

Applying Stable Isotope Techniques to Assess Nutrient and Carbon Dynamics in Salt Marshes

Dissertation

zur Erlangung des Doktorgrades
an der Fakultät für Mathematik, Informatik und
Naturwissenschaften im Fachbereich Biologie
der Universität Hamburg

vorgelegt von

Thi Hai Do

aus Thanh Hoa, Vietnam

Hamburg, 2018

Erstgutachter: Prof. Dr. Kai Jensen

Zweitgutachterin: Prof. Dr. Annette Eschenbach

Tag der Disputation: 14.12.2018

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Summary

Zusammenfassung

Summary

Coastal salt marshes are areas vegetated by herbs, grasses or low shrubs, bordering saline water bodies, and are subject to periodic flooding as a result of fluctuations (tidal and non-tidal) in the level of the adjacent water bodies. Forming at the interface between land and sea, salt marshes are described as very highly dynamic ecosystems that make the assessment of ecological processes challenging. The introduction of stable isotopes as a technique in ecology in the last three decades has made it possible to address plenty of research questions in ecological studies, which could not be studied before, especially in dynamic systems such as salt marshes.

The overall aim of this thesis was therefore to apply these stable isotope techniques to assess small-scale nutrient and large-scale carbon dynamics in salt marshes. **Chapter 1** provides a general introduction to the stable isotope techniques and their applications in salt marsh studies at different scales.

The origin of salt marsh soil organic carbon was investigated in **Chapter 2** utilizing natural abundances of carbon isotopes. Furthermore, Chapter 2 addresses the question how geomorphological factors including elevation, the distance to the sediment source, and habitat management affects the relative contributions of allochthonous vs. autochthonous organic carbon sources to the top soil organic carbon pool of two European salt marshes. The results show a negative effect of distance to the creek (i.e. sediment source) on the relative contribution of allochthonous organic carbon at only one of the two salt marshes. Moreover, livestock grazing affected the relative contribution of allochthonous organic carbon in salt marsh soils via indirect effects on sediment deposition and biomass production. The relative contribution of allochthonous organic carbon increased with increasing distance to the marsh edge in ungrazed, while it decreased in grazed treatments. The findings suggest that spatial factors and land use are important drivers affecting the origin of organic carbon in salt marshes. This knowledge has important implications for estimating carbon sequestration rates.

In the second study of this thesis (**Chapter 3**) addresses the importance of the physiological integration of *Elymus athericus* for establishing in salt marshes at low elevations by applying enriched nitrogen isotope technique. Furthermore, the growth performance of connected (physiological integration allowed) and severed (physiological integration prevented) offspring ramets growing in different hydrological conditions (waterlogged or non-waterlogged) of both *Elymus* high marsh (HM) and low marsh (LM) ecotypes were measured. The results revealed that *Elymus* is able to translocate nitrogen between ramets via the rhizome indicated by higher $\delta^{15}\text{N}$ found in the connected compared to severed offspring ramets. Waterlogged soil conditions were found to indeed reduce the growth of *Elymus*, and the low marsh ecotype was found to generally produce higher biomass and growth. The results indicate that *Elymus* uses physiological integration to improve its photosynthetic capacity leading to higher biomass production. Moreover, LM *Elymus* changes its biomass allocation to elongating aboveground biomass and this is a potential mechanism to survive under prolonged periods of flooding.

The synthesis (**Chapter 4**) summarizes the key findings and discusses the advantages, assumptions and limitations of the application of stable isotope techniques in each study of this thesis. The linkages between these studies and future research perspectives were also considered in this chapter.

Zusammenfassung

Küstensalzmarschen sind Gebiete, die an salzige Gewässer angrenzen und auf Grund von Wasserstands-Schwankungen (tidebeeinflusst oder nicht tidebeeinflusst) dieser angrenzenden Gewässer regelmäßig überflutet werden. Die Vegetation von Küstensalzmarschen besteht vor allem aus krautigen Pflanzen, Gräsern und niedrigen Sträuchern. Auf Grund ihrer Position im Übergangsbereich zwischen Land und Meer sind Salzmarschen äußerst dynamische Ökosysteme, was auch ein Grund dafür ist, dass ökologische Prozesse hier nur schwer zu erfassen sind. Die Einführung der Analyse stabiler Isotope als neue Technik in der Ökologie in den letzten drei Jahrzehnten ermöglicht es jedoch viele ökologische Fragestellungen zu untersuchen, die bisher, vor allem in dynamischen Ökosystemen wie Salzmarschen, nicht untersucht werden konnten.

Das übergreifende Ziel dieser Arbeit ist es, die Analyse stabiler Isotope zu nutzen, um die kleinräumige Nährstoff- und großräumige Kohlenstoffdynamik in Salzmarschen zu untersuchen. **Kapitel 1** gibt zuerst eine generelle Einführung in die Analyse stabiler Isotope und ihrer Anwendung in Salzmarschen auf verschiedenen räumlichen Skalen.

In **Kapitel 2** wird die Abundanz stabiler Kohlenstoffisotope genutzt, um die Herkunft des organischen Kohlenstoffs im Boden zu untersuchen. Des Weiteren wird in Kapitel 2 die Frage untersucht, inwiefern geomorphologische Faktoren, wie etwa Geländehöhe, Abstand zur Sedimentquelle und das Habitatmanagement, den relativen Anteil von allochthonem bzw. autochthonen organischen Kohlenstoffquellen zum organischen Kohlenstoff des Oberbodens in zwei europäischen Salzmarschen beeinflussen. Die Ergebnisse zeigen, dass der Abstand zum Priel als Sedimentquelle nur in einer der beiden untersuchten Marschen einen negativen Einfluss auf den Anteil des allochthonen organischen Kohlenstoffs hat. Außerdem wurde festgestellt, dass Beweidung den relativen Anteil des allochthonen organischen Kohlenstoffs indirekt über den Sedimenteintrag und die Produktion von Biomasse beeinflusst. Der

relative Beitrag des allochthonen organischen Kohlenstoffs nahm mit zunehmendem Abstand zur Marschkante in unbeweideten Marschen zu, während er in beweideten Marschen abnahm. Dies deutet darauf hin, dass räumliche Faktoren und Landnutzung wichtige Faktoren sind, welche die Herkunft des organischen Kohlenstoffs in Salzmarschen beeinflusst. Diese Erkenntnis hat wichtige Auswirkungen auf die Schätzung von Kohlenstofffestlegungsraten.

Die zweite Studie dieser Arbeit (**Kapitel 3**) betrachtet die Bedeutung der physiologischen Integration bei der Ausbreitung von *Elymus athericus* in tiefliegende Bereiche der Salzmarschen durch die Anwendung angereicherter Stickstoff Isotope. Des Weiteren wurde die Wachstumsleistung verbundener Rameten (physiologische Integration zugelassen) und getrennter Rameten (physiologische Integration unterbunden) unter verschiedenen hydrologischen Bedingungen (staunass oder nicht staunass) für beide *Elymus*-Ökotypen (Obere Marsch-Typ und Untere Marsch-Typ) untersucht. Die Ergebnisse zeigen, dass *Elymus* in der Lage ist, Stickstoff über das Rhizom zu anderen Rameten zu transportieren. Dies konnte durch ein höheres $\delta^{15}\text{N}$ in verbundenen im Vergleich zu getrennten Rameten nachgewiesen werden. Staunasse Bedingungen führen außerdem zu einer Verringerung der Wuchsleistung von *Elymus*, wobei der Untere Marsch Ökotyp jedoch generell mehr Biomasse produzierte. Die Ergebnisse zeigen außerdem, dass *Elymus* physiologische Integration nutzt um ihre Photosynthesekapazität zu verbessern, um somit mehr Biomasse zu produzieren. Überdies ändert der Untere Marsch Ökotyp von *Elymus* sein Allokationsmuster dahingehend, dass es zu einer Streckung der oberirdischen Biomasse kommt. Dies könnte ein Anpassungsmechanismus an das Habitat mit langandauernden staunassen Bedingungen sein.

Die Synthese (**Kapitel 4**) fasst dann abschließend die wichtigsten Erkenntnisse zusammen und diskutiert Vorteile, Annahmen und Einschränkungen der Analyse stabiler Isotope, die in den jeweiligen Kapiteln dieser Arbeit eingesetzt wurden. Überdies werden in diesem Kapitel Zusammenhänge zwischen den einzelnen Studien und zukünftige Forschungsperspektiven erläutert.



1

General Introduction

1.1 Stable isotope techniques

1.1.1 What are stable isotopes?

The term 'isotope' was first suggested in 1913 by Margaret Todd, a medical doctor from the United Kingdom. It means that isotopes of an element all occupy the same (*iso*) place (*topos*) in the Periodic Table of the elements. Each atom is composed of a nucleus, including protons (Z) and neutrons (N), surrounded by electrons (e). Isotopes are forms of the same element that differ in the number of neutrons in the nucleus (Fry 2006). Isotopes are divided into two types based on their stability and origin, namely radioactive isotopes and stable isotopes. Radioactive isotopes are those which decay spontaneously, emitting *alpha* or *beta* particles and sometimes also *gamma* rays, to become stable isotopes. ^{14}C isotopes, for example, decay spontaneously into stable ^{14}N emitting a beta particle (Kendall & Caldwell 1998). In contrast, stable isotopes are defined as those that are energetically stable and do not decay (Sulzman 2007). For nuclides of low atomic mass with N or Z below ~ 25 , an isotope tends to be stable when the number of N and number of Z are quite similar ($N/Z \leq 1.5$). For nuclides of high atomic mass with N or Z greater than ~ 25 , an isotope is stable when it has an 'even' Z number (Criss 1999).

Stable isotopes can be divided into two types based on their origin. Stable isotopes which form as products of radioactive decay are called radiogenic stable isotopes, and their abundance is purely a function of time (Newton 2016). Another type of stable isotopes is non-radiogenic, as their abundance is determined by stellar nucleosynthesis at the birth of the solar system (Newton 2016). These non-radiogenic stable isotopes are most useful in ecological studies, especially those of the five elements Hydrogen (H), Carbon (C), Nitrogen (N), Oxygen (O) and Sulfur (S) which comprise most of the mass present in organic materials. These non-radiogenic stable isotopes have large relative mass differences between heavier and lighter isotopes, which cause their distinct physical and chemical behavior in all natural processes, resulting in changes of the stable isotope ratio. This is the most important reason making stable isotopes so useful for ecological studies (Sulzman 2007, Newton 2016).

1.1.2 How stable isotopes are measured and reported

Stable isotopes are usually measured using a stable isotope mass spectrometer. For the measurements, the samples are first combusted and transformed by an elemental analyzer, a gas chromatograph, or a laser into a form, such as e.g. a gas, that is suitable for the specific mass spectrometer. In the next step, the gas is inserted into the mass spectrometer where it is ionized, accelerated and separated based on its mass. In the end, computers tally up the counts from the multiple collectors and calculate the final isotope values.

Isotope values are normally reported using the standard δ -notation. The δ value denotes a sample measurement relative to an internationally agreed standard, according to the following equation:

$$\delta^H X = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000$$

where X is the element of interest; the superscript H gives the heavy isotope mass of that element; R is the ratio of the heavy isotope to the light isotope of a particular element X. For clarity, δ values are multiplied by a thousand to give units in ‰ (per mil). By definition, standards have a δ value of 0 ‰. A positive δ value indicates that the sample contains more of the heavy isotope than the standard whereas a negative δ value indicates that the sample contains less than the standard.

1.1.3 Applications of stable isotopes in ecology

Stable isotope measurement is often considered a new technique, but the idea and/or conception of the existence of the “isotope” has a long history over a century (Budzikiewicz & Grigsby 2006). The use of stable isotopes in ecological research, however, began in the 1960s (Menzies et al. 1961, Parker 1964) and gathered pace in the 1980 and 1990s. By now, it is a fundamental tool for ecologists. Ecological stable isotope studies have mainly focused on five lightweight elements, namely C, H, O, N, and S, as those represent major building blocks of all components of the biosphere, hydrosphere and atmosphere (Fry 2006). Other elements such as silicon (Si), calcium (Ca), and iron (Fe) are also increasingly used, yielding important results for ecological studies (Clementz et al. 2003, Varela et al. 2004, Rouxel et al. 2005, Cooke et al. 2016).

Stable isotopes provide a natural way to directly follow and trace details of element cycling. This tracing of elements can be used to elucidate many ecological problems and thus stable isotopes have proved to be an extremely valuable and powerful tool (West et al. 2006, Ehleringer & Dawson 2007). Stable isotopes have been used to disentangle complex processes taking place in a diversity of ecosystems at a wide range of scales. For instance, by applying stable isotope techniques, ecologists are now able to study processes reaching from the small scale of metabolic change in leaves to the large-scale net ecosystem flux partitioning (Werner et al. 2012). These ecological studies can be divided into two categories based on whether they use stable isotopes at naturally occurring levels (called “natural abundance”) or levels outside the natural range of values due to the addition of labelled substances (called “enriched” levels) (Peterson & Fry 1987).

1.1.3.1 Utilizing natural abundances of stable isotopes

The natural abundance of stable isotopes can be used as integrators, indicators, recorders and tracers of various fundamental ecological processes. As integrator, stable isotopes permit ecologists to integrate many ecological processes that vary both spatially and temporally. For example, Craine et al. (2009) reviewed that plant $\delta^{15}\text{N}$ values increased with increasing nitrogen availability and potential nitrogen mineralization across natural nitrogen supply or nitrogen availability gradients. Wang et al. (2017) found that plant isotopic values were more closely associated with belowground communities than soil isotopic values in the Mongolian Plateau.

As indicators, stable isotopes provide information on the presence and magnitude of key ecological processes. As many ecological processes produce a distinctive isotope value, the presence or absence of such processes and their magnitude in relation to other processes are indicated by the stable isotope values (Kónya & Nagy 2012). For example, partition of the atmospheric N_2O sources between nitrification and denitrification (Yoshida & Toyoda 2000), origin of nitrate pollution (Kreitler 1979), and the contribution of C source in soils (Biggs et al. 2002) were indicated by C and N stable isotopes.

As recorders, stable isotopes reveal biological responses to changing environmental conditions. For example, stable isotope ratios of tree rings, animal hair

and ice cores can be used as a record of system response to changing environmental conditions or a proxy record for environmental change (Loader et al. 2007, Cerling et al. 2009, Savard 2010, Alley 2011).

As tracers, stable isotopes allow ecologists to follow the fates and transformations of key elements and substances. For example, stable isotopes are used to determine the proportional contributions of several sources to a mixture, such as carbon sources to soil organic matter, (Phillips & Gregg 2003) or the migration and diets in animal ecology (Hobson & Wassenaar 2008, Hopkins & Ferguson 2012).

1.1.3.2 Applying enriched levels of stable isotopes

In contrast to studies utilizing natural abundances of stable isotopes, it is also possible to add enriched levels of a stable isotope to an ecological system as a tracer. The addition to a system (plant, soil, etc.) of a quantity of a stable isotope with a δ value significantly different from any natural background level, followed by the observation of its fate, allows the study of its fluxes and/or transformations in undisturbed conditions. The addition of an enriched substance acts as a powerful tracer allowing ecologists to study in situ processes at the individual scale (Winning et al. 1999, Lepoint et al. 2004, Mutchler et al. 2004, Vonk et al. 2008) or community scale (Dudley et al. 2001, Pombo et al. 2005). For example, ^{15}N has been used to reveal the translocation of nitrogen between ramets of clonal plants such as *Sasa palmate* (Saitoh et al. 2006). By measuring the $\delta^{15}\text{N}$ label between interconnected pairs of ramets, the translocation of nitrogen was clearly demonstrated, particularly under heterogeneous resource conditions.

1.2. Applications of stable isotope techniques in salt marsh studies

The introduction of stable isotopes as new techniques in the last three decades has made it possible to address research questions in ecological studies, which could not be studied before, especially in dynamic systems such as salt marshes. Salt marshes are areas vegetated by herbs, grasses or low shrubs, bordering saline water bodies, and are subject to periodic flooding as a result of fluctuations (tidal and non-tidal) in the level of the adjacent water bodies (Adam 1993). Salt marshes have long been

considered important sources, sinks and/or transformers of biologically vital elements in the coastal ecosystems (Tobias & Neubauer 2009). Element cycling in salt marshes involve many different ecological processes which are affected by a wide range of environmental factors. For example, as salt marshes form at the interface between land and sea, they often have a high rate of sedimentation. The sedimentation rate in salt marshes depends on suspended sediment concentrations, tidal range, vegetation, proximity to the sediment source, and hydroperiod (Friedrichs & Perry 2001). After deposition, sediment and associated organic carbon could be decomposed by microorganisms (Bianchi 2011), buried (Duarte et al. 2013, Lovelock et al. 2014) or even exported to adjacent ecosystems (Chen et al. 2015). The fate of organic carbon depends on many factors such as geological set up, hydrodynamics, microbial communities, and anthropogenic activities (Zhou et al. 2006, Macreadie et al. 2013, Saintilan et al. 2013, Ouyang & Lee 2014, Rosencranz et al. 2016). Those processes, however, have not been fully understood. The use of stable isotopes in salt marsh studies now enables us to determine the source of elements, as well as to track them as they cycle in ecosystems. Moreover, the advantages of stable isotope techniques nowadays allow us to study biogeochemical processes of salt marshes in greater detail and at different scales.

In this thesis, stable isotopes techniques have been applied to reveal information on important processes acting at two different scales. At the large scale, the natural abundance of carbon stable isotopes was utilized to assess spatial patterns of the relative contribution of the allochthonous organic carbon source to the soil organic carbon pool. At the small scale, the enriched nitrogen isotope technique was applied to follow the transport of N-rich compounds within clonal plants.

1.2.1 Applying natural abundance of stable isotope in large scale C-transport

Knowledge on the C cycle of salt marshes is becoming increasingly important, as tidal wetlands such as salt marshes were found to play a critical role in C-sequestration (Chmura 2013). So far, most work on C- sequestration in tidal wetlands has focused on estimating sequestration rates and C pools (Chmura et al. 2003, Duarte et al. 2013, Ouyang & Lee 2014), while few studies focus on the origin of organic C (OC) stored in the ecosystem (Bauer et al. 2013). Yet, the origin of OC may have important

implications for C crediting and greenhouse gas (GHG)-offset activities. The OC feeding the soil OC pool either derives from in situ assimilation of CO₂ through primary production (autochthonous; Au OC) or from external sources (allochthonous; Al OC), i.e. phytoplankton derived sedimentary OC. To assess the origin of OC, the analysis of $\delta^{13}\text{C}$ is commonly used in coastal environments (Thornton & McManus 1994, Lamb et al. 2007, Kemp et al. 2010, Pilarczyk et al. 2012) as the stable C isotope value differs between autochthonous and allochthonous sources. The relative contribution of Al OC versus Au OC to the soil OC pool can vary considerably across tidal wetlands (Middelburg et al. 1997). It can be assumed that the geomorphology of tidal wetlands and also habitat management might play key roles in the relative importance of Al OC versus Au OC inputs. Geomorphological factors, including the elevation of the marsh, and the distance to the sediment source (Fagherazzi et al. 2012), could affect the spatial pattern of sediment and associated organic carbon deposition which is the main pathway for Al OC input at the ecosystem scale in salt marshes (Figure 1.1). Habitat management, for example livestock grazing, reduces vegetation height (Elschot et al. 2013), and therefore sediment deposition might be also decreased (Nolte et al. 2015) resulting in lower Al OC contribution. Grazing also reduces aboveground biomass production (Morris & Jensen 1998) and increases belowground biomass production (Elschot et al. 2015), which is the source of Au OC (Figure 1.1).

1.2.2 Applying enriched stable isotope in small scale N translocation

In addition to these large scale C fluxes, nutrient cycling can also play an important ecological role at the small scale. Individual plants which grow clonally were found to transport nutrients from the parent ramet to the offspring ramet via the rhizome. This process is called physiological integration. Even though the nutrient translocation here only encompasses some centimeters to meters, the nutrient support could give the offspring ramets a significant benefit. This benefit might enable plant species to outcompete other species in nutrient poor environments or expand their distribution range to unsuitable habitats. This small scale nutrient transport might for example be an explanation for the invasion of *Elymus athericus* into otherwise unsuitable habitats in Wadden Sea salt marshes. In Wadden Sea salt marshes, *Elymus* is mainly

distributed in the higher salt marsh zone where it forms extended mono-specific stands. The occurrence and dominance of *Elymus* were found to be strongly related to the elevation and drainage conditions of a salt marsh (Veeneklaas et al. 2013). However, in recent years *Elymus* extended its distribution into frequently flooded areas of the low marsh zone, even below the mean high tide water level (Bockelmann & Neuhaus 1999). It is unclear why *Elymus* is now able to spread to lower marshes even though this habitat was expected to be unsuitable. *Elymus* was found to vary in morphology and phenotype depending on site and habitat (Bockelmann et al. 2003). High marsh habitat *Elymus* (hereafter referred to as the high marsh ecotype, HM), with dense and tall stems, and a grey-blue color of leaves, grows in the higher parts of the marsh. Low marsh habitat *Elymus* (hereafter referred to as the high marsh ecotype, LM), in contrast, grows in the lower zones of the marsh and has a light green color, more ramets and spikelets per spike. The application of enriched nitrogen isotope allows to follow the fate of nutrients at the individual scale and could deliver direct evidence for the nutrient support among ramets of *Elymus* (Figure 1.1).

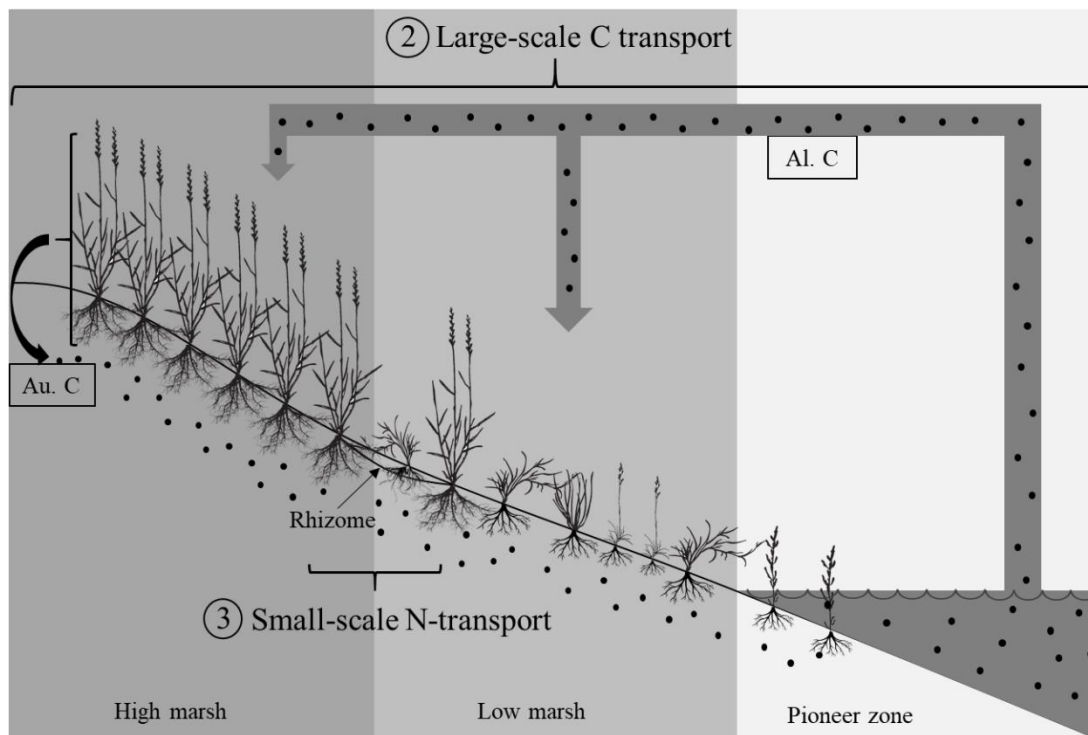


Figure 1.1 Large scale carbon transport along marine-terrestrial gradients may affect the relative contribution of allochthonous versus autochthonous organic carbon in salt marsh soils (**Chapter 2**). Small scale nitrogen transport (**Chapter 3**) between ramets of salt marsh plants might expand their range to otherwise unsuitable habitats.

1.3 Objective and chapter outline

The overall aim of this thesis was to assess small-scale nutrient and large-scale carbon dynamics in salt marshes by means of stable isotope techniques.

Chapter 2: Origin of soil organic carbon in Wadden Sea salt marshes

The study had two key aims

- (i) to identify the effects of geomorphological factors on the contributions of allochthonous organic carbon to the soil OC pool.
- (ii) to investigate the impact of habitat management on the contribution of allochthonous organic carbon to the soil OC pool.

Chapter 3: Physiological integration enhances invasion of two ecotypes of *Elymus athericus*

The study had two key aims:

- (i) to determine whether ramets of *Elymus* are physiologically integrated by analyzing the translocation of labeled heavy nitrogen (^{15}N).
- (ii) to determine the growth performance of connected (physiological integration allowed) and severed (physiological integration prevented) offspring ramets growing in different water stress conditions (waterlogged or non-waterlogged) of both *Elymus* HM and LM ecotypes.



2

Origin of soil organic carbon in Wadden Sea salt marshes

2.1 Introduction

Salt marshes and other tidal wetlands play an important role in climate change mitigation because they are more efficient at storing soil organic carbon (OC) than most terrestrial systems (Chmura et al. 2003, Mcleod et al. 2011). Additionally, and in contrast to other wetland ecosystems, sulfate inputs through seawater effectively suppress methane production and consequently methane emissions in many tidal wetlands, thereby further improving their greenhouse-gas (GHG) balance in relation to other ecosystems (Poffenbarger et al. 2011). The outstanding capacity of tidal wetlands for long-term carbon sequestration, which is estimated to be as much as 3 - 50 times higher than in rainforests (Howard et al. 2017), results from three main factors, namely (1) high rates of OC input, (2) reduced rates of decomposition, and (3) constant burial of organic material (Bridgham et al. 2006, Mcleod et al. 2011). Tidal wetlands have high rates of OC input because they have high net primary productivity (Duarte et al. 2013), and they are effective in trapping particulate OC from both allochthonous (Al; marine) and autochthonous (Au; terrestrial) sources (Kennedy et al. 2010). Moreover, reduced conditions of wetland soils are assumed to inhibit microbial metabolism and slow down the decay of organic matter (OM) such that carbon accumulates in soils and remains stable over centuries and millennia (Mcleod et al. 2011). As tidal wetland soils accrete vertically with rising sea level, they do not become carbon saturated like most terrestrial soils. Rates of carbon sequestration can therefore be maintained over long time scales, potentially millennia, as evident by deep, carbon rich deposits found in tidal-wetland ecosystems worldwide (Mcleod et al. 2011).

The recognition of the important role these coastal ecosystems play in the global carbon (C) cycle has led to the concept of 'blue carbon' and a strong interest to include these ecosystems in C crediting programs. For instance, protection, restoration, or construction of blue C ecosystems could be credited as GHG-offset activities in the context of climate-change policy (Nellemann et al. 2009, Callaway et al. 2012). Such measures, however, need to be based on a thorough understanding of the processes driving carbon sequestration in these ecosystems.

Most work on carbon sequestration in tidal wetlands has focused on estimating sequestration rates and carbon pools (Chmura et al. 2003, Duarte et al. 2013, Ouyang & Lee 2014), while few studies focus on the origin of OC stored in the ecosystem

(Bauer et al. 2013). Yet, the origin of OC may have important implications for C crediting and GHG-offset activities. The OC feeding the soil OC pool either derives from in situ assimilation of CO₂ through primary production (autochthonous; Au OC) or from external sources (allochthonous; Al OC), i.e. phytoplankton derived sedimentary OC. To assess the origin of OC, the analysis of $\delta^{13}\text{C}$ is commonly used in coastal environments (Thornton & McManus 1994, Lamb et al. 2007, Kemp et al. 2010, Pilarczyk et al. 2012) as the stable C isotope value differs between autochthonous and allochthonous sources. In the latter case, however, the alternative fate of Al OC if it had not accumulated in the tidal wetland is unclear. If the externally produced Al OC could be also sequestered outside the tidal wetland (i.e. in the deep sea or in tidal flats), it is questionable whether C credits can be attributed to the tidal wetland. Yet, the relative contribution of Al OC versus Au OC to the soil OC pool can vary considerably across tidal wetlands (Middelburg et al. 1997). However, the factors directly and/or indirectly affecting the contribution of Al OC versus Au OC inputs are largely unknown. It can be assumed that the geomorphology of tidal wetlands and habitat management might play key roles in the relative importance of Al OC versus Au OC inputs. Likewise, studies assessing the spatial relative distribution of Al OC versus Au OC inputs within systems are scarce.

Numerous studies investigated which geomorphological factors affect the spatial pattern of sediment deposition in tidal wetlands, which is the main pathway for Al OC input. Those factors include the elevation of the marsh, and the distance to the sediment source (i.e. distance to the creek, distance to the marsh edge) (Fagherazzi et al. 2012). In general, lower elevations and closer proximity to the sediment source result in higher sediment deposition (Esselink et al. 1998, Temmerman et al. 2003, Chmura & Hung 2004). Additionally, vegetation height is another potential factor which can increase sediment deposition as tall vegetation was found to reduce water flow velocity during tidal inundations (Fagherazzi et al. 2012). As Al OC is transported with sediment suspended in the flooding water, elevation, distance to the marsh edge, and distance to the nearest creek might be the main factors which directly affect the spatial pattern of the relative contribution of Al OC to tidal wetland soils. In addition, as mentioned above, vegetation height could mediate the relative contribution of Al OC to tidal wetland soils as taller vegetation can catch more sediment (Morris et al. 2002), potentially resulting in more Al OC in the salt marsh soil compared to shorter vegetation.

Vegetation height may additionally be influenced by nature management of tidal wetlands, such as livestock grazing in salt marsh ecosystems (Elschot et al. 2013, Nolte, Müller, et al. 2013). Livestock grazing is common in European salt marshes, but also in other regions like China and South America. Livestock grazing reduces vegetation height (Elschot et al. 2013), and therefore sediment deposition might be also decreased (Nolte et al. 2015) resulting in lower Al OC contribution. On the contrary, grazing also affects biomass production (Morris & Jensen 1998, Di Bella et al. 2014), which is the source of Au OC. Both reduction in aboveground biomass production (Morris & Jensen 1998) and increases in belowground biomass production (Elschot et al. 2015) under livestock grazing have been reported. Overall, an understanding of the effects of livestock grazing on the relative contribution of Al OC versus Au OC inputs is missing so far.

The present study assesses the spatial distribution of Al OC in two salt marshes at the Wadden Sea mainland coast of Germany. We expect that the relative contribution of Al OC to the soil OC pool is driven by geomorphological factors and additionally mediated by livestock grazing. Specifically, we hypothesize first that a higher Al OC contribution would be found in lower elevations within the tidal frame. We hypothesize second that the Al OC contribution would decrease with distance to potential sediment sources, namely the distance to creek and the distance to marsh edge. Furthermore, we assess the impact of livestock grazing on the contribution of Al OC to the soil OC pool. We expect that grazing affects vegetation height and biomass production and thus alters both the input of Al OC and of Au OC to the soil OC pool.

2.2 Materials and methods

2.2.1 Study sites and sampling design

The study was conducted from December 2014 to September 2015 in two salt marshes at the mainland coast of the Schleswig-Holstein Wadden Sea National Park, Germany (Figure 2.1 A). The salt marshes within the National Park cover an area of 122 km² (Stock et al. 2005, Esselink et al. 2017). Both study sites, Dieksanderkoog (DSK) and Sönke-Nissen-Koog (SNK), are minerogenic, and shallow depositional salt marshes. In both sites, the presence of a rectangular network of creeks, ditches and levees reveal the anthropogenic origin of the salt marsh formation during land

reclamation in the beginning of the 20th century (Müller et al. 2013). The DSK (53°58'N, 8°53'E) is located in the southern part of the National Park at the mouth of the Elbe Estuary, while the SNK (54°38'N, 8°50'E) is situated in the north, ca. 35 km south of the Danish border (Figure 2.1 A). Traditional land use of the two sites was sheep grazing until 1988 and 1992 in the SNK and DSK, respectively. At these years, a long-term grazing experiment was established at the two marsh sites including a grazed and an ungrazed treatment (Stock et al. 2005).

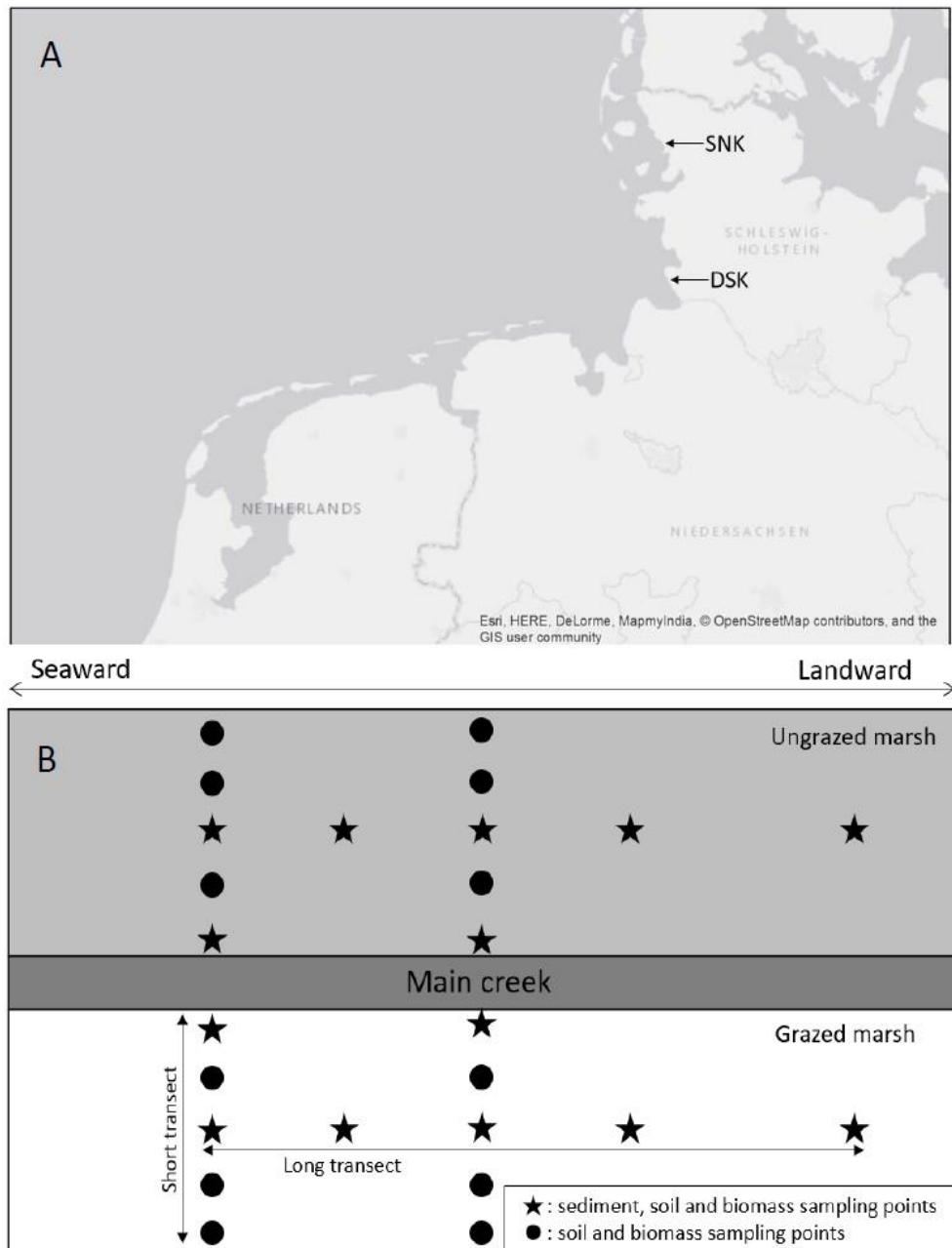


Figure 2.1 A Location of study sites in the Wadden Sea; B Sampling design. Sampling points were organized along short and long transects in each nature management treatment in both sites. Soil and biomass samples were collected at all points while sediment samples were only collected at points marked with an asterisk.

At both study sites, we used grazed and ungrazed treatment areas located next to each other and separated by a straight main creek. In each treatment area, sampling points were placed along short and long transects (Figure 2.1. B). At SNK, the livestock is unable to access the pioneer zone (marsh zone flooded twice a day), and therefore we only consider the marsh platform excluding the pioneer zone in both sites. Furthermore, the width of the pioneer zone differs between sites. To improve comparability we therefore do not use the absolute distance to the marsh edge (Temmerman et al. 2003), but the length along the transect from seaward to landward points. The long transect consisted of five points, starting from 0 m to 731 m in DSK and to 622 m in SNK, but with an almost constant distance to the main creek (62 m in DSK; 54 m in SNK). As long transect distance mainly represents the absolute distance to marsh edge within this study, we hereafter use the term “distance to marsh edge” to improve readability. We also installed two short transects in each treatment, which were perpendicular to the main creek and thus represent the distance to the creek. Each short transect included five points with different distances to the main creek (2 - 107 m in DSK; 3 - 104 m in SNK). The middle points of the short transects are also part of the long transects (Figure 2.1 B). This sampling design resulted in N = 13 sampling points per treatment and a total of N = 52 sampling points for both marsh sites. Surface elevation relative to the German ordnance datum (NHN = Normalhöhennull) of sampling points was measured using a laser leveling device (Trimble, Sunnyvale, California, USA) and a nearby fixed benchmark with known elevation.

2.2.2 Sample collection

We used C stable isotope technique to differentiate between Al and Au OC sources. To assess the $\delta^{13}\text{C}$ of the Al OC source, freshly deposited sediments were collected using circular sediment traps (Temmerman et al. 2003, Nolte, Koppelaar, et al. 2013). The sediment traps were built of plastic plates with an internal diameter of 18.9 cm and a rim of 3 cm, and were equipped with a floatable lid to protect the deposited sediment in the trap from heavy rains and other disturbances (Temmerman et al. 2003). Traps were fixed to the marsh surface with a plastic stick of 100 - 120 cm length. The stick further allowed the lid to move up and down during inundation

(Butzeck et al. 2015). Sediment traps were emptied after a storm event in December 2014. During storm-induced high tides, the whole marsh platform is flooded and thus sediment is transported and deposited on the marsh (Butzeck et al. 2015). The sediment samples were brought to the laboratory within one day. As we assumed the $\delta^{13}\text{C}$ of the freshly deposited sediment to be similar throughout the marsh, we only deployed sediment traps at five sampling points in the long transect and at two sampling points nearest to the creek in the two short transects in each treatment and site (Figure 2.1. B), resulting in the total number of 28 sediment traps.

Above- and belowground plant biomass, as Au OC source, was sampled at all sampling points at the end of the growing season 2015. Aboveground biomass was harvested at each sampling position in an area of 100 cm², put in plastic bags, transferred to the laboratory and stored at -20 °C until processing for further analysis. Belowground biomass of the uppermost (5 cm) soil layer was collected using a soil corer (Ø 2.5 cm). Dual cores were taken at each sampling position and samples were subsequently pooled. These soil samples containing the belowground biomass were also transferred to the laboratory and stored at -20 °C until further processing.

To assess the $\delta^{13}\text{C}$ of the organic matter in the soil, soil samples of the uppermost 5 cm soil layer were collected at all sampling positions using a soil corer (Ø 2.5 cm) in December 2014. Our study investigated the origin of OC in only the uppermost 5 cm of the soil because belowground biomass is usually concentrated at this depth in Wadden Sea salt marshes (Bartholdy et al. 2014) and down-core fractionation due to microbial activities and degradation are commonly reported (Van de Broek et al. 2018). Samples were transferred to the laboratory and stored at -20 °C until further processing.

2.2.3 Sample processing and analysis

Sediment and soil samples were dried at 60 °C to constant weight, and plant roots and coarse organic debris (> 2 mm) were removed. Afterwards, samples were ground and homogenized using pestle and mortar. A sub-sample (1 g) of each soil and sediment sample was placed into a glass vial and treated with 10% hydrochloric acid to remove carbonates. Afterwards, sub-samples were dried again at 60 °C to constant weight.

The top 5 cm of leaves from each aboveground biomass sample was clipped, thawed, and rinsed under tap water to remove sediment and dust from the surface of the leaves. Subsequently, the leaf tips were rinsed with deionized water. Similarly, the belowground biomass samples were cleaned and separated from the soil using tap water, and afterwards samples were rinsed with deionized water. Both above- and belowground biomass samples were dried at 60 °C until constant weight. Dried biomass samples were then homogenized and ground for stable isotope analysis.

$\delta^{13}\text{C}$ of dried and homogenized bulk soil OM, sediment OM and fresh above- and belowground plant biomass were determined using an isotope ratio mass spectrometer (Nu Horizon, Nu Instruments Limited, UK). Samples were analyzed in tandem with both laboratory (BBOT 2,5-Bis-(5-tert-butyl-2-benzo-oxazol-2-yl) thiophene) and international standards (IAEA-600 Caffeine). Precision of isotopic analysis was < 0.1‰ for $\delta^{13}\text{C}$. The isotopic compositions of all samples are reported using the standard δ -notation versus Vienna PeeDee Belemnite (VPDB) as parts per thousand (‰) according to the following equation:

$$\delta^{13}\text{C}(\text{‰}) = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000 \quad (1)$$

where R is the ratio of $^{13}\text{C}/^{12}\text{C}$.

The relative proportions of the Al OC and Au OC were determined by applying a two-end-member mixing model based on the stable isotope composition of OC (Hedges et al. 1988, Jasper & Gagosian 1990, Westerhausen et al. 1993, Schubert & Calvert 2001, Winkelmann & Knies 2005, Belicka & Harvey 2009):

$$f_{\text{Al}}(\%) = \frac{\delta^{13}\text{C}_{\text{Soil}} - \delta^{13}\text{C}_{\text{Au}}}{\delta^{13}\text{C}_{\text{Al}} - \delta^{13}\text{C}_{\text{Au}}} \quad (2)$$

where: f_{Al} (%) is the percentage contribution of the Al OC in soil; $\delta^{13}\text{C}_{\text{Soil}}$ is $\delta^{13}\text{C}$ measured in the soil; $\delta^{13}\text{C}_{\text{Au}}$ is $\delta^{13}\text{C}$ of the Au OC source (plant biomass); and $\delta^{13}\text{C}_{\text{Al}}$ is $\delta^{13}\text{C}$ of Al OC.

The calculated contributions of Al OC vs. Au OC sources depend on the type of plant biomass (i.e. above- vs. belowground biomass) considered for the mixing-model calculations. As the contributions of above- vs. belowground plant biomass to the soil OM pool are uncertain, we calculated f_{Al} (%) under the assumption of three different end-member terms for Au $\delta^{13}\text{C}$: 1.) $\delta^{13}\text{C}$ of the aboveground biomass (Model A), 2.) $\delta^{13}\text{C}$ of the belowground biomass (Model B); and 3.) the calculated $\delta^{13}\text{C}$ of a 1:1 mixed contribution of above- and belowground biomass (Model M).

2.2.4 Statistical analyses

Two-way ANOVA was used to test for differences in the isotopic composition of sediment and soil between sites and treatments. Three-way ANOVA was used to test for the effects of site, treatment, and type of plant tissue (above- vs. belowground biomass) on plant $\delta^{13}\text{C}$, and subsequent two-way ANOVAs were conducted to test for the effects of treatment and site on the $\delta^{13}\text{C}$ of above- and belowground biomass separately. Tukey's HSD test was used for pairwise comparisons.

ANCOVA was conducted to test for effects of site, treatment, distance to the marsh edge, distance to the creek and relative elevation (elevation in comparison to mean surface elevation at each site) on the contribution of Al OC to the soil. Only pairwise interactions of factors were considered in the models. As model simplification is an essential part of analysis of covariance, the most insignificant parameters were dropped step by step, and as few parameters as possible were kept in the model (Crawley 2005). The more complex models were retained only if the p-value (ANOVA based) comparing the two models was < 0.05 (Crawley 2005). To exclude that identified effects on the contribution of Al OC are artefacts driven by spatial variability in the plant isotopic signature, we additionally conducted the same ANCOVA procedure with the three Au end-member types considered here (i.e. aboveground, belowground, 1:1 mixed). Statistical analyses were carried out using the software package R (R version 3.3.1 (2016) The R Foundation for Statistical Computing 2016).

2.3 Results

2.3.1 $\delta^{13}\text{C}$ of Al OC, Au OC and soil samples

$\delta^{13}\text{C}$ of Al OC in freshly deposited sediment differed significantly between sites ($F = 88.6$, $p < 0.0001$), with Al OC being more depleted in ^{13}C at DSK ($-24.22 \pm 0.35\%$) vs. SNK ($-23.10 \pm 0.25\%$) (Table 2.1). Within sites, no significant differences in $\delta^{13}\text{C}$ of the Al OC were detected between grazed and ungrazed treatments (Table 2.1). There was no significant interaction effect of site and treatment on the $\delta^{13}\text{C}$ of Al OM ($p > 0.4$). Therefore, the mean $\delta^{13}\text{C}$ of each site was chosen for $\delta^{13}\text{C}_{\text{Al}}$ in Equation 2.

Table 2.1 $\delta^{13}\text{C}$ of two organic carbon sources (‰) (allochthonous organic carbon and autochthonous organic carbon) and $\delta^{13}\text{C}$ (‰) of soil samples in grazed and ungrazed treatments of two Wadden Sea salt marsh sites (Dieksanderkoog (DSK) and Sönke-Nissen-Koog (SNK)). Significant differences at $p < 0.05$ based on Tukey's HSD test for pairwise comparisons. Values within one column were not connected by the same letters. Value are means and \pm SD

Site	DSK		SNK	
Treatment	Grazed	Ungrazed	Grazed	Ungrazed
Allochthonous OC	$-24.29^a \pm 0.42$	$-24.14^a \pm 0.26$	$-23.08^b \pm 0.20$	$-23.12^b \pm 0.31$
Autochthonous OC				
<i>Aboveground biomass</i>	$-27.81^a \pm 0.74$	$-27.62^{a,b} \pm 0.76$	$-26.86^{a,b} \pm 1.16$	$-26.45^b \pm 1.58$
<i>Belowground biomass</i>	$-27.38^a \pm 1.16$	$-26.60^{a,b} \pm 0.77$	$-27.10^a \pm 1.05$	$-25.60^b \pm 0.95$
<i>Mixed biomass</i>	$-27.60^a \pm 0.98$	$-27.11^a \pm 0.91$	$-26.97^a \pm 1.09$	$-26.03^b \pm 1.35$
Sample (soil)	$-27.04^a \pm 0.57$	$-26.46^{a,b} \pm 0.59$	$-25.94^b \pm 0.96$	$-25.28^b \pm 0.58$

The $\delta^{13}\text{C}$ values of Au OC differed between sites ($F = 17.1$, $p < 0.0001$), treatments ($F = 11.6$, $p < 0.001$), and type of plant tissue ($F = 5.9$, $p < 0.05$). There was a significant interaction effect of treatment and type of plant tissue on $\delta^{13}\text{C}$ of Au OC ($F = 4.0$, $p < 0.05$). The subsequent two-way ANOVAs showed the effects of site and treatment on $\delta^{13}\text{C}$ of aboveground biomass, belowground biomass and the 1:1 mixed above- and belowground biomass (Table 2.1). The aboveground biomass $\delta^{13}\text{C}$ values were significantly different between sites ($F = 11.5$, $p < 0.005$), with being more depleted in ^{13}C at DSK ($-27.72 \pm 0.74\%$) vs. SNK ($-26.65 \pm 1.38\%$) (Table 2.1). Treatment had no effect on $\delta^{13}\text{C}$ of aboveground biomass. There was also no interaction of site and treatment effect on the $\delta^{13}\text{C}$ of aboveground biomass. $\delta^{13}\text{C}$ values of belowground biomass were affected significantly by site ($F = 6.0$, $p < 0.05$), being more depleted in ^{13}C at DSK ($-27.00 \pm 1.05\%$) vs. SNK ($-26.32 \pm 1.24\%$) (Table 2.1). $\delta^{13}\text{C}$ of belowground biomass were constantly more depleted under

grazing ($F = 16.5$, $p < 0.001$) at both sites. The interaction of site and treatment had no effect on belowground biomass $\delta^{13}\text{C}$. Similarly, the 1:1 mixed biomass $\delta^{13}\text{C}$ was affected significantly by site ($F = 15.8$, $p < 0.0005$) and treatment ($F = 9.7$, $p < 0.01$), but not by the interaction of site and treatment. These differences in $\delta^{13}\text{C}$ between above- and belowground biomass, grazed and ungrazed treatments, and sites did not allow to use any sort of mean $\delta^{13}\text{C}$ value for the Au end-member term in the mixing model. Instead, we used the specific $\delta^{13}\text{C}$ values of above-, belowground and mixed biomass of each sampling point for the respective mixing-model calculations (Equation 2).

Soil OC had $\delta^{13}\text{C}$ values varying from -28.00‰ to -23.24‰ and differed significantly by site ($F = 34.7$, $p < 0.001$). DSK soil OC ($-26.75 \pm 0.35\text{‰}$) was more depleted in ^{13}C than SNK soil OC ($-25.61 \pm 0.85\text{‰}$). Treatments had an effect on soil OC $\delta^{13}\text{C}$, ($F = 10.4$, $p < 0.01$), with more depleted in ^{13}C under grazing ($-26.49 \pm 0.95\text{‰}$) compared to ungrazed treatments ($-25.87 \pm 0.82\text{‰}$) (Table 2.1). There was no significant interaction effect between site and treatment on soil OC $\delta^{13}\text{C}$ values.

2.3.2 Contribution of AI OC to salt marsh soils

The three mixing models in which different Au OC end-member assumptions (only aboveground plant biomass (A), only belowground plant biomass (B), or 1:1 mixed plant biomass (M)) were used to calculate the contribution of AI OC to the salt marsh soil OC yielded different results (Figure 2.2).

When comparing the outcomes of the three models, AI OC contribution in grazed treatments ranged from 17.72 ± 3.85 (%) to 23.64 ± 4.84 (%). In comparison, a higher range was found in ungrazed treatments (from 11.79 ± 4.95 to 38.99 ± 5.31 (%)). Here, we found that a mixing model using the aboveground $\delta^{13}\text{C}$ resulted in much higher contributions of AI OC to the soil compared to using the belowground $\delta^{13}\text{C}$.

The ANCOVA results for the three mixing models, which were based on different Au OC end-member assumptions (see above) also differed (Table 2.2). For further interpretation, we therefore considered only those effects as robust which were found in at least two models (Table 2.2).

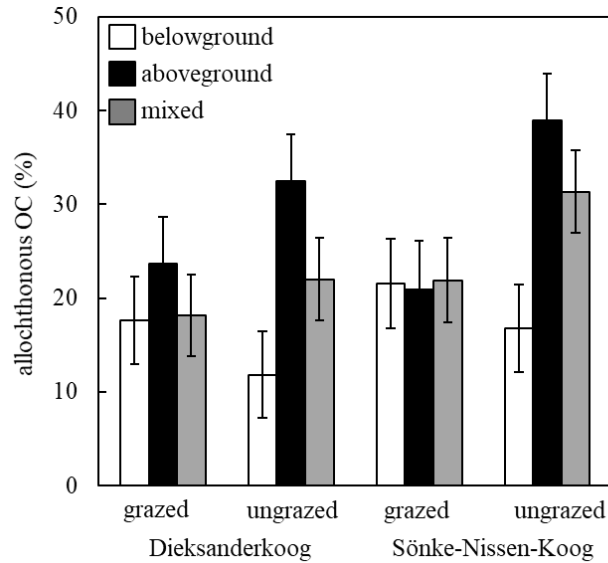


Figure 2.2 Allochthonous organic carbon contribution (%) to the salt marsh soil organic carbon in grazed and ungrazed treatments of two Wadden Sea salt marsh sites (Dieksanderkoog and Sönke-Nissen-Koog). The calculations are based on three end-member assumptions for Au OC $\delta^{13}\text{C}$. 1) $\delta^{13}\text{C}$ of the aboveground biomass; 2) $\delta^{13}\text{C}$ of the belowground biomass, and 3) the calculated $\delta^{13}\text{C}$ of a 1:1 mixed contribution of aboveground and belowground biomass. Values are means \pm SE.

Table 2.2 Results of ANCOVA analyses of the three mixing models based on three autochthonous organic carbon end-member assumptions. 1) $\delta^{13}\text{C}$ of the aboveground biomass (Model A), 2) $\delta^{13}\text{C}$ of the belowground biomass (Model B); and 3) the calculated $\delta^{13}\text{C}$ of a 1:1 mixed contribution of aboveground and belowground biomass (Model M). (-) indicates that factor dropped out of the ANCOVA model.

Models	A		B		M	
	<i>F value</i>	<i>p</i>	<i>F value</i>	<i>p</i>	<i>F value</i>	<i>p</i>
site	0.276	ns.	1.068	ns.	3	<0.1
treat	9.413	<0.01	1.583	ns.	3	0.1
Dm	0.008	ns.	0.029	ns.	0	ns.
Dc	5.12	<0.05	0.461	ns.	3	<0.1
Rel.elv	-	-	3.07	<0.1	4	<0.05
site*treat	-	-	-	-	-	-
site*Dm	-	-	3.517	<0.1	-	-
site*Dc	9.212	<0.01	-	-	4	<0.05
site*Rel.elv	-	-	-	-	-	-
treat*Dm	5.707	<0.05	3.415	<0.1	5	<0.05
treat*Dc	-	-	-	-	-	-
treat*Rel.elv	-	-	4.928	<0.05	-	-
Dm*Dc	-	-	-	-	-	-
Dm*Rel.elv	-	-	-	-	-	-
Dc*Rel.elv	-	-	-	-	-	-

Notes: Site [site], treatment [treat]; distance to marsh edge [Dm]; distance to creek [Dc]; relative elevation [Rel.elv].

The interaction effect between site and distance to the creek was found to be significant in model A and model M (Table 2.2). In DSK, we found the highest Al OC contributions close to the creek (Figure 2.3 A). With increasing distance to the creek, there was a steep drop until a more or less stable value was reached. In contrast, the contribution of Al OC in SNK showed a different pattern and remained quite stable with increasing distance to the creek.

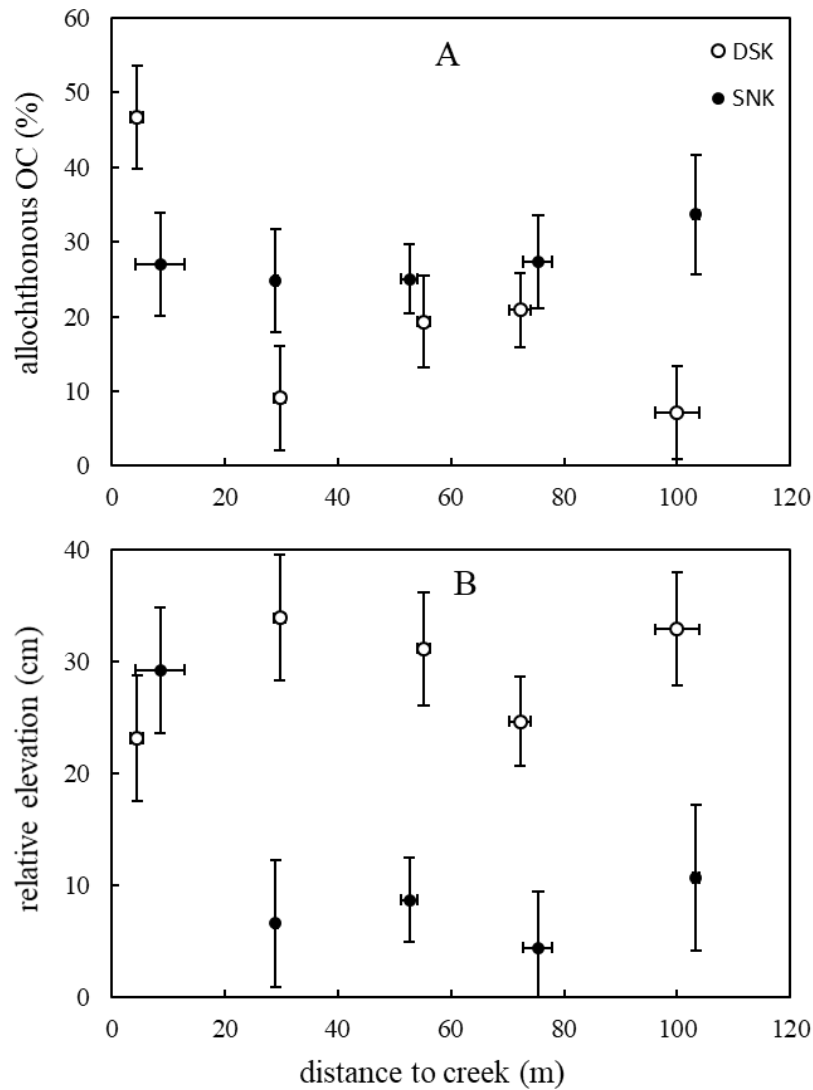


Figure 2.3 **A** Allochthonous organic carbon contribution to the salt marsh soil organic carbon in relation to distance to the creek in Dieksanderkoog (DSK) and Sönke-Nissen-Koog (SNK) salt marshes in model M; **B** Relative elevation of the sampling positions in relation to distance to the creek in DSK and SNK salt marshes. Values are means \pm SE.

The interaction between treatment and distance to the marsh edge also had significant effects on the contribution of Al OC in model A and model M (Table 2.2). Close to the marsh edge (0 to 300 m), the percentages of Al OC contribution were quite similar in both treatments (Figure 2.4 A). In the area of greater distance to the marsh edge, however, we found opposite patterns for the two treatments. Here, the percentage of Al OC increased with increasing distance to the marsh edge in ungrazed treatment, while it decreased in the grazed treatment (Figure 2.4 A).

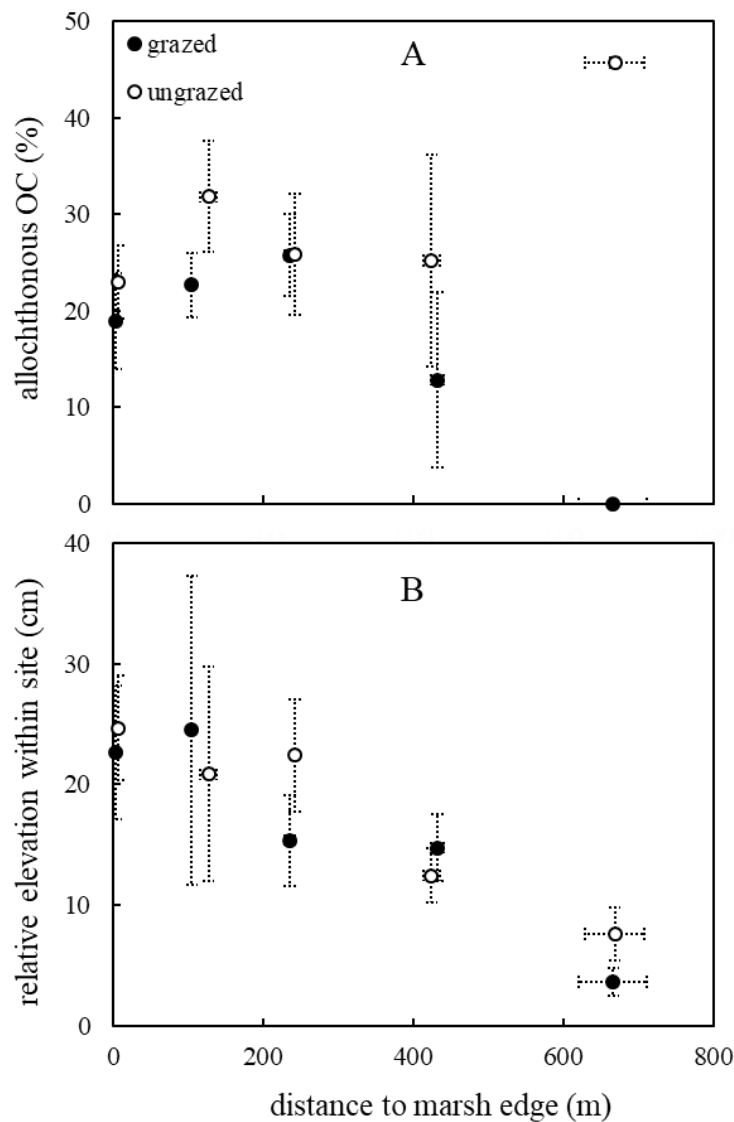


Figure 2.4 A Allochthonous organic carbon contributions to the salt marsh soil organic carbon in relation to distance to the marsh edge in DSK and SNK salt marshes in model M; **B** Relative elevation of the sampling positions in relation to distance to marsh edge in DSK and SNK salt marshes. Values are means \pm SE.

The ANCOVA results for three Au end members considered here (i.e. aboveground, belowground, mixed) showed neither interaction effects of distance to marsh edge and treatment, nor of site or distance to creek on any of Au end member $\delta^{13}\text{C}$, excluding that the identified effects on the Al OC contribution to the soil are artefacts driven by spatial variability in the plant $\delta^{13}\text{C}$ (Table 2.3).

Table 2.3 Results of ANCOVA analyses of three autochthonous organic matter types. (-) indicates that factor dropped out of model because of insignificant effect.

Al OM Factors	Aboveground biomass		Belowground biomass		1:1 mixed biomass	
	<i>F value</i>	<i>p</i>	<i>F value</i>	<i>p</i>	<i>F value</i>	<i>p</i>
site	11.415	<0.01	6.449	<0.05	16.033	<0.001
treat		-	17.759	<0.001	12.07	<0.01
Dm		-	0.224	ns.	2.569	ns.
Dc		-		-		-
Rel.elv	1.651	ns.		-		-
site*treat		-		-		-
site*Dm		-	6.121	<0.05		-
site*Dc		-		-		-
site*Rel.elv	3.862	<0.1		-	3.385	<0.1
treat*Dm		-		-		-
treat*Dc		-		-		-
treat*Rel.elv		-		-		-
Dm*Dc		-		-		-
Dm*Rel.elv		-		-		-
Dc*Rel.elv		-		-		-

Notes: Site [site], treatment [treat]; distance to marsh edge [Dm]; distance to creek [Dc]; relative elevation [Rel.elv].

2.4 Discussion

Elevation in relation to the tidal frame and proximity to the sediment source are well known geomorphological factors explaining spatial pattern of sediment deposition in salt marshes (Friedrichs & Perry 2001, Temmerman et al. 2003, Fagherazzi et al. 2012), and thus are potentially affecting the relative contribution of Al OC to the top soil. Accordingly, we hypothesized that the relative contribution of Al OC would be higher in lower elevations within the tidal frame and with lower distances to potential sediment sources (i.e. creek, marsh edge). In the following, we discuss effects on the relative contribution of Al OC to the top soil that were significant under at least two of the different mixing-model assumptions (Table 2.2).

In contrast to our hypothesis, an effect of relative elevation as a single factor on the relative contribution of Al OC to the top soil could not be clearly identified. We did, however, identify a negative effect of distance to the creek on the relative contribution of Al OC to the top soil. Yet, this effect was only found at DSK and not at SNK. We argue that the different response between sites can be explained by the differences in relative elevation patterns within sites (Figure 2.3 B). At DSK, sampling points closest to the creek are also characterized by the lowest relative elevation which probably had the highest inundation frequency, leading to the highest Al OC input. The other points with larger distance to the creek and with a higher relative elevation had lower inundation frequencies, and consequently lower Al OC input. In contrast, the micro-topographic structure at SNK differs, resulting in different sediment deposition, and consequently Al OC contribution pattern. Here, the existence of a natural levee along the creek only allowed sediment transport to the marsh platform once the tidal height was higher than the elevation of the levee. As a consequence, the inundation frequency was similar over the marsh platform, resulting in equal Al OC input. We therefore conclude, that geomorphological factors such as elevation and the distance to the creek as sediment factors which have been found to determine the spatial patterns of sediment deposition (Esselink et al. 1998, Temmerman et al. 2003, Chmura & Hung 2004), also are important factors determining the relative contribution of Al OC to the soil. However, we do not know the extent to which changes in the Au OC input may have contributed to this finding. That is, biomass production in salt marshes is strongly controlled by elevation,

affecting both hydrology and nutrient supply (Callaway et al. 1997, Morris et al. 2002).

Besides distance to the creek, distance to the marsh edge is a second parameter describing the proximity of a given point to a potential sediment and thus Al OC source. We indeed demonstrate a significant effect of distance to marsh edge. This effect, however, differed significantly between grazed and ungrazed treatments (Table 2.2). Specifically, our data suggest that the relative contribution of Al OC decreased with increasing distance to the marsh edge under grazing, whereas the opposite pattern was found in the ungrazed treatments (Figure 2.4 A). We argue that the unexpected increase of the Al OC contribution with distance to the marsh edge and thus the expected sediment source in ungrazed treatments can be explained by elevational patterns of the marsh platform that are typically found across Wadden Sea salt marshes and elsewhere. That is, the landward decrease in elevation resulting from ditching, diking and grazing processes in salt marshes (LeMay 2007, Stock 2011, Müller-Navarra et al. 2016, Esselink et al. 2017). As a consequence, landward areas of the marsh platform are flooded first and more frequently, despite greater distance to the marsh edge, potentially explaining the pattern displayed in Figure 2.4 A. Although the grazed treatment shows the same decrease in elevation with distance to the marsh edge (Figure 2.4 B), this did not translate into higher contributions of Al OC. In fact, the opposite trend was observed (Figure 2.4 A): At 0 - 300 m long-transect distance, the Al OC contributions were more or less similar between treatments. Here, the grazing pressure in the grazed treatment was presumably low, because livestock tends to remain close to the freshwater source near the seawall (Kiehl et al. 2001). As a consequence, also the vegetation height is similar between treatments (Nolte unpubl. data) in this area, leading to no clear effect on the sediment deposition, and thus resulting in no difference in Al OC contribution. With further distance to the marsh edge, however, the Al OC contribution drastically decreased in grazed treatment. At least two non-exclusive mechanisms could have caused this effect. First, grazing pressure is highest close to the seawall leading to drastically changed vegetation structure, most remarkably decreasing vegetation height (Bos et al. 2002, Elschot et al. 2013), and therefore possibly indirectly reducing sediment deposition (Yang et al. 2008) and Al OC contribution. Second, grazing can also affect biomass production (Morris & Jensen 1998, Di Bella et al. 2014), which represents the availability of the Au source. Greater belowground biomass stocks under grazing have been reported by

Elschot et al. (2015). Thus, the observed pattern can also be driven by a greater Au OC contribution under grazing.

Large differences were found in the estimates of Al OC contribution when the three mixing model approaches based on different plant end-members assumptions were compared, particularly in the ungrazed treatments. Here, mean Al OC contributions ranged from 11.79 ± 4.95 (%) to 32.45 ± 5.33 (%) and from 16.77 ± 5.04 (%) to 38.99 ± 5.31 (%) in DSK and SNK, respectively (Figure 2.2). We therefore want to highlight the importance to consider differences in the isotopic composition of different plant tissues in mixing-model approaches used to calculate the relative contributions of Al vs. Au OC, which is surprisingly left unconsidered in many studies (but see Kelleway et al. (2018)). In the present study, we found differences in $\delta^{13}\text{C}$ between above- and belowground biomass (Table 2.1). Because the relative contribution of above- vs. belowground biomass to the top soil is unknown for our study sites, also the absolute values presented here need to be considered with caution. For more accurate budgets of Al OC vs. Au OC contributions to tidal-wetland ecosystems, future studies should combine C stable-isotope techniques with other stable isotope techniques ($\delta^{15}\text{N}$, $\delta^{34}\text{S}$, compound-specific C isotopic analysis), biomarker approaches (e.g., glomalin, n-alkalenes) (Prahl et al. 1994, Adame et al. 2012) or environmental DNA assessments (Reef et al. 2017).

The main goal of this study was not to calculate precise budgets of Al OC vs. Au OC to the salt marsh soil, but to identify important factors controlling the relative distribution of Al OC vs. Au OC. In order to exclude artefacts caused by spatial variability in the plant C isotopic signature, we also tested for effects of site, treatment, distance to the marsh edge, distance to the creek and relative elevation on the Au end-member $\delta^{13}\text{C}$ (plant end-member). We found neither interaction effects of distance to marsh edge and treatment, nor of site and distance to creek on Au end member $\delta^{13}\text{C}$. We therefore conclude that the identified interaction effects on the Al OC contribution to the soil are not artefacts driven by spatial variability in the plant $\delta^{13}\text{C}$ (Table 2.3). We argue that such sensitivity analyses are crucial to assess the reliability of conclusions derived from stable-isotope mixing-model approaches.

2.5 Conclusions

To conclude, our present study partly supported previous research showing that more Al OC is found closer to the sediment sources and in lower elevations (Middelburg et al. 1997, Spohn et al. 2013, Hansen et al. 2017, Yuan et al. 2017). However, while previous studies have shown that the contribution of Al OC to soil OC varies among different zones of tidal marshes (Spohn et al. 2013) or different tidal marshes (Middelburg et al. 1997, Hansen et al. 2017, Yuan et al. 2017), our study is the first to demonstrate small scale variability in Al OC distribution within the marsh platform. In addition, our study provided first evidence that livestock grazing affects the relative contribution of Al OC vs. Au OC in salt marsh soils. Our study shows that spatial factors and land use are important drivers shaping salt marsh OC pools by their interaction effects on the origin of OC in salt marshes. This has important implications as the origin of OC and its quality (Khan et al. 2015) affects the microbial community (Mueller et al. 2017), thereby influencing C turnover rate and C sequestration. Further research is however needed to assess the extent to which Al OC contributes to total soil OC stocks across blue-carbon ecosystems.

2.6 Acknowledgments

We would like to thank Christoph Reisdorff for his methodological advice and help with lab work. We thank Dr. Martin Stock and our project partners of the Wadden Sea National Park Schleswig-Holstein for cooperation. This project was funded by the Bauer-Hollmann Stiftung and the Rudolf und Helene Glaser Stiftung in the framework of the research project INTERFACE (Interaction of fish, plants, carbon and sediment: management and ecosystem functions of Wadden Sea salt marshes).



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Effects of physiological integration and waterlogging on the growth and physiology of two ecotypes of *Elymus athericus* in salt marshes

3.1 Introduction

Coastal salt marshes are areas vegetated by herbs, grasses or low shrubs, bordering saline water bodies, and are subject to periodic flooding as a result of fluctuations (tidal and non-tidal) in the level of the adjacent water bodies (Adam 1993). Salt marshes are highly dynamic ecosystems and form at the interface between land and sea. They provide a wide range of ecosystem services to human population, such as the protection of the coastal shoreline against storm surges and erosion via wave attenuation, sediment stabilization and floodwater adsorption (Feagin et al. 2009, Gedan et al. 2011, Shepard et al. 2011, Möller et al. 2014). Furthermore, salt marshes support coastal fisheries by providing suitable habitat for reproduction, nursery, shelter and food (Barbier et al. 2011). They also provide a unique habitat for a wide range of salt-tolerant plant and animal species (Bakker et al. 2005, Barbier et al. 2011). Another important function of salt marshes is their role in nutrient cycling due to high rates of nutrient retention and transformation, i.e. via denitrification in their sediments (Jordan et al. 2003, Beutel et al. 2009, Mitsch & Gosselink 2015). Furthermore, salt marshes are considered to be more valuable than other wetlands as sink for carbon because of their high carbon sequestration rates and low greenhouse gas emissions (Chmura et al. 2003, Bridgham et al. 2006).

Despite these valuable ecosystem services, salt marshes have been declining globally due to anthropogenic impacts, such as land conversion for agriculture or development (Adam 2002, Valiela et al. 2009), climate change impacts (i.e. accelerated sea-level rise and increased storm intensity) (Craft et al. 2009, Crosby et al. 2016), pollution, and invasive species (Gedan et al. 2011). According to the Global Biodiversity Outlook 3, 25 percent of the total global salt marshes has been lost and one to two percent of salt marsh area is lost annually (Secretariat of the Convention on Biological Diversity 2010). The recognition of the importance of salt marshes has led, however, to changes in wetland management and conservation policies in many countries and to the protection of existing salt marshes. In the last decades, many salt marshes in Europe for example have been designated as nature reserves or parts of National Parks (Stock 2003). Furthermore, the salt marshes of the Wadden Sea are now recognized as part of a UNESCO world heritage site.

The establishment of National parks led to a cessation of livestock grazing which has previously been very common in Wadden Sea salt marshes (Bakker et al. 1993).

This cessation of grazing, in turn, resulted in the expansion of the competitive grass *Elymus athericus* (Link) Kerguelen (Poacea) (hereafter referred to as *Elymus*) and a lower plant species richness on the plot level (Bockelmann & Neuhaus 1999, Esselink et al. 2000, Bakker et al. 2003). The dominance of *Elymus* can be observed in several European marshes, for example at Mont Saint Michel in France (Valery et al. 2004) and Schleswig Holstein in Germany (Stock et al. 2005, Esselink et al. 2009). The mechanisms whereby *Elymus* outcompetes other species could include the ability for both sexual and clonal reproduction, dense rhizomes and roots that leave little space for neighbors, strong competitive ability in nutrient acquisition, and tall dense canopies that absorb light (Bockelmann & Neuhaus 1999). In addition, *Elymus* may be favored by enhanced soil aeration in artificial mainland salt marshes due to the dense drainage systems (Rupprecht et al. 2015).

The vegetation in salt marshes can be classified into distinct zones along an elevation gradient (Suchrow et al. 2012) representing the flooding frequency, with the lowest zone being flooded every day, while the highest zone is only flooded periodically. *Elymus* is mainly distributed in the higher salt marsh zone where it forms extended mono-specific stands. The occurrence and dominance of *Elymus* were found to be strongly related to the elevation and drainage conditions of a salt marsh (Veeneklaas et al. 2013). When abiotic conditions, i.e. inundation frequency depending on elevation and drainage, are optimal for *Elymus*, this species can dominate the marsh within a few years but when drainage is neglected, this dominance cannot be observed (Veeneklaas et al. 2013). Apparently, *Elymus* does not prefer frequently flooded conditions as its low incidence in the waterlogged areas show (Schröder et al. 2002, Veeneklaas et al. 2013). However, in recent years *Elymus* extended its distribution into frequently flooded areas of the low marsh zone (Bockelmann & Neuhaus 1999). It today even occurs below the mean high tide water level (Bockelmann & Neuhaus 1999). It is unclear why *Elymus* is now able to spread to lower marshes even though this habitat was expected to be unsuitable.

A possible explanation for the spread of *Elymus* into frequently flooded, previously assumed unsuitable habitats may be a phenomenon called physiological integration. Physiological integration enables the movement of essential resources such as water, nutrients and photo-assimilates between connected ramets (Pitelka & Ashmun 1985), allowing clonal plants to colonize unfavorable habitats. Physiological integration could also be crucial for *Elymus*' ability to spread vegetatively via

rhizomes into the low marsh zone. This could be a competitive advantage in otherwise too harsh conditions. Despite the abundance of literature that provides evidence in several species for the translocation of certain nutrients, water, and photosynthates among connected ramets (Hester et al. 1994, Saitoh et al. 2006, Roilola et al. 2014, You et al. 2014, Zhou et al. 2014), there are no published studies to date that document the physiological integration in *Elymus*.

Elymus might also have become capable to deal with the harsher conditions of the low marsh by adaptation processes resulting in the differentiation of a respective ecotype. Indeed, *Elymus* was found to vary in morphology and phenotype depending on site and habitat (Bockelmann et al. 2003). High marsh habitat *Elymus* (hereafter referred to as the high marsh ecotype, HM) grows in the higher parts of the marsh which are less frequently flooded. It has dense and taller stems, and a grey-blue color, growing in wide meadows (Bockelmann et al. 2003). In contrast, low marsh habitat *Elymus* (hereafter referred to as the low marsh ecotype, LM), has a light green color, more ramets and spikelets per spike, and grows in a more patchy distribution. It grows in the lower zones of the marsh where it regularly experiences waterlogging and thus anaerobic soil conditions.

In this study, we firstly aim to test experimentally whether (1) ramets of *Elymus* are physiologically integrated. We do so by tracing the translocation of labeled heavy isotope nitrogen (^{15}N). As *Elymus* is a clonal plant, we hypothesize that (1A) connected offspring ramets show higher values of $\delta^{15}\text{N}$ than severed offspring ramets. Additionally, we hypothesize that (1B) connected offspring ramets under water stress (waterlogging) require more support from parent ramets than connected offspring ramets under ambient condition. Because the LM ecotype is presumably adapted to higher flooding stress, we hypothesize that (1C) LM connected offspring ramets need less support from parent ramets than HM connected offspring ramets. Secondly, we aim to determine (2) the growth performance of connected (physiological integration allowed) and severed (physiological integration prevented) offspring ramets growing in different water stress conditions (waterlogged or non-waterlogged) of both *Elymus* HM and LM ecotypes. Specifically, we hypothesize that (2A) non-waterlogged offspring ramets grow better than waterlogged offspring ramets; (2B) the LM ecotype under waterlogged condition shows a higher growth performance than the HM ecotype in the same condition; and (2C) connected offspring ramets grow better than severed offspring ramets under waterlogged conditions.

3.2 Materials and methods

3.2.1 Plant material

The study was carried out with individuals of *Elymus athericus*, a clonal grass native to salt marshes and distributed on continental European salt marshes along the coast from southern Denmark to northern Portugal, and along the southern coasts of the British Isles (Veeneklaas et al. 2013). This species has various synonymous taxonomic names, e.g. *Elymus pycnanthus* (Gordon) Melderis, *Elytrigia pungens* (Pers.) Tutin, *Agropyron pycnanthum* (Godron) Godron & Gren. *Elymus* is a tall, hexaploid, wind-pollinated species with the ability of reproducing clonally as well as sexually (Bockelmann et al. 2003). As mentioned in the introduction, the population morphology and phenotype of *Elymus* vary depending on site and habitat (Bockelmann et al. 2003). Plant material of both ecotypes were collected from their natural habitats in the salt marsh of the Dutch island Schiermonnikoog in April 2015. The plants were transferred into pots and were kept outdoors for one year at University of Hamburg, during which clonal plant growth led to the emergence of new individuals. The phenotype of the two ecotypes continued to be different even after one year of similar growing conditions.

3.2.2 Experimental design

The experiment was conducted in the greenhouse of the Institute for Plant Science and Microbiology at University of Hamburg in summer 2016 for 84 days. Forty parent ramets of each ecotype that developed a rhizome of 8 to 10 cm length were selected. These ramets were planted separately in plastic pots (10 x 10 x 12 cm), filled with homogenized soil from the German salt marsh at Dieksanderkoog (Schleswig-Holstein). Each plastic pot was connected with a silicone tube to another plastic pot of the same size. The rhizomes of each plant were put through the silicone tube and into the soil of the other pot where a new ramet (offspring ramet) established after 3 to 5 days.

All parent pots were subjected to labeling with the heavy nitrogen isotope (^{15}N) by adding 20 ml of a 0.7 mM ammonium nitrate solution containing 98 atom % ^{15}N of $^{15}\text{NH}_4^{15}\text{NO}_3$. This chosen concentration of ^{15}N in the labeling solution is sufficient to detect the potential transport of ^{15}N from the parents to their connected offspring

ramets. As a control, 20 ml unlabeled 0.7 mM NH_4NO_3 solution was added to the offspring pots.

To test the effect of physiological integration, half of the ramet pairs of each ecotype were randomly selected and their rhizome was severed in the pot of the parent ramet. The part of the rhizome sticking in the silicone tube remained intact. Both sides of the silicon tube were then filled with silicone sealant to prevent water transfer through the tube.

Furthermore, half of the offspring ramets from each integration treatment of each ecotype were randomly selected for a waterlogging treatment. We used synthetic sea salt (Aqua Medic Meersalz, City, Germany) to create salt water with 10‰ salinity, which was added to cover the soil surface (1 cm). To avoid salt build-up, fresh water was added to replace evaporation in the offspring pots during the experimental period. All other parent and offspring pots were well watered every one or two days depending on the weather conditions.

The experimental design thus consisted of three factors in a full-factorial design, namely integration (connected vs. severed rhizome), waterlogging (waterlogged vs. non-waterlogged offspring), and ecotype (low marsh ecotype vs. high marsh ecotype) (Figure 3.1) in the resulting eight treatment combinations and were replicated nine times.

The experiment was performed in a greenhouse chamber under artificial or natural day/night light cycle. The pots were arranged randomly and their positions were changed every week to avoid possible effects of potential differences in light-availability within the chamber. The treatments of the experiment began in summer 2016 and continued for 84 days.

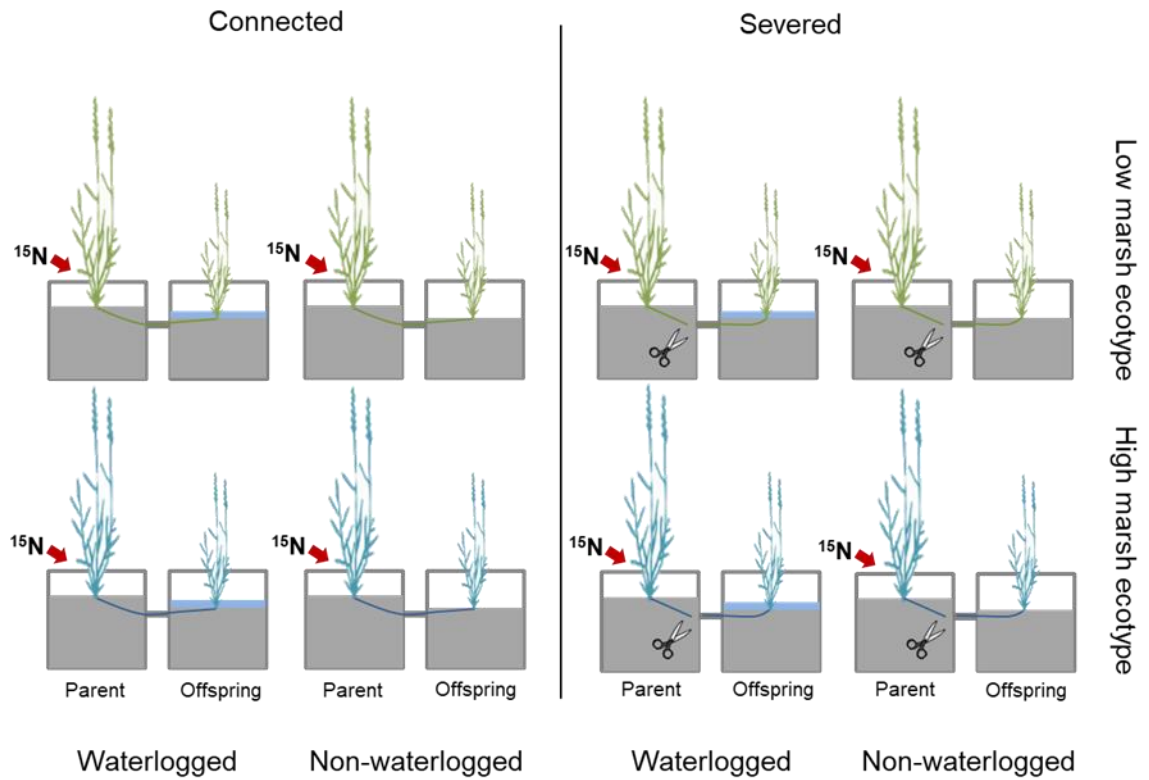


Figure 3.1 Schematic representation of the three-factorial experiment with the factors: integration (connected vs. severed), waterlogging (waterlogged vs. non-waterlogged; light-grey pots for well-watered, light-blue cover layer pots for waterlogged), and ecotype (low marsh ecotype in green vs. high marsh ecotype in grey-blue). Parent ramets were ^{15}N labeled in the beginning of the experiment with $0.7 \text{ mM } ^{15}\text{NH}_4^{15}\text{NO}_3$ (98 atom % ^{15}N). Each treatment combination was replicated nine times.

3.2.3 Measurements

3.2.3.1 Growth measurements

Number of leaves, number of shoots and length of shoots (cm) of offspring ramets were measured in the beginning and at the end of the experiment. Above- and below-ground biomass of offspring were harvested separately at the end of experiment. Biomass was dried to constant mass at 60 °C and weighed. Based on this, the ratio of above- and belowground biomass (ABR) of offspring ramets was determined

3.2.3.2 Elemental and isotope analysis

The top 2 cm of leaves (parent and offspring ramets) was clipped for stable isotope analysis. Additionally, a random subsample of root material of all ramets was collected. All samples for the analysis of C and N concentration and of C and N stable isotope composition were frozen at -20 °C until further analysis. Samples were rinsed with deionized water, dried at 60 °C until constant weight and then homogenized. C and N concentration and the C and N stable-isotope ratios of dried and homogenized leaves and roots samples were determined using an isotope ratio mass spectrometer (nu Horizon, Nu Instruments Limited, UK) linked to an elemental analyser (EURO-EA 3000, Euro Vector, Italy) in continuous flow configuration (set-up by HEKAtech, Germany). Isotope and mass calibration was conducted by the use of the certified standards IAEA-600 Caffeine ($\delta^{13}\text{C} = -27.771\text{‰}$ VPDB, SD 0.043; $\delta^{15}\text{N} = +1.0\text{‰}$ air N_2 , SD 0.2‰), IAEA-NO-3 Potassium Nitrate ($\delta^{15}\text{N} = +4.7\text{‰}$ air N_2 , SD 0.2‰) and 2,5-bis(5-tert-butyl-2-benzoxazol-2-yl) thiophene (6.51% N; 72.52% C; HEKAtech, Germany), respectively. The N mass of each offspring ramets (above- and below-ground nitrogen mass) was calculated based on N concentration and dried biomass. The isotopic compositions of all samples are reported using the standard δ -notation versus Vienna PeeDee Belemnite (VPDB) as parts per thousand (‰).

3.2.4 Statistical analysis

We tested for differences in $\delta^{15}\text{N}$ between connected and severed offspring ramets (Hypothesis 1A) using a non-parametric test (Wilcoxon's rank-sum test) because the data were not normally distributed within groups. To test for the effects of waterlogging and ecotype on $\delta^{15}\text{N}$ of connected offspring ramets (Hypothesis 1B + C), two-way ANOVAs were conducted separately for above- and belowground biomass with waterlogging and ecotype as main factors. Subsequently, Tukey's HSD tests were used for pair-wise comparisons.

The effects of physiological integration (connected vs. severed rhizome), waterlogging (waterlogged vs. non-waterlogged offspring) and ecotype (low marsh ecotype vs. high marsh ecotype) on growth performance of offspring ramets were tested by the following tests: Since the variables 'leaves' (number of grown leaves between day 1 and day 84), and 'shoots' (number of grown shoots between day 1 and day 84) were count data, a GLM with a Poisson distribution was used (Zuur et al. 2009). Over-dispersion was detected in the model for the variable 'leaves' and therefore the standard errors were corrected using a quasi-GLM model (Zuur et al. 2009). The most insignificant parameters were dropped step by step, and as few parameters as possible were kept in the optimal model (Zuur et al. 2009).

Three-way ANOVA were conducted to test for effects of physiological integration (connected vs. severed rhizome), waterlogging (waterlogged vs. non-waterlogged offspring), and ecotype (low marsh ecotype vs. high marsh ecotype) on ramet length, above- and belowground biomass, the ratio of above- and belowground biomass, and nitrogen mass of offspring ramets. Subsequently, Tukey's HSD post hoc tests were used for pair-wise comparison. All statistical analyses were carried out using the software package R (R version 3.5.0 (2018), The R Foundation for Statistical Computing 2018).

3.3 Results

3.3.1 $\delta^{15}\text{N}$ of offspring ramets

$\delta^{15}\text{N}$ in connected ramets (Mdn = 343.55‰) was significantly higher than in severed ramets (Mdn = 13.11‰), $W = 6400$, $p < 0.0001$, $r = 0.87$ (Figure 3.2). This proves the underlying assumption that a significant amount of ^{15}N was translocated from parent ramets to offspring ramets via the rhizome. The following analyses regarding ^{15}N were therefore performed with a dataset containing only connected offspring ramets.

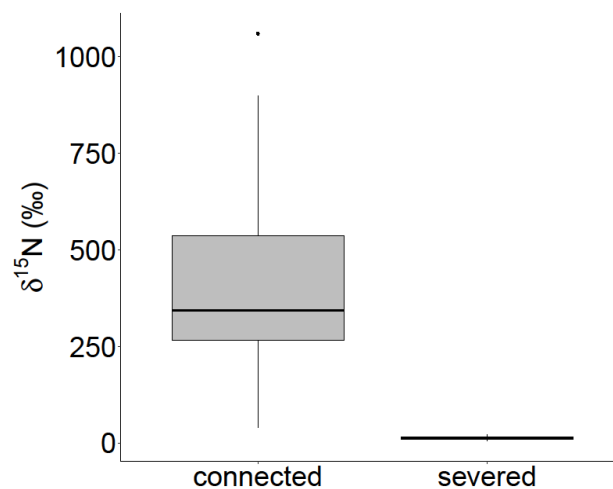


Figure 3.2 Boxplot of $\delta^{15}\text{N}$ of connected and severed offspring ramets of *Elymus athericus* at the end of experiments (84 days) (Wilcoxon's rank-sum test $W = 6400$, $p < 0.0001$, $r = 0.87$).

Aboveground biomass $\delta^{15}\text{N}$ of connected offspring ramets was positively affected by waterlogging (Table 3.1). Aboveground biomass was more enriched in ^{15}N under waterlogged ($522.16 \pm 208.89\text{‰}$) vs. non-waterlogged ($369.15 \pm 220.98\text{‰}$) conditions. Similarly, there was a significant effect of waterlogging on $\delta^{15}\text{N}$ of belowground biomass (Table 3.1). Offspring ramets under waterlogged conditions ($411.04 \pm 170.36\text{‰}$) were more enriched in ^{15}N than non-waterlogged offspring ramets ($290.37 \pm 153.78\text{‰}$). Ecotype and the interaction effect between waterlogging and ecotype had no effect on $\delta^{15}\text{N}$ of both above- and belowground biomass (Table 3.1). Pair-wise comparisons showed a significant difference in $\delta^{15}\text{N}$ of aboveground biomass between the waterlogging treatments, but only in the low marsh ecotype (Figure 3.3). No other significant differences in $\delta^{15}\text{N}$ of belowground biomass between treatments were found (Figure 3.3).

Table 3.1 Results of two-way ANOVA for analyses of differences in $\delta^{15}\text{N}$ of above- and belowground biomass of connected offspring ramets of *Elymus athericus* with waterlogging and ecotype.

Effects	df	Aboveground biomass		Belowground biomass	
		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Waterlogging	1	5.147	0.029	5.519	0.024
Ecotype	1	0.024	0.878	0.445	0.509
Waterlogging x Ecotype	1	2.603	0.115	1.482	0.231
Error	36				

Significant effects ($P < 0.05$) are presented in bold. df, degrees of freedom.

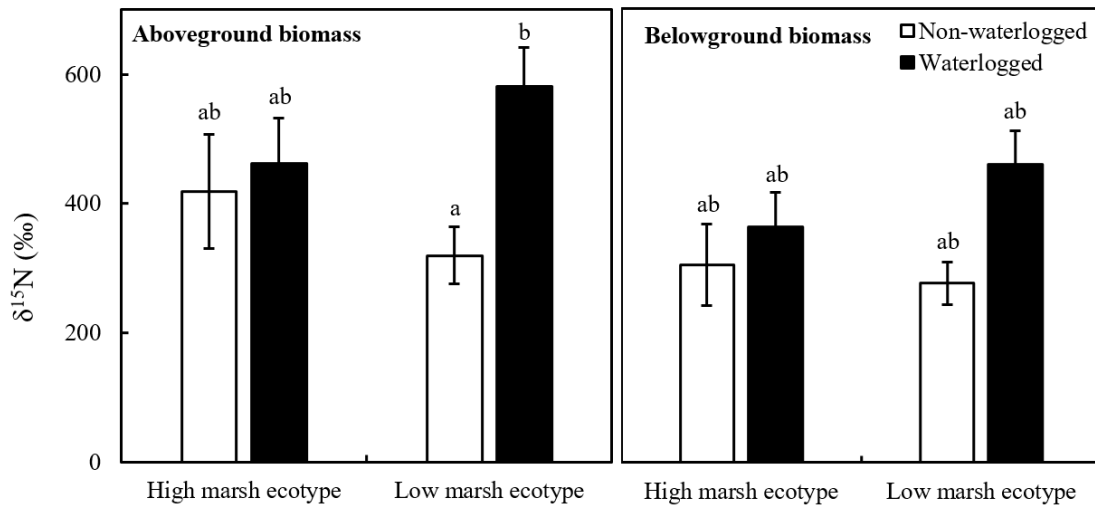


Figure 3.3 Above- and belowground biomass $\delta^{15}\text{N}$ of connected offspring ramets of high marsh and low marsh ecotypes of *Elymus athericus* under waterlogged and non-waterlogged treatments. Significant differences at $p < 0.05$ based on Tukey's HSD test for pairwise comparisons are indicated by different letters. Values are mean and \pm SE.

3.3.2 Growth performance of offspring ramets

3.3.2.1 Number of grown shoots and grown leaves

The difference in number of shoots between day 1 and day 84 of the experiment was affected by clonal integration (Table 3.2). Connected offspring ramets produced significantly more shoots (3.24 ± 1.51) than severed offspring ramets (2.2 ± 1.47) (Figure 3.4).

Table 3.2 GLM results for the effects of integration, waterlogging and ecotype and their interaction on shoot and leave growth of offspring ramets.

Effects	df	Shoot growth		Leave growth
		<i>p</i>		
Integration (I)	1	<i>p</i>	< 0.01	ns.
Waterlogging (W)	1	<i>p</i>	-	< 0.05
Ecotype (E)	1	<i>p</i>	-	-
I x W	1	<i>p</i>	-	< 0.05
I x E	1	<i>p</i>	-	-
W x E	1	<i>p</i>	-	-
I x W x E	1	<i>p</i>	-	-

Significant effects ($P < 0.05$) are presented in bold; (-) indicates that factor dropped out of the GLM model.

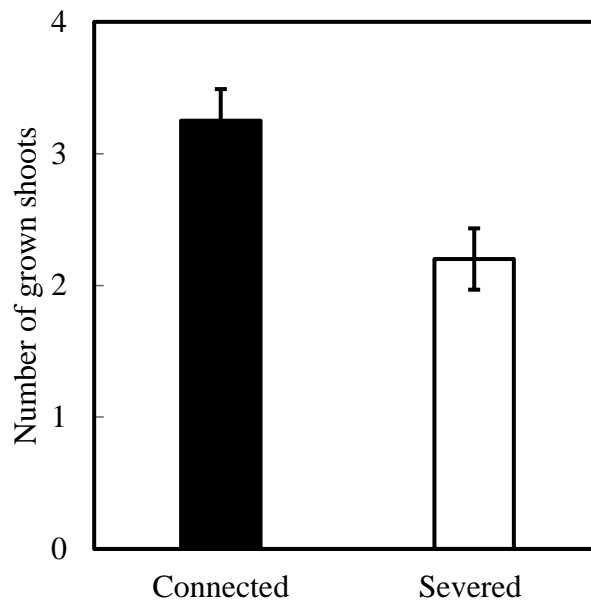


Figure 3.4 Number of grown shoots of *Elymus athericus* offspring ramets (difference between day 1 and day 84). Values are mean and \pm SE.

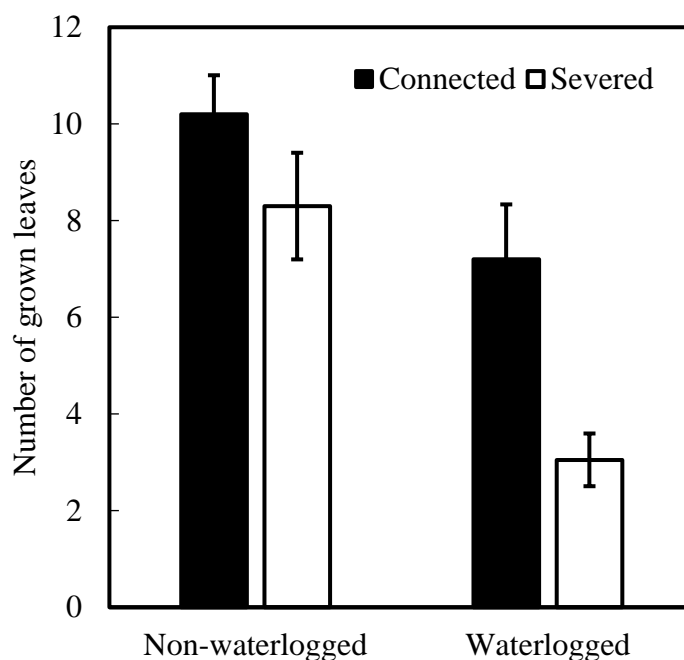


Figure 3.5 Number of grown leaves of *Elymus athericus* offspring ramets (difference between day 1 and day 84). Values are mean and \pm SE.

The differences in number of leaves between day 1 and day 84 of the experiment, however, was affected by the waterlogging treatment and the interaction between integration and waterlogging (Table 3.2). Connected offspring ramets under non-waterlogged treatment grew the highest number of leaves (10.2 ± 0.8), while severed ramets under non-waterlogged treatment produce less leaves (8.3 ± 1.1) (Figure 3.5). Similarly, under waterlogging, connected offspring ramets produce more leaves (7.2 ± 1.1) than severed offspring ramets (3.1 ± 0.5) (Figure 3.5).

3.3.2.2 Length growth

Waterlogging and ecotype had significant effects on the length growth of offspring ramets between day 1 and day 84 (Table 3.3), while integration had no effect. Non-waterlogged ramets grew taller (47.69 ± 13.01 cm) than waterlogged ramets (36.92 ± 13.34 cm) (Figure 3.6 B). The low marsh ecotype ramets grew taller (47.25 ± 13.94 cm) than the high marsh ecotype ramets (37.36 ± 12.74 cm) (Figure 3.6 C).

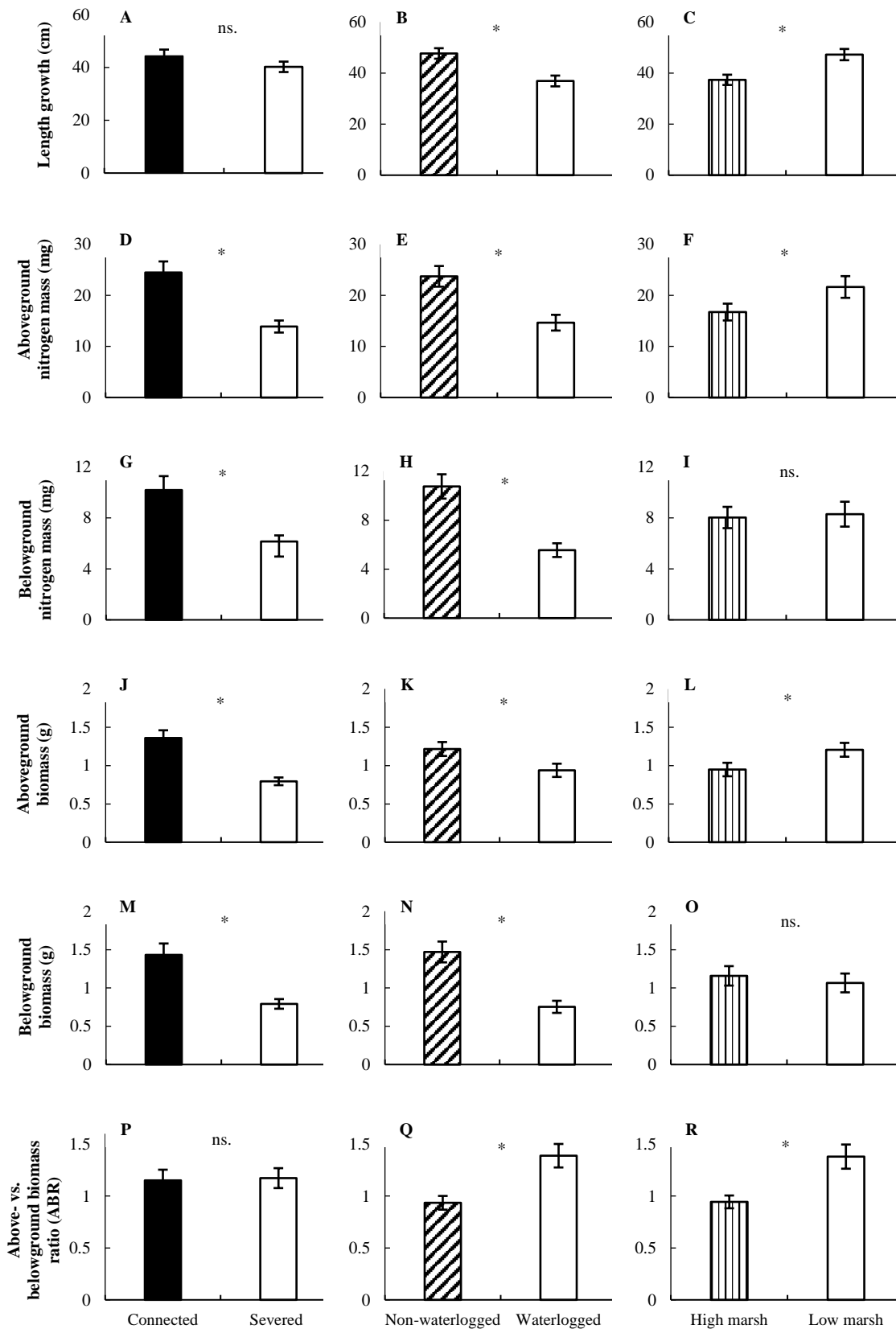


Figure 3.6 Mean (\pm SE) length growth (cm), above- and belowground nitrogen mass (mg), above- and belowground biomass (mg), and above- vs. belowground biomass ratio (ABR) of offspring ramets in the integration (connected vs. severed), waterlogging (non-waterlogged vs. waterlogged) and ecotype (high marsh vs. low marsh) treatments.

Table 3.3 Three-way ANOVA for analyses of differences in length growth, above- and belowground biomass, above/belowground biomass ratio, and above- and belowground nitrogen mass of offspring ramets with integration, waterlogging and ecotype as main factors.

Effects	df	Length growth		Aboveground biomass		Belowground biomass		Shoot root ratio		Aboveground nitrogen mass		Belowground nitrogen mass	
		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
		Connection (C)	1	2.243	0.139	28.154	< 0.001	20.701	< 0.001	0.031	0.861	22.787	< 0.001
Waterlogging (W)	1	15.685	< 0.001	6.796	0.011	25.897	< 0.001	13.345	< 0.001	16.767	< 0.001	24.630	< 0.001
Ecotype (E)	1	13.228	< 0.001	5.831	0.018	0.430	0.514	12.335	< 0.001	4.904	0.030	0.062	0.804
C x W	1	0.495	0.484	0.064	0.801	2.391	0.126	0.276	0.601	0.095	0.759	1.209	0.275
C x E	1	1.925	0.170	0.135	0.715	0.372	0.544	0.315	0.576	0.179	0.673	0.024	0.876
W x E	1	0.834	0.364	1.184	0.280	0.187	0.667	0.576	0.450	2.033	0.158	1.173	0.282
C x W x E	1	0.775	0.382	0.893	0.348	0.951	0.333	1.248	0.268	0.848	0.360	3.077	0.084
Error	72												

Significant effects ($P < 0.05$) are presented in bold. df, degrees of freedom.

3.3.2.3 Above- and belowground nitrogen mass

Integration, waterlogging and ecotype had significant effects on aboveground nitrogen mass (Table 3.3). The aboveground nitrogen mass in connected ramets (24.49 ± 13.72 mg) was significantly higher than in severed ramets (13.91 ± 7.48 mg) (Table 3.3). Offspring ramets grown under waterlogged conditions had lower nitrogen mass (14.66 ± 9.76 mg) than the ones grown under non-waterlogged conditions (23.74 ± 12.81 mg) (Table 3.3). Furthermore, low marsh ecotype offspring ramets had more nitrogen mass (21.66 ± 13.45 mg) than high marsh ecotype offspring ramets (16.75 ± 10.41 mg) ($F = 4.90$, $p < 0.05$). No interaction effects among treatments were found in aboveground nitrogen mass.

Integration and waterlogging effects were detected in belowground nitrogen mass (Table 3.3). The belowground nitrogen mass in connected ramets (10.16 ± 6.97 mg) were significantly higher than in severed ramets (6.14 ± 3.02 mg). Offspring ramets grown under waterlogged conditions had lower nitrogen mass (5.55 ± 3.55 mg) than those grown under non-waterlogged conditions (10.75 ± 6.28 mg). There were no interaction effects among treatments on belowground nitrogen mass (Table 3.3).

3.3.2.4 Above- and belowground biomass

Similar to aboveground nitrogen mass, aboveground biomass of offspring ramets were significantly affected by the factors integration, waterlogging and ecotype (Table 3.3). Integration enhanced aboveground biomass by on average 500 mg. Non-waterlogged ramets also produced more aboveground biomass (1217.01 ± 576.45 mg) than waterlogged ramets (938.83 ± 547.59 mg). Furthermore, low marsh ecotype offspring ramets built more aboveground biomass (1206.76 ± 572.02 mg) than high marsh ecotype offspring ramets (949.08 ± 557.29 mg). No interaction effects among factors were found in aboveground biomass.

Integration and waterlogging effects were also detected to be significant in belowground biomass, while ecotype had no significant effect (Table 3.3). The belowground biomass in connected ramets (1432.25 ± 939.76 mg) was significantly higher than in severed ramets (790.75 ± 398.78 mg). Offspring ramets grown under waterlogged conditions had lower belowground biomass (752.75 ± 500.74 mg) than

the ones grown under non-waterlogged conditions (1470.25 ± 859.33 mg). There were no interaction effects among treatments on belowground biomass (Table 3.3).

3.3.2.5 Above- vs. belowground biomass ratio

Waterlogging and ecotype had significant effects on above- vs. belowground biomass ratio, while no effect of integration was detected. More biomass was allocated to aboveground biomass under waterlogged conditions (Figure 3.6 Q). The low marsh ecotype allocated more resources to aboveground biomass than high marsh ecotype (Figure 3.6 R). There were no interaction effects among treatments on above- vs. belowground biomass ratio (Table 3.3).

3.4 Discussion

The higher $\delta^{15}\text{N}$ found in the connected compared to severed offspring ramets clearly indicates that *Elymus* is able to translocate nitrogen between ramets via the rhizome. Furthermore, the translocation of nitrogen was found to be especially high if offspring ramets were growing in waterlogged and thus probably unfavorable conditions. Physiological integration furthermore enhanced growth performance of offspring ramets in terms of number of shoots, above- and belowground biomass and above- and belowground nitrogen mass. Under waterlogged condition, offspring ramets produce shorter leaves, less leaves, biomass and nitrogen biomass. The two ecotypes, however, were only found to differ in leaf length growth and aboveground biomass and nitrogen mass.

Our findings in $\delta^{15}\text{N}$ values of offspring ramets support our first hypothesis that ramets of *Elymus* are physiologically integrated. To our knowledge, this is the first stable isotope study to demonstrate the physiological integration between ramets in *Elymus athericus*. Our results are in accordance with previous labeled isotope studies investigated physiological integration of other clonal plant species (Jónsdóttir & Callaghan 1990, Alpert 1996, Derner & Briske 1998, Saitoh et al. 2006). Furthermore, connected offspring ramets under water stress (waterlogging) were found to receive more support from parent ramets than connected offspring ramets under ambient condition as a response to stress. However, we did not find any significant differences in $\delta^{15}\text{N}$ between offspring ramets of the two ecotypes.

The growth-parameter results also indicate that indeed, waterlogging seems to cause a certain stress for the offspring ramets, as the non-waterlogged offspring ramets performed better than waterlogged offspring ramets. Waterlogging reduced growth performance of *Elymus* which was observed in all measured growth parameters except for number of newly produced shoots. It is generally assumed that flooding-tolerant species could remain unchanged or even could do better in growth performance in waterlogged conditions (Naidoo & Naidoo 1992, Rubio et al. 1995). In contrast, the growth performance of flooding-intolerant species decrease under waterlogged conditions (Dias-Filho 2002). Therefore, *Elymus* could be considered as not entirely flooding-tolerant and we can certainly say that *Elymus* prefers less frequently flooded habitats with well aerated soils. This observation is in line with results of Veeneklaas et al. (2013) who found that the occurrence of *Elymus* is

strongly negatively related to bad drainage conditions of salt marshes. The negative effect of waterlogging on growth parameters found here could indicate a weak performance of flooding-tolerant mechanisms in *Elymus*. Nevertheless, most offspring ramets were able to establish successfully and survive during the entire experiment (84 days) under these extreme conditions. The shift in biomass allocation to aboveground biomass, as indicated by the significantly increased ratio of above- vs. belowground biomass, under waterlogged conditions might be an adaptation to limited oxygen availability in the soil. The relatively higher allocation to aboveground tissue could be related to the effort of plants to provide oxygen to the waterlogged parts via gas transport as found in other flooding-tolerant species (Naidoo & Naidoo 1992, Rubio et al. 1995, Blom & Voeselek 1996, Grimoldi et al. 1999, Webb et al. 2012). Therefore, we would conclude that *Elymus* is a moderately flooding-tolerant species.

When comparing the ecotypes, the LM ecotype generally showed a better growth performance than the HM ecotype. Specifically, shoots of the LM ecotype grew taller than the HM ecotype, and the LM ecotype stored more nitrogen in aboveground parts and produced more aboveground biomass than the HM ecotype. These differences in growth performance and allocation pattern between the two ecotypes could represent the underlying mechanisms of their different adaptation to waterlogging. The increasing aboveground biomass in the LM ecotype is mainly related to longer shoots, while the number of newly produced shoots and leaves remains similar. Similar results were found by Reents (2018), who tested the response of the two ecotypes of *Elymus athericus* to different flooding frequencies. The increasing biomass allocation to aboveground biomass could be a possible mechanism by which the LM ecotype is adapted to lower marsh zones. Allocating more biomass to elongating shoots can maintain effective gas exchange between aerial and belowground parts (Naidoo & Naidoo 1992), and avoid light dissipation through water (Grace 1989, Blanch et al. 1999), enable them to survive in waterlogged conditions (Webb et al. 2012). Interestingly, the LM ecotype consistently produced more aboveground biomass than the HM ecotype, independently of waterlogging or integration. Therefore, the aboveground biomass responses could be considered as an adaptation of the ecotype irrespective of its current growing conditions which might enable it to expand into the more stressful conditions of the lower marsh zones (Bockelmann & Neuhaus 1999, Veeneklaas et al. 2013).

Physiological integration improves the growth performance of offspring ramets at both waterlogged and non-waterlogged conditions. Specifically, we found physical integration to increase newly produced shoots and leaves, above- and belowground nitrogen mass, and above- and belowground biomass. These results indicated that *Elymus* uses physiological integration to improve its clonal reproduction irrespective of the conditions. This gives *Elymus* the advantage over other species in suitable as well as unsuitable habitats, like flooded marsh areas as observed by Bockelmann & Neuhaus (1999). Amsberry et al. (2000) conducted a study to explore factors influencing the expansion of *Phragmites australis* into low marsh habitats. Their results suggested that *Phragmites* may invade into lower, less favorable habitats using clonal integration. Thus, our results confirm previous studies which show the benefits of physiological integration for the clonal plants in harsh conditions (Roiloa & Retuerto 2012, You et al. 2014, Zhou et al. 2014, Lechuga-Lago et al. 2016) and exploring new habitat and rapid expansion (Amsberry et al. 2000). However, while we could prove the existence of physiological integration in *Elymus* using labeled ^{15}N , we did not find waterlogged offspring ramets to get more support from parent ramets compared to non-waterlogged offspring ramets. Therefore, it was not possible to conclude that clonal plant *Elymus* benefits from physiological integration of nitrogen particularly under stressed conditions.

3.5 Conclusion

The invasion of *Elymus athericus* in Wadden Sea salt marshes after the cessation of livestock grazing is of current conservation concern. *Elymus* is a native species in Wadden Sea salt marshes, but its rapid expansion, even to the lower marshes where it has hitherto been absent, has largely occurred over past decades. Our results show that waterlogged soil conditions indeed reduce the growth of *Elymus*, as previously found in other studies (Reents 2018). We also found physiological integration to be a potential mechanism by which *Elymus* is able to outcompete other species. Furthermore, the low marsh ecotype was found to generally produce higher biomass and growth, which might be a crucial adaptation to invade into low marsh habitats. We suggest that *Elymus* uses physiological integration to improve its photosynthetic capacity leading to higher biomass production. *Elymus* changes its biomass allocation to elongating aboveground biomass and this is a potential mechanism of *Elymus* to survive and stay in extreme habitat with an extreme stress level. In order to get more precise insight, however, other factors such as drainage conditions, inundation frequency, soil salinity, soil redox, and nutrient supply should be considered in further studies regarding the spread of *Elymus*. Moreover, the underlying mechanisms of *Elymus* adaptations to unfavorable habitat need to be investigated regarding morphology, physiology, and molecular or genetic levels.

3.6 Acknowledgements

We would like Jan van den Burgh for collecting the *Elymus* plants in the field and Florian Rink for assistance with the preparation of the material for this experiment. This project was partly funded by the Bauer-Hollmann Stiftung and the Rudolf und Helene Glaser Stiftung.



4

Synthesis

In the first part of this synthesis, the key findings of this thesis are summarized and related to the research objectives outlined in chapter 1. In the second part, I will discuss advantages, assumptions and limitations of stable isotope techniques in salt marsh ecological studies. I will close this synthesis by outlining future research perspectives.

4.1 Key findings

The two main objectives of this study were (1) to trace the origin of soil organic carbon in Wadden Sea salt marshes and to identify the main factors driving the relative contribution of Al OC vs. Au OC sources to the soil carbon pool, and (2) to investigate the role of physiological integration during the invasion of two ecotypes of *Elymus athericus*. The key findings of this study include:

- 1.1) The relative contribution of Al vs. Au OC to the top 5 cm salt marsh soil varied between sites and grazing treatments.
- 1.2) Geomorphological factors are important drivers determining the relative contribution of Al OC to the soil. Distance to the creek had a negative effect on the relative contribution of Al OC to the top soil at DSK. Distance to the marsh edge had a negative effect on relative Al OC contribution under grazing, whereas the opposite pattern was found in the grazed treatment.
- 1.3) Livestock grazing, in the interaction with spatial factors, affected the relative contribution of Al OC vs. Au OC in salt marsh soil.

- 2.1) *Elymus* is able to translocate nitrogen between ramets via the rhizome. Waterlogging increases $\delta^{15}\text{N}$ in above- and belowground biomass of connected offspring ramets.
- 2.2) Physiological integration promoted growth performance of offspring ramets in terms of number of shoots, above- and belowground biomass and above- and belowground nitrogen mass.
- 2.3) Waterlogging had negative effects on the length of leaves, number of leaves, biomass and nitrogen mass of offspring ramets.
- 2.4) The two ecotypes differed in leaf length growth and aboveground biomass and nitrogen mass.

4.2 Stable isotope techniques in salt marshes ecological studies

In this thesis, stable isotope techniques were applied at both levels, natural abundance and “enriched” levels, to reveal information on important processes acting at two different scales. At large scale, the origin of organic matter in the salt marsh soil was traced, and the relative contribution of AI OC and how it is affected by spatial factors as well as land use management was studied. At the small scale, transport of N-rich compounds within a clonal plant was recorded. We could prove that parent ramets support daughter ramets during early establishment and/or development, especially under unfavorable conditions.

Assessing salt marsh OC dynamics in general and specifically the OC origin are difficult as salt marshes are extremely dynamic on a variety of temporal and spatial scales. Stable isotope analysis offered a comprehensive approach which allowed us to differentiate the isotopic composition of potential sources and clarify carbon flow pathways in salt marshes. To determine the relative contribution of each source, mixing models have been developed (Schubert and Calvert, 2001; Westerhausen et al., 1993) based on stable isotope measurements. The ability to identify the origin and relative contribution of each source to the salt marsh soil is a great strength of the applied natural abundance tracer approach. A second strength is the fact that the approach is useful at large spatial scales. However, the application of this approach and the interpretation of the obtained results need to be conducted cautiously, as the mixing model approach relies on several assumptions and has its limitations.

First, the precision of the mixing-model outputs strongly decreases with the differences in $\delta^{13}\text{C}$ between sources. In our study system, the difference between AI OC and Au OC sources were only moderate (about 3‰), yet there was no overlap in $\delta^{13}\text{C}$ between the two sources. To increase precision of our mixing-model calculations, we used the specific $\delta^{13}\text{C}$ values of each point for the Au OC source. This approach improves the frequently used less precise method of applying a mean value or even literature values (Bouchard et al. 1998, Winkelmann & Knies 2005, Freese et al. 2008, Watanabe & Kuwae 2015, Yuan et al. 2017). Second, source values can differ seasonally and spatially (Fry 2006). It was reported that plant isotopic variability changes seasonally during plant growth (Sherr 1982, Hedges et al. 1988, Cloern et al. 2002). Therefore, we deliberately collected Au samples in the end of the growing season when aboveground biomass almost died off. We assume that

isotope values at this point represent plant material which will be ready to accumulate in the soil. Third, variability of $\delta^{13}\text{C}$ within one of the sources such as between aboveground and belowground biomass can be large (Cloern et al. 2002). This, however, is often left unconsidered in mixing-model approaches in coastal systems (Kelleway et al. 2017). The ratio of aboveground to belowground biomass contributions to the soil are usually unknown and it is therefore advisable to perform mixing-model calculations under different assumptions in order to assess the robustness of the obtained results (Wolf et al. 2007, Mueller et al. 2016). Therefore, precise budgets of AI OC vs. Au OC to the salt marsh soils can only be calculated when the relative contributions of above- vs. belowground plant biomass to the soil OM pool are known.

In the second study, labeled nitrogen was applied to reveal nutrient dynamics on the small scale in a salt marsh plant. In Wadden Sea salt marshes, the native salt marsh plant species *Elymus athericus* has spread rapidly in the past decades. Nitrogen is commonly assumed to be a limiting factor in coastal marine ecosystems, hence, we studied whether nitrogen is translocated from parent ramets to daughter ramets. We did indeed demonstrate that parent ramets support daughter ramets via rhizomes leading to an increased growth.

The use of enriched isotope compounds have obvious advantages over radioactive tracers in tracer studies because labeled isotopes are not toxic, less expensive and do not require licensing. They are generally used to investigate the routing of materials and chemical transformations which occur along the way. The use of enriched isotope compounds, however, also has some assumptions and limitations. Firstly, non-labeled and labeled materials are assumed to have the same chemical behavior. Secondly, the addition of the isotope does not stimulate rates of transformations. Thirdly, the source pool is uniformly labeled by the isotope. Finally, the loss of labelled compounds in the system (if material flows into the source pool because of dilution or if there is outflow from the sink pool(s)) need to be accounted for (Stark 2000) when calculating the rates of flow.

The two studies presented in this thesis give an insight into processes at two different scales. Potentially, these processes also interact or affect other processes in the ecosystem. As demonstrated in chapter 2, it was found that AI OC could be affected by vegetation height and structure, which in this case was reduced by grazing. Consequently, the described spread in *Elymus* could potentially also

indirectly affect the AI OC input, as *Elymus* grows in tall dense stands and might thus increase AI OC input. Thereby, the small-scale transport of nitrogen observed in chapter 3 could potentially affect these larger scale processes. The source of OC in the soil, in turn, has a strong effect on the microbial community structure (Mueller et al. 2017). Specifically, higher contributions of AI OC favor relative bacterial over fungal abundance (Mueller et al. 2017). These connections between carbon source and microbial community structure can in turn play an important role in carbon sequestration. Furthermore, the invasive plant could also alter the soil microbial community, e.g. through changes in litter production, root exudates, or nutrient acquisition (Yarwood et al. 2016). Those indirect effects on other processes should be studied further.

In summary, the results reported in this thesis demonstrate that stable isotope analysis is a powerful tool, capable of answering many wide-ranging questions in the fields of ecology at a variety of scales. However, we also showed that researchers must use this method with caution and combine it with other methods that allow us to understand ecological processes.

4.3 Future research perspectives

The results of this study show the advantages of using stable isotope techniques to assess carbon and nutrient dynamics in Wadden Sea salt marshes at different scales. The results also raise new research questions regarding different aspects of ecological processes that could be studied using stable isotope techniques in combination with other methods.

Results from chapter 2 show that spatial factors and land use are important drivers shaping salt marsh OC pools by their interaction effects on the origin of OC in salt marshes. The origin of OC and its quality affects the microbial community (Mueller et al. 2017), thereby influencing C turnover rate and C sequestration. Future research needs to address the accurate budgets of Al OC vs. Au OC and their contribution to total soil OC stocks across blue-carbon ecosystems. In order to answer these questions, other processes that affect the C pool such as organic matter decomposition, aging or stabilization need to be taken in account. In these cases, C stable-isotope techniques should be used combined with other stable isotope techniques ($\delta^{15}\text{N}$, $\delta^{34}\text{S}$, compound-specific C isotopic analysis), biomarker approaches (e.g., glomalin, n-alkalenes) (Prahl et al. 1994, Adame et al. 2012) or environmental DNA assessments (Reef et al. 2017). Moreover, the evidence for a direct link between microorganisms and salt marsh soil OM and the underlying mechanisms needs to be corroborated in further experiments. Even though the role of Al and Au OC as OC sources for microorganisms in tidal wetlands has long been demonstrated (Boschker et al. 1999), little is known about the linkage between soil OC and microorganisms in those ecosystems. The application of stable isotope methods would allow researchers to trace the fluxes of carbon and nutrient in biogeochemical cycling by microorganisms (Radajewski et al. 2000). This would be a key step to improve our understanding of carbon and nutrient dynamics in these ecosystems.

The study of chapter 3 provides insights into the effects of physiological integration on the performance of *Elymus* regarding biomass production under unsuitable conditions, and this could be a good explanation for its invasion in Wadden Sea salt marshes. The spread and formation of large monospecific *Elymus* induced by cessation of grazing could alter the Au OC and Al OC input as demonstrated in chapter 2. This phenomenon could also indirectly alter the soil biological community locally through changes in litter production, root exudates, changes in nutrient

acquisition and cycling, and changes in root architecture and function. Those linkages between plant and microorganisms in salt marshes should be studied further.

Furthermore, it is well known that stable isotopes are excellent tools to study spatial and temporal changes in food web structures (Layman et al. 2012), especially in coastal ecosystems. Carbon and nitrogen are the most commonly employed elements in food web studies. While carbon isotopes are often used to determine origin sources of dietary carbon, nitrogen isotopes are useful in studying the trophic position of different groups (Fry 2006). Further studies should be conducted in Wadden Sea salt marshes to reveal the salt marsh food web structure, contributing to a more complete knowledge about salt marsh carbon and nutrient cycles. Moreover, as intertidal ecosystems, salt marshes could play a role as an energy source for marine transient and resident animals, especially fishes. In order to answer those research questions, multiple stable isotopes analysis are powerful tools. When possible, stable isotope analysis should be used in conjunction with other methods such as gut content analysis, feeding behavior, fatty acid tracers, or compound-specific isotopes, which will provide new insights into food web structure.

Stable isotope techniques undoubtable will continue to provide new opportunities for the advancement of ecological studies at a variety of spatial and temporal scales. To old questions, stable isotope techniques provide new novel approaches, for example niche size in food web studies (Bearhop et al. 2004). Combining stable isotope methods with other approaches such as genetic techniques (Vogt et al. 2016), microsatellites (Feyrer et al. 2007) gives great chances to address ecological research questions which were previously difficult or impossible to answer.



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Acknowledgements

I still remember the day I came to Hamburg from a country very far away in South-East Asia to experience a very new life. Since then five years have gone by, my journey with a PhD study is coming to an end, and I would like to take this opportunity to thank all people supporting me on my way.

First of all, I would like to express my deep sense of thanks and gratitude to my supervisor Prof. Dr. Kai Jensen for giving me an opportunity to study in Germany. Thank you very much for your continuous support, patience, motivation and immense knowledge. Your guidance helped me in all the time of research and writing of this thesis. I could not have imagined having a better advisor and mentor for my Ph.D study. I would further like to thank to Prof. Dr. Annette Eschenbach for agreeing to be on my thesis-committee as well.

My heartfelt appreciation goes to Dr. Stefanie Nolte. Without your guidance and persistent help this dissertation would not have been possible. Thank you very much for your support, guidance and encouragement along this hard journey. I am very grateful that you truly consider me as your real friend, that I can share feelings, emotions, and every happy and sad moments of my life.

My research project was part of the “Interaction of fish, plants, carbon and sediment: management and ecosystem functions of Wadden Sea salt marshes” (INTERFACE). Special thanks to Dr. Peter Müller for long-term support, insightful comments and constructive discussion. I furthermore would like to acknowledge the work of Julia Friese, Dr. Andreas Dänhardt, Dr. Antonia Wanner, and Prof. Axel Temming. I would like to thank Dr. Martin Stock and our project partners of the Wadden Sea National Park Schleswig-Holstein for cooperation. I also sincerely appreciate the help of Prof. Chris Smit (University of Groningen) by sending the *Elymus* from The Netherlands.

There are many colleagues at the university I would like to thank. I am deeply grateful to Dr. Christoph Reisdorff for technical support, fruitful discussions, and advice on experimental design. I also would like to offer my special thanks to Claudia Mählmann for technical and administrative support. Thank you very much for the tea-kitchen coffee management, which significantly helped me to complete my writing. I would also like to thank all other colleagues of the group: Dr. J. Erhardt, Dr. C. Butzeck, Dr. J. Dorendorf, Dr. F. Rupprecht, Dr. S. Suchrow, Dr. S. Thomsen, Dr. K. Ludewig, Damian Tom-Dery, Anna Sandner, Hao Tang, Dennis Schulze, Svenja Rents, Dirk Granse, Nikola Lenzewski, Heather Alyson Shupe, Anastasia Leonova, Eva Ostertag, Salomé Gonçalves and everybody else for their helpful assistance, friendship and hospitality. I also sincerely appreciate the valuable help of various technical assistants: Detlef Böhm, Sigrid Mörke, Marion Klötzl, Maren Winnacker, Jutta Krüger. A further thanks goes to all Hiwis and BTA-students for assistance in the field and laboratory work.

I would also like to express my gratitude to the Vietnamese Government, the Bauer-Hollmann Stiftung and the Rudolf und Helene Glaser Stiftung for their financial support.

Last but not the least, I would like to thank my family: my parents and to my brothers and sisters-in law for supporting me spiritually throughout my study period and my life in general. To my son, Le Ngoc Hai Anh: you are the most amazing thing I have ever had in my life. I know five years without your mom beside you were not easy for you. Thank you for your understanding and unconditional love.

Heather Alyson Shupe

Platenstrasse 2a

22609, Hamburg

Email: Heather.Alyson.Shupe@uni-hamburg.de

24th October 2018

To whom it may concern,

As a native English Speaker, I do hereby declare that the PhD thesis:
“Applying Stable Isotope Techniques to Assess Nutrient and Carbon Dynamics
in Salt Marshes” has been written in concise and correct English (US).

Sincerely,



Heather Alyson Shupe

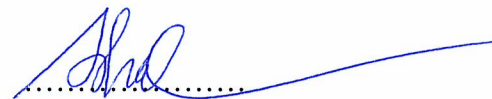
Declaration

I hereby declare, on oath, that I have written the present dissertation by myself and have not used other than the acknowledged resources and aids referenced.

Eidesstattliche Versicherung

Hiermit erkläre ich an Eides statt, dass ich die vorliegende Dissertationsschrift selbst verfasst und ausschließlich die angegebenen Quellen und Hilfsmittel verwendet habe.

Hamburg, den 25.10.2018

A handwritten signature in blue ink, consisting of a stylized 'D' followed by 'o' and 'Hai', with a long horizontal flourish extending to the right.

Do, Thi Hai