axis Figure 4.4).

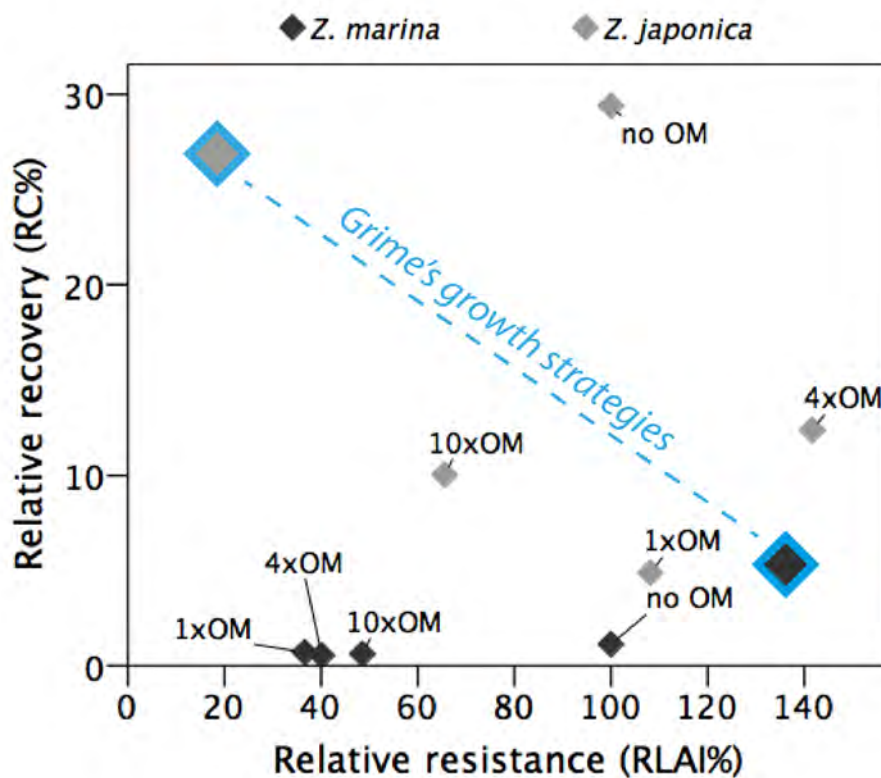


Figure 4.4: Ecosystem engineering alters the expected response to stress and disturbance as based on Grime's theory (blue line). Data represent the correlation between relative resistance to stress (RLAI, calculated as a function of controls, so no OM is set at 100%) and relative recovery from disturbances for both species. Labels represent the different levels of OM addition used to create the anoxia gradient. Grime's theory (in blue) suggests that fast-growers would have a high recovery but a low resistance to stress, while slow-growers would have a low recovery and a high resistance. Our results show that with a strong ecosystem engineering strategy, the fast-grower (light grey markers) can increase its resistance to stress while maintaining a high recovery from disturbances. On the other hand, because of a weak ecosystem engineering strategy, the slow-grower (dark markers) has a low recovery from disturbances and a low resistance under stress.

From our experiment, we observed that the fast-growing species showed high resistance to low and medium OM addition (i.e., same position along X-axis Figure 4.4), but not to high OM levels (i.e., shifted position along X-axis Figure 4.4). As expected, the fast-growing species showed a relatively high recovery from above-ground removal, as long as OM-levels were low (Y-axis Figure 4.4).

When OM addition was high, the recovery of the fast growing plants decreased, although it remained higher than that of the slow growing species at all levels of OM addition, including the control. In contrast to our null-hypothesis based on Grime's theory, the slow-growing species did not show a good resistance to stress. That is, both the resistance and recovery of the slow-growing species was very low due to OM addition, when compared to the fast-grower. The high recovery and high resistance to stress of the fast-growing species confirms our alternative hypothesis of a strong potential ecosystem engineering capacity, altering the expected response to stress and disturbance based on Grime's theory (Figure 4.4).

### Discussion

#### **Growth rates vs. ecosystem engineering as strategies for resilience**

In our study, the fast-growing species presented both a higher recovery from disturbances and a higher resistance to stress than the slow growing species. The latter was in contrast to our null-hypothesis sensu Grime (1977), that the slow growing species would be more stress-resistant. This indicates that ecosystem engineering may in some cases override the expected outcomes based on the well-recognized growth strategies identified following Grime (Figure 4.4). We hypothesize that ecosystem engineering and growth strategies may be directly related via the costs related to the ecosystem engineering; low cost ecosystem engineering may short-cut the relationship between costs and growth strategy (Figure 4.5).

The construction of relatively impermeable roots by the slow growing species (*Zostera marina*; (Pedersen and Kristensen 2015)) seems to fit a slow-growth strategy of investing in expensive tissue (Grime 1977; Klap et al. 2000; Lamers et al. 2013). As the plant also presents a slow growth rate, this 'impermeability strategy', allows it to better preserve its resources by not letting any toxin in (e.g. sulphide) nor any assets out (e.g. oxygen) (Hasler-Sheetal and Holmer 2015; Pedersen and Kristensen 2015). *Sensu*

Grime (1977), this strategy may hence be considered as competitive in stressful environments by making the plant more resistant. These adaptations however also inherently restricts the species' ecosystem engineering capacity by Radial Oxygen Loss (Jovanovic et al. 2015). For this species (*Zostera marina*), oxygen leakage can only be measured at its root tips (about <8% of the root surface) as opposed to other species that can release oxygen through 33% of their root surface, thereby providing a much better detoxification potential (Frederiksen et al. 2006; Jovanovic et al. 2015; Pedersen and Kristensen 2015). Therefore, if the stresses increase, a slow-growing – resource-efficient – species may present a lowered resistance to stress, due to a limited ecosystem engineering detoxification potential. Ultimately, this might create a negative feedback between the plant and its environment (i.e. lowered ecosystem engineering leading to lowered resistance to stress and/or lowered recovery from disturbance and vice-versa), further reducing its resilience. As seen in our study, the resistance of the slow-growing species, as well as its recovery, was very low, showing neither an effective stress tolerance (i.e., tolerance for sulphide-rich sediments by impermeable roots, cf. Frederiksen et al. 2006; Pedersen and Kristensen 2015) nor a stress avoiding strategy (i.e., detoxify the sediment by oxygen release, cf. Greve et al. 2003).

In contrast, the fast-growing species presented a higher overall resilience in having both a higher resistance to stress and a higher recovery from disturbance. This confirms our alternative hypothesis implying that ecosystem engineering may enhance a species' resilience to higher stress levels than what could be expected based on the growth-rate only. That is, ecosystem engineering potentially alters the expected responses of a species to outgrow stresses and disturbances as suggested by Grime (Figure 4.4). This result shows that low cost ecosystem engineering may short-cut the relationship between costs and growth strategy (Figure 4.5).

The capacity of the fast-growing species in our study to be resilience and to recover from an additional disturbance under stress seems to be due to the combination of being capable of fast growth and the construction of cheap, and thereby also leaky roots. This shows how growth strategy (Grime 1977) an ecosystem engineering can together explain fast recovery and resilience (Figure 4.5). This strategy might be broader present, as for example shown for *Ruppia maritima*, which also is a fast grower and efficient in releasing oxygen to detoxify a larger area around the roots and thus, ameliorate the stress (Jovanovic et al. 2015; Pedersen and Kristensen 2015). This strategy has its advantages in stressful environments, where a

strong ecosystem engineer can successfully resist and maintain its habitat (Crain and Bertness 2006; van Wesenbeeck et al. 2007) at low cost in terms of investment in tissues (Figure 4.5).

### **Do we know which types of ecosystem engineering are 'cheap' versus 'costly'?**

The concept of ecosystem engineering describes how organisms can modify their environment by their presence (autogenic ecosystem engineering) versus their action/activity (allogenic) (Jones et al. 1994). To our knowledge, the link between ecosystem engineering and its costs for the organism has not been clearly described. Plants can present various ecosystem-engineering capacities related to their structural traits (autogenic ecosystem engineering) or physiological activity (allogenic). We could speculate that ecosystem engineering capacities that act through physical – or structural - processes may be especially related to slow-growers. As slow growing plants tend to invest more in their tissues (Grime 1977) they may become physically stronger (Bouma et al. 2010), which can be a requirement for autogenic ecosystem engineering processes such as e.g. wave attenuation (Bouma et al. 2005). Such ecosystem engineering is then indirectly costly, as it requires relative high investments in strong tissues. The same may apply for tree or bush species that by ecosystem engineering (i.e. enhance water infiltration into the soil) can enhance their resistance to drought (Jones et al. 1997; Hille Ris Lambers et al. 2001).

In our study, the ecosystem engineering investigated was related to oxygenation of the rhizosphere, which is primarily related to physiological activity. In general terms, it may be speculated that ecosystem engineering depending on physiological activity may be especially related to fast-growing species, with cheaper tissues (low investment/cost) but high activity. The combination of low cost investment in growth while having ecosystem engineering that alleviate stress, hence creates a short cut for resilience by being both fast in recovery (fast growth rate) and more resistant (ecosystem engineering) (Figure 4.5). We do not think that current knowledge is advanced enough to link the strength of ecosystem engineering to a plants growth rate. However, the conceptual framework presented here might provide a stepping-stone to advance our thinking into this direction.

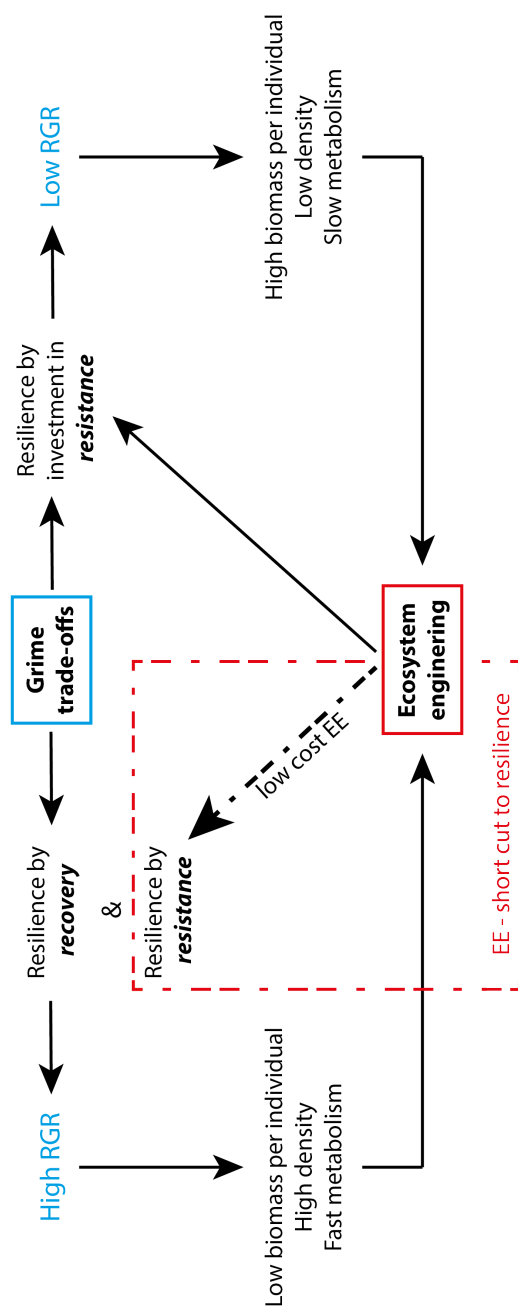


Figure 4.5: conceptual model illustrating the relationship between Grime's growth strategies and ecosystem engineering and how both can influence a species' resilience. Full arrows represent the current state of knowledge on Grime's plant trade-offs. A low relative growth rate (RGR) is associated with more investment in tissues and traits increasing the plant's resistance; a high RGR is associated with cheap tissues but fast metabolism increasing the plant's resilience by recovery. The dotted arrow and dotted red box represent the study's findings and short cut to resilience explaining how ecosystem engineering (EE) can alter the expected response to stress by making a fast-growing plant more resistant to stress at low cost and without affecting its relative growth rate (RGR).

### Implications for the resilience of ecosystems

Ecosystem engineers create positive feedback which often leads to bi-stability, implying hysteresis between two alternative stable states (van der Heide et al. 2007, 2010; Scheffer et al. 2009; Eklöf et al. 2011; Dakos et al. 2011). The presence of a stress gradient can affect the resilience of the system and slow down its capacity to recover from an additional disturbance (van Nes & Scheffer 2007; Scheffer et al. 2009; Dakos et al. 2011). Indeed, the low resilience of the slow-growing species is more likely to lead to collapse (Chisholm & Filotas 2009), as the species cannot cope with sulphide increase any longer (Scheffer et al. 2001; Eklöf et al. 2011).

The fast-growing species that we studied, presented no trade-offs between its resilience and ecosystem engineering capacity; rather its resistance and its recovery potential were both higher with a strong positive plant-environment feedback, as compared to the slow growing species even at high stress levels. We explain this by having low costs involved in ecosystem engineering (Figure 4.5). The interaction between stress levels and recovery from a disturbance can however become determinant for the fast-grower: when disturbances were combined with stress, the resilience of the fast-growing species (*Zostera Japonica*) tended to diminish with increasing stress levels (= OM addition strengths). The most likely reason for this effect is that ROL requires active above-ground tissues (Greve et al. 2003). Without having shoots, the plants thus can no longer engineer their environment by releasing oxygen in the sediment, to escape the toxic sulphide effect (Terrados et al. 1999; Koch et al. 2007; Perez et al. 2007; Pulido and Borum 2010; van der Heide et al. 2012).

### Conclusion

Ecosystem engineers provide many ecosystem services due to their activity (allogenic engineers) or presence (autogenic engineers) (Bertness and Callaway 1994; Jones et al. 1994, 1997; Bruno et al. 2003). It was already known that their efficiency and the services they provide were also related to physiology and growth-strategies (Bouma et al. 2005, 2009, 2010). This study experimentally demonstrates that growth rates and ecosystem engineering capacity form two essential strategies at the organism level, both having a strong influence on the system resilience. In addition, our results indicate that ecosystem engineering can alter the resilience of slow vs. fast-growing plants as suggested by Grime (Grime 1974, 1977), by changing the plant-environment feedback loop. We

hypothesize that it is only low-costs ecosystem engineering that can alter the outcome of the growth strategy-stress interaction proposed by Grime. Hence, both growth strategies and ecosystem engineering need to be accounted for to evaluate a species' resilience to stress as well as its capacity to maintain its habitat.



## CHAPTER 5

### The influence of short-term stress events on seagrass seasonal carbon storage: implications for their long-term resilience in a changing environment

*Submitted*

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#### Abstract

Seagrass meadows form highly valuable ecosystems and habitats in the marine environments. They are, however, threatened and declining worldwide at an alarming rate, raising the need to better understand their resilience in a globally changing environment. Throughout the year and related to their climatic settings, seagrass meadows are exposed to different levels of stresses: (i) expected seasonal changes, such as low temperatures and low light levels during winter; (ii) and the rather unpredictable stress events from nutrient enrichment or small-scale disturbances. Carbohydrate reserves in seagrass rhizomes are stored over their growing season and have been defined as a good indicator for chances for winter survival. Using *Zostera noltei* meadows as a model, we firstly assessed how carbohydrate reserves relate to seasonal changes, using a climatic latitudinal gradient in Western Europe to vary winter intensity. Subsequently we tested through a manipulative field experiment at a single latitude, whether small-scale disturbance under nutrient enrichment may affect carbohydrate reserves, and thereby affect the seagrass capacity to withstand long-term seasonal changes.

We observed a positive relationship between rhizomal carbohydrate reserves at the beginning of the growing season and past winter temperatures hence lower rhizomal carbohydrate reserves when winters were the coldest. At the end of the growing season, we also

observed a linear increase of carbohydrate reserves along the latitudinal gradient from south to north. Our small-scale field experiment revealed that carbohydrate reserves may be strongly reduced by the combination of both nutrient enrichment and above-ground removal. This implies that the long-term winter survival in northern latitudes is highly sensitive to unpredictable stress events from eutrophication or small-scale disturbances. Our results have strong implication in a context of climate change and increasing threats to seagrass ecosystems, particularly in the most sensitive – not evergreen – northern populations.

## Introduction

Seagrasses are flowering plants, adapted to the marine environment (Les et al. 1997), forming extensive and highly productive meadows worldwide (Short et al. 2007). Throughout the year, temperate seagrass meadows face various levels of stresses related to their environmental or climatic settings: expected seasonal changes related to variations in light and temperature controlling their presence and seasonal growth (Dennison 1987, Duarte 1991, Olesen and Sand-jensen 1993, Ochieng et al. 2010); or more unpredictable stress events such as human-induced stresses and disturbances (i.e. eutrophication, plant removal, overgrazing), jointly affecting their resilience and survival (Burkholder et al. 2007, Macreadie et al. 2014).

Seagrass meadows are able to react and to recover from stresses and disturbances (Charpentier et al. 2005), depending on their clonal growth strategy (i.e. potential rhizome elongation rate; Macreadie et al. 2014), their seasonal growth (Soissons et al. 2016), their seed production (van Tussenbroek et al. 2016) and their ecosystem engineering capacity (Bos et al. 2007, van Katwijk et al. 2010). They can acclimate to changing conditions and adapt their morphological, physiological and biomechanical traits (Peralta et al. 2005, 2006, Cabaço et al. 2009, de los Santos et al. 2010, 2013, La Nafie et al. 2013), making them more resilient under threats. However, in the current context of climate change and increasing anthropogenic pressure on coastal ecosystems, there is a possibility that the combination – being either synergistic, additive or antagonistic – of stressors might gradually lower the resilience of seagrass ecosystems, favouring the conditions for a potential collapse and loss of their ecosystem services (Scheffer et al. 2001, 2009, Carr et al. 2012). The effect of unpredictable stressors on resilience could be contributing to the worldwide decline of seagrasses (Orth et al. 2006, Waycott et al. 2009). In this study we question if unpredictable these stress events might affect the seagrass capacity to withstand (relative predictable) seasonal changes in this era of global change.

To overcome both stressful low photosynthetic (low temperature and low light) periods like the winter months (Govers et al. 2015) and short-term disturbances (Burke et al. 1996), seagrasses exploit the carbon reserves gained during their growing season in the form of non-structural carbohydrates (i.e. starch and/or sucrose) (Madsen 1991, Alcoverro et al. 1999, Olivé et al. 2007, Lee et al. 2007). The carbohydrate reserves are usually stored when photosynthesis exceeds the carbon demand for growth

and respiration (Madsen 1991). The amount of carbohydrates needed for seagrass survival over stressful periods depends on abiotic factors such as i.e. temperature and light availability; but also on internal factors affecting the carbon balance, such as respiration and growth (Madsen 1991, Alcoverro et al. 2001, Govers et al. 2015). Chances of survival during these periods thus depend on the plants' capacity to build up their carbohydrate reserves during summer (Govers et al. 2015) and to maintain a positive carbon balance.

The carbon balance of seagrasses has been the focus of various studies, suggesting that light irradiance, daylight hours (photoperiod), temperatures, weather and hydrodynamics were factors influencing the ability of seagrasses to store carbohydrates (Marsh et al. 1986, Burke et al. 1996, Alcoverro et al. 2001, Olivé et al. 2007, Govers et al. 2015). To our knowledge, these previous studies only focused on the partitioning of carbon or on the gain vs. depletion of the non-structural carbohydrate reserves throughout the growing season, but did not include a climatic dimension on the storage of non-structural carbohydrates. The range of climatic conditions that a seagrass species encounters along its latitudinal distribution gradient may alter its capacity to store carbohydrate reserves through a positive carbon balance, or lead to different responses to short-term stresses and disturbances and thus affect their resilience (positively or negatively). The human-induced threats undergone by seagrass meadows in a short-term throughout the year are, for instance, rather unpredictable in intensity and length. We hence wondered if such unpredictable short-term stress events, might affect the seagrass capacity to withstand seasonal changes and how this would affect their long-term resilience and survival in a globally changing environment (Unsworth et al. 2014).

In the present study, we aimed at (i) evaluating the influence of climatic settings (i.e. the intensity of the winter period) along a latitudinal gradient, on the non-structural carbohydrate reserves of a model seagrass species: *Zostera noltei* along their growing season. Since carbohydrate reserves in autumn have already been identified as a good indicator for winter survival (Govers et al. 2015), we also aimed at (ii) examining, through a manipulative experiment on two *Z. noltei* meadows located at a single latitude, the effect of nutrient enrichment and short-term disturbance (i.e. above-ground removal) on their capacity to store carbohydrates along their growing season. From this, we discussed the implications for seagrass long-term survival and resilience depending on their climatic setting.

## Material and methods

### Study sites

In order to cover a large climatic gradient (variation in intensity of the winter period, i.e. winter temperatures), seagrasses were sampled at 12 sites along the European coastline, at 3 different times over the growing season: at the beginning, the peak and the end of the growing season (Table 5.1). Sites were selected following a latitudinal gradient from south (warmer) to north (colder), being: E1. Cadiz (Spain); E2. & E3. Mondego estuary (Portugal); E4. Santander (Spain); E5. Bidasoa estuary (France); E6. & E7. Arcachon Bay (France); E8. Noirmoutier (France); E9. St-Jacut-de-la-mer (France); E10. & E11. The Oosterschelde (Netherlands); and E12. Sylt (Germany) (Figure 5.1, Table 5.1). The samples were taken each time and at all sites on healthy and expanded *Zostera noltei* meadow found in intertidal areas (as known by local experts). Sampling dates were not identical between locations, as growth and the length of the growing season are dependent on local conditions such as temperature and light availability depending on latitude. In order to get a comparable set of data depending on the stage in the growing season (i.e. beginning, peak and end), the exact sampling dates were determined by local experts (Table 5.1).

### Experimental design

#### The influence of climate settings on seasonal patterns in carbohydrate reserves

For each sampling date at each site (Table 5.1), seagrass samples (n=5) were collected by using 10 cm diameter PVC cores inserted into the sediment. The seagrass samples were briefly washed on site and stored in wet tissues for preservation during transportation to the NIOZ in Yerseke, Netherlands. In the laboratory, the samples were carefully washed a second time with freshwater to remove all remaining sediment, algae and epiphytes. Then, for the whole sample, rhizomes were carefully separated from the roots and leaves, and subsequently freeze-dried for dry biomass measurements and further carbohydrate (in rhizomes) analysis.

Table 5.1: Site characteristics, past winter temperatures (averaged over the winter months 2013-2014), sampling dates and average ( $\pm$  SE) water temperature as measured by the HOBO-pendant loggers over one month around the sampling dates at all sites (data collected in 2014).

Site number, names and coordinates		Start-End experimental dates			Temperature (°C)				Evergreen Y/N
		Beginning	Peak	End	past winter	Beginning	Peak	End	
E1	Cadiz Bay, Spain N 36 30' W 6 10'	02-Jul	09-Sep	06-Nov	13.45	-	26.1 ± 0.08	20.6 ± 0.08	Y
E2	Upstream - Mondego N 40 8' estuary, Portugal W 8 50'	28-May	13-Aug	10-Nov	10	17.6 ± 0.1	20.8 ± 0.09	18.8 ± 0.07	Y
E3	Downstream - Mondego N 40 8' estuary, Portugal W 8 50'	28-May	13-Aug	10-Nov	10	17.3 ± 0.1	20.6 ± 0.1	18.7 ± 0.08	Y
E4	Santander, Spain N 43 25' W 003 48'	13-Jun	11-Aug	08-Oct	11.5	17.2 ± 0.06	22.6 ± 0.03	19.9 ± 0.03	Y
E5	Bidasoa estuary, France N 43 21' W 001 46'	29-May	07-Aug	11-Oct	9.7	-	23.3 ± 0.1	20.6 ± 0.1	Y
E6	Germanan - Arcachon Bay, N 44 42' France W 001 8'	27-May	13-Aug	27-Oct	8.7	17.1 ± 0.09	23.2 ± 0.07	18.9 ± 0.08	Y
E7	Hautebelle - Arcachon Bay, N 44 43' France W 001 09'	27-May	13-Aug	27-Oct	8.7	17.1 ± 0.09	23.2 ± 0.07	19.0 ± 0.08	Y
E8	Noirmoutier, France N 46 98' W 002 21'	27-Jun	25-Jul	11-Sep	9.5	20.4 ± 0.09	21.3 ± 0.09	21.1 ± 0.07	N
E9	St-Jacut-de-la-mer, France N 48 36' W 002 11'	25-Jun	29-Jul	20-Aug	7.8	17.3 ± 0.1	20.4 ± 0.1	20.1 ± 0.2	N
E10	Oostdijk - Oosterschelde, N 51 26' Netherlands E 004 05'	11-Jun	19-Aug	26-Sep	6.8	19.2 ± 0.1	20.3 ± 0.1	19.0 ± 0.1	N
E11	Dortsman - Oosterschelde, N 51 34' Netherlands E 003 59'	12-Jun	20-Aug	25-Sep	6.8	18.0 ± 0.1	20.2 ± 0.08	18.3 ± 0.07	N
E12	Sylt, Germany N 54 54' E 008 19'	-	30-Jul	12-Sep	3.3	-	22.2 ± 0.1	17.9 ± 0.09	N

Water temperature was monitored over the study period at all sites. Water temperatures were measured using two HOBO Pendant Temperature loggers (64k – UA-002-64, ONSET) at a frequency of 1 measurement every 30 minutes. Two loggers were placed within the study area for each site.

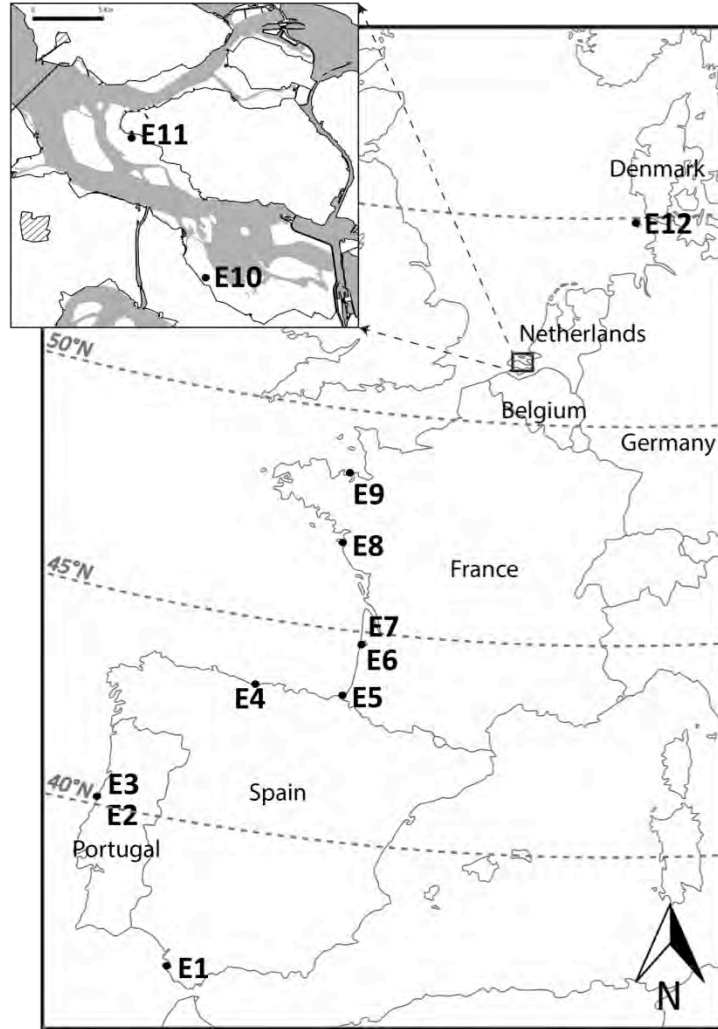


Figure 5.1: Localisation of the studied sites along the Western European coast. Zoom in the case study sites (E10 and E11).

The influence of short-term nutrient enrichment and small-scale disturbances on carbohydrate reserves

In order to evaluate the response to short-term stresses and disturbances on carbohydrate reserves, a manipulative experiment was implemented at two sites located at the same latitude in the Oosterschelde, The Netherlands (Sites E10 and E11; Figure 5.1, Table 5.1). The two sites mainly differed in their exposure to hydrodynamics (i.e. E10 is considered as sheltered and E11 as exposed; pers. obs.). This study at two sites consisted of a factorial experiment with four treatments (n=5 for each treatment): Undisturbed-no nutrient control [C]; Undisturbed with nutrient [N]; Disturbed-no nutrient [D]; Disturbed with nutrient [DN]. It was designed and identically implemented at both sites 3 times over the growing season (at the beginning: 14 May – 12 June; the peak: 22 July – 20 August and the end: 27 August – 26 September 2014).

Plot installation: At each site, plots were randomly allocated in areas located in the middle of the seagrass meadow to prevent from edge effects, and providing a distance of at least 5 m between plots to avoid contamination due to the nutrient enrichment treatments. All plots included an inner circle of 30 cm diameter for the treatments, delimited by 10 metal sticks placed around the circles at the edge. The experimental areas were different for each of the three experimental periods, which were at both sites independent from each other.

Nutrient enrichment: Nutrient enrichment consisted of placing small bags of slow release fertilisers (Osmocote, N:P:K = 15:9:12) on top of the sediment around the [N] and [DN] 30 cm diameter plots by using the metal sticks as anchors. The fertiliser bags were made of panty hoses and containing 10 g of slow release fertiliser each. A total of 10 bags per plot were placed and fixed on top of the sediment with the metal sticks. In total each plot with a nutrient treatment (n=10 per experiment) received 100 g of slow-release fertiliser, hence an enrichment of 15 g N, 9 g P and 12 g K.

Small-scale disturbances: Disturbances of above-ground biomass were created by clipping the leaves, leaving the below-ground and sheaths in place inside the [D] and [DN] (n=10) 30 cm diameter circles. This type of disturbance was chosen to mimic the effect of over-grazing or anchoring creating gaps in seagrass meadows and allowing direct regrowth measurements within the 4 weeks-long experiments. All seagrass material removed at gap creation was kept in individual bags for biomass measurements.

Rhizomes around the inner circles were cut for all plots (including controls) to limit both lateral carbon transfer and shoot recovery on the basis of their reserves outside the gap, in order to measure regrowth independently from the surrounding meadow.

Seagrass samples: At the start of each experiment (at sites E10 and E11 only), seagrass samples (n=5) were collected within the area where the experiment was implemented by using 10 cm diameter PVC cores inserted into the sediment. At the end of each experimental period, all plots were harvested by using the same 10 cm diameter PVC cores inserted into the sediment randomly within the inner 30 cm diameter circle. Following harvest or clipping at the start and the end of the experiment, the seagrass samples were washed and processed the same way as for the samples collected along the latitudinal gradient.

### **Measuring Carbohydrate reserves in rhizomes**

Seagrass rhizomes store most of the non-structural carbon reserves as carbohydrates (Burke et al. 1996, Zimmerman and Alberte 1996, Olivé et al. 2007, Govers et al. 2015). Non-structural carbohydrate reserves were evaluated in rhizomes from control samples for all pan-European sites, to investigate the potential of each site to store carbon reserves over their growing season. Carbohydrate reserves were measured in freeze-dried and grinded rhizomes of the control samples from the beginning, peak and end of the growing season of all twelve field sites. In addition, the carbohydrates in the rhizomes samples from all treatments at the Oosterschelde sites (site E10 and E11) were also analyzed to investigate their response to treatments.

For carbohydrate measurements, soluble sugars – glucose, fructose and sucrose - were extracted out of the plant material by using an 80% ethanol solution. The residue was hydrolyzed with diluted hydrochloric acid (3% HCL) to convert sugar compounds into carbohydrates. Subsequently, the fraction was boiled at 100°C for 30 minutes. Rhizomal carbohydrate reserves were measured in mg carbohydrate per gram dry plant material by anthrone assay standardized to d-glucose (Yemm and Willis 1954). All samples were measured in duplicate and a new calibration curve was prepared for every series of measurements.

In order to evaluate the carbohydrate response to treatments for the two Oosterschelde sites (E10 and E11), a response variable,  $\Delta$ Carbohydrate, was calculated by computing the carbohydrate reserves measured in treated plots at the end of the experiment, as a function of

control data for: response to nutrient enrichment (=N/C), response to disturbance (=D/C) and response to the combination of nutrient enrichment and disturbance (=DN/C).

### Statistical analysis

The variation in carbohydrate reserves and above-ground biomass during the growing season was assessed with a 1-way-ANOVA testing the influence of the factor 'experimental period' (beginning, peak and end) on data pooled by sites. The effect of climate settings on carbohydrate reserves was tested by looking at the linear relationship between past winter temperatures (averaged from December 2013 until February 2014 for each site; data available from local weather stations) or latitude with carbohydrate reserves at the beginning and the end of the growing season. The linear regressions were separately performed for the two experimental periods. The relationship between water temperatures as measured from the loggers on site and latitude over the experimental period was tested with a general linear model. The effect of short-term stress events (nutrient enrichment and above-ground removal) on carbohydrate reserves was tested with a mixed effects model using 'sites' (E10 and E11), 'treatment' (C, CN, D, DN) and 'experimental period' (beginning, peak and end) as fixed factors and replicates (n=5) as a random factor. Differences between treated plots (CN, D and DN) and control (C) were obtained with following post-hoc Tukey tests. Response variables (i.e. calculated by computing the carbohydrate reserves measured in treated plots at the end of the experiment, as a function of control data) were used as standardized values to test for differences in carbohydrate reserves between the two sites (E10 and E11) for each experimental period by using independent samples T-tests. Normal distribution of all the data was tested with the Shapiro Wilk test on the data. All statistical analyses were realized with R version 3.1.3 (2015-03-09).

## Results

Water temperatures as measured from the loggers reached their maximum at the peak of the growing season ( $F=24.28$ ;  $p<0.001$ ) at most sites with a range from  $20.2 \pm 0.08^{\circ}\text{C}$  at site E11 up to  $26.1 \pm 0.08^{\circ}\text{C}$  at site E1 (Table 5.1). No significant differences in water temperatures were found between sites over the experimental period ( $F=2.17$ ;  $p=0.07$ ).

### Seasonal variations in carbohydrate reserves: effects of winter intensity and latitude

Carbohydrate reserves in rhizomes significantly increased over the growing season (Figure 5.2a) along with the seasonal growth of seagrass leaves and above-ground biomass (Figure 5.2b). At the end of season, the amount of non-structural carbohydrates in rhizomes reached values of up to 400 mg.g DW<sup>-1</sup> (Figure 5.2a).

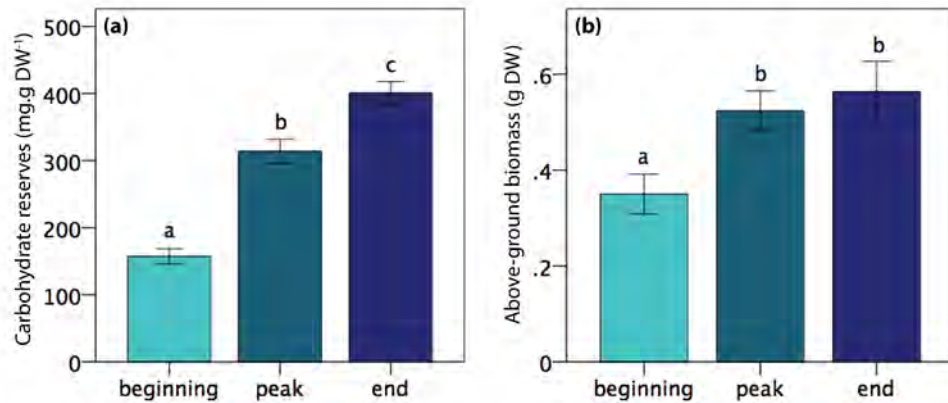


Figure 5.2: Increase of carbohydrate reserves in rhizome (a) and of above-ground biomass (b) over the season (three experimental periods) for all European sites (pooled). Small letters a and b represent the statistical groups from post-hoc Tukey tests.

At the beginning of the season, carbohydrate reserves were positively related to the average past winter temperature ( $F = 13.46$ ,  $R^2 = 0.1219$ ,  $df = 97$ ,  $p < 0.001$ ) (Figure 5.3a). This result suggested that the cold winter temperatures of 2013-2014 were correlated with the depletion of carbohydrate reserves, as seen from the lower carbohydrate reserves measured at sites experiencing colder winters. At the end of the growing season, carbohydrate reserves were positively related to latitude ( $F = 15.02$ ,  $R^2 = 0.1403$ ,  $df = 92$ ,  $p < 0.001$ ) (Figure 5.3b). This indicated that the *Z. noltei* beds at the northern sites had greater carbohydrate reserves before the winter months (colder) than the southern ones. Thus, factors (temperature or light availability) during the photosynthetic-active growing season cause a greater increase of the carbohydrate reserves in the north of Europe compared to the south.

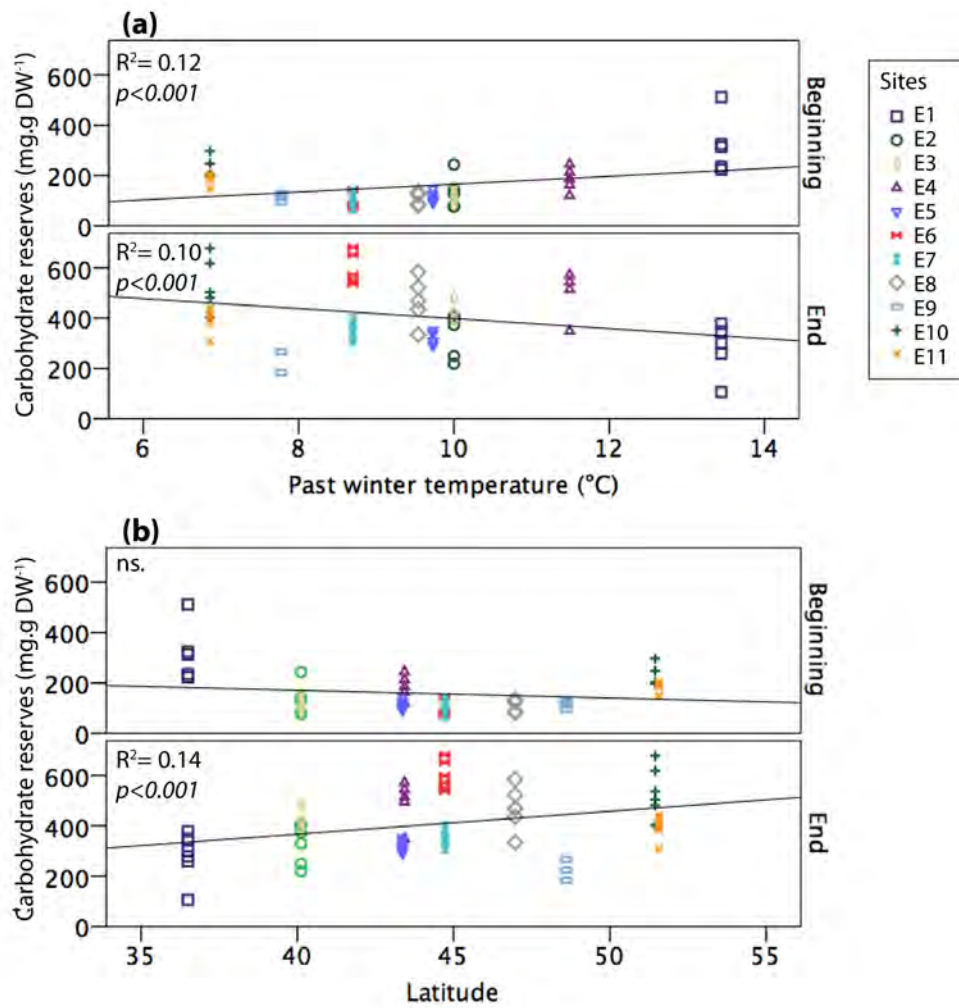


Figure 5.3: The influence of past winter temperatures (a) and of latitude (b) on carbohydrate reserves at the beginning and the end of the growing season for all sites.

### The effect of nutrient enrichment and small-scale disturbance on carbohydrate reserves

To investigate the effect of short-term stress events such as nutrient enrichment, small-scale disturbance and their combination on carbohydrate

reserves, two sites located at the same latitude (sites E10 and E11) were submitted to manipulative experiments.

Table 5.2: The effect of short-term stress events (treatments) on carbohydrate reserves at two sites (Sites E10 and E11) at different experimental periods and their interactions. Stars (\*) symbolize a significant effect of the factor, when  $p < 0.05$ .

	F	<i>p-value</i>
experimental period	17.47	<0.001*
treatment	7.685	0.018*
site	1.669	0.15
experimental period * treatment	1.179	0.42
experimental period * site	3.589	0.05*
treatment * site	4.492	0.05*
experimental period * treatment * site	1.899	0.08

Statistical differences were found between sites depending on the treatment and the experimental period (interaction effects, Table 5.2). In controls, carbohydrate reserves were significantly higher at site E10 as compared to site E11, particularly at the end of the growing season (Figure 5.4).

In terms of response to treatments, nutrient enrichment at site E11 had no significant effect on the carbohydrate reserves in rhizomes, while a significant negative effect on the carbohydrate reserves was observed at site E10, both at the beginning and at the peak of growth (Figure 5.5a). Adding a small-scale disturbance to the plots did again not have a significant effect on carbohydrate reserves at site E11 (Figure 5.5b), whereas it had a negative effect at site E10; be it only at the peak of the growing season (Figure 5.5b). The combination of disturbance and nutrient enrichment always tended to reduce carbohydrate reserves, as seen by a reduced response at both sites (Figure 5.5c), which was significant only at site E10 both at the peak of growth and at the end of the growing season.

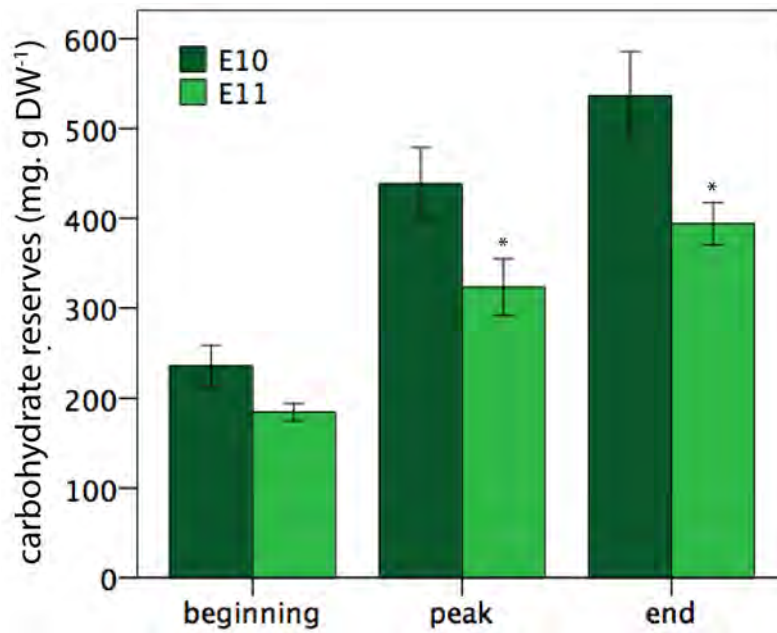


Figure 5.4: Carbohydrate reserves in controls at both sites for the three experimental periods along the growing season. Stars (\*) represent significant differences between sites for each experimental period.

## Discussion

Seagrass meadows experience various levels of stresses throughout the year. Some are relatively predictable, i.e. expected seasonal changes related to their climate settings; but others are rather unpredictable such as eutrophication (through nutrient enrichment or short-scale disturbances (Orth et al. 2006). With our study, we showed how carbohydrate reserves in seagrass rhizomes vary along a latitudinal gradient and that this variation is related to past winter temperatures. We also demonstrated the influence of short-term unpredictable stress events on the resilience of seagrass meadows, by disturbing the seasonal storage of carbon reserves, needed for growth and survival.

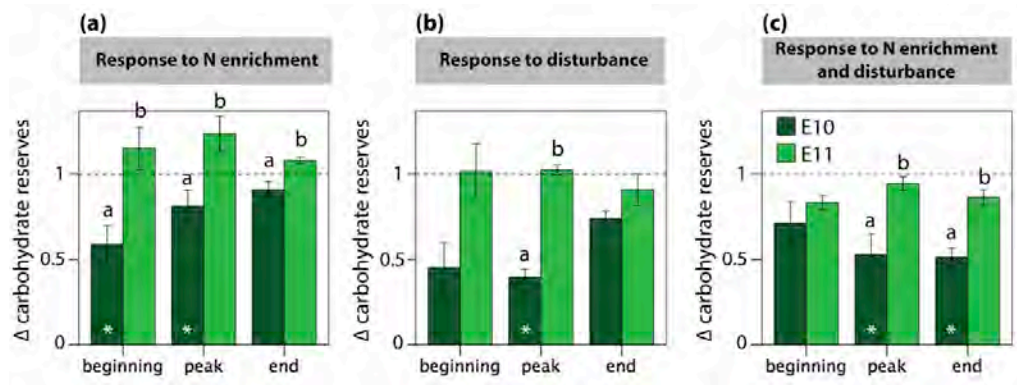


Figure 5.5: Carbohydrate reserves response ( $\Delta$ ) to treatments along the growing season for both studied sites (E10 and E11). (a) represents the response to nutrient enrichment (=N/C); (b) the response to disturbance (=D/C); and (c) the response to both nutrient enrichment and disturbance (=DN/C). Small letters (a, b) illustrate statistical differences between sites at each timing. Stars (\*) represent statistical differences between the response variable and the control value. Control values are represented by the dotted lines at  $\Delta = 1$ .

### The influence of seasonal changes

In our study, climate settings such as winter intensity or latitude were both playing a significant role in regulating rhizomal carbohydrate reserves. Our results showed that carbohydrates reserves were highest in northern seagrass populations at the end of the growing season. Though these northern seagrass population also showed a higher depletion of carbohydrate reserves over winter (i.e., highest depletion = lowest reserves in northern seagrass populations at the beginning of the growing season).

In southern Europe, winter temperatures are milder and daylight hours longer with higher daily doses (less clouds), allowing a higher photosynthetic production than in northern Europe (Touchette and Burkholder 2000a, Olivé et al. 2007). For dwarf eelgrass meadows in northern temperate areas in winter, only the below ground biomass – with limited leaves cover – and seed banks remain until spring when new shoots grow again, while southern seagrass population can form evergreen meadows (Pérez-Lloréns and Niell 1993, Auby and Labourg 1996, Vermaat and Verhagen 1996, Plus et al. 2003). Thus, cold winter months lead to a

greater depletion of the non-structural carbohydrate reserves than milder winters, since they form a higher stress level for seagrass meadows, thereby explaining the patterns observed in northern latitudes. The opposite however happens in summer, during the seagrass growing season, when temperatures are milder and daylight hours longer in northern Europe as compared to southern Europe. Both milder temperatures and higher daylight doses might hence stimulate the photosynthetic production of northern seagrass populations with a positive growth-respiration balance (Madsen 1991, Alcoverro et al. 1999, Brun et al. 2003a). Consequently, especially in northern populations, the carbohydrate reserves in rhizomes increase extensively during the summer months, also due to lower stress linked to warm temperatures (i.e. heat waves in the south) or excessive daily light doses (Schubert et al. 2015). This increase in carbohydrate reserves might then help the seagrass beds to better endure the stressful winter period. On the opposite, due to higher light availability (daily doses) and milder winter temperatures favouring growth, southern populations can survive winter with a positive primary production (Madsen 1991). This constant growth hence leads to higher carbohydrate reserves at the beginning of the next growing season in southern as opposed to northern seagrass populations. Therefore seagrass populations in the north benefit from the milder summer conditions (high daily doses, no stress caused by extreme temperatures) while southern seagrass populations benefit from milder winter conditions, favouring constant growth (developing evergreen populations).

These patterns of higher carbohydrate reserves in northern populations before winter, and stronger depletion during winter, coincide with a higher reproductive effort found in northern – not evergreen – populations (Van Tussenbroek et al. 2016). Thus, the southern (evergreen) seagrass population may be considered as less dependent on their carbohydrate reserves due to constant clonal growth (Coyer et al. 2004, Zipperle et al. 2011, Ribaudó et al. 2016) and photosynthetic production; but potentially submitted to higher stress levels related to their climate settings (temperature, heat waves) in summer. In contrast, northern seagrass populations experience stronger seasonal dynamics due to colder winter and milder summer temperatures. Thus, they may be considered as being in a perpetual colonizing phase (Peralta et al. 2005): yearly population initiation depending on their carbohydrate reserves in dormant rhizomes to

survive the cold winter conditions, and on seed production (van Tussenbroek et al. 2016) and establishment, for the next growing season.

### **The influence of unpredictable short-term stress events**

Short-term stress events, being rather unpredictable, can occur at any time over the seasonal growth of seagrasses, independently from their climate settings. These stress events range from intense herbivory (Christianen et al. 2014) to dredging (Erftemeijer and Lewis 2006), trampling (Eckrich and Holmquist 2000) or wastewater discharge (Cabaço et al. 2007) and can severely affect seagrass meadows. Our study showed that the synergistic effect of short-term stress events, i.e. short-term nutrient enrichment and above-ground removal, resulted in decreased rhizome carbohydrate reserves at one of the two studied sites, although both located at the same latitude. This suggests that at that particular site – sheltered from hydrodynamics – the carbon balance was disturbed (i.e. reduced carbon storage to favour regrowth), hence carbohydrate reserves decreased.

When short environmental stresses are experienced, seagrasses use simple saccharides rather than carbohydrate reserves to overcome the short stress events, because of the lower energy costs (Burke et al. 1996). We thus found that regardless of the benefits of simple saccharides usage during short stress events, carbohydrate reserves may also play a key role to overcome small-scale stresses, such as nutrient enrichment and disturbances that generates leaf loss. This insight corroborates that soluble carbohydrate concentrations may not only be a valid indicator for growing success after long-time stress periods (Govers et al. 2015), but also for seagrass resilience dynamics. It is however important to note that the dependence on these carbohydrates varied between sites. Only one site, the most sheltered from hydrodynamics, showed a significant decrease in carbohydrate content. This indicates that the amount of carbohydrates needed to overcome a small-scale disturbance may vary depending on the environmental quality at a location, and that at some locations the available soluble carbohydrate concentrations may be sufficient. Furthermore, it is noted that the quantity and quality of non-structural carbohydrate reserves may vary among seagrass species (Ralph et al. 2007), as well as the inherent clonal growth rates. As both aspects are key mechanisms for recovery, (Hemminga and Duarte 2000; Borum et al. 2004; Neckles et al. 2005), the

amount of carbohydrates needed to overcome a small-scale disturbance may vary among species as well.

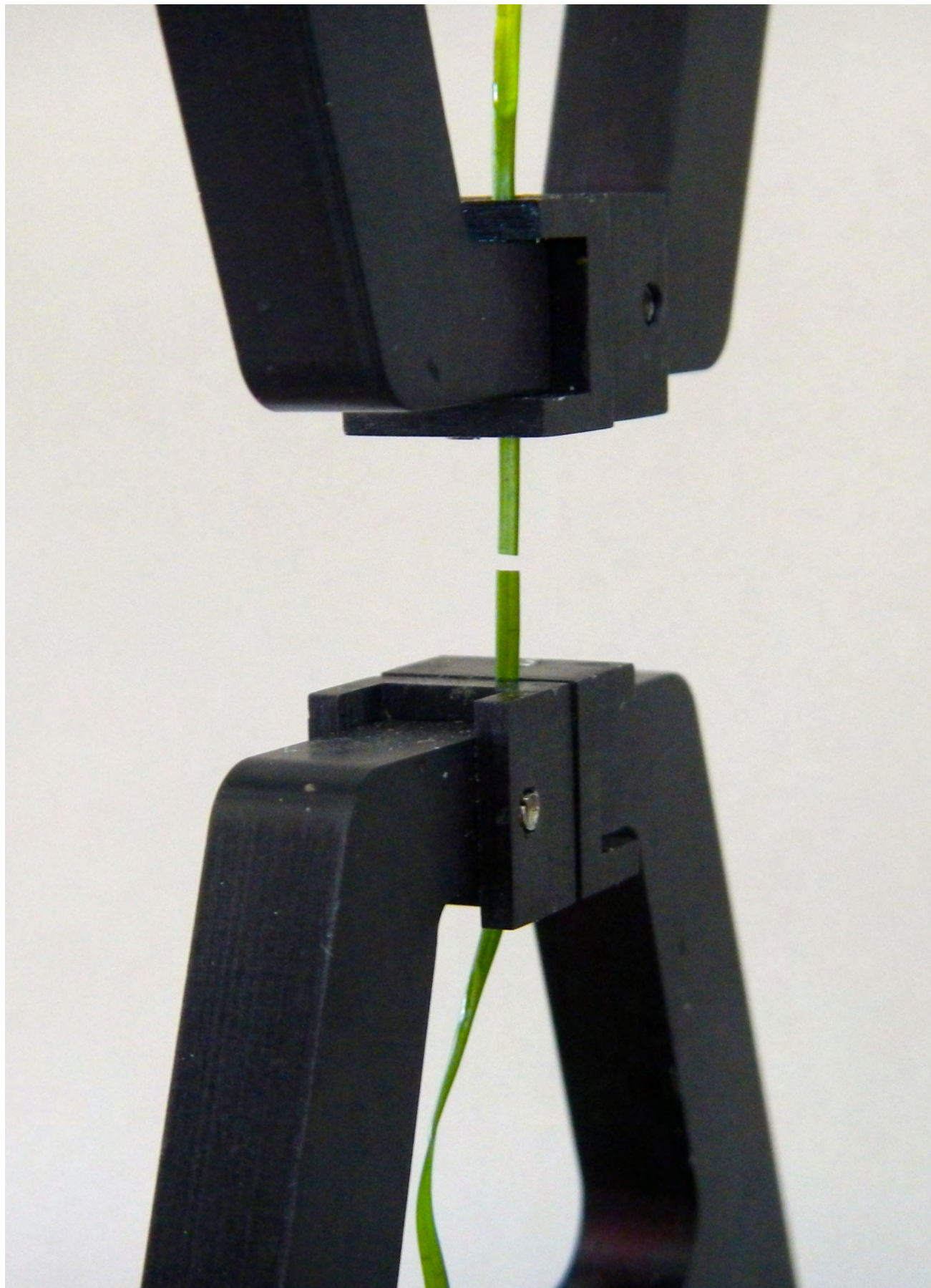
### **Consequences for seagrass meadows in a globally changing environment**

Integrating the results of the latitudinal gradient with those of the disturbance experiments, indicate that northern seagrass populations might be more sensitive to short-term stress and disturbance events, because such stress and disturbance events might reduce the carbohydrate reserves that are actually needed to withstand colder winter settings at higher – colder – latitudes. A short-term stress or disturbance happening at the end of the growing season might hence be expected to have the strongest negative influence on the plant's capacity to preserve their carbohydrate reserves, necessary for wintering.

The difference in carbon storage between southern and northern seagrass populations could equally lead to a lowered resilience under the influence of short-term stress events. In our study, we evaluated the effect of short-term stress events in northern and not directly in southern seagrass populations. However we observed that southern seagrass meadows did not store as much carbohydrate reserves as northern seagrass meadows during their growing season. It may well be that this lower carbohydrate reserve is due to an unbalanced carbon demand (Touchette and Burkholder 2000a): carbon is used for growth and the maintenance of evergreen meadows. But when short-term stress events occur, resulting too low carbohydrate reserves could disturb the constant growth or reduce the resistance of the seagrass meadow to other stress events (related to their climate settings like summer temperatures and heat waves for instance). Therefore, this decrease could gradually push the system towards a collapse (van Nes and Scheffer 2007, Scheffer et al. 2009).

Present findings bare implications for our globally changing environment, where unpredictable (stochastic) events due to climate change (Short and Neckles 1999, Easterling et al. 2000, Thomson et al. 2015) or the increase of anthropogenic stresses on coastal ecosystems (Halpern et al. 2008, Duarte 2014) are expected to become more frequent. Indeed, although seagrasses present the capacity to adapt to their climate or environmental settings (Peralta et al. 2005, Cabaço et al. 2009, Staehr and Borum 2011), as also seen in our study, unpredictable stresses could disturb the carbohydrate reserves needed for overwintering. Previous

studies showed that sufficiently high carbohydrate reserves were key to overwintering at northern latitudes (Govers et al. 2015). Hence such reduction of carbohydrate reserves may ultimately lead to a complete collapse of seagrass meadows, by becoming less resilient to stresses (Scheffer et al. 2001, Scheffer and Carpenter 2003).



## CHAPTER 6

### Seasonal and latitudinal variations in seagrass mechanical resistance to eutrophication

*Submitted*

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#### Abstract

Seagrasses are marine flowering plants distributed worldwide but are threatened, mostly due to the increase of human activities, affecting their survival. Seagrasses have the capacity to adapt their morphological, physiological and mechanical traits to their local conditions. Mechanical traits have been identified as a good tool to investigate a plant-species capacity to withstand physical forces or disturbances. They are, however, still sparsely studied in seagrasses. With this study, we aimed to assess how the mechanical traits of a broadly spread seagrass species vary along a climatic gradient in relation to its morphological plasticity and nutrient status. We found that seagrasses acclimate their mechanical traits in relation to their physiological or morphological traits, both over the growing season and across a climatic latitudinal range: leaves were weaker and thinner in northern areas, particularly at the end of the growing season. Moreover, we showed that leaves mechanical traits change depending on their nutrient status: leaves were stronger and stiffer in oligotrophic conditions as compared to more eutrophic conditions, which presented more stretchy (extensible) leaves. Overall, our study showed for the first time how seagrass mechanical traits vary across a large gradient of seagrass meadows and how their traits change depending on their local conditions. Our results have strong implication in the assessment of seagrass resilience to threats such as eutrophication as their capacity to withstand physical forces (i.e. mechanical traits) depend on their nutrient status.

## Introduction

Seagrasses are marine flowering plants widely distributed worldwide (Short et al. 2007), providing highly valuable ecosystem services for coastal areas (Orth et al. 2006). Their development and distribution depend on various conditions such as light and nutrients availability (Duarte 1991, Grice et al. 1996, Wicks et al. 2009), sufficiently sheltered hydrodynamic conditions (varies per species), and sediment characteristics (Koch 2001, de Boer 2007, Eriksson et al. 2010). Seagrass meadows are facing severe declines worldwide, due to the increase of human activities (Orth et al. 2006, Halpern et al. 2008, Waycott et al. 2009), threatening their resilience and survival. Being sessile organisms, seagrasses can to some extent adapt their morphological and physiological traits (Touchette and Burkholder 2000b, Peralta et al. 2005, Lee et al. 2007, Cabaço et al. 2009, de los Santos et al. 2010, 2013) to their local conditions, and thus also to face natural and human-induced stress and disturbance (Short and Wyllie-Echeverria 1996).

There are several examples of seagrass morphological responses to acclimate to abiotic conditions. When light is limiting, seagrasses have been shown to reduce their investment in below-ground biomass to maintain their photosynthetic production (Peralta et al. 2002; Lee et al. 2007, de los Santos et al. 2010). In contrast, in highly dynamic environments with no limiting light, seagrasses can adapt their morphology by allocating more energy in their below-ground structures. This allows them to reinforce their anchorage capacity into the sediment to prevent dislodgement and reduce drag due to smaller above-ground structure (Peralta et al. 2005, Wicks et al. 2009, de los Santos et al. 2010). Only very recently it has also been shown that seagrasses can modify their mechanical traits in response to external forcing (de los Santos et al. 2013).

Mechanical traits have been identified as a good tool to investigate a plant-species capacity to withstand physical forces or disturbances (Onoda et al. 2011, Puijalon et al. 2011, de los Santos et al. 2013). They are evaluated by measuring the strength, stiffness or extensibility of tissues before breakage and have been the focus of several studies on freshwater (Puijalon et al. 2011), terrestrial plants (Onoda et al. 2008, 2011) as well as marine macroalgae (Harder et al. 2006, Demes et al. 2013). However, for seagrass species, there are still only a very limited number of studies available on mechanical traits / strength of leaves and reproductive shoots (Patterson et al. 2001). Available studies have focussed on comparing species-specific traits (de los Santos et al., 2016) and on quantifying the

effect of specific abiotic conditions such as the effect of nutrient enrichment (La Nafie et al. 2012, 2013), wave exposure (de los Santos et al. 2010, La Nafie et al. 2012), and the spatial and temporal variability in abiotic conditions (de los Santos et al. 2013). The effect of how stresses may affect seagrass properties on a larger scale, like across a climatic gradient and over the season, has not been resolved, despite the importance for understanding seagrass resilience to global warming in combination with other human induced stresses (Short and Neckles 1999, Duarte 2014).

In the present study, we aimed to assess how the mechanical traits of a broadly spread seagrass species vary along a climatic (latitudinal) gradient. Using *Zostera noltei* as a model species, we focussed our study on two critical moments in their growing season: the peak of growth, when their productivity and biomass reaches a maximum; and at the end of growing season, when the biomass decreases before the winter period. We hypothesize that mechanical traits along the latitudinal gradient are mainly due to morphological plasticity, as a recent study demonstrated that leaf width was the most important factor affecting leaf-strength across species (de los Santos et al. 2016). We furthermore hypothesize that the nutrient status of a seagrass meadow lead to altered mechanical properties (La Nafie et al. 2012). That is, for the same species, we expect plants at oligotrophic locations to be mechanically stronger than those at eutrophic locations.

## Material and methods

### Study area and experimental design

*Zostera noltei* meadows can be found in intertidal areas along the European coastline (Valle et al. 2014). To evaluate the large-scale spatial and seasonal variation of seagrass mechanical traits, 12 well studied *Zostera noltei* meadows were selected and sampled at two different time periods corresponding to the peak and the end of the seagrass growing season (*i.e.*, based on local expertise). Meadows were selected following a latitudinal gradient from South to North, being: 1. Cadiz (Spain); 2. & 3. Mondego estuary (Portugal); 4. Santander (Spain); 5. Bidasoa estuary (France); 6. & 7. Arcachon Bay (France); 8. Noirmoutier (France); 9. St-Jacut-de-la-mer (France); 10. & 11. The Oosterschelde (Netherlands); and 12. Sylt (Germany) (Figure 6.1, Table 6.1). For some meadows, two close sites were selected in order to account for local variability and to compare sites according to their health status (*i.e.* Mondego estuary sites 2. Upstream and 3. Downstream),

their elevation (*i.e.* Arcachon Bay sites 6. Germanan and 7. Hautebelle) and their exposure to hydrodynamics (*i.e.* Oosterschelde sites 10. Oostdijk and 11. Dortsman) (see Figure 6.1 and Table 6.1 for background information on all selected seagrass meadows). Sampling dates were not identical between meadows since the growth and the length of the growing season are dependent on local conditions such as temperature, light availability and latitude. To better account for the seagrass growing season, the exact locations, different for each meadow, as well as the starting and ending dates, were all determined by local experts (Table 6.1). Water temperature was monitored over the study period at all sites using HOBO Pendant Temperature loggers (64k – UA-002-64, ONSET) at a frequency of 1 measurement every 30 minutes. Two loggers were placed within the study area and the temperatures averaged over one month around the sampling date for each site (Table 6.1).

*(next page)*

Table 6.1: Selected meadows names and characteristics with coordinates, sampling time, shoot density at sampling time and temperature (averaged on 1 month around sampling time) for both the peak and the end of the growing season.

Meadow number and names	Coordinates	Sampling time		Shoot density (n.m <sup>-2</sup> )		Temperature (°C)	
		Peak	End	Peak	End	Peak	End
E1	Cadiz Bay, Spain N 36 30' W 006 10'	09-Sep	06-Nov	12561 ± 1350	5172 ± 732	26.1 ± 0.08	20.6 ± 0.08
E2	Upstream - Mondego estuary, Portugal N 40 8' W 008 50'	13-Aug	10-Nov	6573 ± 1243	7108 ± 458	20.8 ± 0.09	18.8 ± 0.07
E3	Downstream - Mondego estuary, Portugal N 40 8' W 008 50'	13-Aug	10-Nov	6420 ± 1357	12739 ± 1406	20.6 ± 0.1	18.7 ± 0.08
E4	Santander, Spain N 43 25' W 003 48'	11-Aug	08-Oct	1580 ± 590	2191 ± 338	22.6 ± 0.03	19.9 ± 0.03
E5	Bidasoa estuary, France N 43 21' W 001 46'	07-Aug	11-Oct	5580 ± 870	4892 ± 578	23.3 ± 0.1	20.6 ± 0.1
E6	Germanan - Arcachon Bay, France N 44 42' W 001 8'	13-Aug	27-Oct	9146 ± 1061	10904 ± 1273	23.2 ± 0.07	18.9 ± 0.08
E7	Hautebelle - Arcachon Bay, France N 44 43' W 001 09'	13-Aug	27-Oct	9045 ± 2661	9197 ± 588	23.2 ± 0.07	19.0 ± 0.08
E8	Noirmoutier, France N 46 98' W 002 21'	25-Jul	11-Sep	5478 ± 1554	6548 ± 1167	21.3 ± 0.09	21.1 ± 0.07
E9	St-Jacut-de-la-mer, France N 48 36' W 002 11'	29-Jul	20-Aug	2752 ± 398	2038 ± 593	20.4 ± 0.1	20.1 ± 0.2
E10	Oostdijk - Oosterschelde, Netherlands N 51 26' E 004 05'	19-Aug	26-Sep	10318 ± 1052	7389 ± 1495	20.3 ± 0.1	19.0 ± 0.1
E11	Dortman - Oosterschelde, Netherlands N 51 34' E 003 59'	20-Aug	25-Sep	6115 ± 772	5427 ± 971	20.2 ± 0.08	18.3 ± 0.07
E12	Sylt, Germany N 54 54' E 008 19'	30-Jul	12-Sep	6064 ± 668	9809 ± 838	22.2 ± 0.1	17.9 ± 0.09

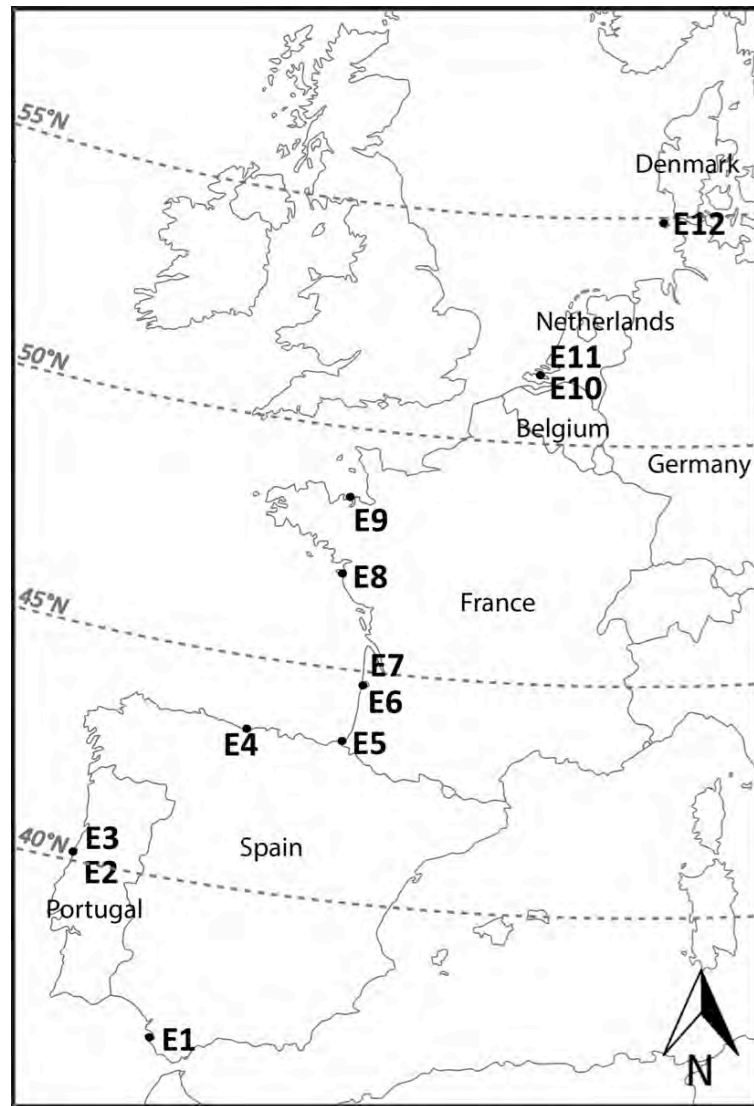


Figure 6.1: Studied sites along the Western-European latitudinal gradient.

During each sampling time, 5 seagrass samples were collected at all sites by using a 10 cm diameter PVC tube, inserted into the sediment randomly within the area. Samples were labelled, carefully washed a first time on site, stored into wet tissues and shipped via express shipment (<24 h) for further analysis to the Royal Netherland institute of Sea Research (NIOZ) in Yerseke, the Netherlands.

### Seagrass traits measurements

Upon arrival at the NIOZ and prior to mechanical measurements, the samples were carefully washed to remove epiphytes from the plant material. The total number of shoots was firstly noted for shoot density (Table 6.1). Then, from each sample, 5 apical shoots were randomly selected for traits analysis. The third leaf from each apical shoot was then cut off at the junction between the sheath and the blade and placed into seawater at room temperature. The rest of the apical shoot was kept and split between above-ground (leaves) and below-ground parts (roots and rhizomes) for further tissue analyses.

Morphological traits: Morphological traits (Table 6.2) such as leaf length ( $L_0$ , mm), width ( $L_W$ , mm) and thickness ( $L_T$ , mm) were measured by using a calliper and dial thickness gauge (Mitutoyo®, precision  $\pm 0.01$  mm). They were used to calculate the cross-sectional area ( $CA$ ,  $\text{mm}^2$ ) (Table 6.2). For leaves longer than 60 mm, a leaf blade fragment of 60 mm was excised from the lower part of the leaf (i.e. originally the closest to the leaf sheath) and this new length was considered as the new  $L_0$  for mechanical measurements.

Mechanical traits: Measures of mechanical traits of the leaves in tension were conducted by using a tensometer (custom made electric actuator with a rated capacity of 5 kN and an accuracy of 0.01 mm of displacement; Instron® universal testing machine) and the Bluehill software (version 3.0). The tensile tests were performed using a load cell of 10 N with pneumatic action grips of 5 N (model 2712). The leaf fragments were individually clamped between the action grips, parallel to the main axis with the mounting 10 mm apart. During the test, the leaf fragments were stretched at a constant velocity of  $10 \text{ mm} \cdot \text{min}^{-1}$ . The extension and the load were recorded every 0.1 seconds until breakage, where the maximum load ( $F_{TA}$ , N) and extension ( $\delta_{TA}$ , mm) were recorded. These two direct measurements of tissue properties were defined as (1) the breaking stress

( $F_{TA}$ , N), the maximum force each individual fragment can bear before breakage; and (2) the absolute extension ( $\delta_{TA}$ , mm), the maximum extension relative to the fragment before breakage (Table 6.2).

Table 6.2: List of leaf characteristics measured and calculated with relative units.

Leaf characteristics	Name	Measure/formula	Units
Morphological traits	Length	$L_0$	mm
	Width	$L_W$	mm
	Thickness	$L_T$	mm
	Cross-sectional area	$CA = L_W \times L_T$	$\text{mm}^2$
Mechanical traits			
(1) Tissue properties	Breaking stress	$F_{TA}$	N
	Absolute extension	$\delta_{TA}$	mm
	Extensibility	$\delta_{\max} = (\delta_{TA}/L_0) \times 100$	%
(2) Material properties	Strength	$F_{TS} = F_{TA}/CA$	$\text{N} \cdot \text{mm}^{-2}$
	Stiffness	$E_T = (L_0/CA) \times (F_{TA}/\delta_{TA})$	$\text{N} \cdot \text{mm}^{-2}$
Physiological traits	C:N-ratio	$C:N = C/N$	

From the recorded measurements, and the morphological traits of each leaf fragment, three additional mechanical traits were calculated (Table 6.2): (3) the extensibility ( $\delta_{\max}$ , %) corresponding to the increase in length ( $\delta_{TA}$ ) from the original specimen length ( $L_0$ ) that occurs before it breaks; (4) the tensile strength ( $F_{TS}$ ,  $\text{N} \cdot \text{mm}^{-2}$ ) which is the maximum force per unit of cross-sectional area needed to break the leaf fragment; and (5) the stiffness, given by the Young's modulus of elasticity in tension ( $E_T$ ,  $\text{N} \cdot \text{mm}^{-2}$ ) and representing the leaf's resistance to deformation (the higher the  $E_T$ , the stiffer the leaf fragment).

Mechanical traits used in this study were separated into two categories describing (1) the tissue properties, relative to the absolute mechanical property of the leaf fragment (i.e. breaking stress, absolute extension and extensibility); and (2) the material properties, a standardized mechanical property accounting for the leaf morphological traits (i.e. strength, stiffness) (Table 6.2).

Physiological traits: Physiological traits were determined by considering the C:N-ratio in leaves' tissues. To measure the C:N-ratio in leaves, total Carbon and Nitrogen content were measured on freeze-dried and grinded leaves samples using an Element Analyzer (EA) Flash 1112, Thermo Scientific. Lyophilized and grinded samples were combusted at 1020 °C in oxic conditions. The nitrous oxides were reduced to N<sub>2</sub> with elementary copper at 650 °C. Water was removed by trapping. After separation on a Haysep Q column, CO<sub>2</sub> and N<sub>2</sub> were detected on a TCD detector. The C:N-ratio was thus calculated by dividing the total Carbon content by the total Nitrogen content obtained.

### Statistical analysis

The seasonal variation of leaves morphological (length  $L_0$ , CA), and physiological (C:N-ratio) traits, as well as tissue ( $F_{TA}$  and  $\delta_{max}$ ) and material ( $F_{TS}$  and  $E_T$ ) properties across a latitudinal gradient were evaluated with a mixed effects model accounting for the effect of latitude (fixed factor) and timing in the season (random factor). A K-means cluster analysis was performed to discriminate the sites according to their C:N-ratio in three groups, which centre was calculated as mean  $\pm$  SE, and tested through an ANOVA. Afterwards, differences between the obtained C:N clusters were then tested for all mechanical traits through a mixed effect model accounting for the effect of C:N clusters (fixed factor) and timing in the season (random factor). In order to test the relationship between mechanical traits and both morphological traits (CA) and physiological traits (C:N-ratio), linear regression models were applied on tissue properties ( $F_{TA}$ ,  $\delta_{max}$ ) and material properties ( $F_{TS}$ ,  $E_T$ ). Moreover, a stepwise regression was used to test the relative influence of morphological traits (CA), physiological traits (C:N-ratio) and tissue properties ( $F_{TA}$ ) on material properties ( $F_{TS}$ ). For all tests, normality of the data was previously checked along with interactions between factors. All statistical analyses were performed with R version 3.1.3 (2015-03-09).

## Results

### Seasonal variation of leaves characteristics across a European gradient

Morphological traits: Seagrass cross-sectional area (CA, mm<sup>2</sup>) decreased between the peak and the end of the growing season (Table 6.3, Figure 6.2a), while leaf length (L<sub>0</sub>, mm) did not show any significant changes along the growing season (Table 6.3, Figure 6.2b). Leaf length was also the only morphological parameter that did not present any relation with latitude (Table 6.3, Figure 6.2b) reaching its highest values at the peak of growth at a southern site (E3 Mondego-downstream) and at a northern site (E10 Oostdijk), of  $126 \pm 14$  and  $124 \pm 7$  mm respectively. The lowest values were found at the southernmost site (E1 Cadiz) with a leaf length at the peak of growth of  $61 \pm 4$  mm. Other parameters (L<sub>w</sub>, width and L<sub>T</sub>, thickness) expressed as the cross-sectional area CA (mm<sup>2</sup>) generally decreased with latitude, with however still high values in the mid-latitudes (Figure 6.2a).

Tissue properties (absolute mechanical traits): Breaking stress (N) significantly decreased by 3.5 fold along the latitudinal gradient from south to north (Figure 6.2c, Table 6.3), whereas the leave's extensibility (%) did not significantly differ along the latitudinal gradient (Figure 6.2d, Table 6.3). Both traits were, however, presenting significantly lower values at the end of the season when compared to the peak of growth, particularly in higher latitudes (Table 6.3, Figure 6.2c and 6.2d).

Material properties (standardized mechanical traits): Standardized mechanical traits were calculated from absolute mechanical measurements as a function of leaves morphology (L<sub>0</sub>, CA). Hence they define the material properties. The leaves' strength (N.mm<sup>-2</sup>), as well as the stiffness (N.mm<sup>-2</sup>), were generally lower in northern locations (Table 6.3, Figure 6.2e and 6.2f), meaning that the plants from northern sites were less stiff and easier to break than in the south. Although the leaves' strength was reduced of about 1.5 fold in the north, it did not show any significant changes along the growing season (Table 6.3, Figure 6.2e). On the opposite, leaf stiffness significantly increased along the growing season, particularly in northern sites (Table 6.3, Figure 6.2f).

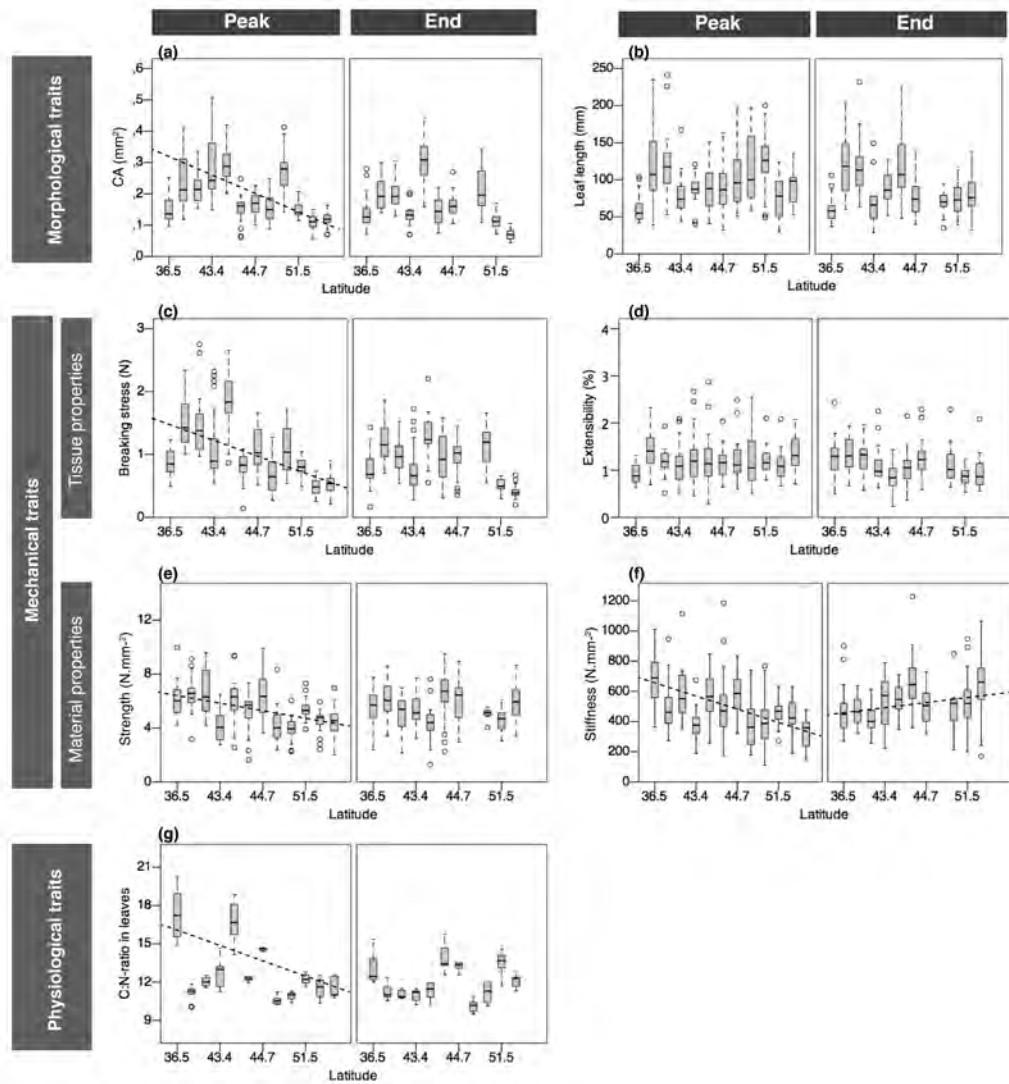


Figure 6.2: Box plots representing leaves morphological (a and b), mechanical (c to f) and physiological (g) traits along the latitudinal gradient at the peak and end of their growing season. Mechanical traits are divided between tissue properties (c and d; integrated measure relative to the specimen dimensions) and material properties (e and f; standardized measure). The dotted lines illustrate a significant linear relationship between the leaf characteristic and latitude ( $p < 0.001$ ).

Table 6.3: Results (F and p) from the mixed effect model testing the seasonal (timing) and latitudinal (latitude) variations in morphological, physiological and mechanical traits of the seagrass meadows studied.

	Latitude		Timing	
	F	p	F	p
Leaf length ( $L_0$ , mm)	0.1	0.752	1.389	0.239
CA ( $\text{mm}^2$ )	57.407	<0.001*	28.889	<0.001*
Breaking stress ( $F_{TA}$ , N)	111.117	<0.001*	27.609	<0.001*
Extensibility ( $\delta_{\max}$ , %)	0.017	0.896	5.319	0.022*
Strength ( $F_{TA}$ , $\text{N}\cdot\text{mm}^{-2}$ )	30.911	<0.001*	0.457	0.499
Stiffness ( $E_T$ , $\text{N}\cdot\text{mm}^{-2}$ )	8.529	0.004*	10.148	0.002*
C:N-ratio	55.257	<0.001*	31.195	0.001*

Physiological traits (C:N-ratio): C:N-ratio in leaves decreased along the latitudinal gradient (from south to north) as well as along the growing season (Table 6.3, Figure 6.2g) with values ranging from 9.5 to 20.2. The clusters created by using leaves C:N-ratio at the peak of growth in controls allowed to separate the sites between 3 groups ( $p < 0.001$ ): (1) Sites with a low C:N-ratio (cluster centre =  $11.5 \pm 0.06$ ), i.e. eutrophic ( $\text{C:N} < 12$ ; Duarte 1990); (2) sites with an intermediate C:N-ratio (cluster centre =  $13.6 \pm 0.2$ ) and (3) sites with a high C:N-ratio (cluster centre =  $17.03 \pm 0.3$ ) (Figure 6.3).

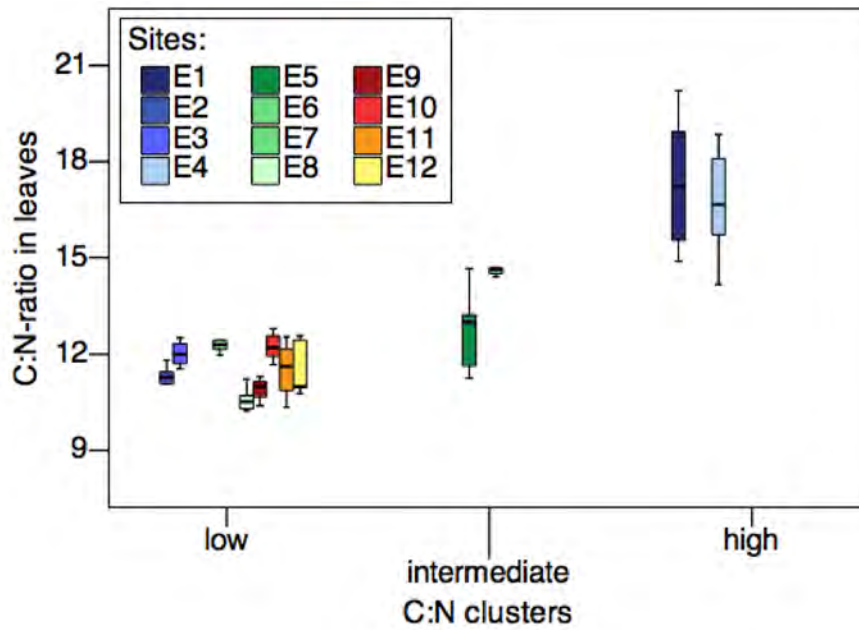


Figure 6.3: Boxplot representing the three clusters (x-axis) created from the C:N-ratio measured in leaves at the peak of growth. Data are represented per site.

### The influence of morphological and physiological traits on leaves mechanical traits

Mechanical traits showed to be linked with the C:N-ratio measured in leaves, as confirmed by a positive linear relationship between C:N-ratio in leaves and material properties such as the tensile strength ( $F_{TS}$ ,  $N \cdot mm^{-2}$ ) and stiffness ( $E_T$ ,  $N \cdot mm^{-2}$ ) (Figure 6.4).

Sites with high C:N-ratios showed significantly higher breaking stress ( $F_{TA}$ , N) and stiffness ( $E_T$ ,  $N \cdot mm^{-2}$ ) (Figure 6.5a and 6.5d) as compared to the two other categories. In contrast, leaves' extensibility (%) was the lowest at sites with high C:N-ratios (Figure 6.5b).

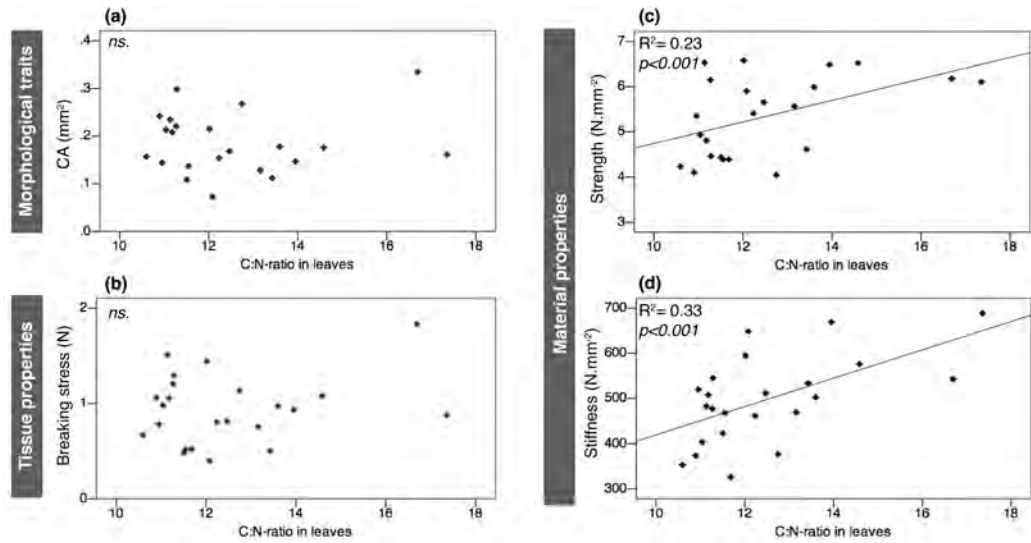


Figure 6.4: The relationship between C:N-ratio in leaves and (a) leaves morphological traits (CA), (b) tissue properties (breaking stress), and (c) and (d) material properties (strength and stiffness). Data obtained for all seagrass meadows selected along the European gradient at both the peak and end of the growing season.

Material properties (standardized mechanical traits): Standardized mechanical traits were calculated from absolute mechanical measurements as a function of leaves morphology ( $L_0$ , CA). Hence they define the material properties. The leaves' strength (N.mm<sup>-2</sup>), as well as the stiffness (N.mm<sup>-2</sup>), were generally lower in northern locations (Table 6.3, Figure 6.2e and 6.2f), meaning that the plants from northern sites were less stiff and easier to break than in the south. Although the leaves' strength was reduced of about 1.5 fold in the north, it did not show any significant changes along the growing season (Table 6.3, Figure 6.2e). On the opposite, leaf stiffness significantly increased along the growing season, particularly in northern sites (Table 6.3, Figure 6.2f).

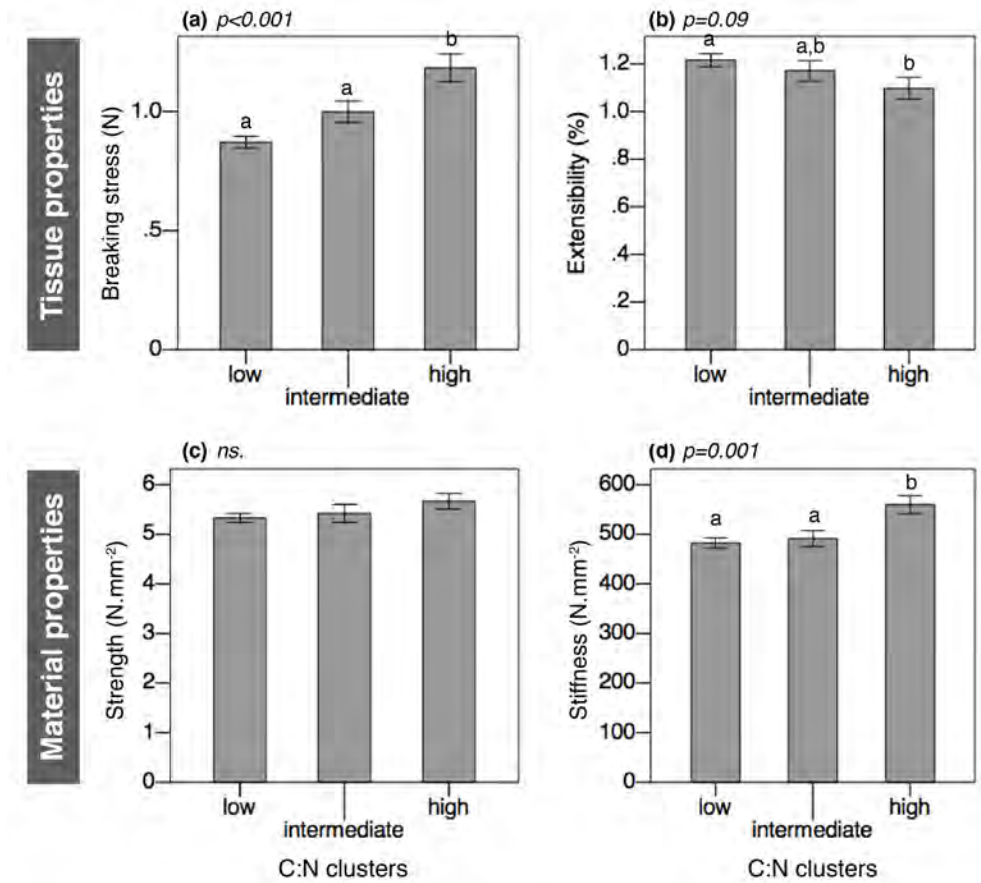


Figure 6.5: Differences in tissue (a and b) and material (c and d) properties depending on the C:N clusters. The p-values represent significant differences between C:N clusters for each variable. Small letters (a, b) represent the statistical groups from the post-hoc Tukey tests and illustrate significant differences between C:N clusters for each variable.

## Discussion

Seagrasses are sessile organisms able to acclimate their physiological and morphological traits to face the various stresses and disturbances they are submitted to (Short and Wyllie-Echeverria 1996). Mechanical traits of plant material are important to confer protection and resistance to leaves against high currents, waves or herbivory (Read and

Stokes 2006; Onoda et al. 2011). However these traits remain scarcely understood for seagrasses (La Nafie et al. 2012, 2013; de los Santos et al. 2013). The present study reveals that seagrasses have the capacity to acclimate their mechanical traits in relation to their physiological or morphological traits, both over the growing season and across a climatic latitudinal range: leaves were weaker and thinner in northern areas, particularly at the end of the growing season. Moreover, we showed that leaves mechanical traits could change depending on their nutrient status: leaves were stronger and stiffer in oligotrophic conditions (high C:N-ratio) as compared to more eutrophic conditions (low C:N-ratio), which presented more stretchy (extensible) leaves.

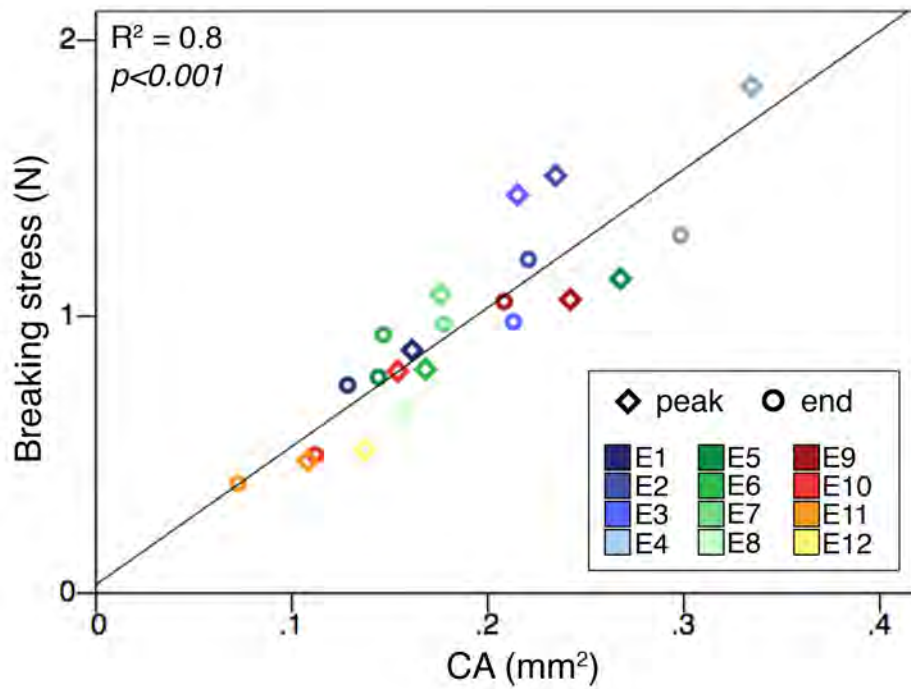


Figure 6.6: Linear regression between breaking stress (FTa; tissue property) and CA (morphological trait). Data represent all sites at both the peak and the end of the growing season.

### Seagrass mechanical traits along a seasonal and latitudinal gradient

Firstly, our study along the European coastline showed that seagrass meadows (*Zostera noltei*) located in northern areas presented mechanically weaker leaves with a lower C:N-ratio when compared to southern populations. In contrast, morphological traits such as leaf length did not vary across the gradient. This observation may be related to the seagrass seasonal dynamics (Pérez-Lloréns and Niell 1993; Auby and Labourg 1996; Vermaat and Verhagen 1996; Hansen and Reidenbach 2013) or acclimation to local environmental conditions (Peralta et al. 2005; de los Santos et al. 2013).

Most mechanical traits decreased along the growing season, particularly the one relative to tissue properties such as breaking stress ( $F_{TA}$ ), i.e. the specific force needed to break a leaf, and the leaf extensibility ( $\delta_{max}$ ). This was particularly the case in northern seagrass meadows, where leaves get thinner (i.e. reduced CA due to reduced leaf width and thickness) before wintering and, as seen in our study, weaker and easier to break. Indeed, in northern – not evergreen – seagrass populations, only the below-ground biomass with limited leaf cover (mostly one leaf left per shoot) remain for the winter period (Hemminga and Duarte 2000; Larkum et al. 2006). The reduced force for breakage related to a lower leaf width and thickness (i.e. reduced CA) at the end of the season seems then a logical trait for these northern seagrass populations before wintering (i.e. limited growth due to a decrease in light and temperature). In contrast, the constantly growing (evergreen) southern seagrass population presented mechanically stronger leaves, linked to their higher leaves width and thickness as seen with the strong correlation between leaf cross-sectional area and breaking stress.

### Changes in seagrass mechanical traits depending on their nutrient status

The relationship between mechanical (leaves strength and stiffness) and physiological (C:N-ratio in leaves) traits demonstrates the importance of the local environmental status on seagrass mechanical traits. Indeed, seagrass meadows with a higher C:N-ratio in their leaves, i.e. oligotrophic sites (Duarte 1990), presented stiffer, stronger but less extensible leaves than seagrass meadows with a low or intermediate C:N-ratio. Although the variation in mechanical resistance correlated to changes in C:N-ratio may be due to the plants morphotype and growth rate (Peralta et al. 2000), these

results confirm previous observations on terrestrial plants, which leaves are stiffer in low nutrient conditions (Onoda et al. 2008). It also confirms previous findings on tropical seagrasses (La Nafie et al. 2012, 2013) in which reduced leaves strength and stiffness was reported when subjected to high nutrients levels (i.e. low C:N-ratio) but a higher extensibility. This higher leaf extensibility under eutrophication might be explained as a way to counteract their reduced strength: extensible leaves are more prone to deformation which might slow down breakage (Onoda et al. 2011; La Nafie et al. 2013).

Indeed, as seen along the latitudinal gradient, seagrass mechanical traits were related to their nutrient status. It may well be that, because of their capacity to adapt to their local high or low nutrient status, these seagrass meadows might be able to also withstand more 'extreme' nutrient variations (Puijalon et al. 2008; de los Santos et al. 2013; Kohlmeier et al. 2014). Therefore, as observed for other seagrass species (*Cymodocea nodosa*) under different hydrodynamic conditions (de los Santos et al. 2013), it could be hypothesized that *Zostera noltei* presents a plasticity in their mechanical traits depending on their nutrient status.

### **The influence of morphological traits on tissue and material properties**

Seagrasses differ in their size or leaf dimensions depending on their local conditions, making them more or less structurally resistant to face the local stress and disturbances they undergo (Peralta et al. 2005). In a comparative study over one third of world seagrass species, de los Santos et al. (2016) showed how mechanical traits varied in seagrass species depending on their geographical region (tropical vs. temperate) and their morphological traits, particularly leaf width. In our study, we also found a strong positive relationship between leaf cross-sectional area and their tissue properties, expressed as breaking stress. In terrestrial plants, it has been shown that a high leaf mass per area could define a more structurally resistant plant capable to achieve a longer lifespan, hence mechanically more resistant (Read and Stokes 2006). In our study, we found that seagrass leaves in southern populations and oligotrophic sites presented both wider and thicker leaves (high CA) and stronger tissues. These results hence confirm previous observations in terrestrial plants (Read and Stokes 2006) and also in other seagrass species (de los Santos et al. 2016).

**Implications for the resilience of temperate seagrass meadows**

Overall, our study reports an important aspect of seagrass ecology by showing how they acclimate their mechanical traits along a latitudinal – climatic – gradient, with leaves being weaker in northern areas, particularly at the end of the growing season; and how their mechanical traits relate to their morphological (leaf cross-sectional area, CA) and physiological traits (C:N-ratio in leaves), with leaf being stronger and stiffer in oligotrophic conditions as compared to more eutrophic conditions. The relationship between leaves physiological and mechanical traits is important when considering the potential effect of eutrophication on seagrass meadows. As shown in experimental lab-studies, leaves mechanical traits can be reduced under stressful nutrient enrichment levels (La Nafie et al. 2012, 2013). The meadows presenting a low C:N-ratio, i.e. eutrophic conditions, already showed signs of affected morphological and mechanical traits (longer but thinner leaves, easier to break despite their slightly higher extensibility). Further eutrophication may likely affect the width and thickness or strength of the leaves and subsequently reduce the resistance to other stressors such as waves, currents or herbivory (Read and Stokes 2006; Puijalon et al. 2008, 2011; Onoda et al. 2011). This is highly important in the context of increasing human activities and threats on coastal areas and climate change. The repeated and growing occurrence of eutrophication stress events, potentially reducing leaf mechanical traits might reduce the resilience of seagrass meadows and potentially lead to the collapse of the system (Scheffer et al. 2009; Carr et al. 2012).



## CHAPTER 7

## Pollen limitation may be a common Allee effect in marine hydrophilous plants: implications for decline and recovery in seagrasses

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### Abstract

Pollen limitation may be an important factor in accelerated decline of sparse or fragmented populations. Little is known whether hydrophilous plants (pollen transport by water) suffer from an Allee effect due to pollen limitation or not. Hydrophilous pollination is a typical trait of marine angiosperms or seagrasses. Although seagrass flowers usually have high pollen production, floral densities are highly variable. We evaluated pollen limitation for intertidal populations of the seagrass *Zostera noltei* in The Netherlands and found a significant positive relation between flowering spathe density and fruit-set, which was suboptimal at <1200 flowering spathes m<sup>-2</sup> (corresponding to < 600 reproductive shoots m<sup>-2</sup>). A fragmented population had ≈35 % lower fruit-set at similar reproductive density than a continuous population. 75% of all European populations studied over a large latitudinal gradient had flowering spathe densities below that required for optimal fruit-set, particularly in Southern countries. Literature review of the reproductive output of hydrophilous pollinated plants revealed that seed- or fruit-set of marine hydrophilous plants is generally low, as compared to hydrophilous freshwater and wind-pollinated plants. We conclude that pollen limitation as found in *Z. noltei* may be a common Allee effect for seagrasses, potentially accelerating decline and impairing recovery even after environmental conditions have improved substantially.

## Introduction

Allee effects, or positive density dependence of the fitness of individuals in a population, may accelerate decline of sparse or fragmented populations and may impair recovery of disturbed populations (e.g. Scheffer et al. 2001). Allee effects due to pollen limitation are frequently reported in animal-pollinated land plants. In contrast, surprisingly little is known about water plants. This lack of knowledge becomes even more remarkable if one takes into account that the communities of marine submerged plants (seagrasses) belong to the most valuable ecosystems of our planet (Duarte et al. 2008). Valuable seagrass populations are rapidly decreasing worldwide (Orth et al. 2006, Waycott et al. 2009); and restoration of declining populations is difficult (Valdemarsen et al. 2011, van Katwijk et al. 2016). Seagrass populations under threat often have decreased densities or may become fragmented or patchy (Bell et al. 1999, Apostolaki et al. 2009). Reduced seed- or fruit-set due to pollen limitation is a demographic mechanism of an Allee effect, causing reproductive impairment (Aguilar et al. 2006). Recolonization of gaps and recovery of such seagrass populations can be partly or fully dependent on recruitment from seed (Ouborg et al. 1999). Allee effects from pollen limitation may thus impair recovery of (meta-) populations as well as recovery from localized damage such as formation of gaps. Hence there is need for more knowledge on Allee effects due to pollen limitation in aquatic species like seagrasses.

Hydrophily is an abiotic pollination mechanism where pollen is transported by water, and differs from pollen transport by biotic vectors such as insects, bats or birds (zoophilous pollination). Abiotic pollination in terrestrial systems occurs by wind (anemophilous) or rarely by rain. Anemophilous plants are thought to suffer less from pollen limitation (sensu Ashman et al. 2004, Knight et al. 2005) than zoophilous ones, because they produce copious amounts of pollen and do not depend on potentially fluctuating populations of pollinators for successful seed-set (e.g. Friedman and Barrett 2009), though pollen limitation may occur in marginal conditions (Davis et al. 2004, Hesse and Pannell 2011). Similar to anemophilous flowers, hydrophilous flowers produce a lot of pollen, resulting in high pollen:ovule ratios (e.g.  $10^4:1$  for *Zostera*: Ackerman 2006). But tremendous amounts of pollen can be lost due to high pollen dispersion in the water, and there is little information whether pollen transport limits seed-set of submersed species (Titus and Hoover 1991). Reduced fruit- or seed-set may be due to failure in pollination due to rapid

dilution of pollen and unpredictable hydrodynamic forces (Verduin et al. 2002, Ackerman 2006, Van Tussenbroek et al. 2009). Anecdotal evidence suggests that, for plants with hydrophilous pollination, deviations from optimal conditions may result in pollen limitation, as is generally the case for zoophilous land plants (Burd 1994, Ashman et al. 2004). Specifically, pollen limitation in seagrasses has been established for female-dominated populations of the dioecious *Phyllospadix* spp (Shelton 2008, Buckel et al. 2012), populations with limited flowering shoots of monoecious *Zostera marina* (Reusch 2003), and under reduced abundance of male flowers of dioecious *Thalassia testudinum* (Van Tussenbroek et al. 2009). However, it has as yet to be established whether pollen limitation under water is a more common phenomena; and if so, this would have important implications for management of seagrass populations.

We evaluate, firstly, whether the successful pollination of hydrophilous plants, with the marine angiosperm *Zostera noltei* as a model species, is depended on the density of reproductive structures (Allee effect). We determine the critical reproductive density for optimal pollination success and whether habitat fragmentation may exacerbate reproductive failure. Secondly, the reproductive density of *Z. noltei* was determined across Europe, to evaluate whether this density is above the critical level for optimal pollination success. We discuss possible consequences of pollen limitation for the preservation of the European *Z. noltei* populations. Lastly, we reviewed the literature to verify whether low seed- or fruit-set is generally common in hydrophilous plants, and may thus contribute to the accelerated worldwide decline of seagrasses.

## Materials and methods

### Model species: *Zostera noltei*

*Zostera noltei* typically grows on tidal mudflats or in the shallow subtidal, forming large continuous meadows or growing in patches throughout the European and Northern-African Atlantic coastline and the Baltic, Mediterranean, Black, Caspian and Azov Seas. In its southern distribution area, this species is leaf-bearing throughout the year (Buia and Mazzella 1991, Peralta et al. 2000, 2005, Cabaço et al. 2009, 2012), but in north Atlantic Europe (including the study area Oosterschelde) the plants overwinter as small rhizome fragments with few or no leaves (Vermaat and Verhagen 1996). In spring, seeds or overwintering rhizome sections initiate patches through vigorous vegetative expansion, which may eventually form

large continuous meadows under favorable conditions. Spathes bearing 6-10 male and 2-5 female flowers are clearly visible later in the growing season (June - September/October: Auby and Labourg 1996, Vermaat and Verhagen 1996, Brun et al. 2003b, Zipperle et al. 2009a, 2009b), with one reproductive shoot producing up to 8 spathes (usually less) per reproductive season. This species is protogynous, and filamentous pollen is taken to the female flowers of neighboring spathes through the water column. Median pollen dispersal distance in the Waddenzee is 1.8-3.2 m (Zipperle et al. 2011). The fruit is a nutlet with a membranous transparent testa containing one seed. The small seeds (1-2 mm long) are negatively buoyant and form a seed bank, for periods <1 to > 3 y (Hootsmans et al. 1987, Zipperle et al. 2009a).

### Study areas

Density dependent reproductive success was determined for intertidal *Z. noltei* populations in the Oosterschelde (the Netherlands) at 8 locations (Figure 7.1). The Oosterschelde is a former arm of the river Scheldt delta that became a semi-enclosed sea-arm, following large-scale civil engineering work (for more information see Suykerbuyk et al. 2012). These works, consisting of dam constructions and the creation of storm-surge barrier at the mouth of the estuary, reduced the inflow of freshwater and nutrients; thus increasing the salinity of its eastern compartments (Wetsteyn and Kromkamp 1994). The Oosterschelde is a relatively sheltered system with little wind fetch and small waves. The tidal range varies between 2.4 and 3.5 m; maximum current velocities range from around 0.3 m s<sup>-1</sup> in the shallow areas to 1-1.5 m s<sup>-1</sup> in the tidal channels, and waves are generated within the system by wind (Louters et al. 1998). The air exposure time of the studied meadows ranges between 50-70% of the tidal cycle (See Suykerbuyk et al. 2015 for further information on the 8 study sites). Oostdijk presents at large continuous well-established bed at an average elevation of 0.4 m above sea level, with an average cover of 70-80% (maximum cover 100%). The seagrass meadow in Goesse Sas presents an average elevation of 0.35-0.40 m above sea level (Amsterdam Ordnance Datum: NAP) with total surface area of 1.5 ha with an average seagrass cover of 15-20% with maximum cover of 70% in the continuous meadow, but this site also presents fragmented meadows.

Spathe density of *Z. noltei* was also determined for 12 seagrass populations across Europe on intertidal flats along the Western-European coast from Southern Spain to Northern Germany (Figure 7.1, Table 7.1).

### Density-dependent reproductive success

Density-dependent variability in reproductive success among populations was determined for 8 sites representing intertidal *Zostera noltei* meadows across the Oosterschelde; visited from 28 August until 1 September 2014 (Figure 7.1). Between 27 and 56 reproductive shoots were collected haphazardly at each site, and their density was estimated by counting them in 10 haphazardly chosen areas of 10x10 cm. The spathes were classified according to reproductive phases as follows: 1) buds (with male and female flower buds), 2) male-anthesis (male flowers opening-up, anthers protruding from the spathe-sheath), 3) female-anthesis (female flowers opening up, fresh stigmas protruding from spathe-sheath), 4) female-post-anthesis (female flowers with oxidized stigmas but without indications of ovule development), 5) fruit-bearing (fruits with unripe or ripe seeds), 6) aborted (spathes with only aborted fruits, often in deteriorated condition). Spathe-set was determined as the proportion of fruit-bearing spathes (phase 5) of the total number of fruit-bearing and aborted spathes (phases 5 and 6).

Density-dependent variability in reproductive success within seagrass populations was subsequently (10-Sep-2014) determined along a tidal gradient in the continuous well-established bed at Oostdijk as follows: 1. channel: minor tributary tidal channels,  $\approx 0.5$  m lower than the typical continuous meadow sections, with permanently submersed *Z. noltei*, 2. continuous: typical section of the large well-established continuous meadow at on average 0.38m above sea level, 3. elevated: elevated section,  $\approx 0.2$  m higher than the typical continuous meadow, intersected by minor tidal channels. In addition, we sampled a fourth area that was a fragmented (patchy) meadow section at Goesse Sas, with patch sizes of  $\approx 25$  m<sup>2</sup>, at tidal level similar to that of the typical continuous population at Oostdijk. The seagrasses were sampled with a corer (10 cm diameter). On each site, 5 samples were collected within a more or less homogenous area of  $\approx 25$  m<sup>2</sup>, except in the channels that were usually not wider than 1 m. The samples were collected approximately 2 m apart, as this distance corresponds with the median pollen dispersal distance for this species reported by Zipperle et al. (2011). The samples were placed in a sieve, washed separating the seagrass plants from mud and other materials and placed in plastic bags for transport to the laboratory. In the laboratory, the plants were cleaned and tissues were separated in above-ground (leaves, sheaths and spathes) and below-ground (rhizomes and roots) tissues.

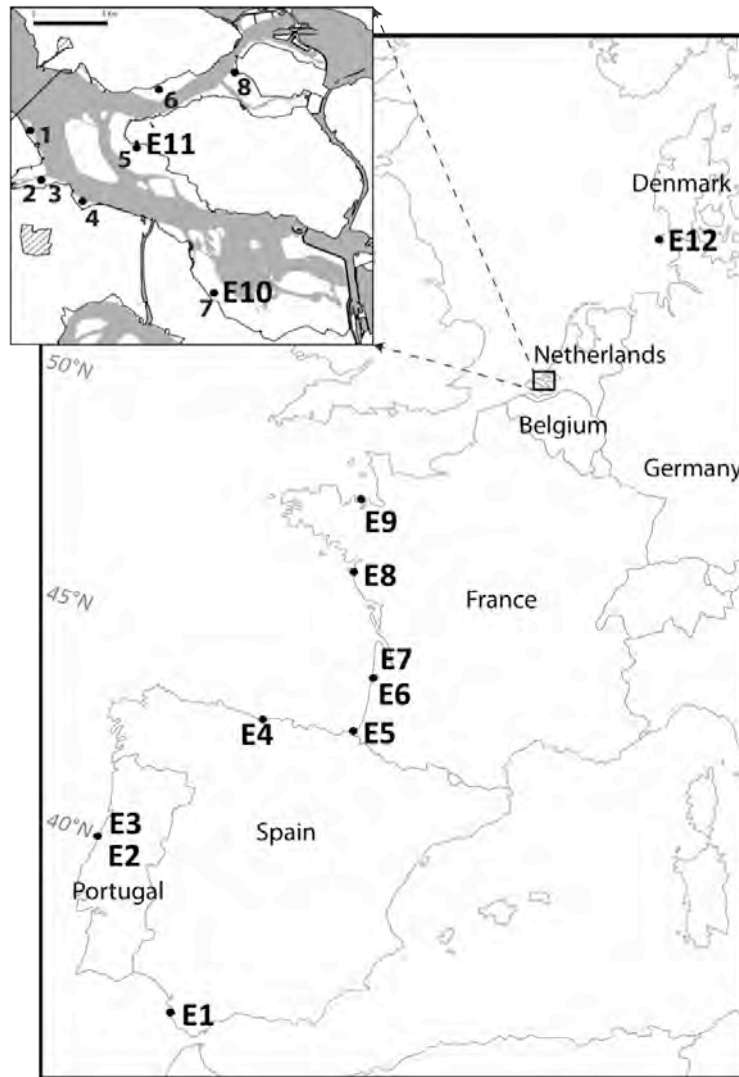


Figure 7.1: Map of Europe with detailed map of the Oosterschelde indicating sampling sites. See Table 1 for coding of European sites. The numbers in the inserted maps of the Oosterschelde indicate the following sampling sites: 1. Slikken van Kats, 2. Zandkreek-area A, 3. Zandkreek-area B, 4. Goesse Sas (continuous meadow), 5. Dortsman, 6. Viane West, 7. Oostdijk, 8. Krabbenkreek.

Table 7.1: European sampling: Latitude, sampling date (2014), and shoot density for all sites. na: not analyzed

Station	Site	Latitude	Sampling time			Shoot density (shoots m <sup>-2</sup> )		
			Start	Peak	End	Start	Peak	End
E1	Spain, Cadiz Bay	N 36°30'	02-Jul	09-Sep	06-Nov	15643 ± 1525	12561 ± 1350	5172 ± 732
E2	Portugal, Mondego Estuary 1	N 40°8'	28-May	13-Aug	10-Nov	6548 ± 1243	6573 ± 1243	7108 ± 458
E3	Portugal, Mondego Bay 2	N 40°8'	28-May	13-Aug	10-Nov	6675 ± 559	6420 ± 1357	12739 ± 1406
E4	Spain, Santander	N 43°25'	13-Jun	11-Aug	08-Oct	2013 ± 277	1580 ± 590	2191 ± 338
E5	France, Bidasoa Estuary	N 43°21'	29-May	07-Aug	11-Oct	2573 ± 283	5580 ± 870	4892 ± 578
E6	France, Arcachon-Germanan	N 44°42'	27-May	13-Aug	27-Oct	6599 ± 1443	9146 ± 1061	10904 ± 1273
E7	France, Arcachon- Hautebelle	N 44°43'	27-May	13-Aug	27-Oct	10293 ± 1246	9045 ± 2661	9197 ± 588
E8	France, Noirmoutier	N 46°98'	27-Jun	25-Jul	11-Sep	5783 ± 1577	5478 ± 1554	6548 ± 1167
E9	France, St-Jacut-de-la-Mer	N 48°36'	25-Jun	29-Jul	20-Aug	3363 ± 1120	2752 ± 398	2038 ± 593
E10	Netherlands, Oosterschelde 1	N 51°26'	11-Jun	19-Aug	26-Sep	6318 ± 498	10318 ± 1052	7389 ± 1495
E11	Netherlands, Oosterschelde 2	N 51°34'	12-Jun	20-Aug	25-Sep	3745 ± 640	6115 ± 772	5427 ± 971
E12	Germany, Sylt	N 54°54'	na	30-Jul	12-Sep	na	6064 ± 668	9809 ± 838

For each reproductive shoot, the spathes were classified according to the six reproductive phases as described above, with an additional phase of decayed spathes, which consisted of remnants of the peduncles; occasionally with decomposed scales. All spathes were dissected under a binocular microscope, and the number of developed and aborted fruits was counted to determine fruit-set. Reproductive success was determined as spathe-set (see above) or fruit-set (the proportion of seed-bearing fruits of all fruits either seed-bearing or aborted). Density dependent successful reproduction was expressed as: 1) spathe-set vs density of reproductive shoots and 2) fruit-set vs density of flowering spathes. The density of flowering spathes (at the time of pollination) was not determined directly, and was considered to be equivalent to the density of the potential seed-bearing spathes at the time of sampling, because these spathes most likely underwent female anthesis at approximately the same time. We assessed annual seed production from the product of total number of spathes of all phases and the seed-bearing fruits per spathe. This assessment assumed that seed production per spathe did not vary throughout the reproductive season, and no more spathes would be formed after our sampling. This is reasonable, because very few reproductive shoots would be formed after our sampling, as the reproductive season finishes in September/October (also see Results: Spathe Density across Europe).

### Spathe density across Europe

*Z. noltei* plants were sampled with cores of 10 cm diameter ( $n=5$ ) at the 12 locations at the beginning, the peak and the end of the seagrass growing season (total  $n=15$  per site). The choice of the sampling time was site-specific and tuned to the growing season length for each site (Table 7.1). Following collection, the seagrass samples were washed on site with freshwater and stored in wet tissues for preservation during transportation to the Royal Netherlands Institute for Sea Research (NIOZ) in Yerseke, Netherlands. The number of foliar shoots and spathes were counted to determine respectively shoot and spathe density (no.  $m^{-2}$ ). Only fully-grown spathes with flowers or fruits (phases 2-5) were considered.

### Statistical analysis

Potential differences in biomass and density of reproductive shoots among meadow sections were analyzed with ANOVA and posthoc Tukey test. We applied a linear regression to below-ground biomass and number of fruits of the pooled data of the four sampling areas, to verify whether the

production of seed-bearing fruits depended on internal reserves (which are stored in the rhizomes). Generalized Additive Models (GAM) with a logit link function (for binary data) and polynomial smoothing of x-variable (x being the number of reproductive shoots) were applied to test for significant trends of spathe-set and density of the reproductive shoots for all pooled data. Subsequently, a separate GAM analysis was applied to the data of the three meadow sections along a tidal gradient at Oostdijk (channel, continuous and elevated) with meadow section as a cofactor, to test whether density-dependent probabilities of spathe fertilization differed with the position along the tidal level. A separate GAM analysis was applied to test for differences in density-dependent spathe-set in the continuous population at Oostdijk and the fragmented population at Goesse Sas. These GAM-analyses were also realized for fruit-set vs the density of flowering spathes.

A two-way ANOVA, followed by a posthoc Tukey test, was realized to test for differences in spathe density during the different sampling times and between the different European sampling sites. All statistical analyses were realized with R 2.15.3 (R core Team 2013).

### Literature search

We searched the literature for manuscripts on the reproductive output and success of all plant species known to have hydrophilous pollination and recorded data on fruit- and seed-set.

## Results

### Density-dependent pollination success in *Zostera noltei*

At the Oosterschelde in between the end of August - beginning of September, *Zostera noltei* exhibited spathes at every reproductive phase (Figure 7.2a). The number of spathes varied considerably among the 8 sampling sites, and we found a clear positive relation between the densities of reproductive shoots and spathe-set (Figure 7.2b).

*Z. noltei* had fewer reproductive shoots and spathes in the minor tidal channels than in the typical continuous and elevated meadow sections at Oostdijk, despite the high leaf biomass exhibited (Tables 7.2-4, Figure 7.3). The fragmented population at Goesse Sas had similar density of reproductive shoots and spathes as those at the typical and elevated meadow sections of the continuous meadow at Oostdijk (Figure 7.3). The

production of fruits (of the pooled samples) showed no relationship with below-ground biomass ( $F=3.439$ ,  $p=0.688$ ,  $df = 58$ ,  $R^2=0.040$ ; Figure 7.4a).

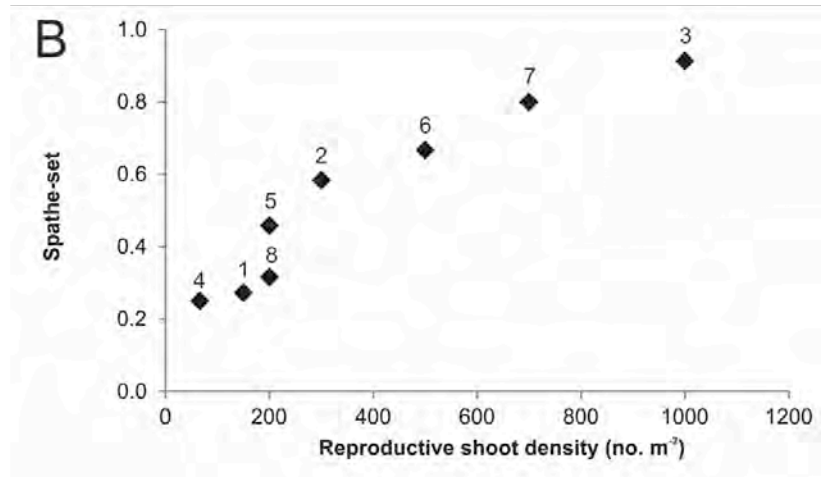
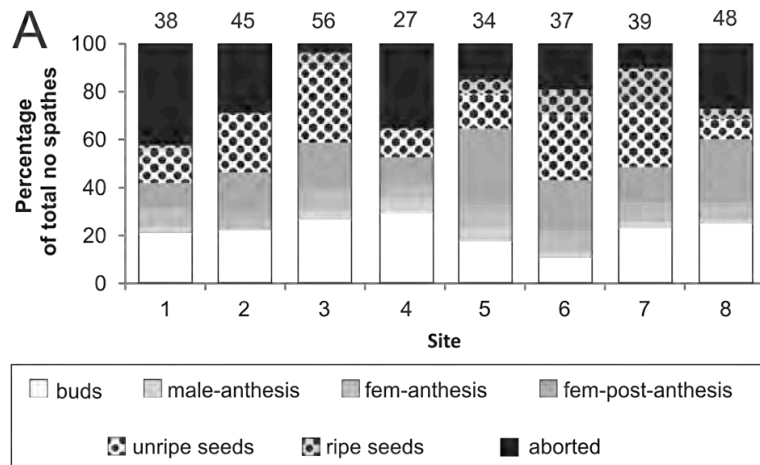


Figure 7.2: Reproduction of intertidal *Zostera noltei* throughout the Oosterschelde. Samples were collected in between 28-Aug and 01-Sep-2014. A. Phases of the spathe per site. The numbers above the bars indicate total number of collected spathe. B. Spathe-set (proportion of the number of fruit-bearing spathe of the number of flowering spathe) vs. density of reproductive shoots (median density measured in 10 quadrats of 10x10cm). The numbers above the data points indicate the sampling sites. See Fig 1 for site location map.

Table 7.2: Average ( $\pm$ SE) spathe density (no. m<sup>-2</sup>) of *Zostera noltei* in the Oosterschelde on 10-Sep-2014.

Meadow	Place	N	Bud	Male-anthesis	Female-anthesis	Female-post-anthesis	Seed-bearing	Aborted	Decayed	Total
Channel	Oostdijk	10	127 $\pm$ 76	25 $\pm$ 17	0	51 $\pm$ 27	51 $\pm$ 28	89 $\pm$ 34	38 $\pm$ 19	381 $\pm$ 178
Continuous	Oostdijk	20	407 $\pm$ 103	45 $\pm$ 17	45 $\pm$ 17	324 $\pm$ 93	859 $\pm$ 219	134 $\pm$ 30	1120 $\pm$ 295	2934 $\pm$ 646
Elevated	Oostdijk	10	293 $\pm$ 71	89 $\pm$ 33	64 $\pm$ 34	242 $\pm$ 58	458 $\pm$ 109	165 $\pm$ 33	904 $\pm$ 292	2215 $\pm$ 460
Fragmented	Goese Sas	20	274 $\pm$ 47	38 $\pm$ 23	51 $\pm$ 24	267 $\pm$ 41	866 $\pm$ 77	261 $\pm$ 20	567 $\pm$ 206	2324 $\pm$ 325

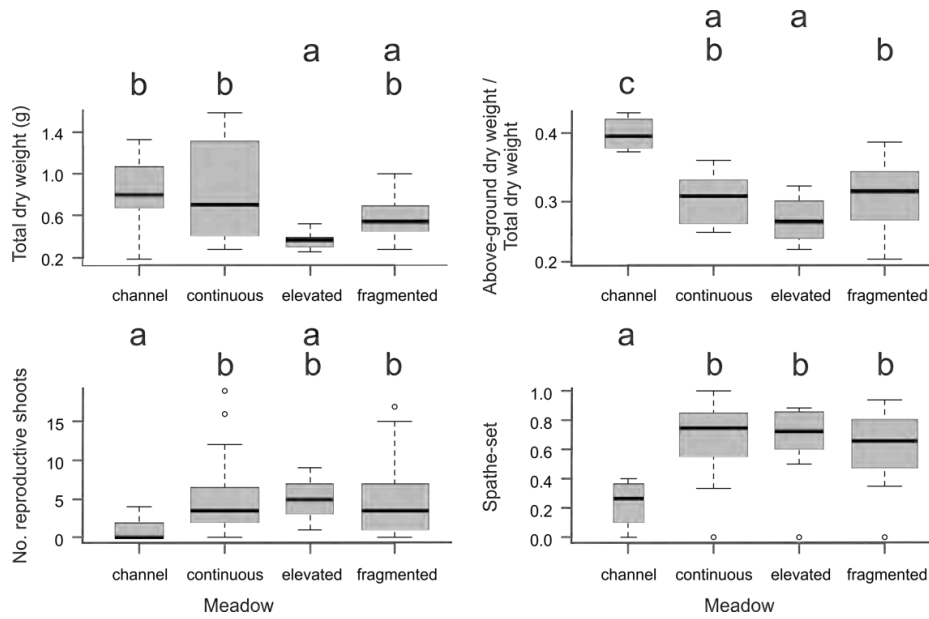


Figure 7.3: Boxplot of selected parameters per sample (10 cm diameter, N=5) of *Zostera noltei* in different meadow sections at Oostdijk and Goesse Sas in the Oosterschelde sampled on 10-Sep-2014, indicating median (line in box), upper and lower quartile (box), 1.5 \* inner quartile spread (whiskers) and outliers (circles). The letters above the graphs indicate significantly different groups identified by the Tukey posthoc test (significantly different at  $\alpha = 0.05$ ).

The GAM of the pooled data showed a significant relationship between spathe-set and density of the reproductive shoots ( $X^2 = 43.66$ ,  $p < 0.0001$ ,  $R^2 = 0.657$ ,  $n = 60$ ). The density-dependent spathe-set was similar across all tidal levels (channel, typical continuous and elevated) in the continuous population at Oostdijk (channel vs typical continuous:  $z = 1.482$ ,  $p = 0.138$ , channel vs elevated:  $z = 0.440$ ,  $p = 0.660$ ,  $n = 40$ ; Figure 7.4b). The comparison between typical continuous and fragmented populations showed that spathe-set was significantly lower at similar reproductive shoot density when the population was fragmented ( $z = 5.960$ ,  $p < 0.0001$ ,  $n = 40$ ; Figure 7.4b). Similar results were found for fruit-set related to flowering spathe density: the probability of fruit-set for all pooled data increased significantly with increasing density of flowering spathes ( $X^2 = 80.79$ ,

$p < 0.0001$ ,  $R^2 = 0.587$ ,  $n = 60$ ), and fruit-set was significantly lower in the fragmented than in the typical continuous population ( $z = 5.311$ ,  $p < 0.0001$ ,  $n = 40$ ; Figure 7.4c). Spathe- or fruit-set approached saturation at 5 reproductive shoots (Figure 7.4b) or 10 flowering spathes (Figure 7.4c) per sample, corresponding with respectively  $\approx 600$  reproductive shoots  $m^{-2}$  or  $\approx 1200$  flowering spathes  $m^{-2}$ .

The estimated annual seed production in the channel was low ( $\approx 200$  seed  $m^{-2}$ ), medium in the fragmented population and elevated meadow sections (respectively  $\approx 3100$  and  $3500$  seeds  $m^{-2}$ ), and high in the typical continuous meadow ( $\approx 5600$  seeds  $m^{-2}$ ; Table 7.4).

Table 7.3: Results of the One-Way ANOVA ( $df = 3$ ) for differences in dry weight and reproductive shoot density of *Zostera noltei* among meadows in the Oosterschelde. Results of the posthoc Tukey test are depicted in Fig. 3.

Parameter	F	p
Total dry weight	5.612	0.002
Above-ground /total dry weight	21.47	<0.0001
No. reproductive shoots	2.854	0.0452
Spathe-set	3.185	0.0324

Table 7.4: Summary (average  $\pm$  SE) for *Zostera noltei* populations in the Oosterschelde sampled on 10<sup>th</sup> September 2014. Total spathe density: density of spathes of all phases (including decayed ones). N number of samples

Meadow	N	Total vegetative biomass	Leaf biomass	Reproductive biomass	Foliar shoot density	Reproductive shoot density	Total spathe density	Estimated annual seed production	Rounded-up values of seed production
Channel	10	(g dry m <sup>-2</sup> ) 106.7 $\pm$ 12.9	(g dry m <sup>-2</sup> ) 43.2 $\pm$ 5.57	(g dry m <sup>-2</sup> ) 1.7 $\pm$ 1.23	(no. m <sup>-2</sup> ) 1194 $\pm$ 1294	(no. m <sup>-2</sup> ) 115 $\pm$ 55	(no. m <sup>-2</sup> ) 381 $\pm$ 178	(no. m <sup>-2</sup> ) 167 $\pm$ 92	(no. m <sup>-2</sup> ) 200
Continuous	20	103.9 $\pm$ 13.0	32.6 $\pm$ 4.6	4.6 $\pm$ 1.3	6958 $\pm$ 894	687 $\pm$ 148	2934 $\pm$ 714	5571 $\pm$ 1549	5600
Elevated	10	47.1 $\pm$ 3.5	12.9 $\pm$ 1.1	2.7 $\pm$ 0.5	3973 $\pm$ 558	586 $\pm$ 116	2215 $\pm$ 460	3529 $\pm$ 943	3500
Fragmented	20	75.6 $\pm$ 2.4	22.4 $\pm$ 0.8	4.8 $\pm$ 0.5	4927 $\pm$ 395	662 $\pm$ 82	2324 $\pm$ 325	3064 $\pm$ 667	3100

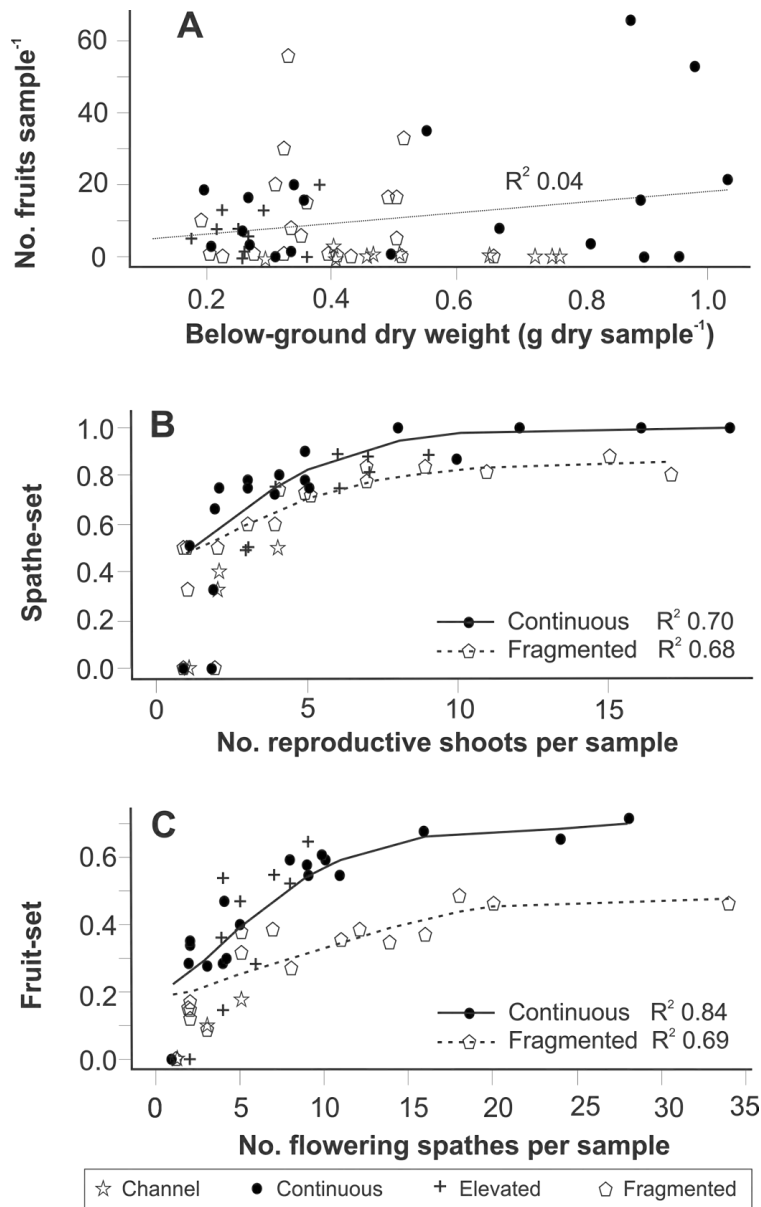


Figure 7.4: Reproduction of *Zostera noltei* in the Oosterschelde: A. Relationship between below-ground biomass and production of seed-bearing fruits, B. Density-dependent spathe-set, C. Density dependent fruit-set. Sample size=10 cm diam, N=5.

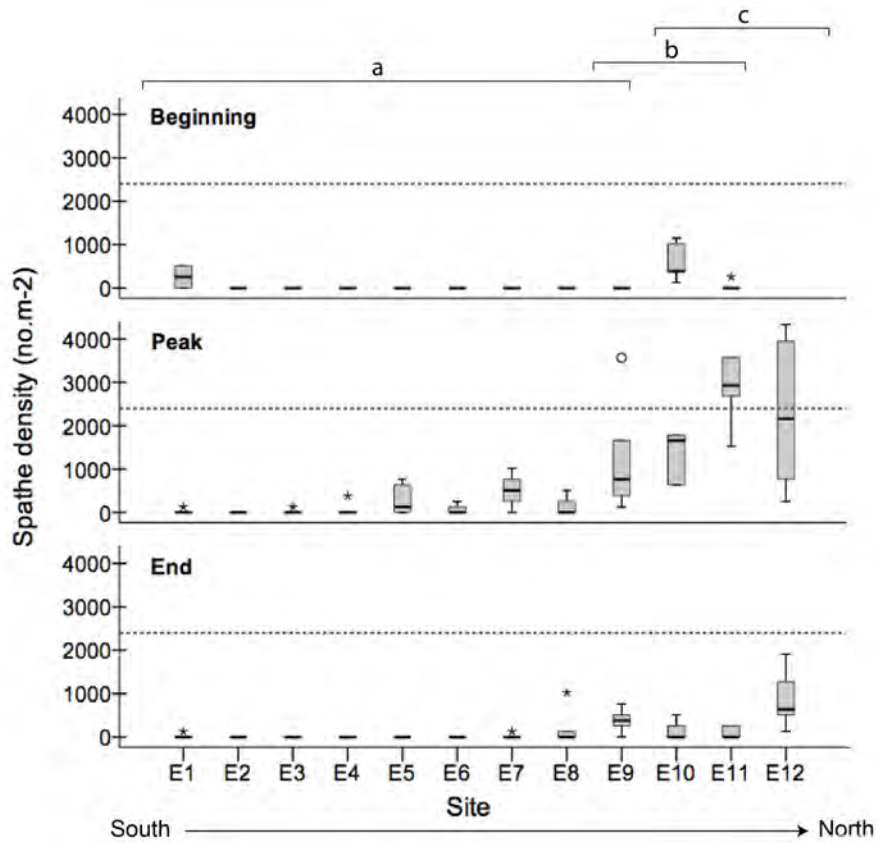


Figure 7.5: Spathe density of *Zostera noltei* in intertidal populations across a latitudinal gradient at different stages of the growing season (beginning, peak and end): Boxplots represents the spathe density for all sites (N=5); sites are displayed from South to North according to the initials assigned in Table 1. The dotted grey line represents the pollen limitation threshold defined at 1200 flowering spathes m<sup>-2</sup> ( $\approx 2400$  total no. spathes m<sup>-2</sup>).

### Spathe density across Europe

The density of spathes of *Z. noltei* at intertidal flats along the European Atlantic coast showed a significant temporal variation ( $F=21.771$ ,  $df=11$ ,  $p<0.001$ ), exhibiting a clear peak in the growing season (Figure 7.5). Spathe densities also varied significantly among sites ( $F=8.227$ ,  $df=11$ ,  $p<0.001$ ), and showed a remarkable trend of higher spathe densities at more northern sites, dividing the latitudinal range in two homogeneous subsets

(Tukey:  $p < 0.001$ ; Figure 7.5); being: Southern locations (from E1 to E8) and Northern locations (from E10 to E12). The highest spathe density was found in the Oosterschelde (Figure 7.1, site E11) at  $3133 \pm 565$  spathes  $m^{-2}$ , which was 6-120 times higher than at southern sites.

### Seed/fruit set in hydrophilous pollinated plants

Extensive literature search into the reproductive output of all hydrophilous pollinated plants reveals that the seed- or fruit-set (expressed as Seed:Ovule and Fruit:Flower ratio, respectively) of marine hydrophilous plants (seagrasses) is generally low in comparison with abiotic pollinated land plants with wind as the vector of pollen transport (Table 7.5). The number of ovules per female flower is low (1 to 9, but often 1 or 2) similar to anemophilous plants (Friedman and Barrett 2009). The density of the male shoots and ovules varies considerably among and within the species.

## Discussion

Pollen limitation may be an important factor in accelerating the decline of sparse or fragmented vegetation, and may impair recovery. We showed pollen limitation in the hydrophilous pollinated seagrass *Zostera noltei* at  $< 600$  reproductive shoots  $m^{-2}$ , a density that is only reached in northern 25% of the meadows studied across a large latitudinal gradient during the peak season. In addition, fragmentation contributed to a reduced fruit-set in one of the populations. Literature study revealed that pollen limitation might be a common phenomenon in seagrass species.

### Suboptimal reproductive output in *Zostera noltei*

We found that spathe- or fruit-set is generally reduced in *Z. noltei*, and that this decreased reproductive output is related to the density of reproductive shoots or spathes. Reduced seed-set can be due to (i) resource limitation, (ii) overproduction of ovules, (iii) reduced pollen quality or (iv) insufficient pollen production (Ashman et al. 2004, Knight et al. 2005). In our case, we discarded resource limitation, because we did not find a clear relationship between below-ground biomass and the total number of produced seed-bearing spathes or seeds, and rhizomes are the principal storage organs (Vermaat and Verhagen 1996).

An excess of ovule production (bet-hedging) may be a response to stochastic pollination (Burd 1994, Holland and Chamberlain 2007), and water is not always a reliable vehicle for pollen transport as strong

hydrodynamics may result in pollen dilution (Smith and Walker 2002, Verduin et al. 2002, Van Tussenbroek et al. 2009). In dioecious *Phyllospadix* species that grow in environments with high hydrodynamics on rocky shores, bet-hedging was suggested as a reason for the high female dominance (Buckel et al. 2012). However, this strategy doesn't seem necessary on monoecious plants such as *Z. noltei*, as they can rely on self-pollination as a mechanism of reproductive assurance (e.g. geitonogamy has been reported for the congeneric monoecious eelgrass *Z. marina*: Reusch 2001).

Self-pollination may result in reduced pollen quality, which is a third possible cause for reduced seed- or fruit-set. Balestri and Cinelli (2003) reported 87% of the seed loss due to abortions in the Mediterranean hermaphrodite *Posidonia oceanica*, attributed to a possible combination of limited pollination, resource limitation and inbreeding depression. Insufficient pollen quality due to self-incompatibility (geitonogamous selfing) was registered for the monoecious seagrass *Z. marina* by Reusch (2001). However, in a subsequent study, Hämmerli and Reusch (2003) found that this species had significant outcrossing independent of its genetic neighborhood, suggesting that this species may have a self-incompatibility system. Zipperle et al. (2011) reported that 88% of the offspring of *Z. noltei* in the Wadden Sea was outcrossed. More than half of the ovules in their population were aborted, which they assumed were selfed offspring that had failed to develop. But spathe or ovule abortions due to selfing would not decrease at increased flowering density as was found in this study. Seed:ovule ratios of *Z. noltei* in the Oosterschelde were maximally 0.7, and it is possible that geitonogamous selfing was responsible for 30% of the ovule abortions. But, the increased possibility of spathe- or fruit-set at respectively increased reproductive shoot or flowering spathe density in present study can only be explained by pollen limitation at lower densities, suggesting a demographic Allee effect.

We determined successful reproduction both as spathe- or fruit – set, which may be subjected to different pressures to assure reproduction (Holland and Chamberlain 2007). Holland and Chamberlain (2007) reported that low seed:ovule ratios of cacti were explained by excess (variable) ovule production and not by pollen limitation, whereas fruit:flower ratios were explained by equilibrium between resource and pollen limitation. The ovule production per spathe in *Z. noltei* is fairly constant (Table 7.4) and we found density dependent reproductive success for both spathe-set and fruit-set, suggesting that they were both pollen-limited.

### Latitudinal gradient

We found that high floral densities ( $>1200$  flowering spathes  $\text{m}^{-2}$  or  $> 600$  reproductive shoots  $\text{m}^{-2}$ ) were required for optimal pollination in *Z. noltei*. The total mature spathe density (bearing flowers or fruits) throughout western European stations, varied from no spathes to  $6624 \text{ spathes m}^{-2}$  for the upper limit, with an average of  $847 \text{ spathes m}^{-2}$  during the peak flowering season. Assuming that approximately half of these spathes were flowering at the same time and potentially cross-pollinate each other, as was registered for the Oosterschelde (Figure 7.2a), only 3 out of the 12 sampling sites (25%) had upper densities above this critical limit of  $1200 \text{ flowering spathes m}^{-2}$  ( $\approx 2400$  total spathe density) for optimal pollination during the peak of the flowering season. At other times flowering spathe densities were almost always below this limit (Figure 7.5). Thus, intertidal populations of this species across Western Europe are usually pollen limited.

Spathe density increased from south to north along a latitudinal gradient in Europe (Figure 7.5). The more southern populations were evergreen (they had green leaves throughout the year), although seasonal fluctuations in biomass could be considerable (Duarte 1989). Lower reproductive frequency in the more southern *Z. noltei* populations had also been documented by Buia and Mazzella (1991) in Italy, by Peralta et al. (2005) in Portugal, by Peralta et al. (2000) and Brun et al. (2003b) in Spain. This difference between northern and southern populations seems to be reflected in their genetic structure. The sizes of the genets tend to be smaller in northern than southern Europe: in N-Europe on the tidal flats they vary between  $1\text{-}10 \text{ m}^2$  (generally  $< 3 \text{ m}^2$ , Coyer et al. 2004), but in S-Europe they are up to  $50 \text{ m}$  in length (Coyer et al. 2004, Ruggiero et al. 2005). Thus, the Northern-European populations may be considered to be in a perpetually colonizing phase (sensu Peralta et al. 2000, 2005) with yearly recurrent population initiation by sexual and asexual propagules (seeds and dormant rhizome fragments), and clonal extension of the creeping rhizomes (Vermaat and Verhagen 1996, Zipperle et al. 2009a). In contrast, the southern, evergreen populations of *Z. noltei* depend much more on clonal propagation (Coyer et al. 2004).

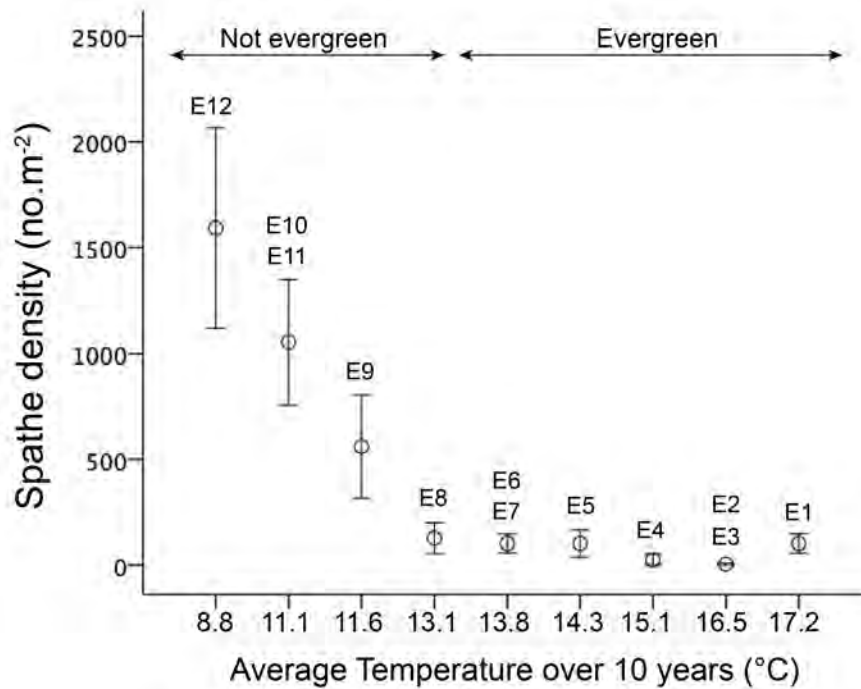


Figure 7.6: Spathes density ( $n=5$ ) and seasonal conditions of the populations (not evergreen=leaves and many rhizome sections dying-off during the winter or evergreen) against the average temperatures at the European sampling sites

The balance between vegetative and generative reproductive modes in clonal plants is thought to be mainly affected by factors suppressing sexual reproduction (Eckert 2002, Silvertown 2008). Models that have examined the conditions under which sexual and asexual reproduction coexist have found that sexual reproduction will only persist if temporal variability in resource supply is such that vegetative growth is (temporarily) not possible (Weeks 1993). This may explain why evergreen populations of *Z. noltei* are more common in the south than in the north with its colder winters, and we found a clear relationship between average air temperature and spathe density in *Z. noltei* (Figure 7.6). In a global warming scenario, northern populations may become more clonal when temperatures rise. However, stochastic climate extremes are also expected due to global warming (IPCC 2014), and the reduced sexual reproduction

even in the northern intertidal populations, may result in less genetic variability and therefore a smaller variable genetic pool to allow for genetic adaptations to changing conditions of this seagrass species throughout its whole distribution range. To a certain extent, this may be counteracted by possible increased reproductive effort that plants display under various forms of stress and disturbances (Alexandre et al. 2005, 2006, Cabaço and Santos 2012), but that will depend on the frequency and intensity of the climatic changes.

### **Are hydrophilous plants generally pollen limited?**

Very little is known concerning reproductive success of hydrophilous plants, and we could only find data for seed- or fruit set for 14 out of the > 100 species exhibiting true hydrophily (including  $\approx$  40 species of *Najas*, Haynes 1997). The low number of ovules per flower in the hydrophilous plants (Table 7.5; although *Halophila* spp. may have up to 60 ovules: Kuo et al. 1993) may be related with an abiotic pollination syndrome, as Friedman and Barrett (2009) suggested that the low cost of producing flowers in wind pollinated plants may favor having more flowers per plant with few ovules. This may enlarge the spatial distribution of the flowers and thereby enhancing pollen capture.

Pollination success in the freshwater *Najas marina* (Huang et al. 2001) and seed-set in *Zannichellia palustris* (Table 7.5) are relatively high; but the seed- or fruit-set of marine hydrophilous plants are generally much lower (Table 7.5). Although absolute seed-, or fruit-set do not indicate pollen limitation which can be assessed through pollen addition experiments (e.g. Burd 1994, Friedman and Barrett 2009) or density-dependent seed-, or fruit-set (e.g. this study), the generally low value implies that the Allee effect found in our study for *Z. noltei* may be common for marine angiosperms. Friedman and Barrett (2009) found a seed-set varying between 0.61-0.89 for ten herbaceous anemophilous land plants; and only one out of the ten studied plants had increased seed-set after artificial pollen addition, suggesting that these plants are generally not pollen limited. In contrast, Burd (1994) found significant pollen limitation in 62% out of 258 animal-pollinated species, based on comparisons of natural- and hand-pollinated plants. Further research into the relation between reproductive success and floral density in seagrasses, or pollination experiments is needed to confirm whether pollen-limitation in marine environments is common.

### Pollen limitation due to habitat fragmentation

In addition to density-dependent pollen limitation, we found that fragmented populations suffered more from pollen limitation, than continuous ones at similar flowering density (Figures 7.4b and 7.4c). Reusch (2003) also demonstrated for *Z. marina* that seed-set was 22% lower in isolated vegetation patches as compared to continuous populations, and Vermaat et al. (2004) found reduced seed-set in more fragmented meadows of *Enhalus acoroides*. Qin et al. (2014) found reduced seed-set of *Z. marina* in fragmented populations and at meadow margins in comparison with the centers of continuous meadows in China. This suggests that seed-set is not only positively affected by proximity of other reproductive shoots at a local (cm) scale, but also at larger scale proximity (patch scale, i.e. 1-10 meters). Thus, the recovery potential and colonization capacity will decline more than linearly when populations become fragmented or patchy which is commonplace when seagrass populations are under threat (Bell et al. 1999, Apostolaki et al. 2009). This increased Allee effect in fragmented populations may partially be compensated by higher investment in reproductive structures (Alexandre et al. 2005, Cabaço and Santos 2012).

### Implications for conservation and restoration

Though they are clonal plants, seagrasses depend on generative reproduction for recovery after disturbance, colonization of new areas and to maintain genotypic variability (Ouborg et al. 1999), thus for the longer-term resilience of the populations. For conservation measures, the reproductive density can be considered as an indicator for the reproductive capacity of the population. In our model species *Zostera noltei*, below 600 reproductive shoots  $\text{m}^{-2}$  (1200 flowering spathes  $\text{m}^{-2}$ ), seed production decreases more than linearly, which may reduce resilience and maintenance of genetic variation.

Reduced successful reproduction due to Allee effects may particularly threaten the conservation of northern *Z. noltei* populations, where recovery and recolonization processes by seed are part of the year-to-year maintenance of the population, though in our studied populations the densities of reproductive shoots was still sufficiently high. In contrast, southern populations depend less on seed recruitment, but are likely experiencing strong demographic Allee effects, making them vulnerable for large-scale disturbances where vegetative recolonization may be slow.

Pollen limitation, shown in our study in the model species *Z. noltei*, will result in non-linear population responses to disturbance; even more so

as fragmentation was shown to strengthen this Allee effect. Density dependent feedback (in our case depending of density of reproductive shoots, but also the 'density' of the patches) is self-reinforcing; when the population is decreasing, it leads to accelerated decline and impaired recovery. It should be realized that in the case of pollen limitation, which is a demographic Allee effect, recovery will be impaired even when environmental conditions improve. This is also the case with genetic Allee effects (shown in *Z. marina* by Reusch 2003, Hughes and Stachowicz 2009). In contrast, environmental Allee effects such as density dependent reduction of turbidity (van der Heide et al. 2007, Carr et al. 2010), toxicity (van der Heide et al. 2008, Govers et al. 2014a), or nutrition (Williams 1990, Jensen et al. 1998), will disappear when the environment is sufficiently improved. For example, if the water is sufficiently clear, density-dependent reduction of turbidity is not relevant anymore.

Our literature review suggests a low seed- or fruit-set is likely common in seagrass populations worldwide; this Allee effect may thus help to explain the accelerated global seagrass decline and limited recovery as witnessed during recent decades (Waycott et al. 2009, van Katwijk et al. 2016).

(next page)

Table 7.5: Summary of successful reproduction (seed:ovule ratio or fruit:flower ratio) of plants with true hydrophilous pollination. Fl.: Flower type (M: monoecious, D: dioecious). If the species are dioecious, the density of male reproductive shoots or inflorescences is mentioned. Values within brackets correspond to the average value. \* As *Heterozostera tasmanica* in publication, \*\*data for continuous population. \*\*\* part of seed-set due to self-pollination. The data for wind pollinated land plant are of 10 species of herbaceous plants of the genera *Carex*, *Rumex* and *Thalictrum* (from Friedman and Barrett 2009).

Species	Fl.	Place	No. male reproductive shoots m <sup>-2</sup>	No. male inflorescence m <sup>-2</sup>	No. ovules / flower	No. ovules m <sup>-2</sup>	Seed:Ovule ratio	Fruit:Flower ratio	Reference
<b>FRESHWATER</b>									
<b>Zannichelliaceae</b>									
<i>Zanichellia palustris</i>	M	Switzerland	na	na	01-Sep	na	0.56***		(Guo et al. 1990)
<i>Zanichellia palustris</i>	M	Utah, USA	na	na	01-Sep	na	0.91***		(Guo et al. 1990)
<b>SEAGRASSES</b>									
<b>Cymodoceaceae</b>									
<i>Syringodium filiforme</i>	D	Mexico	0-310 (77)	0-310 (77)	2	0-4972 (845)	0.02-0.5		(van Tussenbroek and Muhlia-Montero 2013)
<b>Hydrocharitaceae</b>									
<i>Enhalus acoroides</i>	D	Philippines	0-2			0-18		0-0.7	(Vermaat et al. 2004)
<i>Thalassia testudinum</i>	D	Mexico	01-Jul		02-May	3-88 (35)	0.5-0.7		(Van Tussenbroek et al. 2010)
<b>Posidoniaceae</b>									
<i>Posidonia oceanica</i>	M	Italy		56-137	1	233-574	0.02		(Balestri and Cinelli 2003)
<i>Posidonia australis</i>	M	SW Australia		46	1	736	0.27		(Smith and Walker 2002)
<i>Posidonia sinuosa</i>	M	SW Australia		42	1	420	0.31		(Smith and Walker 2002)
<i>Posidonia coriacea</i>	M	SW Australia		01-Mar	1	18-70	0.2-0.24		(Campey et al. 2002)
<b>Zosteraceae</b>									
<i>Phyllospadix torreyi</i>	D	SW-USA	0-100	0-800	1	2767-9756		0.6-1.0	(Williams 1995)
<i>Phyllospadix scouleri</i>	D	NW-USA	<1		1		0.02-0.7		(Shelton 2008)
<i>Phyllospadix serrulatus</i>	D	NW-USA	<1		1		0.004-0.37		(Shelton 2008)
<i>Phyllospadix torreyi</i>	D	SW-USA	<1-16		1	22-208	0.02-0.71		(Buckel et al. 2012)
<i>Zostera tasmanica</i> *	M	W-Australia		0-5	1	0-67	0.89		(Campey et al. 2002)
<i>Zostera marina</i>	M	NE-USA	53	403	1	265-2544	0.20-0.72		(Churchill and Riner 1978)
<i>Zostera marina</i>	M	NE-USA	303-424 (353)	353-2471	1	1765-15532	0-0.87 (0.21)		(Silberhorn et al. 1983)
<i>Zostera marina</i> **	M	Germany-Baltic S.	Jan-27				0.48-0.63		(Reusch 2003)
<i>Zostera marina</i>	M	China-Moon Lake	21-650	61-2210	1	648-56421	0.54-0.85		(Qin et al. 2014)
<i>Zostera noltei</i>	M	Portugal	126	252-1008 (529)	1	2600	0.12	0-0.75 (0.22)	(Alexandre et al. 2005)
<i>Zostera noltei</i>	M	Germany-Wadden S	310		1	1240	<0.5		(Zipperle et al. 2009b)
<b>LAND PLANTS</b>									
Wind pollinated	M&D	Canada	na	na	often 1	na	0.61-0.89		(Friedman and Barrett 2009)



## CHAPTER 8

### Discussion: The resilience of temperate seagrass meadows in a changing environment

#### Context and aim of the thesis: a recap

##### *Introduction*

Temperate seagrass meadows form highly valuable ecosystems in the marine environment. They are considered as ecosystem engineers, as their presence modifies the environment, creating suitable habitats for many organisms and promoting biodiversity (Jones et al. 1994, Bos et al. 2007, Bouma et al. 2009). They are, however, exposed to an increasing number of threats, mostly linked to human activities (Halpern et al. 2008). The main threats to seagrasses in temperate systems come from eutrophication (Taylor et al. 1995, Burkholder et al. 2007) and plant removal (Alexandre et al. 2005, Cabaço et al. 2005, Erftemeijer and Lewis 2006, Cabaço and Santos 2007, Eklöf et al. 2008a, 2008b), individually or jointly affecting their resilience. As a consequence, seagrasses have been declining at an alarming rate over the past decades, threatening the diversity and stability of coastal ecosystems (Orth et al. 2006, Waycott et al. 2009). In order to preserve coastal ecosystems and seagrass meadows from collapse, we need to better understand their resilience and chances for survival under threats.

##### *Aim and objectives*

The present thesis aimed at understanding the resilience of temperate seagrass meadows in a globally changing environment in order to prevent collapse. To answer this question we looked into: (i) indicators of resilience, needed for monitoring; (ii) strategies and mechanisms of resilience allowing seagrasses to resist stresses and to recover from disturbances; and (iii) the influence of climatic – latitudinal – gradients as drivers of seagrass traits, resilience and indicators (see Box 8.1 for questions and answers relative to this thesis).

**Box 8.1** Questions and answers

		Question	Answer
INDICATORS	Chapter 2	How does the response of two seagrass indicators differ under threat (nutrient enrichment)?	Both traditional (cover) and theoretical (CSD-recovery) indicators give an opposite response to threat: the higher the cover, the lower the recovery; potentially due to a physiological imbalance in seagrass leaves following nutrient addition.
	Chapter 3	What is the effect of timing of a disturbance on seagrass resilience and indicators of resilience?	Timing of the disturbance is essential for the resilience of temperate seagrass meadows and the response of indicators: recovery is lowest at the peak of growth, when cover is at its highest. The response of indicators is negatively correlated during the growing phase and positively correlated at the end of the growing season.
STRATEGIES	Chapter 4	What is the relative importance of seagrass ecosystem engineering vs. growth rate as strategies for their resilience?	Ecosystem engineering can alter the expected response of plants as based on Grime's growth strategies by making fast-growing plants physiologically more resistant to sulfide stress and a faster recoloniser, hence more resilient than a slow grower.

## THE INFLUENCE OF GLOBAL GRADIENTS

Chapter 5	<ul style="list-style-type: none"> <li>• How do unpredictable short-term stress events affect the seagrass capacity to withstand seasonal changes?</li> <li>• How this can affect seagrass long-term resilience and survival in a globally changing environment?</li> </ul>	<p>Short-term stress events reduce seagrass resilience and their capacity to store the carbon reserves they need to withstand seasonal changes such as the winter period.</p> <p>This is particularly important for northern seagrass populations that rely on the carbon reserves they store over their growing season.</p>
Chapter 6	<ul style="list-style-type: none"> <li>• How do seagrass mechanical traits vary along a latitudinal and seasonal gradient?</li> <li>• What is the influence of seagrass morphological and physiological traits on their mechanical response to eutrophication?</li> </ul>	<p>Seagrass mechanical traits vary along a latitudinal gradient, with tougher and stiffer leaves in southern Europe than in northern Europe. This variation is also linked to the seagrass meadow nutrient status, shaping their response to nutrient enrichment.</p>
Chapter 7	<p>Is there an Allee effect in hydrophilous plant, i.e. seagrass populations along the Atlantic European coast?</p>	<p>There is an Allee effect due to pollen limitation in seagrasses and a strong influence of temperatures on their reproductive success.</p>

### *Methodology*

The whole thesis was based on experimental data gained through the implementation of various disturbance-recovery field experiments in temperate seagrass meadows located in China (Shandong province; **Chapters 2, 3 and 4**) and across Western Europe (along the Atlantic coast from Spain to Germany; **Chapters 5, 6 and 7**). The manipulative experiments were implemented by creating a stress to mimic the effect of eutrophication on seagrass resistance through nutrient addition by adding slow-release fertilizers (**Chapters 2, 5, 6**) or organic matter, in the form of cellulose (**Chapter 4**). Recovery was measured as the plant regrowth during the experimental period following an additional disturbance created by removing the above-ground biomass through leaves clipping, leaving only the below-ground and sheaths in place (**Chapters 2, 3, 4, 5**). All experiments were full factorial, enabling us to investigate the individual and joint effects of stress and disturbance, in short-term experiments (all manipulative experiments never lasted more than 6 weeks) but with potential long-term impacts (i.e. absence of recovery, physiological traits affected). Part of the thesis was also based on monitored data, collected across Western Europe (**Chapters 5, 6 and 7**).

### Indicators of resilience

The increasing decline of seagrass meadows over the past decades raised the need to find good indicators for their resilience and health status. Various indicators looking at multiple seagrass traits ('multivariate indexes') have been identified, and monitoring programs are being established in an increasing number of areas (Duarte et al. 2004a, Krause-Jensen et al. 2004).

With this thesis, we raised the question on the proper interpretation of such indicators to estimate seagrass health and resilience, and how to ideally monitor seagrass ecosystems in order to predict collapse. To do so, we compared the response of two indicators: Cover, a traditionally used indicator for seagrass health status and measured by calculating the leaf area index (LAI); and critical slowing down (CSD), a more theoretical and new insight, as an indicator for seagrass resilience. According to the alternative stable state theory, a CSD is measured when the resilience of the system is reduced close to a transition, i.e. bifurcation or tipping point (van Nes and Scheffer 2007, Chisholm and Filotas 2009). In this thesis, CSD was measured by looking at the rate of recovery after an additional disturbance;

reduction of this recovery rate would indicate a lowered resilience of the system.

Our results revealed that, on the short-term, both indicators were having an opposed response: recovery decreased when cover was higher (**Chapter 2**). This opposed response was measured particularly during the seagrass growing phase, independently from local conditions such as hydrodynamics or nutrient status (**Chapter 3**). Furthermore, we found that timing of the disturbance during the seasonal growth of seagrasses was crucial in determining their resilience and the response of indicators. Thus, when cover peaked, resilience (expressed as relative recovery from disturbance) of the meadows was indicated to be lowest.

This conclusion was confirmed after the occurrence of short-term green tides at the peak of growth at one studied seagrass meadow (**Chapter 3**). Despite the additional stress caused by the green tide, cover was not affected. Recovery, in contrast, was strongly reduced, potentially due to the presence of algae preventing from regrowth for instance (Burkholder et al. 2007, Martínez-Lüscher and Holmer 2010, Han et al. 2015). It might be speculated that the plants, under disturbance and additional stress (green tides/algae shading) might experience a trade-off between recovering from the disturbance through re-growth (Rasheed 1999, Macreadie et al. 2014) and maintaining their below-ground stock for better chances to survive the winter period (Vermaat and Verhagen 1996, Govers et al. 2015). Such trade-off might explain the difference in recovery rates. Likewise, a potential explanation of the mechanisms underlying the conflicting response between indicators in **Chapter 2** may result from the unimodal response of plants to nutrient enrichment: increased plant dimensions following fertilization (Short 1983, Marschner 1995) but decreased growth and physiological imbalance due to nutrient toxicity (Pearson and Stewart 1993, Touchette and Burkholder 2000b, Burkholder et al. 2007). Along this eutrophication trajectory, prior to collapse, increasing plant cover thus may coincide with increasing physiological imbalance, which likely diminishes plant recovery potential.

The two indicators investigated were indicators related to seagrass health status and resilience at the population level. All in all, we concluded that their opposite response might be misleading as a good estimate of a seagrass meadow health status and resilience. The response of the two indicators was dependent on the effect of eutrophication (**Chapter 2**), timing within their growing season (**Chapter 3**), but also species-specific

traits (**Chapter 4**) and their capacity to adapt to their environment (**Chapters 5 to 7**). In order to fully understand the health status and resilience of a particular seagrass meadow, other parameters need to be evaluated such as species-specific traits or environmental gradients and conditions (Dale and Beyeler 2001, Fitch et al. 2014). Moreover, seagrasses have the capacity to adapt to their environment by changing their morphological, physiological and mechanical traits. In a changing environment or under threats, this plasticity or capacity to adapt to their environment becomes essential for their resilience. We identified, in this thesis, several strategies and mechanisms related to their growth rates, ecosystem engineering capacity, carbon storage and mechanical traits that can make them more or less resilient to stress and disturbances, and preserve them from collapse.

## Strategies and mechanisms of resilience

### *Growth rate and ecosystem engineering as strategies*

Seagrasses can be characterized by different growth strategies, related to their inherent growth and recovery rates, shaping their response to stresses and disturbances. As stated by Grime (1977), plants can be divided into three main growth strategies: stress-tolerant species, being slow-growers with relatively 'expensive' leaves, competitive and ruderal, being both fast-growers with 'cheaper' leaves (Grime 1977). Seagrasses are also considered as ecosystem engineers, implying the existence of feedback loops fundamental for the stability of coastal ecosystems (Suding et al. 2004, van der Heide et al. 2007, 2011, Carr et al. 2010, Suykerbuyk et al. 2012). Indeed, their canopy properties and below-ground structures drive several self-sustaining feedbacks, also useful for other organisms and referred to as their ecosystem engineering capacity.

We demonstrated experimentally that ecosystem engineering is an essential strategy to cope with stressful and disturbed environment by making some seagrass species more resilient (**Chapter 4**). Moreover, we showed that ecosystem engineering could alter the expected response to stress and disturbances as based on Grime's growth strategies by changing the plant-environment feedback loop.

Depending on the nature of the ecosystem engineering capacity (i.e. based on physiological or structural traits), as well as the nature of the environment (i.e. stimulating growth or stimulating the formation of physically strong tissues), plants can be strong or weak ecosystem

engineers (Jones et al. 1994, 1997, Widdows et al. 2008, Bouma et al. 2010). Ecosystem engineering hence forms an important strategy for seagrass resilience in a changing environment, in addition to their inherent growth strategy. Stress-tolerant slow-growing species (e.g. *Zostera marina*) might be more competitive in stable environments (Jovanovic et al. 2015). By investing more of their resources in strong structural traits (morphological and mechanical), and less in regrowth (Grime and Hunt 1975, Grime 1977), they become more resilient to the physical stresses they undergo (Widdows et al. 2008). In contrast, fast-growers (ruderal or competitor) might be more competitive when conditions change, as observed for *Zostera japonica* (**Chapter 4**) and *Zostera noltei* (**Chapter 5**) by having a faster physiological turnover and spending their resources in regrowth instead of expensive tissues (Grime 1977). Indeed, fast-growers are usually smaller with mechanically weaker leaves (La Nafie et al. 2013, de los Santos et al. 2016) making them more vulnerable to physical stresses. But by being capable of fast recolonisation and being strong physiological ecosystem engineer (**Chapter 4**), they become more resilient in a changing environment.

#### ***The use of carbon reserves***

During their seasonal growth, seagrasses build up carbon reserves, in the form of non-structural carbohydrates (i.e. starch and/or sucrose) (Alcoverro et al., 1999; Lee et al., 2007; Madsen, 1991; Olivé et al., 2007). They are usually stored through photosynthesis when the carbon demand (for growth and respiration) is balanced (Madsen 1991). It is well known that, to overcome both stressful low photosynthetic periods like the winter months (Govers et al. 2015) and short-term disturbances (Burke et al. 1996), seagrasses can exploit their carbohydrate reserves (Alcoverro et al., 1999; Lee et al., 2007; Madsen, 1991; Olivé et al., 2007). Hence, the consumption of their reserves allows them to react to changes and quickly recover from disturbances (**Chapter 5**). However, as they also need these reserves for winter survival, such use might lead to a resource depletion and influence their long-term survival, particularly in case of chronic stresses and disturbances (**Chapter 5**). The amount of carbohydrates needed for seagrass survival over stressful periods depends on abiotic factors such as temperature and light availability; but also on internal factors affecting the carbon balance, such as respiration and growth (Madsen 1991, Alcoverro et al. 2001, Govers et al. 2015).

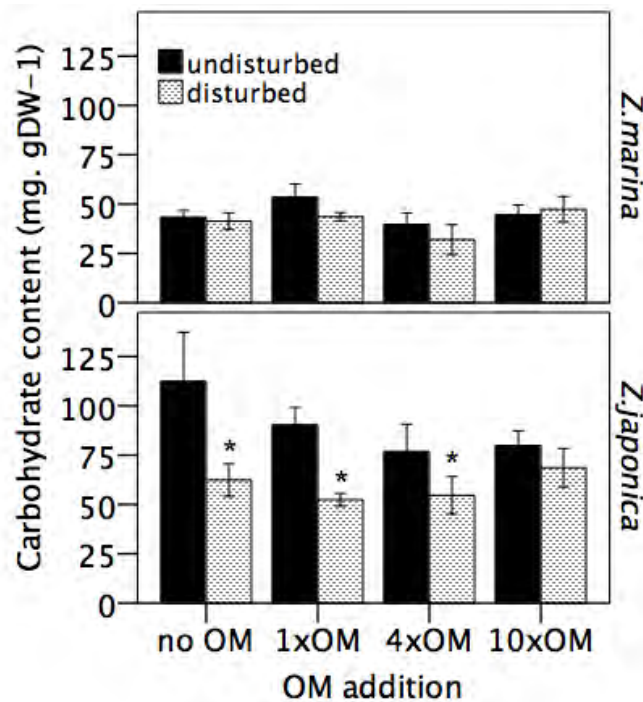


Figure 8.1. Bar plots representing the use of carbohydrate reserves for two seagrass species: The slow-growing resource efficient *Zostera marina* (top graph) and the fast-growing *Zostera japonica*. Results are based of samples described in Chapter 4, where *Z. japonica* is using its carbohydrate reserves for recovery along an OM-stress gradient, as seen by the decrease in its reserves when disturbed (above-ground removal) whereas *Z. marina* did not.

In our study (**Chapter 4**), we show that a strong ecosystem engineering capacity can be beneficial for plants to resist stress, but it can also be costly, depending on the species. In **Chapter 3**, looking at recovery rates along the growing season for the species *Zostera marina*, a slow-growing resource efficient species, we showed that carbohydrate reserves in rhizomes varied between sites and over the growing season. Yet these reserves were not affected by the experimental small-scale disturbances applied throughout the growing season. Thus they could not explain the low recovery during mid-growing season (**Chapter 3**). However, when looking at other species such as *Zostera japonica* (Figure

8.1) or *Zostera noltei* (**Chapter 5**), both fast-growing species, we observed a reduction in their carbohydrate reserves following a disturbance (**Chapter 5**). Such differences between species, mostly due to their growth strategy (Grime 1974, 1977), create disparities in their response to stress or disturbances, hence their overall resilience (Holling 1973, Scheffer et al. 2001, Beisner et al. 2003, Carr et al. 2012). It can be speculated that on the short-term, being a fast-grower is beneficial for the plants but on the long-term the over-consumption of their reserves to recover from disturbance might be enough to tip the system and lead to collapse, particularly when done at the end of the season (**Chapter 5**).

Overall, the use of carbohydrate reserves as a resilience mechanism depends on the species (growth strategies; unpublished results from **Chapter 4**, Figure 8.1), the environmental conditions (i.e. exposure to hydrodynamics, nutrient status; **Chapter 3 and 5**), the climatic settings (length and strength of winter; **Chapter 5**) and the occurrence of short-term stress events during their growing season (**Chapter 5**).

#### ***Leaf mechanical resistance to breakage***

Mechanical traits of seagrass leaves are important to consider when looking at the potential resistance a leaf can have when facing high currents, waves or herbivory (Onoda et al. 2011). They are evaluated by measuring the strength, stiffness or extensibility of tissues before breakage and have been the focus of several studies on freshwater, terrestrial plants or macroalgae (Onoda et al. 2008, 2011, Puijalon et al. 2008, Demes et al. 2013). However they remain still scarcely studied in seagrasses (Patterson et al. 2001, La Nafie et al. 2012, 2013, de los Santos et al. 2013, 2016).

The mechanical resistance of seagrass leaves depends on their species-specific traits (i.e. growth strategies) (de los Santos et al. 2016), their distribution along a seasonal or latitudinal gradient (**Chapter 6**), and their environment (de los Santos et al. 2010, La Nafie et al. 2012, 2013) such as their nutrient status (**Chapter 6**). In eutrophic conditions, seagrasses presented more brittle leaves, easier to break, potentially due to nutrient toxicity as opposed to oligotrophic conditions where leaves were stiffer (**Chapter 6**). But the leaves from eutrophied sites were also more extensible, as seen in the work of (La Nafie et al. 2013) following nutrient addition. This response was explained as a way to counteract their reduced strength: extensible leaves are more prone to deformation, which might slow down breakage. Such plasticity in their leaves mechanical traits (de los

Santos et al. 2013), also depending on their nutrient status (**Chapter 6**), hence help seagrass meadows to be more or less resistant to other stresses such as physical forces due to waves and currents (Puijalon et al. 2011).

## Drivers of resilience: the influence of global gradients

### *The effect of timing*

Seagrasses can be found in temperate systems along a large gradient of environmental and climatic conditions (Short et al. 2007), controlling the length of their seasonal growth and their population dynamics. In this thesis we have seen that timing of the disturbance along their growing season played an important role in the evaluation of indicators of resilience (**Chapter 3**). The seasonal dimension of seagrass growth in temperate systems hence plays a major driving role for seagrass resilience, particularly the winter period (**Chapter 5**) and the peak of growth (**Chapter 3**). It is along their growing season that seagrasses store their carbohydrate reserves (Madsen 1991, Alcoverro et al. 1999, Olivé et al. 2007, Lee et al. 2007), essential for their growth, resilience and survival over winter (Govers et al. 2015) (**Chapter 4 and 5**). The production and development of seeds also depends on this seasonal growth (Meling-Lopez and Ibarra-Obando 1999, Alexandre et al. 2006) (**Chapter 7**). Any stochastic disturbance or stress during their growing season might then affect the resilience of seagrass meadows, as seen in this thesis, with: a lowered recovery due to nutrient enrichment in their growing phase (**Chapter 2**) or at the peak of growth (**Chapter 3**); a higher consumption of carbohydrate reserves due to short-term stress events at the end of the season when they should be the highest (**Chapter 5**); and reduced leaf mechanical strength at the end of the season (**Chapter 6**).

### *The influence of climatic – latitudinal - gradients*

Global gradients related to – relatively predictable - climate settings are known to play a major role in seagrass distribution (Short et al. 2007, van der Heide et al. 2009, Valle et al. 2014). With this thesis we demonstrated that these gradients could also play an important driving role on their traits such as their reproductive success, carbon reserves and storage, physiological and mechanical traits (**Chapters 5 to 7**) (Figure 8.2).

The range of climatic conditions found along a latitudinal gradient, such as the European Atlantic coast, can strongly influence seagrass seasonal dynamics and growth strategies. Yearly temperatures and daylight hours are on average higher in the south than in the north, influencing the length of the seagrass growing season (i.e. longer growing season in the south; Figure 8.2). In southern Europe, temperatures are milder and daylight hours longer in winter, allowing a higher photosynthetic production than in northern Europe (Touchette and Burkholder 2000a, Olivé et al. 2007). Evergreen seagrass meadows can hence be found in southern latitudes (Pérez-Lloréns and Niell 1993, Auby and Labourg 1996) (Figure 8.2). In northern Europe, winters are usually very cold, with low daily light doses, while summers present milder temperatures with long daylight hours. In order to cope with such strong seasonal dynamics, in winter, only the below ground biomass – with limited leaf cover – and seed banks remain until spring when new shoots grow again (Vermaat and Verhagen 1996).

To recolonise after winter, northern seagrasses hence use the carbon reserves stored in their rhizomes during their growing season (Madsen 1991, Govers et al. 2015), as seen with a larger depletion of carbohydrate reserves at the beginning of the growing season found in northern populations (**Chapter 5**). Then, as the conditions for growth and respiration are optimal during their growing season (high daily light doses and mild temperatures), the carbohydrate reserves of seagrass meadows located at high latitudes thus increase (**Chapter 5**; Figure 8.2). This carbon storage allows the seagrass beds to better endure the stressful winter period and regrow from their reserves for the next growing season. These patterns of higher carbohydrate storage by northern populations before winter, and stronger depletion during winter, coincide with a higher reproductive effort found in Northern – deciduous – populations (**Chapter 7**; Figure 8.2).

Seagrasses are marine flowering plants. It has been shown that under various forms of stress and disturbances, seagrass increase their reproductive effort (Alexandre et al. 2005, Cabaço and Santos 2012). In this thesis we show that at lower temperatures, i.e. in northern areas, the density of seed-bearing shoots was the highest (**Chapter 7**; Figure 8.2). Similarly to carbon storage, northern seagrass population hence adapted their seed production to cope with the more stressful conditions they experience, particularly during the winter period. It might furthermore be

speculated that the higher temperatures and increasing occurrence of heat waves in the south during summer form an additional stress for seagrass meadows. Under such stress, the energy demand for respiration might be too high (Marsh et al. 1986, Hansen et al. 2000), hence reducing storage of carbohydrate reserves during their growing season.

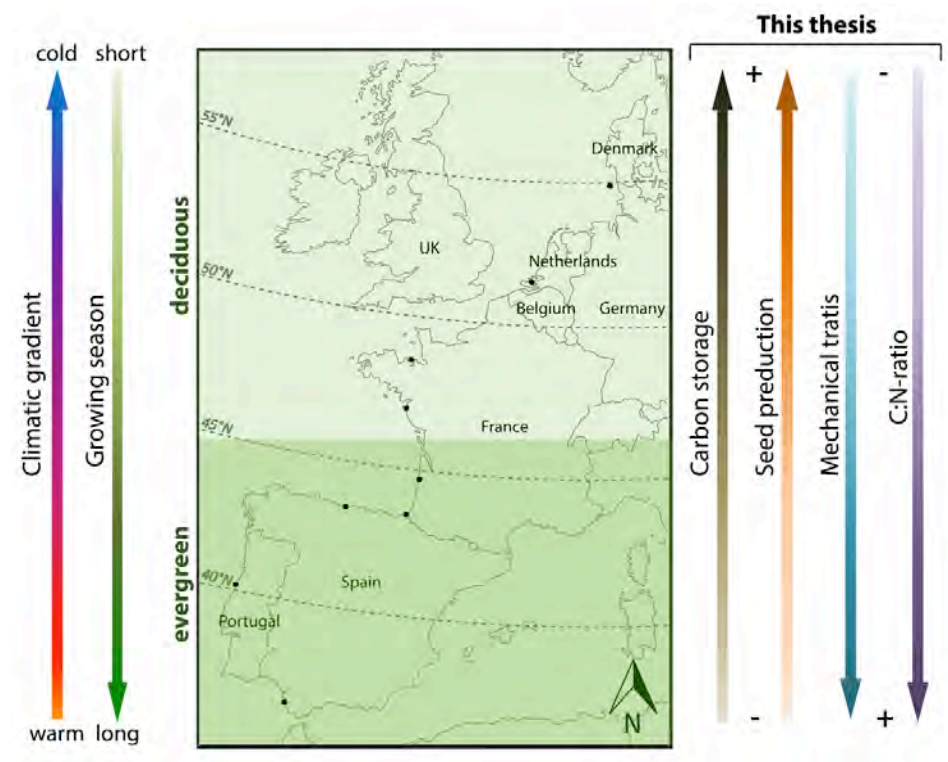


Figure 8.2. The influence of global gradients on seagrass traits. Along a Western European climatic gradient from south to north, the range of conditions such as light and mostly temperatures influence the seasonal dynamics of seagrass meadows. The growing season is hence longer in the south than in the north, making these meadows evergreen (constant growth throughout the year). With this thesis (arrows on the right hand side of the map), we observed that the use of carbohydrate reserves and their storage, as well as the production of seed bearing shoots over the growing season were higher in the north than in the south. In contrast we observed a decrease in mechanical traits (strength, breaking stress, stiffness) from north to south in combination to a decrease in leaves C:N-ratio.

The influence of latitude (and thus climatic gradient) was also important for leaf mechanical and physiological traits, with stronger and stiffer leaves with a higher CN ratio in the south than in the north (**Chapter 6**). This difference can be ascribed to their growth strategy, with southern populations being evergreen, hence more prone to invest in strong tissues than deciduous northern populations.

In conclusion, the northern-European populations may be considered to be in a perpetually colonising phase (*sensu* Peralta et al. 2000, 2005) with yearly recurrent population initiation by sexual propagules (seeds) (**Chapter 7**), making them genetically more diverse (Provan et al. 2007); and by asexual – clonal – extension of the dormant rhizomes relying on their carbon reserves after the winter period (Vermaat and Verhagen 1996; Zipperle et al. 2009a; **Chapter 5**). In contrast, the southern, evergreen populations depend much more on clonal propagation (Coyer et al. 2004), hence being productive all year due to good light and temperature conditions for photosynthesis (Bulthuis 1987, Olivé et al. 2007); and relying less on their carbon reserves (**Chapter 5**) or seed production (**Chapter 7**), with mechanically stronger leaves (**Chapter 6**) (Figure 8.2).

Overall, the distribution of seagrass meadows along a latitudinal gradient from south to north forms an important parameter to consider when looking at their resilience and the strategy they might develop to be resilient. As seen with our results, it is mostly their seasonal dynamics (length of growing season, winter intensity, summer temperature stress), or their evergreen vs. deciduous status that play a role in determining their resilience (**Chapters 5 and 7**). In fact, deciduous populations, submitted to colder winter would react in a completely different way to a stress in terms of plasticity or resilience than an evergreen population.

### Implications for the management of temperate seagrass meadows under threats

In a context of climate change and increasing occurrence of stochastic events (i.e. storms, extreme weather) and human-induced stresses (Easterling et al. 2000), it is important to understand how to better preserve coastal ecosystems. With this thesis we aim to provide, not only an insight into general seagrass research, but also a tool to improve the management of seagrass meadows. Our results comparing two indicators

showed that in order to evaluate seagrass resilience, looking at recovery rate, as a potential critical slowing down when submitted to an additional disturbance, could be more appropriate than cover (**Chapter 2 and 3**) on a short-term. That is, measuring a relative regrowth (recovery) as compared to natural conditions (cover) could be a good sign of whether a system is getting close to its bifurcation point. Yet, cover remains a very useful and straightforward indicator for the long-term monitoring of seagrass health status (McKenzie et al. 2003, Duarte et al. 2004a). We also show that it is important to consider timing in the evaluation of seagrass resilience and health status in temperate systems (**Chapter 3**). To effectively protect seagrass beds, conservation management should aim at avoiding disturbances particularly during the peak of the growing season, when resilience is lowest (**Chapter 3**). Furthermore, we established that it is important to understand the local conditions of the seagrass meadow, along a climatic – latitudinal – gradient (**Chapters 5 to 7**). Depending on the distribution along a climatic – latitudinal – gradient and on the condition of the seagrass meadow (i.e. nutrient status, exposure to hydrodynamics), the choice of indicators might be completely different. Managers and stakeholders should then think of different parameters before taking actions that might help preserve seagrass meadows (Box 8.2).

### The resilience of temperate seagrass meadows: conclusions

Understanding or evaluating the health status of a seagrass meadow is a complex task that requires knowledge of the local stressors or disturbances and climatic conditions a meadow experiences. Indicators are needed, but their response can be altered due to the seagrass capacity to adapt to their environment (Figure 8.3). That is, seagrasses use different strategies, involving changes in their morphological, physiological and mechanical traits, to become more resilient. In fact, the response of indicators and strategies involved in the resilience of seagrass meadows depend on their distribution along a climatic gradient (**Chapters 5 to 7**), their seasonal growth (**Chapters 3, 5, 6 and 7**), their plasticity, i.e. capacity to adapt their traits to environmental conditions (**Chapters 2, 5, 6**), their ecosystem engineering capacity and growth strategy (**Chapter 4**) (Figure 8.3).

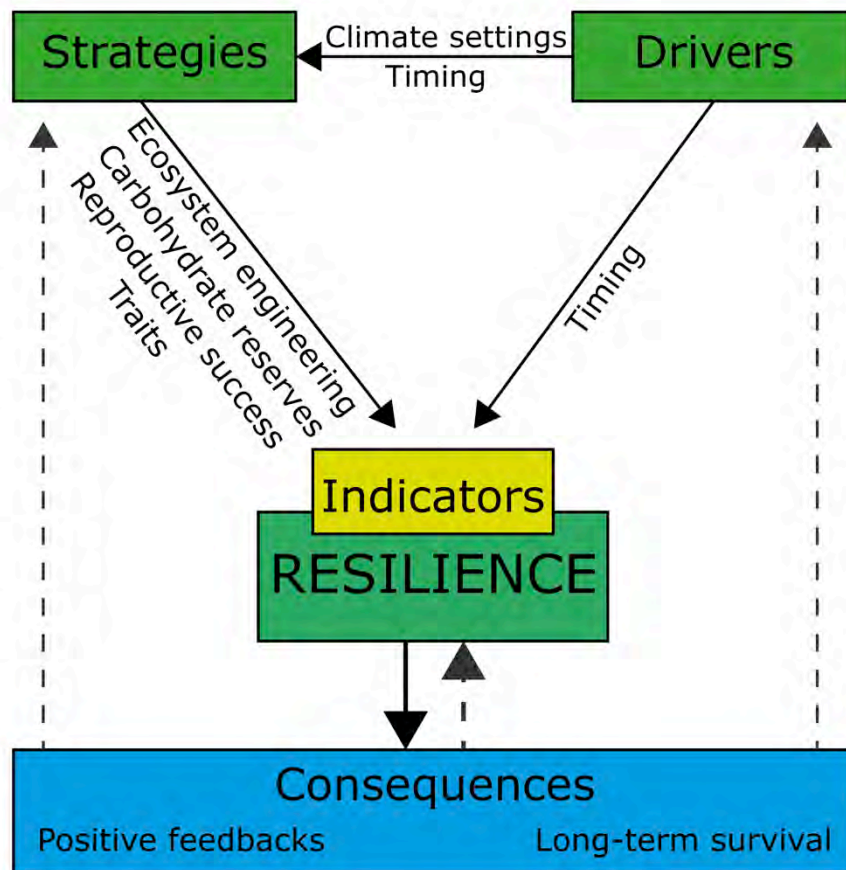


Figure 8.3: Synthetic diagram representing the findings of this thesis. Resilience and health status are measured by using indicators. But seagrasses hold different strategies that can increase their resilience and the response of indicators such as their ecosystem engineering capacity, the use of their carbon reserves, their reproductive success (i.e. production of seed-bearing shoots), or the adaptation of their morphological, physiological or mechanical traits. Such strategies, which can be species-specific, are influenced by global gradients such as climatic settings and seasonal dynamics (timing). The latter also plays a direct driving role on the resilience and response of indicators. The consequences are large, as these drivers and strategies can potentially affect the positive feedbacks and long-term survival of seagrass meadows.

Overall, all the mechanisms involved in seagrass resilience as well as the influence of global gradients have considerable consequences on the seagrass resilience and the stability of coastal ecosystems in the long-term. Seagrasses can adapt to their changing environment, as observed along a climatic – latitudinal – gradient (**Chapters 5 to 7**). But the additional effect of short-term stresses such as nutrient enrichment (to mimic eutrophication) might push the system towards its bifurcation point (Scheffer et al. 2009). When even a small disturbance occurs, the dramatic response of the system might thus lead to its collapse (van der Heide et al. 2007, van Wesenbeeck et al. 2008), by reducing the seagrass resistance to stress and recovery potential after a disturbance. The consequences for coastal ecosystems are large as a lowered resilience of seagrass meadows can affect their efficiency as ecosystem engineers and thus their positive feedbacks (van der Heide et al. 2007, 2011). The maintenance of positive feedbacks for seagrass meadows is essential (van Katwijk et al. 2016), as in turns it also affects their strategies for resilience (for instance, capacity to reduce sulphide stress through their root system, **Chapter 4**) and their capacity to adapt to climatic gradients (Figure 8.3).

Understanding the effects of global gradients (i.e. climatic – latitudinal – gradients) on resilience is clearly important in seasonal and bistable seagrass ecosystems (van der Heide et al. 2010b, Carr et al. 2010, 2012), but may also apply to other bi-stable temperate ecosystems such as e.g. temperate shallow lakes (Scheffer et al., 2001, Scheffer and van Nes 2007); salt-marshes (van Wesenbeeck et al. 2008); temperate reefs (Baskett and Salomon 2010); and temperate floodplains lakes (Chaparro et al. 2014). A time-scale in the evaluation of resilience and potential transition to another stable state (for instance, unvegetated) is to be considered at two levels: (i) recovery might slow down in time as an indicator of a lower resilience (van Nes and Scheffer 2007, Chisholm and Filotas 2009, Dakos et al. 2011) but also (ii) might vary along the growth curve of the system as a function of its growth rate, and seasonal dynamics.

**Box 8.2** Toolbox for management

Three main criteria to help manage a seagrass meadow:

- 1. What are the characteristics/traits of the seagrass meadow?**
- 2. What is the nature of the threat and nutrient status?**
- 3. At what stage of the season am I?**

1. Characteristics of the seagrass meadow

That is, you need to define which species form your seagrass meadow (to know their growth rate and ecosystem engineering capacity), or if the meadow is evergreen or deciduous. Such knowledge will help determine which strategies might play a role in the meadow resilience and chances of survival.

For instance:

- A slow-grower will be able to handle higher hydrodynamic stress than a fast-grower, whereas the fast-grower might be physiologically more resilient and capable to recolonise faster after eutrophication stress.
- A northern deciduous seagrass population will for instance be very sensitive to short-term stress events at the end of their growing season, when its carbon reserves should be highest.
- A southern evergreen seagrass population, naturally pollen limited would rely more on clonal growth. This will make it thus very sensitive to disturbances affecting their below or above-ground biomass, as their seed production could not guarantee their recovery.

2. Nature of the threat and nutrient status

That is, knowing whether the site is more eutrophic or oligotrophic, what type of additional stressors or disturbances is the seagrass meadow experiencing?

This is important in order to decide which indicator to use but also to understand what mechanisms of resilience are going to play a role.

Also the response of the seagrass meadow might differ depending on its nutrient status and the type of stress (chronic or acute).

For instance:

- When facing a physical stressors: indicator = mechanical traits;
- Under eutrophication stress: indicator = CN ratio or recovery or algae cover.

### 3. The stage of the seasonal growth

A stress is a bad as its effect in the most sensitive stage of the seagrass life cycle. Hence, depending on the length of the winter and the stage in the growing season, the seagrass response might have consequences for their future development.

For instance:

- A disturbance applied at the peak of growth when the resilience is lowest, might considerably affect the chances for a plant to store enough carbon reserves to overwinter and to regrow for the next growing season.
- A disturbance clearing large areas in a pollen-limited meadow at the end of the growing season could prevent the seagrasses from future recovery through seedling establishment.





## CHAPTER 9

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Summary (EN)  
Samenvatting (NL)  
Résumé (FR)

References

Acknowledgments

Curriculum Vitae



## SUMMARY

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Despite being highly valuable ecosystems, seagrass meadows are threatened worldwide, mostly by human activities. In order to preserve seagrass meadows from collapse, we need to better understand their resilience in a changing environment. By means of various manipulative field experiment in temperate systems, the present thesis addressed seagrass resilience by looking at (i) indicators of resilience as needed for monitoring; (ii) strategies and mechanisms of resilience allowing seagrasses to resist stresses and to recover from disturbances; and (iii) the influence of climatic – latitudinal –gradients as drivers of seagrass traits, resilience and indicators.

### Indicators of resilience

Indicators are needed in order to monitor seagrass ecosystems and to predict nearness to collapse. In this thesis, we raised the question on the proper interpretation of indicators to estimate seagrass health and resilience. We compared the response of two indicators: i) vegetation cover, which is a traditionally used indicator to assess the seagrass health status; and ii) *critical slowing down* which is a theoretically suggested indicator for seagrass health status in terms of resilience to disturbances, expressed as relative recovery from disturbance. The two indicators showed an opposite response to disturbance: the higher the cover, the lower the relative recovery from disturbance (Chapter 2). This was however only observed during the seagrass growing phase (Chapter 3). Indeed, we noticed that the timing of a disturbance relative to the seagrass seasonal growth period, were crucial for seagrass resilience, with highest recovery at the start of the growing season, and a decreasing recovery with higher cover (Chapter 3). This conflicting response between indicators was observed independently from wave exposure or nutrient status (i.e. same response even under eutrophication stress). These results do emphasize the need to carefully consider timing of monitoring of the indicators, as it forms a fundamental factor to evaluate indicators in terms of resilience. These findings are likely to extend beyond seagrass, also to other temperate seasonal ecosystems.

### Strategies/mechanisms for resilience

Seagrasses have the capacity to adapt to their environment by changing their morphological, physiological and mechanical traits. They moreover present different strategies, related to their growth rate (Grime, 1977), resource allocation or ecosystem engineering that make them more or less resilient to stresses and disturbances and preserve them from collapse.

In this thesis, we observed that the plant's responses to stress and disturbances deviated from the response predicted by Grime's growth strategies, under the influence of ecosystem engineering (Chapter 4). That is, the fast-growing seagrass species was not only a better recoloniser after disturbance as compared to a slower-growing species, but also more resistant to sulphide invasion. This higher resistance was explained by its strong capacity to reduce the stress through ecosystem engineering (i.e. release of oxygen through their root system). Ecosystem engineering hence forms an essential strategy to cope with stressful and disturbed environment by making some seagrass species more resilient. We speculate that this effect depends however on the nature of the ecosystem engineering (i.e. based on physiological or structural traits) as well as the nature of the environment (i.e. stimulating growth or stimulating the formation of physically strong tissues).

Another strategy for resilience evidenced in this thesis was related to their resource allocation and more specifically the use of their carbon reserves for resilience. Being photosynthetic organisms, seagrasses can store carbon reserves in their rhizomes during their seasonal growth. These reserves are stored in the form of non-structural carbohydrates and mostly used for growth or to survive the winter period. We showed that, when experiencing short-term stress events, seagrasses use their carbohydrate reserves to recover and regrow, particularly the fast-growing species (Chapter 4 and 5). Overall, we saw that the use of carbohydrate reserves as a resilience mechanism increased depending on the species (i.e. fast growth strategy), the environmental conditions (i.e. exposure to hydrodynamics, nutrient status), the climatic settings (cold winter temperatures and low daily light doses) and the occurrence of short-term stress events during their seasonal growth. It can be speculated that on the short-term, the use of carbon reserves for regrowth is beneficial for the plants. But on the long-term, the over-consumption of their reserves to recover from disturbance

might be enough to tip the system and lead to collapse, particularly when done at the end of the season (Chapter 5).

Being sessile organisms, seagrasses have the capacity to modify their structure or traits to resist the adverse effect of biotic or abiotic stressors such as waves or currents. It is known that the mechanical resistance of seagrass leaves to physical stress depends on their species-specific traits (i.e. growth strategies). In this thesis we also showed that seagrasses could adapt their mechanical traits depending on their distribution along a seasonal or latitudinal gradient but also depending on their environment such as their nutrient status (Chapter 6). In eutrophic conditions, seagrasses presented more brittle leaves, easier to break but also more extensible than in more oligotrophic conditions. This plasticity and adaptation to their local environmental conditions forms hence another important strategy for resilience.

### **The influence of climatic –latitudinal- gradients**

Seagrasses can be found in temperate systems along a large gradient of environmental and climatic conditions, controlling the length of their seasonal growth and their population dynamics. With this thesis we demonstrated that these gradients could play an important driving role on their traits such as their reproductive success, carbon reserves, physiological and mechanical traits (Chapters 5 to 7). We also observed that the seasonal dimension of seagrass growth in temperate systems plays a major driving role for seagrass resilience, particularly the winter period (Chapter 5) and the peak of growth (Chapter 3). In fact, depending on their local conditions and distribution along a climatic – latitudinal – gradient, seagrasses present different traits, shaping their resilience to external stresses or disturbances. The northern-European populations may be considered to be in a perpetually colonizing phase with yearly recurrent population initiation by sexual propagules (seeds) (Chapter 7), making them genetically more diverse; and by asexual – clonal – extension of the dormant rhizomes relying on their carbon reserves (Chapter 5). In contrast, the southern, evergreen populations depend much more on clonal propagation, hence being productive all year due to suitable light and temperature conditions for photosynthesis; and relying less on their carbon reserves (Chapter 5) or seed production (Chapter 7), with mechanically stronger leaves (Chapter 6).

### **Conclusions and implications**

Overall, all the mechanisms involved in seagrass resilience as well as the influence of global trends have considerable consequences on the seagrass resilience and the stability of coastal ecosystems in the long-term. Seagrasses can adapt to their changing environment, as observed along a climatic – latitudinal – gradient. But additional effects of short-term stresses such as e.g. nutrient enrichment inducing eutrophication, might push the system towards its bifurcation point. In that situation, even a small disturbance may induce a meadow to collapse, due to a reduced resistance to stress and recovery potential. The consequences of such collapse are large for the overall health of the coastal ecosystems. In general, a lowered resilience of seagrass meadows can affect their efficiency as ecosystem engineers and thus the positive feedbacks they induce. The maintenance of positive feedbacks for seagrass meadows is essential, as this affects their strategies for resilience (for instance, capacity to reduce sulphide stress through their root system) and their capacity to adapt to climatic gradients.

Our findings bear implications for the management of seagrass meadows. In a context of climate change and increasing occurrence of stochastic events (i.e. storms, extreme weather) and human-induced stresses, managers, scientists and stakeholders should consider the importance of: when (seasonal growth), where (latitudinal – climatic gradient) and how (nature of the threat, resilience strategies) seagrass resilience might be affected. Considering these parameters is essential to better preserve temperate seagrass meadows from collapse and to maintain the stability of their ecosystems.

## SAMENVATTING

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Zeegrasvelden zijn zeer waardevolle ecosystemen, maar staan desondanks wereldwijd onder druk door menselijke activiteiten. Om te voorkomen dat zeegrasvelden verder verdwijnen is het belangrijk om beter te begrijpen hoe de veerkracht van deze systemen zich gedraagt in een veranderende omgeving. In dit proefschrift beschrijf ik veld experimenten waarin de veerkracht van zeegras wordt onderzocht door te kijken naar (i) indicatoren voor de veerkracht van zeegras die geschikt zijn voor monitoring; (ii) strategieën en mechanismen die zeegras in staat stellen met stress om te gaan en zich te herstellen na een verstoring; en (iii) de invloed van klimaat – breedtegraad – gradiënten op de eigenschappen van zeegras, de veerkracht van zeegrasecosystemen en mogelijke indicatoren voor die veerkracht.

### Indicatoren voor veerkracht

Indicatoren helpen bij de monitoring van zeegrasecosystemen en bij het voorspellen of het systeem op het punt staat om in te storten. In dit proefschrift heb ik mij afgevraagd hoe gezondheids- en veerkrachtsindicatoren van een zeegrasveld geïnterpreteerd dienen te worden. Ik heb twee indicatoren met elkaar vergeleken: i) vegetatiebedekking, een veelgebruikte traditionele indicator om de gezondheid van zeegrasvelden te beoordelen, en ii) relatief herstel na een verstoring (*'critical slowing down'* op basis van theorie over alternatieve stabiele toestanden), een indicator die de veerkracht na verstoring weergeeft. Deze twee indicatoren reageren tegengesteld na verstoring: hoe hoger de bedekking, des te trager het relatieve herstel na verstoring (Hoofdstuk 2). Dit werd echter enkel waargenomen tijdens het groeiseizoen (Hoofdstuk 3). De timing van de verstoring ten opzichte van het groeiseizoen bleek van cruciaal belang voor de veerkracht van het zeegras; het herstel was het grootst aan het begin van het groeiseizoen en nam af wanneer de bedekking toenam (Hoofdstuk 3). Deze tegenstrijdigheid tussen de indicatoren werd onafhankelijk van blootstelling aan golven en hoeveelheid nutriënten waargenomen (zelfs wanneer er sprake was van eutrophiëring). Het moment van monitoring van deze indicatoren was van groot belang voor de evaluatie van de veerkracht van het systeem.. Dit

geldt waarschijnlijk niet alleen voor zeegras, maar ook voor andere seizoensafhankelijke ecosystemen in gematigd klimaat.

### Strategieën/mechanismen voor veerkracht

Zeegrassen kunnen zich aan de omgeving aanpassen door morfologische, fysiologische en mechanische eigenschappen van de plant te veranderen. De planten hebben verschillende strategieën zoals het aanpassen van de groeisnelheid (Grime, 1977), herverdeling van hulpbronnen (nutriënten, koolstof) of actief beïnvloeden van de omgeving ('*ecosystem engineering*') die ze meer of minder veerkrachtig maken tegen stress en verstoring en voorkomen dat het ecosysteem instort.

In Hoofdstuk 4 van dit proefschrift beschrijf ik dat de reactie van de plant op stress en verstoring afwijkt van wat wordt voorspeld door de groeistrategieën van Grime, en dat het verschil kan worden verklaard door het belang van het actief veranderen van de omgeving (Hoofdstuk 4). De snelgroeende zeegrassoort was niet alleen beter in staat het verstoorde gebied te herkoloniseren in vergelijking met de langzaam groeiende soort, maar was ook beter bestand tegen indringend giftig sulfide. Deze verhoogde weerstand kan verklaard worden door de mogelijkheid van de soort om stress te verlagen door middel van '*ecosystem engineering*', meer specifiek de afgifte van zuurstof door de wortels. Actieve beïnvloeding van de omgeving is dus een belangrijke strategie om te overleven in een omgeving met veel stress en verstoring. We speculeren dat dit effect afhangt van het type *ecosystem engineering* (bijvoorbeeld gebaseerd op fysiologische of structurele eigenschappen), maar ook van het type omgeving.

In dit proefschrift is ook bewijs gevonden voor een andere strategie om veerkracht te beïnvloeden, namelijk de herverdeling van grondstoffen. De herverdeling van koolhydraten kan ook gebruikt worden om de veerkracht van de planten te verbeteren. Zeegrassen kunnen door middel van fotosynthese koolhydraten maken en deze opslaan in de vorm van niet-gestructureerde koolhydraten in het rizoom tijdens het groeiseizoen. Deze kunnen naderhand gebruikt worden voor groei of om de winter door te komen. In dit proefschrift laat ik zien dat gedurende korte stressperiodes deze reserves ook aangesproken kunnen worden om te herstellen en terug te groeien, met name bij de snelgroeende soort (Hoofdstuk 4 en 5). Het gebruik van koolhydraten om terug te groeien als veerkrachtmechanisme neemt toe afhankelijk van de soort (snelle groei strategie), de

omgevingsfactoren (blootstelling aan hydrodynamiek, hoeveelheid nutriënten), klimaatomstandigheden (koude winters en weinig licht) en het voorkomen van korte stressperiodes tijdens het groeiseizoen. Het zou kunnen dat op de korte termijn het aanspreken van de koolhydratenreserve de planten ten goede komt, maar op de lange termijn zou het kunnen dat overconsumptie van de reserve om te herstellen van verstoring resulteert in het omslaan van het systeem, zeker wanneer dit gebeurt aan het einde van het groeiseizoen (Hoofdstuk 5).

Zeegras is sessiel en heeft de mogelijkheid om de structuur en eigenschappen aan te passen zodat de schadelijke effecten van biotische en abiotische factoren, zoals golven en stroming, opgevangen kunnen worden. Het is bekend dat de mechanische weerstand van zeegrasbladeren tegen fysische stress afhangt van soortsafhankelijke eigenschappen (groeistrategieën). In dit proefschrift documenteer ik aanpassingen van de mechanische eigenschappen van zeegras, afhankelijk seizoen, breedtegraad en omgevingsfactoren zoals de hoeveelheid aanwezige nutriënten (Hoofdstuk 6). Onder eutrofe omstandigheden hebben zeegrassen tere bladeren, welke makkelijk afbreken, maar ook verder uit kunnen rekken dan bladeren die worden gevonden in meer oligotrofe omstandigheden. De plasticiteit en het aanpassingsvermogen aan de lokale omgeving vormen een belangrijke strategie voor veerkracht.

### **De invloed van klimaat -latitudinale- gradiënten**

Zeegrassen komen voor in gematigde streken over een brede gradiënt aan omgevings- en klimaatomstandigheden, die samen de lengte van het groeiseizoen en de populatiedynamiek reguleren. Deze gradiënten spelen een belangrijke rol in het bepalen van reproductief succes, koolhydratenreserve, en fysiologische en mechanische eigenschappen (Hoofdstuk 5 tot 7). Daarnaast speelt het seizoensafhankelijke aspect een belangrijke rol bij de veerkracht van zeegrasvelden, met name tijdens de winter (Hoofdstuk 5) en tijdens de piek in het groeiseizoen (Hoofdstuk 3). Afhankelijk van de lokale omstandigheden en de verspreiding met betrekking tot de klimaat – latitudinale – gradiënt laten zeegrassen verschillende eigenschappen zien die samen de veerkracht bepalen met betrekking tot stress en verstoringen. De Noord-Europese populatie zeegras is als het ware voortdurend in de kolonisatie fase waarbij elk jaar opnieuw populaties ontstaan vanuit zaden (Hoofdstuk 7). Dit zorgt er voor dat deze populaties genetisch diverser zijn dan zuidelijke populaties. Daarnaast zijn er ook overwinterende rhizomen die asexueel (klonaal)

voortplanten. Hierbij maken de planten gebruik van de reserves die opgeslagen liggen in het rizoom (Hoofdstuk 5). De zuidelijke populaties die het hele jaar door kunnen groeien zijn daarentegen veel meer afhankelijk van klonale voortplanting. Deze planten kunnen door gunstige licht- en temperaturomstandigheden het hele jaar productief zijn en zijn mede daardoor minder afhankelijk van de koolhydratenreserve in het rizoom (Hoofdstuk 5) of van zaad productie (Hoofdstuk 7). Tegelijkertijd hebben deze planten ook sterkere bladeren (Hoofdstuk 6).

### **Conclusies en consequenties**

Door de invloed van wereldwijde trends van druk op de kust, hebben alle mechanismen die betrokken zijn bij de veerkracht van zeegrassystemen aanzienlijke consequenties voor het lot van zeegras en de stabiliteit van kustecosystemen op de lange termijn. Dit proefschrift heeft aangetoond dat zeegrassen zich kunnen aanpassen aan een veranderende omgeving, zoals is waargenomen in de studie langs een klimaat – latitudinale – gradiënt. Bijkomende stress op de korte termijn, zoals eutrofiëring als gevolg van nutriëntenaanrijking, kunnen er echter alsnog voor zorgen dat het systeem omslaat en de vegetatie verdwijnt. Dat kan zelfs het geval zijn bij kleine verstoringen, wanneer er reeds sprake is van een verlaagde weerstand tegen stress en een kleiner herstelvermogen. In het algemeen zorgt een lagere veerkracht van zeegrasvelden dat ze minder goed werken als *ecosystem engineer* en dit verzwakt het in stand houden van de positieve terugkoppeling. Het in stand houden van deze positieve terugkoppelingen is van groot belang, want deze hebben direct invloed op de strategieën om veerkracht op te bouwen (bijvoorbeeld het vermogen om sulfide stress te verminderen met behulp van de wortels) en op het vermogen om zich aan te passen aan verschillende klimaat omstandigheden. Het verdwijnen van zeegrasvelden heeft grote gevolgen voor de gezondheid van het gehele kustecosysteem.

Wanneer klimaatverandering, frequentere verstoring door stochastische gebeurtenissen (storm, extreem weer), en meer stress door menselijk toedoen een rol spelen, moeten beheerders, wetenschappers en belanghebbenden het belang van: het wanneer (seizoensafhankelijke groei), het waar (langs de klimaat – latitudinale – gradiënt) en het hoe (de aard van de dreiging en veerkrachtstrategieën) goed in de gaten houden. Deze parameters vormen de spil om zeegrasvelden, en daarmee de stabiliteit van ecosystemen in gematigde systemen, beter te kunnen beschermen.

## RÉSUMÉ

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Les herbiers d'angiospermes marines forment de précieux écosystèmes, jouant un rôle écologique majeur pour la biodiversité et la stabilité des zones côtières. Ils sont cependant menacés dans le monde entier, principalement par l'intensification des activités humaines. Afin de préserver ces prairies marines et d'empêcher leur disparition, il est nécessaire de comprendre leur capacité de résilience dans un environnement changeant. La présente thèse a pour but, au moyen de diverses expérimentations sur le terrain, d'étudier la résilience des herbiers d'angiospermes marines des milieux tempérés en examinant (i) les indicateurs de résilience nécessaires à leur conservation; (ii) les stratégies et mécanismes de résilience permettant aux herbiers de résister aux stress et de se remettre des perturbations; et (iii) l'influence des gradients climatiques - latitudinaux en tant que moteurs des traits de caractéristique des herbiers marins, de leur résilience et de la réponse des indicateurs.

### Indicateurs de résilience

Le développement d'indicateurs de résilience et de santé est indispensable pour évaluer au mieux l'évolution des écosystèmes marins et prédire un potentiel déclin. Dans cette thèse, nous avons remis en question l'interprétation de ces indicateurs pour estimer l'état de santé et la résilience des herbiers d'angiospermes marines. Nous avons comparé la réponse de deux indicateurs: i) la couverture végétale, qui est un indicateur traditionnellement utilisé pour évaluer l'état de santé des herbiers marins; et ii) le *Critical slowing down* (ou ralentissement critique), un indicateur théorique de l'état de santé des herbiers marins en terme de résilience aux perturbations. Un *Critical slowing down* se mesure par un ralentissement de la récupération de l'herbier après une perturbation. Les deux indicateurs ont présenté une réponse contradictoire après une perturbation: plus la couverture est élevée, plus la récupération est lente/faible (chapitre 2). Cela n'a toutefois été observé qu'au cours de la phase de croissance des angiospermes marines (chapitre 3). En effet, nous avons remarqué que la temporalité de la perturbation par rapport à la période de croissance saisonnière des angiospermes marines était cruciale pour leur résilience. Leur résilience est élevée au début de la saison de croissance alors qu'elle

décroît lorsque la couverture végétale augmente. Cette réponse contradictoire entre les indicateurs a été observée indépendamment de l'exposition des herbiers aux vagues ou de leur état d'eutrophisation (i.e. même réponse contradictoire observée lorsque les plantes sont soumises au même stress d'eutrophisation). Ces résultats mettent l'accent sur la nécessité de considérer attentivement la temporalité du suivi des indicateurs, car elle constitue un facteur fondamental pour évaluer la résilience des herbiers. Ces résultats sont susceptibles de s'étendre au-delà des herbiers marins, à d'autres écosystèmes saisonniers des milieux tempérés.

### **Stratégies / mécanismes de résilience**

Les angiospermes marines ont la capacité de s'adapter à leur environnement en acclimatant leurs traits morphologiques, physiologiques et mécaniques. Elles présentent par ailleurs différentes stratégies, liées à leur taux de croissance (Grime, 1977), à l'utilisation de leurs ressources ou à leur capacité à modifier leur environnement (ingénieurs de l'écosystème), les rendant plus ou moins résilientes aux stress et aux perturbations qu'elles subissent, et les préservant de l'extinction.

Dans cette thèse, nous avons remarqué que la réponse des herbiers au stress et aux perturbations divergeait de la réponse prédite par les stratégies de croissance de Grime, du fait de leur capacité à être des ingénieurs de l'écosystème (chapitre 4). En effet, les angiospermes marines présentant une croissance rapide étaient non seulement plus rapide que celles à croissance lente pour récupérer des perturbations ; mais également plus résistantes au stress, tel que l'augmentation de sulfures dans le sédiment. Cette forte résistance s'explique par la forte capacité de ces plantes ingénieurs de l'écosystème à réduire le stress (i.e. émission d'oxygène par le système racinaire). Être un ingénieur de l'écosystème constitue donc une stratégie essentielle pour faire face à un environnement stressant et perturbé, rendant certaines espèces d'angiospermes marines plus résilientes. Nous présumons cependant que cet effet dépend également de la nature de l'ingénierie de l'écosystème (i.e. basée sur des traits physiologiques ou structurels) ainsi que la nature de l'environnement (stimulant la croissance ou stimulant la formation de tissus physiquement plus solides).

Une autre stratégie de résilience des herbiers mise en évidence dans cette thèse est liée à l'allocation de leurs ressources et plus

précisément à l'utilisation des réserves de carbone pour leur résilience. Étant des organismes photosynthétiques, les plantes marines peuvent, au cours de leur croissance saisonnière, stocker des réserves de carbone dans leurs rhizomes. Ces réserves sont stockées sous forme de carbohydrates non structuraux, utilisés pour la croissance des plantes ou pour survivre à l'hiver. Nous avons démontré que, lorsqu'elles sont soumises à des épisodes de stress courts, les angiospermes marines utilisent leurs réserves en carbohydrates pour récupérer et repousser, en particulier les espèces à croissance rapide (Chapitre 4 et 5). Dans l'ensemble, nous avons constaté que l'utilisation des réserves en carbohydrates en tant que mécanisme de résilience diffère selon les espèces (utilisation plus importante pour les plantes à croissance rapide), les conditions environnementales (i.e. l'exposition à l'hydrodynamique, l'état d'eutrophisation), les conditions climatiques (ensoleillement, durée et intensité de l'hiver) et l'existence d'épisodes de stress à court terme au cours de leur croissance saisonnière. Nous pouvons concevoir qu'à court terme, l'utilisation des réserves de carbone pour la repousse est bénéfique pour les plantes. Mais sur le long terme, la surconsommation de leurs réserves pour se remettre de potentielles perturbations pourrait suffire à faire basculer le système et conduire à la disparition de l'herbier, surtout lorsqu'une perturbation arrive à la fin de la période de croissance (chapitre 5).

Les angiospermes marines sont des organismes sessiles. Elles ont cependant la capacité de modifier leur structure ou leurs traits pour résister à l'effet néfaste des facteurs de stress biotiques ou abiotiques tels que les vagues ou les courants. La résistance mécanique des feuilles d'angiospermes marines aux contraintes physiques dépend des caractéristiques morphologiques et physiologiques propres à l'espèce (c'est-à-dire des stratégies de croissance). Dans cette thèse, nous avons également démontré que les angiospermes marines pouvaient adapter leurs caractéristiques mécaniques en fonction de leur distribution géographique le long d'un gradient latitudinal, mais aussi en fonction de leur environnement, comme leur état d'eutrophisation (chapitre 6). En conditions d'eutrophie, les angiospermes marines présentaient des feuilles plus fragiles, plus faciles à casser mais plus extensibles que dans des conditions d'oligotrophie. Cette plasticité et capacité d'adaptation à leurs conditions environnementales locales constituent donc une autre stratégie importante de résilience.

**L'influence des gradients climatiques -latitudinaux**

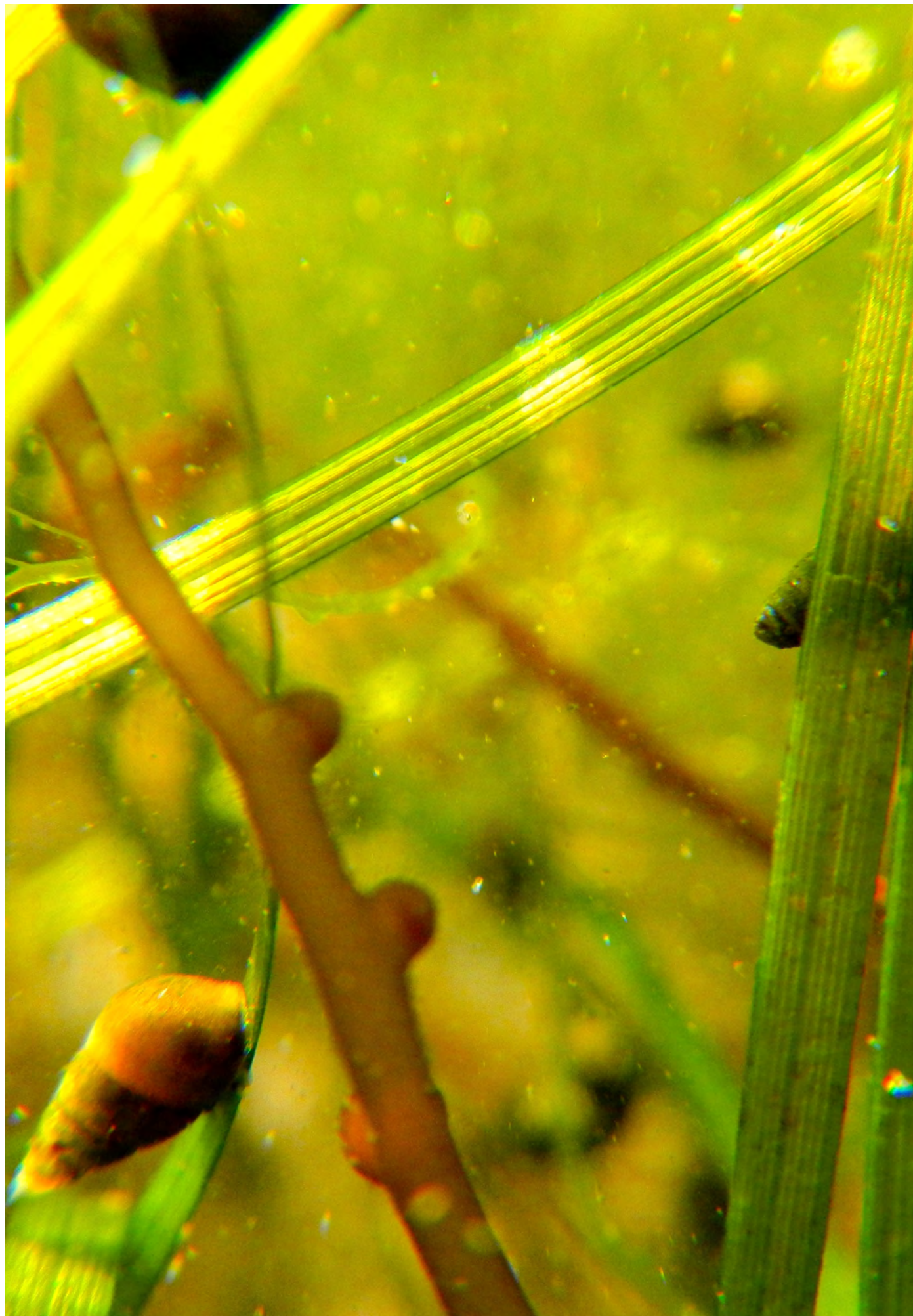
Les herbiers marins des milieux tempérés sont distribués le long de larges gradients environnementaux et climatiques. Ces gradients contrôlent la croissance saisonnière et la dynamique des populations d'herbiers. Dans cette thèse, nous avons démontré que ces gradients pouvaient également jouer un rôle moteur important sur leurs traits tels que leur production de graines, leurs réserves de carbone, leurs traits physiologiques et mécaniques (chapitres 5 à 7). Nous avons également observé que la dimension saisonnière de la croissance des herbiers marins dans les systèmes tempérés joue un rôle moteur majeur pour leur résilience, en particulier la période hivernale (chapitre 5) et le pic de croissance (chapitre 3). En effet, selon leurs conditions locales et leur répartition le long d'un gradient climatique - latitudinal, les herbiers d'angiospermes marines présentent des traits différents, façonnant leur résilience au stress ou aux perturbations externes. Les populations nord-européennes peuvent être considérées comme étant en phase de colonisation perpétuelle avec l'initiation récurrente annuelle de la population par propagules sexuelles (graines) (chapitre 7), ce qui les rend génétiquement plus diversifiées; et une croissance asexuée - clonale des rhizomes dormants en puisant sur leurs réserves de carbone après l'hiver (chapitre 5). En revanche, les populations à feuilles persistantes du sud dépendent beaucoup plus de la propagation clonale. Ces herbiers sont productifs toute l'année en raison de conditions de lumière et de température favorables pour la photosynthèse; (Chapitre 5) produisent peu de graines (chapitre 7), mais possèdent des feuilles mécaniquement plus résistantes (chapitre 6).

**Conclusions et implications**

Dans l'ensemble, tous les mécanismes impliqués dans la résilience des herbiers marins ainsi que l'influence des gradients climatiques peuvent avoir des conséquences considérables sur la résilience des herbiers marins et la stabilité des écosystèmes côtiers à long terme. Les angiospermes marines peuvent s'adapter à leur environnement, comme il a été observé le long d'un gradient climatique - latitudinal. Mais des effets supplémentaires de stress à court terme tels que, par exemple, l'enrichissement en nutriments amenant à l'eutrophisation de l'herbier, pourrait pousser le système vers son point de bifurcation. Dans cette situation, même une petite perturbation peut pousser un herbier au déclin, en diminuant sa résistance au stress et en ralentissant son potentiel de récupération. Les conséquences d'un tel déclin seraient importantes pour la santé globale

des écosystèmes côtiers. D'une manière générale, une réduction de la résilience des herbiers d'angiospermes marines pourrait affecter leur efficacité en tant qu'ingénieur de l'écosystème et donc les *positive feedbacks* qu'ils induisent. Le maintien de ces *positives feedbacks* (i.e. réactions rétroactives) pour les herbiers marins est essentiel, car cela affecte leurs stratégies de résilience (par exemple, la capacité de réduire le stress lié à l'augmentation de sulfures à travers leur système racinaire) et leur capacité à s'adapter aux gradients climatiques.

Nos résultats ont des répercussions sur la gestion des herbiers marins. Dans un contexte de changement climatique, d'occurrence croissante d'épisodes stochastiques (i.e. tempêtes, conditions météorologiques extrêmes) et de stress causés par l'homme, les gestionnaires, les scientifiques et les parties prenantes devraient considérer l'importance de: quand (croissance saisonnière), où (gradient latitudinal-climatique) et comment (nature de la menace, stratégies de résilience) la résistance des herbiers d'angiospermes marines pourrait être affectée. La prise en compte de ces paramètres est essentielle pour mieux préserver les herbiers d'angiospermes marins d'une potentielle disparition et pour maintenir la stabilité de leurs écosystèmes.



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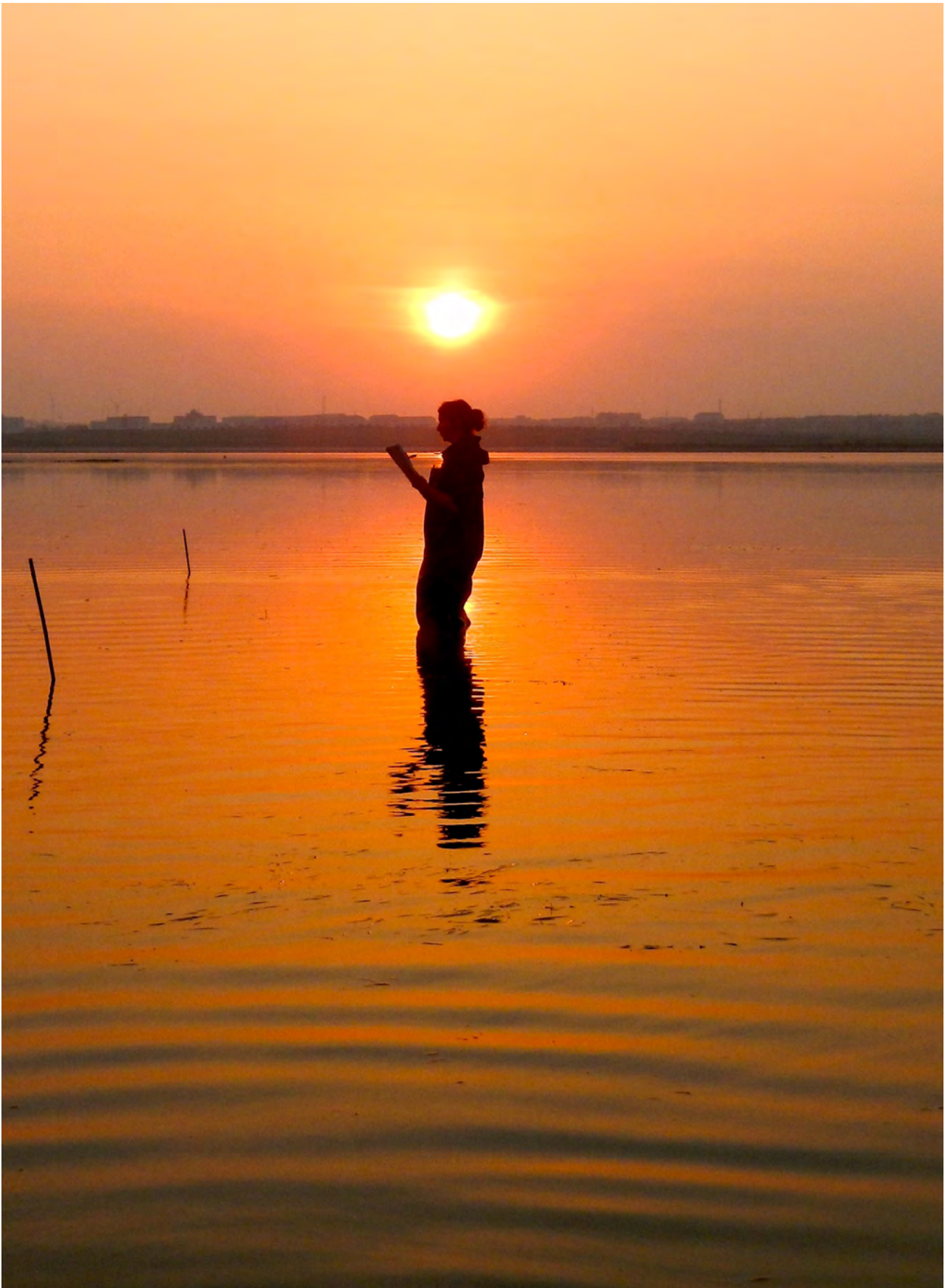
Merci à toi Mathieu, pour tout ce que tu as fait pour moi; les stats, n'en parlons pas, mais aussi pour tout ce que tu m'apportes et ton amour. Merci de m'avoir suivie jusqu'aux Pays-Bas pour y trouver toi aussi un sujet de thèse. Merci de m'avoir supportée dans les moments de doute comme dans les moments de bonheur. Merci de me rendre heureuse tous les jours et pour longtemps encore.

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Laura





## CURRICULUM VITAE

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### ABOUT THE AUTHOR

Laura, Marie Soissons was born in Montpellier (France), on November 14, 1987. Growing up by the Mediterranean sea and sailing with her family, she fell in love with the marine environment. This is why, after graduating high school (Lycée privé La Trinité, Béziers) in 2005, she decided to start a Bsc in Earth and Environmental Sciences at the University of Montpellier II, France. She graduated in 2008 and decided to pursue her dream of becoming a marine researcher by applying to a two-years Master program at the University of Bordeaux I specialising in Ocean Sciences. This is during her first year of Master that she discovered the wonders of seagrass research by doing a three-months internship on the hydrodynamic characterisation of a seagrass meadow in Arcachon Bay. This is also the time she discovered the joy of walking on a mudflat with snowshoes and watching out for the tide! During her second year of Master, she spent a full semester as an Erasmus student at the University of Southampton to study coastal sediment dynamics and English. She came back to France to complete her master thesis at the Ifremer of L'Houmeau, looking at the effect of *Zostera noltei* meadows on sediment dynamics. This internship sealed her passion for seagrasses. She graduated from her Msc in 2010. She then decided to further improve her English level and writing skills by following a second Msc in science communication at the University of the West of England in Bristol. While graduating from this second Msc in 2011, she decided to go back to marine – and seagrass – research and began her PhD at the Royal Netherlands Institute for Sea Research (NIOZ) in Yerseke. From 2011 to 2016 she carried out her PhD project, looking at seagrass resilience to human-induced threats across Europe and at the Yantai Institute for Coastal Zone Research in China. She is currently doing a postdoctoral research looking at biophysical interactions and the optimisation of sand nourishment techniques to restore ecologically valuable tidal flats at NIOZ-Yerseke.

## LIST OF PUBLICATIONS

- Han Q, **Soissons LM**, Liu D, van Katwijk MM, Bouma TJ. (in press). Individual and population indicators of *Zostera japonica* respond quickly to experimental addition of sediment-nutrient and organic matter. *Mar Pollut Bull*.
- van Tussenbroek BI, **Soissons LM**, Bouma TJ, Asmus R, Auby I, Brun FG, Cardoso PG, Desroy N, Fournier J, Ganthy F, Garmendia JM, Godet L, Grilo TF, Kadel P, Ondiviela B, Peralta G, Recio M, Valle M, Van der Heide T, Van Katwijk MM. 2016. Pollen limitation may be a common Allee effect in marine hydrophilous plants: implications for decline and recovery in seagrasses. *Oecologia* 182:595–609.
- Soissons LM**, Li B, Han Q, van Katwijk MM, Ysebaert T, Herman PMJ, Bouma TJ. 2016. Understanding seagrass resilience in temperate systems: the importance of timing of the disturbance. *Ecol Indic* 66:190–8.
- Han Q, **Soissons LM**, Bouma TJ, van Katwijk MM, Liu D. 2015. Combined nutrient and macroalgae loads lead to response in seagrass indicator properties. *Mar Pollut Bull*.
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- Soissons LM**, Han Q, Li B, van Katwijk MM, Ysebaert T, Herman PMJ, Bouma TJ. 2014. Cover versus recovery: Contrasting responses of two indicators in seagrass beds. *Mar Pollut Bull* 87:211–9.
- Under review:*
- Soissons LM**, Li B, Han Q, van Katwijk MM, Ysebaert T, Herman PMJ, Bouma TJ. Ecosystem engineering may alter the expected response to stress and disturbances as based on Grime's growth strategies: need for integrating both concepts?
- Soissons LM**, Haanstra EP, van Katwijk MM, Asmus R, Auby I, Barillé L, Brun FG, Cardoso PG, Desroy N, Fournier J, Ganthy F, Garmendia JM, Godet L, Grilo TF, Kadel P, Ondiviela B, Peralta G, Recio M, Rigouin L, Valle M, Herman PMJ, Bouma TJ. The influence of

unpredictable stress events on seagrass seasonal carbon reserves: implications for their long-term resilience in a changing environment.

**Soissons LM**, van Katwijk MM, Asmus R, Auby I, Barillé L, Brun FG, Cardoso PG, Desroy N, Fournier J, Ganthy F, Garmendia JM, Godet L, Grilo TF, Kadel P, Ondiviela B, Peralta G, Recio M, Rigouin L, Valle M, Herman PMJ, Bouma TJ. Seasonal and latitudinal variation in seagrass mechanical traits across Europe: the influence of local nutrient status.

Li B, Li X, Bouma TJ, **Soissons LM**, Cozzoli F, Wang Q, Zhou Z, Chen L. Analysis of macrobenthic assemblages and ecological health of the Yellow River Delta, China using AMBI & M-AMBI assessment method.

## SUPERVISORY EXPERIENCE

- 2016 Hoogeschool student Jeroen van Kuijk, Helicon  
Thesis: Carbon sequestration in European seagrass meadows
- 2016 Msc student John Bastiaan, Groningen University  
Thesis: The effect of *Venerupis Philippinarum* on sediment erodibility
- 2016 Msc student Martijn Hoolsema, Wageningen University  
Thesis: The effect of priming on macrobenthic communities' recovery
- 2015 Msc student Titus Mens, Utrecht University  
Thesis: Effects of multi stressors on seagrass seed establishment, winter survival and mechanical properties
- 2014 Bsc student Eeke Haanstra, Utrecht University  
Thesis: Seagrass seasonal carbon storage and chances for winter survival under threat
- 2014 Bsc student Macilino Heijnen, Utrecht University  
Thesis: Literature study of European seagrass meadows health status
- 2014 Bsc student, Miriam Loth, Utrecht University  
Thesis: The importance of latitude for European seagrass meadow resilience
- 2014 Bsc student Nynke ten Hoeve, Utrecht University  
Thesis: Seagrass cover along a latitudinal gradient: effect of seasonal light and temperature changes

**PRESENTATIONS/LECTURES**

- 2016 Guest lecturer at Utrecht University, NL  
Title: Understanding the resilience of temperate seagrass meadows in a changing environment.
- 2016 Conference talk: International Seagrass Biology Workshop (ISBW 12), Nant Gwrtheyrn, Wales, UK  
Title: The influence of short-term stress events on seagrass carbon reserves: implications for their resilience.
- 2016 Conference talk: Estuarine Coastal Sciences Association (ECSA 56), Bremen, DE  
Title: The relative importance of ecosystem engineering and growth rate in determining seagrass resilience to eutrophication.
- 2016 Guest lecturer at Wageningen University, NL  
Title: Hydrology meets Ecology: Bio-physical interactions in the marine environment.
- 2015 Conference talk: IWWR annual meeting, Nijmegen, NL  
Title: How to better evaluate resilience? Answers from seagrass experiments in coastal China.
- 2015 NSFC-NWO workshop: NSFC-NWO projects final joint meeting, Beijing, CN  
Title: Drivers of seagrass resilience in temperate systems.
- 2015 Conference talk: Association for the Sciences of Limnology and Oceanography (ASLO), Granada, ES  
Title: How to evaluate seagrass resilience: Timing effects on indicators.
- 2014 Conference talk: Netherlands Annual Ecology meeting, Lunteren  
Title: Contrasting responses of two indicators in seagrass beds: Site and Timing effect.
- 2012 NSFC-NWO workshop: NSFC-NWO projects mid-term meeting, Guangzhou, CN  
Title: Assessing the impact of human activities on seagrass ecosystems health and resilience.

Temperate seagrass meadows form highly valuable ecosystems in the marine environment. They are, however, exposed to an increasing number of threats, mostly linked to human activities, individually or jointly affecting their resilience. In order to preserve seagrass meadows from collapse, we need to better understand their resilience and chances for survival under threats. By means of various manipulative field experiments, the present thesis addresses seagrass resilience by looking at: (1) indicators of resilience; (2) strategies for resilience; and (3) the influence of global gradients on seagrass traits, resilience and indicators.

