Lizenzhinweise:

Ostertag, E. J. M., Jensen, K., Unger, V., and Nolte, S. (2023). Warming experiment indicates that increasing global temperatures may not affect windows of opportunity for salt marsh seedlings. Limnology and Oceanography.

Für die Arbeit wurden die Abbildungen und Tabellen des Artikels renummeriert, um ein einheitliches Bild zu schaffen, sowie die Quellen ans Ende der Arbeit gestellt.

Licence information:

Ostertag, E. J. M., Jensen, K., Unger, V., and Nolte, S. (2023). Warming experiment indicates that increasing global temperatures may not affect windows of opportunity for salt marsh seedlings. Limnology and Oceanography.

For consistency, the figures and tables of the article were renumbered, and the references put at the end of the thesis.

Warming effects on plants
in
Wadden Sea salt marshes

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

vorgelegt von
Eva Julia Marie Ostertag
Hamburg, Oktober 2023

Erstgutachter: Prof. Dr. Kai Jensen

Zweitgutachterin: Dr. Stefanie Nolte

Datum der Disputation: 18.12.2023

Prüfungskommissionsvorsitz: Prof. Dr. Kai Jensen

Contents

Ostertag, E. J. M., Jensen, K., Unger, V., and Nolte, S. (2023). Warming experiment indicates that increasing global temperatures may not affect windows of opportunity for salt marsh seedlings. *Limnology and Oceanography*.
was reprinted (Chapter 4) with the kind permission of John Wiley & Sons and is under CC BY-NC-ND license.

For consistency throughout this thesis, the figures and tables of the paper were renumbered, and all references were summarized at the end of the thesis.

1

General introduction

1.1 Salt marshes

Salt marshes are inundation-driven ecosystems which act as a transition zone between the terrestrial and marine environment. They mainly develop in temperate regions of the world (Allen 2000; Yando et al. 2023) in areas with high sedimentation rates and low wave impact (Adam 1993). In subtropical and tropical regions, they are replaced by mangroves (Tomlinson 1986), but there are also areas where salt marshes and mangroves coexist (Doughty et al. 2015). Salt marshes cover an area of approximately 55,000 km² worldwide (Davidson and Finlayson 2018, 2019; Fig. 1.1). About 400 km², which is ~ 20 % of the European salt marshes (Doody 2008) are located in the Wadden Sea (Esselink et al. 2017).

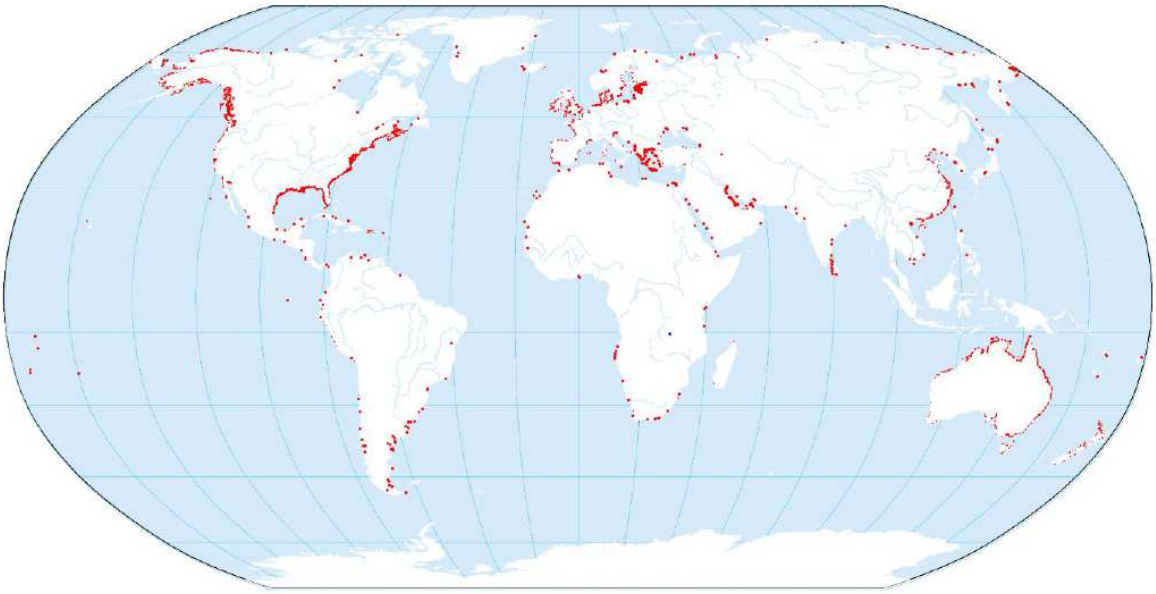

Fig. 1.1: Global distribution of salt marshes. Source: UNEP-WCMC; McOwen et al. 2017; map version 6.1 (2021).

1.2 Salt marsh zonation and vegetation

In Northwestern (NW) Europe, salt marshes show a spatially distinct vegetation pattern (Olff et al. 1997; Bockelmann et al. 2002), consisting of highly adapted grasses, herbs, and small-growing shrubs, which are adapted to high salinity levels (halophytes). This zonation is mostly based on an elevational gradient, with gradually changing environmental conditions such as flooding frequency and duration, waterlogging, salinity, redox potential, soil aeration and nutrient availability (Bockelmann et al. 2002; Ursino et al. 2004; Mueller et al. 2017, 2020). According to their stress tolerance and competitiveness, the NW European salt marsh halophytes inhabit different parts of the marsh (Suchrow and Jensen 2010), leading to three typical marsh zones with distinct communities of vegetation: the pioneer zone, the low marsh, and the high marsh (Bockelmann et al. 2002). The pioneer zone starts at an elevation below mean high tide (MHT), directly adjacent to the non-vegetated mudflats (Fig. 1.2). Due to its position, the pioneer zone is usually inundated semi-diurnally. The typical vegetation includes the succulent *Salicornia europaea* and the grass *Spartina anglica*. Both species are highly tolerant to salt stress and waterlogging because they have morphologically adapted to this stressful environment by developing aerenchyma (Granse et al. 2022) and succulence. The low marsh is situated directly above the MHT level and is usually flooded during spring tides. It is more species rich than the pioneer zone and high marsh, with a mixture of up to 15-20 halophytes and higher abundances of *Puccinellia maritima*, *Limonium vulgare* and *Halimione portulacoides* (Bockelmann et al. 2002; Suchrow and Jensen 2010, Esselink et al. 2017). The high marsh is usually only flooded during storm surges in the autumn and winter months. It is dominated by the grasses *Elymus athericus* or *Festuca rubra* (Suchrow and Jensen 2010; Wanner et al. 2014).

For most salt marshes, the spatial marsh zonation can be translated into temporal marsh development, which is also known as succession (de Leeuw et al. 1993; Olff et al.1997; Mitchel and Adam 1989). The first species to colonize a mudflat are pioneer species, which need to use `windows of opportunity` with a low magnitude of physical stressors to establish (Balke et al. 2011; Hu et al. 2015; Bouma et al. 2016). When the mudflats are successfully colonized, the plants stabilize the sediment with their rooting system (de Battisti et al. 2019), leading to an increased sediment settlement. This causes an increase in surface elevation, which in turn leads to a competitive replacement (Grime 1977) of the pioneer species by low marsh species. With further accretion (the vertical net surface elevation growth of the marsh in mm yr^{-1}; Nolte et al. 2013b), the low marsh vegetation is succeeded by high marsh species.

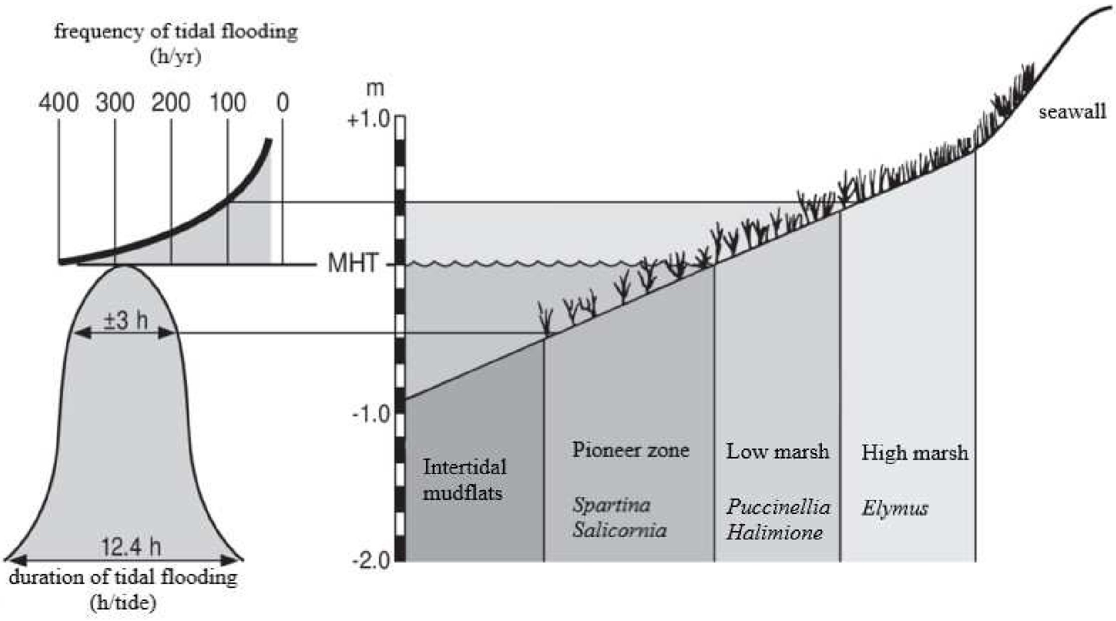

Fig. 1.2: The zonation of Northwestern European salt marshes in relation to flooding duration and frequency. MHT = mean high tide. Modified after Erchinger (1985).

1.3 Human intervention in salt marsh formation

In NW Europe, processes that lead to the formation of new salt marshes have been altered by humans for centuries (Adam 2002). Initially to gain suitable land for livestock and later to prevent flooding, humans have intervened in the Wadden Sea salt marshes by dredging, ditching, and constructing brushwood groynes and dikes. These modifications have changed the hydrological conditions of the marshes and sped up accretion processes (Esselink et al. 2000). Therefore, natural salt marshes are rare along the Wadden Sea mainland coast (Esselink et al. 2017). However, some natural salt marshes (back-barrier marshes) can be found on the islands of the North Sea (Esselink et al. 2017). Most existing mainland coast salt marshes partly formed due to human intervention or are even man-made (Esselink et al. 2000). The salt marshes on the Halligs are remnants of the mainland salt marshes (Esselink et al. 2017). In contrast to artificial salt marsh promotion, there have also been human measures which led to a loss of former salt marshes. The construction of dikes, to protect the land from storms and to convert the former salt marshes into agricultural fields (i.e. embankment), have led to a loss of large former salt marsh areas, due to the absence of flooding. Nowadays, there is an increasing interest in protecting and restoring salt marshes. In the Wadden Sea, this has led to transnational conservation measures and continuous monitoring of salt marshes (Esselink et al. 2017).

1.4 Ecosystem services

Salt marsh ecosystems are not only worth protecting because they are valuable habitats for highly adapted and rare species; they also provide many benefits for human society. Within the ecosystem services concept, salt marshes provide recreational services, supporting and provisioning services, and regulating services for the benefit of humans (Friess et al. 2020). The Wadden Sea National Park is a UNESCO World heritage site, which allows for many recreational activities like hiking or bird watching in designated zones. Salt marshes provide breeding and feeding ground habitat for many bird species (Koffijberg et al. 2016). Additionally, the Wadden Sea salt marshes provide nurseries for commercially used fish (Bolle et al. 2009). Salt marshes also provide regulating ecosystem services such as flood protection, coastal stabilisation, and the protection of the coastline from storm damages by wave attenuation (Shepard et al. 2011; Möller et al. 2014; Schoutens et al. 2021). Salt marsh vegetation mitigates the impact of storm damage by acting as an obstruction to reduce the intensity of the forces from currents and waves (Bouma et al. 2009b). Vegetation also reduces the erosion of sediments by stabilizing the sediments with their roots (de Battisti et al. 2019; Ford et al. 2016). Recently, salt marshes have been increasingly recognized for their role in climate change mitigation due to their ability to sequester and store large amounts of organic carbon (Mcleod et al. 2011; Chmura et al. 2003). The high carbon sequestration capacity of salt marshes and other tidal wetlands can mainly be attributed to high primary productivity (autochtonous organic carbon), trapping of high amounts of marine-derived (allochtonous) organic carbon (Mcleod et al. 2011; Duarte et al. 2013), and low organic matter decomposition rates. Furthermore, salt marshes grow constantly through accretion, therefore the soils do not become carbon-saturated and can accumulate organic carbon over long periods (Mcleod et al. 2011; Kirwan and Megonigal 2013). The salt marsh vegetation plays a direct role in these regulating services (Friess et al. 2020) by trapping and producing organic matter.

1.5 Global change and threats for salt marshes

Like most other ecosystems, salt marshes are affected by global change. Global change is frequently used in scientific literature to refer to several factors that lead to changes affecting the whole planet. While the term can be found in thousands of articles since 1980, a clear definition of it is important (Duarte 2014). In general, global change is a result of both anthropogenic influences and natural drivers like rare asteroid collisions with the earth (Schulte et al. 2010). Hereafter, I refer to the anthropogenic-driven global change as global change since it is the predictable and hence manageable part (to a certain extent; Duarte 2014). Human activities have had an increasing impact on the nature of biosphere processes (Duarte 2014), which can mainly be attributed to land-use change, loss of biodiversity and climate change. These drivers are linked to each other and are a result of two main factors: human population growth and increasing human per capita resource use (Camill 2010; Duarte 2014).

Salt marshes are affected by these drivers in several ways. A couple of global change studies in salt marshes have focussed on the impact of land-use change in terms of livestock management (e.g. Bakker et al. 1985; Nolte et al. 2013a) and its resulting effect on biodiversity (e.g. Wanner et al. 2014; van Klink et al. 2016) or plant community dynamics (e.g. Esselink et al. 2000; Rupprecht et al. 2015). Other studies focused on climate change factors like increased storminess (Donat et al. 2011), sea-level rise (e.g. Kirwan et al. 2009; Morris et al. 2023) and global warming (e.g. Noyce et al. 2019; Smith et al. 2022; Gedan and Bertness 2009; Charles and Dukes 2009).

Land-use change in salt marshes can be considered with different temporal lenses. On a long timescale, the centuries-long history of human impact (Adam 2002; Esselink et al. 2000) had dramatic effects on the formation of salt marshes (section 1.3). Consequently, the salt marshes along the North Sea mainland coast have developed under the impact of human management practices like livestock grazing for centuries. In the last couple of decades, however, land-use change has undergone a different direction in Wadden Sea salt marshes. Under the aspect of nature conservation, grazing intensities have been reduced or grazing has ceased altogether in many areas (Bakker et al. 2003; Esselink et al. 2000). Because of the cessation of grazing, species composition of salt marshes shifted from grazing-tolerant species to species which are more competitive under non-grazed conditions (Esselink et al. 2000; Howison et al. 2015; Rupprecht et al. 2015).

The shift of species or the local loss of species may also affect bio-geomorphological aspects in a salt marsh system (Schepers 2017). The impact of roots on the stability of the sediment (Ford et al. 2016; de Battisti et al. 2019) may play an important role in the aspect of coastal stabilisation under the threat of increased storminess in the last decades (Young et al. 2011).

Another threat salt marshes are confronted with is anthropogenically accelerated sea-level rise (Crosby et al. 2016; IPPC 2023). Due to higher temperatures, thermal water expansion, and the melting of glaciers, the volume of the ocean water mass increases. Average global rates of sea-level rise have been ~3 mm yr^{-1} in the last decades and are expected to increase in pace (Chen et al. 2017; IPCC 2023). In the North Sea, the average sea-level rise was 1.5 mm yr^{-1} between 1900 and 2009, but the rate has increased in the last decades to 4 mm yr^{-1} between 1993 and 2009 (Wahl et al. 2013). The global scenarios of marsh loss due to submergence range from high losses (Crosby et al. 2016; Nicholls et al. 2007) to low losses (Kirwan et al. 2016). To keep pace with sea-level rise and persist in the long term, the accretion rates of salt marshes need to exceed the rates of relative sea-level rise (Nolte et al. 2013b). Accretion and sediment deposition depend on the interplay of sediment availability, tidal amplitude, marsh type (minerogenic vs. organogenic; Niering 1997; Allen 2000; Nolte et al. 2013b), and trapping by vegetation (Fagherazzi et al. 2012; Cahoon et al. 2021). The crucial role vegetation plays in salt marsh development by reducing flow velocities (Bouma et al. 2005) and trapping sediments (Cahoon et al. 2021) has been studied under the aspect of marsh accretion (reviewed in Shepard et al. 2011). The response of vegetation to environmental impacts is an important aspect to consider in order to model the persistence of salt marshes and the services they provide. Since accelerated sea-level rise is an indirect effect of global warming, the direct effect of rising temperatures on the vitality of salt marshes cannot be neglected.

1.6 Global warming

When comparing the last decade (2011-2020) to the period from 1850-1900, the global surface temperature has increased by +1.09 °C (IPCC 2023). This increase is mainly caused by greenhouse gas (GHG) emissions from human activities. Therefore, the rise in temperatures predicted for the coming decades depends on future GHG emissions. Under the current predictions, it is very likely that warming will exceed +1.5 °C and that under very high GHG emissions, global surface temperatures may be up to +4.5 °C (compared to 1850 to 1900) at the end of the century (IPCC 2023). The impact of increasing temperatures on salt marshes is still currently not well understood. It likely depends on the sensitivity of different ecosystem processes to warming, among which plant physiological and vegetation ecological processes are of importance for the overall vulnerability and resilience of marshes. Increasing temperatures can affect plants by directly changing the physical environment and indirectly by altering species composition and thus interspecific competition (Adler et al. 2009; Ives 1995). The role of global warming on the productivity, physiology, and competition/recruitment of plants will be discussed in the following sections.

1.6 The effect of increasing temperatures on plants

Productivity and resource allocation

Meta-analyses on experimental warming in terrestrial plant communities document a general positive influence of warming on plant productivity (Rustad et al. 2001; Lin et al. 2010; Peñuelas et al. 2013). Higher temperatures lead to a higher biomass, but the biomass allocation between roots and shoots is often affected by a decrease in soil moisture (Rustad et al. 2001; Eziz et al. 2017), which typically leads to drought responses in plants. According to the optimal partitioning theory, plants invest more in the structure by which the limiting resource (e.g. nutrients, light, or water) is captured (Bloom et al. 1985). Therefore, most terrestrial plants increase their belowground biomass when experiencing drought stress to access enough water from the soil (Poorter et al. 2012). Tidal wetland plants show the same response to warming: a general increase in biomass due to warmer temperatures (Baldwin et al. 2014). Aboveground biomass in US salt marsh species increased due to both warming treatments and drought treatments at ambient temperatures (Charles and Dukes 2009). However, these studies did not include belowground biomass. As belowground biomass is an important factor in relation to marsh degradation (Turner et al. 2004), sediment stability (de Battisti et al. 2019), and vertical accretion due to biomass accumulation (Nolte et al. 2013b), the effect of warming on root productivity is of special concern. In another warming experiment, salt marsh plants of the US coast showed a non-linear response of belowground allocation due to warming. The belowground biomass peaks under moderate warming (+1.7 °C), then the belowground biomass decreases with increased warming (Noyce et al. 2019). The effect of warming on belowground biomass allocation in European salt marshes may differ from the US coastal marshes to a great extent, since the salt marshes differ in tidal amplitude, flooding frequency, and soil properties. Additionally, the salt marshes along the US Atlantic coast of the mentioned studies are organogenic salt marshes (Niering 1997), while most marshes are minerogenic along the North Sea coast (Allen 2000; Yando et al.

2023). Therefore, the nutrient supply of these marshes may differ. In a mesocosm experiment, belowground productivity increased under +3 °C warming treatments with daily inundation for some Wadden Sea species (Koop-Jakobsen and Dolch 2023), however, this experiment lacks the interaction of warming with natural processes like differing flooding frequency along the elevational gradient. It is yet to be established how the belowground productivity of plants in meso- to macro-tidal salt marshes along the Wadden Sea coast will be affected by warmer temperatures under natural conditions. This knowledge may help to make predictions on marsh stability against erosion along an elevational gradient. The stability of the different marsh zones is of special importance under the projected increase of storm events, which may also lead to higher flooding frequencies in the higher zones of the salt marshes. Additionally, changes in belowground biomass may help to better understand the role of autochthonous organic carbon input into carbon cycling processes (Mueller et al. 2019, 2023) under future warming.

Influence of warming on (eco)physiological processes

The influence of increasing temperatures on physiological processes in plants is determined by both effects of temperature on the plant organ level and effects of changing environmental conditions causing alterations of physiological processes. Physiological processes in plants, such as photosynthesis, photorespiration, and respiration are temperature dependent. The latter two increase with rising temperatures (Brooks and Farquhar 1985; Sharkey 1988; Sage et al. 1990), while there is a temperature optimum for photosynthesis. The optimum lies in the middle of the non-harmful range at which photosynthesis can function, and this differs between photosynthetic pathways (Sage and Kubien 2007). Due to the differing temperature sensitivity, the interplay of these physiological processes has a thermal optimum (Sage and Kubien 2007). However, some terrestrial species can shift their thermal optimum and acclimate to a new temperature regime (Mawson et al. 1986; Atkin et al. 2006b; Gunderson et al. 2010). Terrestrial species of extreme habitats show less acclimation potential (Atkin et al. 2006b).

Decreasing soil moisture due to increasing temperatures and subsequent increases in evapotranspiration, has been observed in several warming experiments across ecosystems (Rustad et al. 2001; Eziz et al. 2017). Decreasing soil moisture often leads to drought stress. Plants experiencing drought stress usually regulate water loss by closing their stomata. This also affects plant photosynthesis by reducing CO_2 diffusion into the leaves, and directly affecting the water use efficiency (WUE; Lambers and Oliveira 2019). The WUE is defined by the amount of water loss in relation to CO_2 fixed in photosynthesis (carbon gain). In plant species with C3 photosynthesis, an increase in WUE due to drought or salt stress conditions is indicated by a decrease of the relative carbon isotope composition ($\delta^{13}C$; Farquhar et al. 1982, 1989). The WUE under experimental warming in salt marshes remains understudied. Permanently waterlogged salt marsh species show a decrease in WUE under warming (Gedan and Bertness 2009), however salt marsh species at higher elevations may experience drought stress due to lower flooding frequencies during the summer months and thus an increase in WUE may be expected. Therefore, the WUE of different species in different salt marsh zones should be studied to get a better picture of the effect of warming under differing environmental conditions.

A second plant trait which has been shown to interact with WUE is the specific leaf area (SLA; Wright et al. 2001; Ackerly 2004): the ratio of the leaf area to leaf dry mass. In a global meta-analysis, SLA of terrestrial plants decreased under decreasing soil moisture conditions (Poorter et al. 2009). Several mechanisms lead to more rigid leaves, which wilt less easily under drought conditions (Wellstein et al. 2017). For example, the expansion rates of leaves are lower under drought conditions (Poorter et al. 2009), resulting in more tightly packed cells with less air spaces (Maximov 1929; Shields 1950). Irrespective of warming, the change of soil (moisture) conditions is an important driver of SLA in salt marshes (Lavorel et al. 2007; Minden and Kleyer 2011). However, it remains unclear how warming will affect SLA in different marsh zones with differing environmental conditions. For example, warming may increase soil salinity through enhanced evapotranspiration, which may decrease SLA in salt marsh species (Lenssen et al. 1995).

Both WUE and SLA may be important ecophysiological traits which help to understand physiological adaptations or adjustments to warming in terms of drought response and nutrient allocation within and across plant species. Furthermore, the influence of possible drought conditions under warmer temperatures along an elevational gradient (represented by vegetation zones) may help to better understand if physiological adaptations may be more driven by drought or if other warming-induced changes at the plant organ have a higher importance in salt marshes.

Species specific reactions and the influence of life cycle stage

Temperature-induced changes in interspecific competition is one of the main effects of global warming on species interactions and can lead to changes in vegetation composition

(Adler et al. 2009; Ives 1995). Under warmer temperatures, the competitiveness and stress tolerance of different species may be affected. While some species perform better under increasing temperatures, others lack adaptability. It has been shown that under warmer temperatures, terrestrial plant communities may shift in their species composition (Chapin et al. 1995; Post and Pedersen 2008; Shi et al. 2015) and that altered nutrient availability (Chapin et al. 1995), invasive species (Dukes and Mooney 1999; Dukes et al. 2009), and herbivores (Post and Pedersen 2008) play an important role in these shifts. Salt marsh communities of the US East coast also showed a shift and a rapid decline in species richness and diversity under warming (Gedan and Bertness 2009). Additionally, the competitive interactions were altered in a way that rare salt marsh forbs became outcompeted by the increasing dominance of tall grasses (Gedan and Bertness 2009, 2010). The encroachment of mangroves into salt marshes at their ecotone has been observed under warmer conditions as well (Guo et al. 2013; Armitage et al. 2015). Effects of warming on the species composition of salt marshes in NW Europe have not been studied. In the Wadden Sea, *Spartina anglica* has been invading the salt marshes for over a century (Nehring and Hesse 2008; Granse et al. 2021). Under increasing temperatures, the performance of *S. anglica* is of major interest, as it has the potential to spread under warming. This has been indicated by a spread of *S. anglica* on Sylt with increasing mean daytime temperatures in spring after 1987 (Loebl et al. 2006) and has altered the salt marsh communities to a great extent (Nehring and Hesse 2008).

Warming-induced shifts in community composition are also most likely affected by dispersal and seedling recruitment. Hence, the availability of seeds and recruitment processes play an important role. Warming can alter the successful establishment of salt marsh seedlings, by altering germination processes of specific species (Ungar 1977; Egan and Ungar 1999; Noe and Zedler 2000). Due to different reactions towards temperature regimes (Egan and Ungar 1999), species dominance may be already altered at the seedling level. The influence of warming on the germination of salt marsh species has mainly been studied under controlled conditions (Ungar 1977; Egan and Ungar 1999; Noe and Zedler 2000; Martin 2017). Survival under natural conditions, and the effect of dispersal and possible shifts in seedling species richness due to warming, however, can only be studied in-situ. While it is known that mangrove seedling survival is not affected by experimental warming (Coldren et al. 2016), in-situ salt marsh seedling survival under experimental warming has not been studied yet. The effect of warming on seedling survival and recruitment may have major implications on how the salt marsh communities in the Wadden Sea may shift under future climate warming.

1.7 Aims and objectives

The aim of this thesis is to study the effect of warming on salt marsh plants under different mediating factors, such as elevation, season, and soil moisture (Fig. 1.3). This information will have further implications as plants and their performance play a crucial role in ecosystem processes and services.

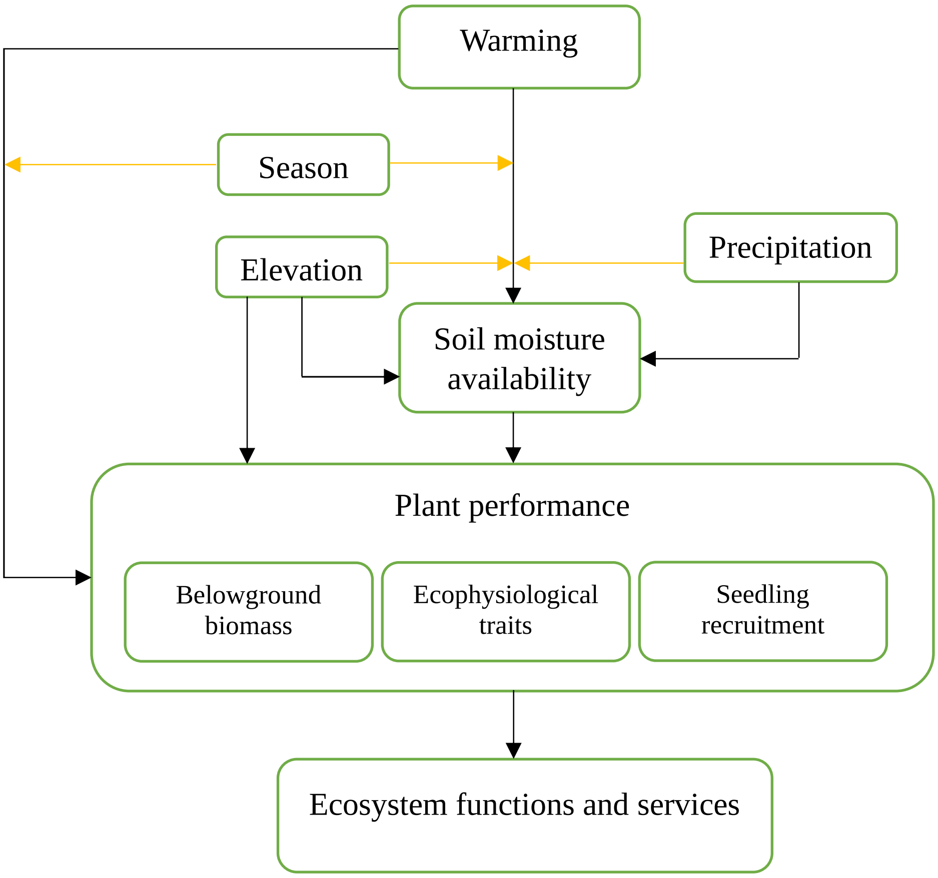

Fig. 1.3: Conceptual diagram of this thesis. The influence of warming is studied with a focus on the indirect effect of warming on plant performance. The effect of warming on soil moisture availability is expected to be mediated by season, elevation (hydrological gradient) and precipitation. The direct effect of warming on plant performance is expected to be mediated by season.

To capture different aspects of plant performance in salt marshes, the following three main research questions were asked:

1. How does warming alter belowground biomass?
2. Does warming lead to drought responses of ecophysiological traits as indicated by WUE and SLA?
3. How is seedling recruitment affected by warming?

The work presented here was conducted in a whole ecosystem warming experiment on the German Wadden Sea coast. The experiment includes ambient temperature, +1.5 °C, and +3.0 °C treatments in a full factorial design across the three typical marsh zones of Wadden Sea salt marshes (pioneer zone, low marsh, and high marsh; see Rich et al. in press). The following sections give a short overview of the objectives of each chapter.

Chapter 2:

The influence of warming on belowground biomass was studied by non-destructive sampling by taking pictures of living roots with a camera system inserted into pre-installed clear tubes over the course of three years (2019-2021) in spring, summer, and autumn. Pictures of roots were taken at two depths and the calculated root biomass was compared between ambient and +3 °C temperature treatments to detect potential effects of warming on belowground productivity. The mediating effect of salt marsh zones and climate on warming effects are discussed.

Chapter 3:

Potential drought responses of different salt marsh species due to warming were measured by measuring their WUE via the $\delta^{13}C$ signature of leaves, collected in spring, summer, and autumn over the course of three years (2019-2021). Additionally, the SLA of the same individuals was used to assess further possible drought adaptations. Potential interactions of temperature treatments (ambient temperature, +1.5 °C, and +3 °C) with season and elevational influences are assessed and compared between years.

Chapter 4:

The influence of warming on seedling emergence and survival was studied from March to mid-September in 2019. In situ seedling numbers were recorded weekly until June (then biweekly) in three temperature treatments (ambient temperature, +1.5 °C, and +3 °C).

Additionally, the lifespan of individual high marsh and low marsh seedlings was recorded. The percentage of surviving seedlings at the end of the vegetation period and the survival probability was compared between temperature treatments. The influence of changing soil conditions along the elevational gradient are discussed under the aspect that the influence of warming on seedlings may differ between salt marsh zones.

2

Response of salt marsh root biomass to experimental warming is mediated by abiotic conditions

Abstract

Ecosystem services provided by salt marshes like coastal protection and carbon sequestration become increasingly important under future climate change. Roots play a crucial role in the salt marsh ecosystem by stabilizing the sediment, which under the projected increase of storms and accelerated sea-level rise, is one important factor for coastal protection. Additionally, roots are a source of autochthonous organic carbon input and thereby closely linked to carbon sequestration in salt marshes. However, the knowledge of belowground processes and root dynamics in salt marshes under climate change scenarios is limited. Here, we studied the effect of warming (+3 °C) on the belowground biomass of salt marsh vegetation in three marsh zones along an elevational gradient (pioneer zone, low marsh, high marsh) in an in-situ warming experiment on the Wadden Sea coast. Root biomass was studied by non-destructive sampling with a mini-rhizotron camera system in ambient temperature and +3 °C temperature treatments for three consecutive years (2019-2021). We took pictures at two soil depths (approx. 8 cm and 20 cm) in different seasons (spring, summer, autumn) of each year to study the influence of warming on root biomass. In general, we found only small effects of warming on root biomass across seasons and years. In 2019, a combination of warming and severe drought led to a shift of root biomass from upper to lower soil depths in the high marsh. Pooled across all treatments, we observed a seasonal development of root biomass with the peak during the main growing season in summer, and less root biomass in spring and autumn. Along the elevational gradient in salt marshes, we found the lowest root biomass in the high marsh and considerably more root biomass in the pioneer zone and low marsh, which are more frequently inundated. Our results indicate that root biomass responses to warming in salt marshes are dependent on abiotic conditions driven by small scale variations in inundation frequencies and climatic impacts like precipitation. Given the small

change of salt marsh root biomass with warming, we argue that the sediment-stabilizing effect of roots may not change under warming scenarios of +3 °C. Furthermore, plants use phenotypic plasticity to adapt their root system to changing environmental conditions.

Introduction

Salt marshes form the transition between marine and terrestrial ecosystems and provide important ecosystem services like carbon sequestration and coastal protection (Mcleod et al. 2011; Shepard et al. 2011; Möller et al. 2014). Due to their ability to efficiently sequester and store large amounts of organic carbon (OC), the term 'blue carbon' has been introduced to highlight the importance of coastal ecosystems like salt marshes in the global carbon (C) cycle (Duarte et al. 2005; Mcleod et al. 2011; Chmura 2013). There are several studies highlighting how salt marshes provide coastal protection by stabilizing the shoreline and decreasing wave energy (Möller and Spencer 2002; Bouma et al. 2009b, 2010; Möller et al. 2014).

The potential of tidal wetlands to produce and store large amounts of autochthonous OC (Kirwan and Megonigal 2013) and trap sediments by reducing the flow velocity (Bouma et al. 2009a; Fagherazzi et al. 2012), is often associated with aboveground biomass (Morris et al. 2002; Schoutens et al. 2021). However, the belowground biomass contributes primarily to soil OC processes (Elschot et al. 2015; Smith et al. 2022), especially as aboveground material may be exported by tidal currents (Boschker et al. 1999). The belowground biomass directly affects the accretion rate by accumulating OC and thereby affects surface elevation height. By exudation of small organic molecules, roots also directly impact the OC amount and composition in salt marsh sediments (Grey et al. 2023). Furthermore, the belowground biomass of plants in salt marshes often exceeds the aboveground biomass (Tripathee and Schäfer 2015; Valiela et al. 1976), which is a typical response in stressful habitats (Jackson et al. 1996; Leuschner et al. 2013). Stress can affect the morphological and architectural characteristics of roots (Bouma et al. 2001; Redelstein et al. 2018a; de Battisti et al. 2019), which in turn affect ecosystem processes such as sediment stabilization (Ford et al. 2016; Wang et al. 2017; de Battisti et al. 2019), and therefore coastal protection. Thus, roots play an important role for salt marsh resistance under accelerated sea level rise and increasing storm events in the future. One important factor for the stability of the sediment against erosion is root biomass (Ford et al. 2016; de Battisti et al. 2019). Therefore, belowground processes and belowground biomass distribution play an essential role in ecosystem feedback mechanisms to climate change as they link abiotic and biotic soil processes with plants via their roots.

The salt marshes along the European North Sea coast are ecosystems with a high variability of abiotic conditions on a small spatial scale, which creates distinct vegetation zones. This zonation is mainly affected by elevation in relation to mean high tide and the associated inundation frequency (Bockelmann et al. 2002), resulting in highly different habitats (pioneer zone, low marsh, high marsh), each with its own typical plant community and soil properties. Consequently, plant species occur along the elevational gradient according to their stress tolerance and competitiveness (Bouma et al. 2001; Suchrow and Jensen 2010). Depending on the position along the gradient, salt marsh plants must cope with either permanently waterlogged soils at the low end or with pronounced competition (Snow and Vince 1984; Pennings and Callaway 1992) and temporal drought at the high end of the gradient. This complex stress gradient leads to different belowground strategies, including root architectural and morphological traits (Bouma et al. 2001; Redelstein et al. 2018a; de Battisti et al. 2019). In the frequently-flooded parts of the marsh, root adaptations to waterlogging and anoxic conditions can be found (Bouma et al. 2001; Redelstein et al. 2018a). Contrastingly, in the higher parts of the gradient, belowground space partitioning to reduce interspecific competition can be observed (Redelstein et al. 2018a). For example, *Spartina anglica,* which often dominates the pioneer zone, develops large root systems to cope with high salinity and anoxia in this regularly flooded zone of the salt marsh (Redelstein et al. 2018a). In the less often flooded low marsh, the roots of *Puccinellia maritima* grow mainly in the top 5 cm of soil (Gray and Scott 1977), whereas the roots of *Halimione portulacoides* grow up to 30 cm deep in the soil (Chapman 1950), and the roots of *Limonium vulgare* reach up to 50 cm deep (Boorman 1967). In the high marsh, most root biomass originates from *Elymus athericus*, which spreads clonally and forms monospecific stands (Bockelmann and Neuhaus 1999). Consequently, these differences in plant communities and belowground plant strategies lead to a zone-specific fine root density (Redelstein et al. 2018a). However, it remains largely unknown how root distribution and root biomass in different salt marsh zones will react to changing environmental conditions like increasing temperatures due to global warming.

Belowground and aboveground productivity in salt marshes have previously been found to increase due to warming (Charles and Dukes 2009; Gedan and Bertness 2009; Coldren et al.

2016). These studies analyzed the effect of warming on salt marsh vegetation using open-top chambers. However, to create a soil temperature profile with ecological significance, feedback-controlled soil warming is necessary, because open top chambers do not warm the salt marsh soil effectively (Carey et al. 2018). Without feedback control, important daily and seasonal variation in temperature dynamics may not be adequately represented. Furthermore, belowground warming can also alter other soil processes in salt marshes (Noyce et al. 2019; Smith et al. 2022; Tang et al. 2023), which may affect the plant-soil-microbial interplay. There is one salt marsh experiment analyzing belowground productivity under experimental feedback-controlled soil warming in an organogenic, microtidal salt marsh on the US Atlantic coast (Noyce et al. 2019). Here, warming increased belowground productivity at warming treatments of +1.7° C, but under higher temperatures, the productivity declined to ambient levels (Noyce et al. 2019). This was explained by different thresholds of nitrogen mineralisation (supply) and increased plant nitrogen demand due to warming (Noyce et al. 2019). However, minerogenic salt marshes may respond differently due to lower nutrient availabilities, particularly at higher elevations (Mueller et al. 2020). A mesocosm experiment at the Wadden Sea coast, with daily flooding in all mesocosms, found an increase in belowground productivity of the pioneer species *S. anglica* and no effect of the belowground productivity of *E. athericus* under +3 °C warming treatments (Koop-Jakobsen and Dolch 2023). However, that experiment did not take into account fluctuations of abiotic conditions due to the tidal cycle. So far, belowground biomass responses to soil warming in minerogenic, meso- to macrotidal salt marshes have not been studied in situ.

As the influence of warming on salt marsh plants may interact with other abiotic factors such as flooding frequency and soil moisture conditions, in-situ experimental approaches are necessary to better understand belowground processes. Particularly, differences in soil moisture conditions between zones may play an important role in determining how plants may react to experimental warming (Pennings and Callaway 1992; Davy et al. 2011). In the lower elevated areas, soils are waterlogged and often anaerobic. This results in lower redox potentials (Davy et al. 2011), which may supress the enhancing effect of warming on soil microbial activity (Wilson et al. 2016; Hopple et al. 2020). In the higher parts of the marsh, soils are better aerated, which may result in a greater effect of climatic conditions on belowground processes (Mueller et al. 2020). Soil moisture and redox differences can vary along the elevational gradient as well as with increasing soil depth. Because of these variations, microbial activity is affected differently (Tang et al. 2023), and thus also root-soil interactions. Moreover, soil moisture, redox potential, and temperature show seasonal variability (Patterson and Mendelssohn 1991), which may lead to season-specific effects of warming on belowground biomass (Bai et al. 2012; Wang et al. 2016). Generally, higher temperatures in summer may result in a higher investment in belowground productivity to avoid growth limitations due to drought effects (Bloom et al. 1985; Hagedorn et al. 2016). In the high marsh, this response may also be expected due to continuous soil desiccation over the course of the vegetation period.

In this study, we analyzed root surface area by taking pictures of living roots in different soil depths with a camera system inserted into pre-installed clear plastic tubes (mini-rhizotrons). Root surface area was converted to root biomass by using an allometric relationship of the root mass per unit length (RML) and the diameter of the roots (Iversen et al. 2008). Mini-rhizotron pictures were taken in spring, summer, and autumn over the course of three years (2019-2021) in a unique in-situ salt marsh warming experiment on the Wadden Sea coast. The MERIT (‘Marsh Ecosystem Response to Increased Temperature’) experiment uses a combination of feedback-controlled active belowground heating and passive aboveground warming with partly foil-covered domes to achieve conditions of +1.5 °C and +3.0 °C above ambient temperatures (Rich et al. in press). We monitored root surface area development in two soil depths (7-9 cm and 19-21 cm) and two treatments (ambient and +3 °C). We hypothesize (1) an increasing positive influence of warming on root biomass with increasing elevation, with little effect in the pioneer zone, but a more pronounced increase in root biomass from low marsh to high marsh. Furthermore, we hypothesize (2) that root biomass and the influence of warming on it will depend on season. The highest root biomass will be found in summer, but the strongest increase due to warming will be found in spring.

Methods

Study area

The study was conducted within the ‘Marsh Ecosystem Response to Increased Temperature’ (MERIT) experiment. MERIT was set up in 2018 on the Hamburger Hallig in the German Wadden Sea (54° 35′ 58″ N, 8° 49′ 8″ E). The site is part of the Schleswig-Holstein Wadden Sea National Park and listed by UNESCO as a World Heritage site. The mean annual temperature between 1991 and 2021 was 9.6 °C, and the mean annual precipitation was 859 mm (Deutscher Wetterdienst, DWD). The tidal range at the site is approximately 3.0 m (Stock 2011). There are three distinct marsh zones which are vegetated by the typical plant communities of the Wadden Sea salt marshes. The pioneer zone (PIO; flooded twice daily) is mainly vegetated by *Spartina anglica* Hubb. and *Salicornia europaea* agg. (L.). The low marsh (LM; flooded during spring tides) is more species rich, with up to 20 species, but higher abundances of *Halimione portulacoides* (L.) Aell. *Limonium vulgare* Mill. and *Puccinellia maritima* (Huts.) Parl. The high marsh (HM; flooded during storm tides) is dominated by dense stands of *Elymus athericus* (Lk) Kerg.

Study design

We set up nine plots in each zone, which make a total of 27 plots in the experiment. The temperature treatment levels are ambient temperature, +1.5 °C, and +3.0 °C. Treatments are replicated three times per zone (n=3). The passive aboveground warming treatment is achieved by domes covered with different amounts of clear plastic foil, which results in warming efficiency similar to other open top chambers (Rich et al. in press). The foils are deployed during spring, summer, and early autumn (Table 2.1). The active feedback-controlled belowground warming is achieved by a combination of vertical resistance pins, reaching 1 m deep into the ground, and horizontally deployed resistance cables on the soil surface. The soil warming is generally switched on in the vegetation period (Table 2.1) and switched off in autumn/winter, as sensors and heating system are vulnerable to damage by heavy storm tides (Rich et al. in press).

Table 2.1: Sampling dates and warming treatment runtimes for 2019-2021.

Year	2019	2020	2021
Foil deployment	Mar 1st* – Sep 25th	Mar 10th - Sep 14th	Apr 9th- Oct 4th
Belowground warming	Mar 14th - Sep 22nd	Mar 15th- Sep 27th	Mar 12th Oct 4th
Spring sampling	May 13th / 15th	May 18th-20th	May 12th-14th
Summer sampling	July 30th-31st	July 13th- 15th	July 20th- 22nd
Autumn sampling	Sep 24th-25th	Sep 16th- 19th	Sep 14th- 16th

*due to storm damage in the pioneer zone 2019, foils were removed from March 19th to April 17th.

Sampling method and sample processing

Destructive root sampling by taking soil cores

In September 2021, we took soil cores using a steel corer with an inner diameter of 5 cm and a depth of 50 cm. We took six cores from each zone, with two cores per treatment (including

+1.5 °C). Collected cores were put in plastic bags and transported in a cool box to the laboratory. The samples were stored frozen until further processing. For washing roots, the soil cores were thawed in the refrigerator for at least 24 h, then put in a bowl of tap water, and all visible roots were picked manually from the surface and stored at + 3 °C in plastic bags until further processing. 128 roots were randomly chosen and scanned at a resolution of 600 dpi (Epson Expression 1680), oven dried at 70 °C, and weighed. From the scans, we measured root surface area and root length, and calculated the mean diameter with the open-source plugin rhizoTRAK (Möller et al. 2019) for FIJI (Schindelin et al. 2012). These data were used to determine the root mass per unit length (RML).

Mini-rhizotron

During each sampling campaign, 36 tubes (mini-rhizotrons) in 18 plots were sampled (ambient and +3 °C treatment). Two clear acrylic tubes of 1 m length were installed in each plot in November 2018. The inner diameter is 6.4 cm, and the outer diameter is 7 cm. The tubes were deployed at approximately 45 °, sealed at the bottom, and could be closed with a lid. The approx. 20 cm of the tubes sticking out of the soil were covered with black tubes to avoid light from entering. Between sampling campaigns, the tubes were closed with a lid and sealed with black tape to avoid moisture and sediment from entering. To take pictures, a mini-rhizotron camera system (VSI-MS16; Vienna Scientific Instruments, Bad Vöslau, Austria) was inserted into the tube and fixed at the same position every sampling date. The pictures were taken and saved on a field notebook with a Raspberry Pi system (LCD Handheld PC). We took pictures at two depths, 8.6 ± 1.09 cm and 20.5 ± 1.09 cm, which are hereafter referred to as upper and lower depth, respectively. Each picture had an area of 3,280 x 2,464 pixels (34.25 x 25.8 mm = 883.7 cm^2, with 95.6 pixel per 1 mm). Since the angle and the protrusion of the tubes vary slightly, the depth (measured for the central point of each picture) has a range of approx. 7-9 cm for the upper and 19-21 cm for the lower depth.

All living roots were measured in each picture. The roots were measured by using intersections (root segments) when the root diameter changed. We measured root length and root diameter of each root segment and calculated the mean root diameter and the root surface area of the segment using the open-source plugin rhizoTRAK (Möller et al. 2019) for FIJI (Schindelin et al. 2012). During most of 2019, we used more intersections per root and picture, which due to time constraints we did not continue in some of the 2019 and all of the 2020 and 2021 pictures. To ensure comparability of processing methods, we repeated 30 pictures of the original labour-intensive method with the method deployed later, and corrected the surface area values of 2019 with the linear equation derived from this validation method.

To calculate the root biomass, the length of each root segment was multiplied by the root mass per unit length (RML), with the value of RML dependent on mean root diameter of the segment (Iversen et al. 2008). The allometric relationship of RML and root biomass (see statistical analyses) was determined from 128 randomly chosen roots from the destructive sampling in September 2021. To calculate the root biomass per soil surface area, we first calculated the root biomass per soil volume by adding a depth-of-field to the mini-rhizotron picture area (Cordeiro et al. 2020). We estimated a depth of field of 2.5 mm. As belowground biomass is the standard unit reported in literature, we used the calculated root biomass per soil surface area to report our results. Since the calculations include some general assumptions, we also report results on root surface area per mini-rhizotron picture. In all models but one (in the lower depth 2019), we observed the same minimal models (Appendix, Table S2.1).

Statistical analyses

To test our hypotheses, we used separate models for the upper and lower depths and for the three study years to describe root biomass per soil surface area and temperature treatment ($n = 6$). We chose separate models as we were not interested in between-years-effects, and we aimed to avoid the non-independence of data from the same tube at different depths. All statistical analysis were carried out using RStudio version 2023.06.1 (RStudio Team 2023), based on R version 4.2.3 (R Core Team 2023) using the additional packages car (Fox and Weisberg 2019), emmeans (Lenth 2022), ggplot2 (Wickham 2016), glmmTMB (Brooks et al. 2017).

Allometric relationship of surface area and root biomass

To determine the allometric relationship of RML and root diameter, we implemented different regression models (logarithmic, exponential, quadratic, and power), compared them, and choose the one which best fit the data based on the lowest Akaike information criterion (AIC). The relationship between RML and root diameter followed a positive power function, where $\text{RML} = 0.77377 * \text{root diameter}^{1.19940}$ (Appendix, Fig. S2.1).

Root biomass response to warming

The calculated root biomass per soil surface area was used as the response variable in the models. Due to non-normality, heterogeneity, highly positively skewness and the large proportion of zero measurements in the data, we built generalized linear mixed models with a Tweedie distribution and log link function using the 'glmmTMB' function. The following categorical explanatory variables were used in the model: zone (three levels: PIO, LM, and HM), temperature treatment (two levels: ambient temperature and + 3 °C) and season (three levels: spring, summer, and autumn). The baseline levels were set as 'PIO' for zone, 'ambient' for treatment and 'spring' for season. Tube ID and plot ID were entered as nested random effects in the 'glmmTMB' model to account for repeated measures. We compared the full models with different random structures via AIC analysis, and found the best fit had the nested random slope model. We implemented the models as three-way-interaction models andperformed a backwards model selection via the AIC to achieve a minimal adequate model. To extract 'anova'-like tables from the models, we used the 'Anova' function from 'car'. To analyse the significance of the main effects and their interactions, we used type-III Wald tests. To do so, it was necessary to change the contrasts of the factorial variables from the R-default dummy coding to deviation coding, using the function 'contr.sum' within the 'glmmTMB' function. To test true differences of significant variables, we did post-hoc pairwise comparisons (Tukey HSD, $p<0.05$) for main effects and for interactions, using the function 'emmeans'.

Results

Along the elevational gradient, we found different responses of root biomass per soil surface area (hereafter referred to as root biomass) to experimental warming mediated by year, season, and soil depth. Mean root biomass pooled across zones and treatments increased slightly throughout the years in the upper depth (7-9 cm; 2019: 105 ± 103 g m^{-2}; 2020: 105 ± 79 g m^{-2}; 2012: 133 ± 80 g m^{-2}). In the lower depth (19-21 cm), we found a more pronounced increase of pooled mean root biomass from 38 ± 53 g m^{-2} in 2019, to 56 ± 68 g m^{-2} in 2020, and 64 ± 60 g m^{-2} in 2021.

Influencing factors on root biomass in the upper depth

In 2019, root biomass in the upper depth was significantly affected by the interaction between zone and treatment (Table 2.2). Here, root biomass decreased in the high marsh under experimental warming (Tukey HSD; p=0.0201). In the low marsh, a similar (but non-significant) decrease of root biomass was found under warming, while the root biomass increased in the warming treatments in the pioneer zone (Fig. 2.1a). In 2020 and 2021, there was no significant effect of treatment or of an interaction between treatment and other factors in the upper depth, but we found a slight trend of increasing root biomass in the pioneer zone under warming (Table 2.2; Fig. 2.1b, c).

In the upper depth we found the highest root biomass in all three zones in summer, but the effect was differently pronounced between years (Fig. 2.2). There was a significant interaction of zone and season in the upper depth in 2019 (Table 2.2). Here, we found a much more pronounced difference of root biomass in the upper depth between spring and summer in the pioneer zone than in the low marsh and high marsh (Fig. 2.2a). Post-hoc pairwise comparisons showed a significant increase in root biomass in the upper depth, 2019, from spring to summer, followed by a decrease in autumn in the pioneer zone. In both the low marsh and the high marsh, we saw a significant lower root biomass in the upper depth in spring compared to summer and autumn (Fig. 2.2a).

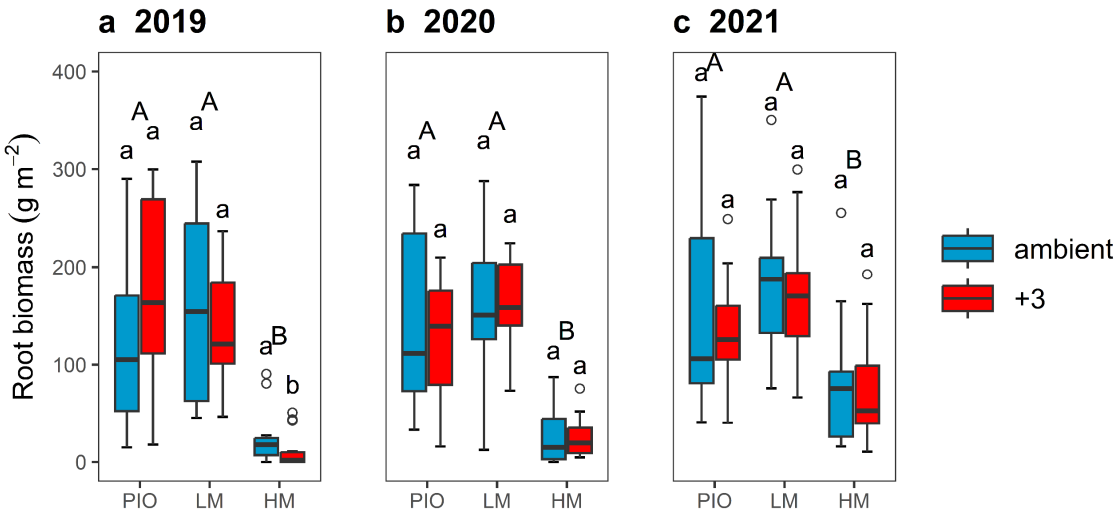

Fig. 2.1: Temperature treatment effect on root biomass in the upper depth (7-9 cm) in three salt marsh zones for (**a**) 2019, (**b**) 2020, and (**c**) 2021. PIO = pioneer zone, LM = low marsh, HM = high marsh. For better visualization of the high marsh values, one outlier in the +3 °C treatment in the pioneer zone 2019 is not displayed (53 g m^{-2}). Different lowercase letters denote significant differences from Tukey HSD ($p<0.05$) between treatments within zones. Significant differences (Tukey HSD, $p<0.05$) between zones are displayed by different uppercase letters. Boxes represent 25% and 75% quartiles from the median, whiskers represent maximum and minimum values (excluding outliers), and circles represent outliers.

Table 2.2: Summary statistics of all GLMMs. The levels of zone are pioneer, low marsh, and high marsh. The temperature treatments are ambient temperature and +3.0 °C. The estimates of the Chi-square distribution are given for significant factors included in the model. Corresponding p values are given for significant outcome ($p < 0.05$). (-) indicates that the factor dropped out of the model, (×) indicates an interaction.

		2019		2020		2021	
		χ^2	p	χ^2	p	χ^2	p
Upper depth	zone	85.35	<0.0001	75.24	<0.0001	25.1	<0.0001
	treatment	0.85	0.3561		-		-
	season	54.01	<0.0001		-	17.82	0.0001
	zone×season	14.95	0.0048		-		-
	zone×treatment	6.73	0.0346		-		-
	treatment×season		-		-		-
	zone×treatment× season		-		-		-
Lower depth	zone	47.23	<0.0001	27.7	<0.0001	35.49	<0.0001
	treatment	0.37	0.5443		-	0.24	0.6215
	season	15.05	0.0005		-	22.40	<0.0001
	zone×season		-		-	1.21	0.5455
	zone×treatment	6.71	0.0349		-	14.32	0.0063
	treatment×season		-		-	3.81	0.1485
	zone×treatment× season		-		-	16.07	0.0029

In 2020, the root biomass was not affected by season (Table 2.2), whereas in 2021, the root biomass was significantly higher in summer than in the other seasons in all zones (Fig. 2.2c). Additionally, root biomass differed between salt marsh zones in the upper depth. Post-hoc pairwise comparisons revealed a significant difference between root biomass of high marsh and low marsh/pioneer zone in the upper depth in all years (Fig. 2.1, Fig. 2.2).

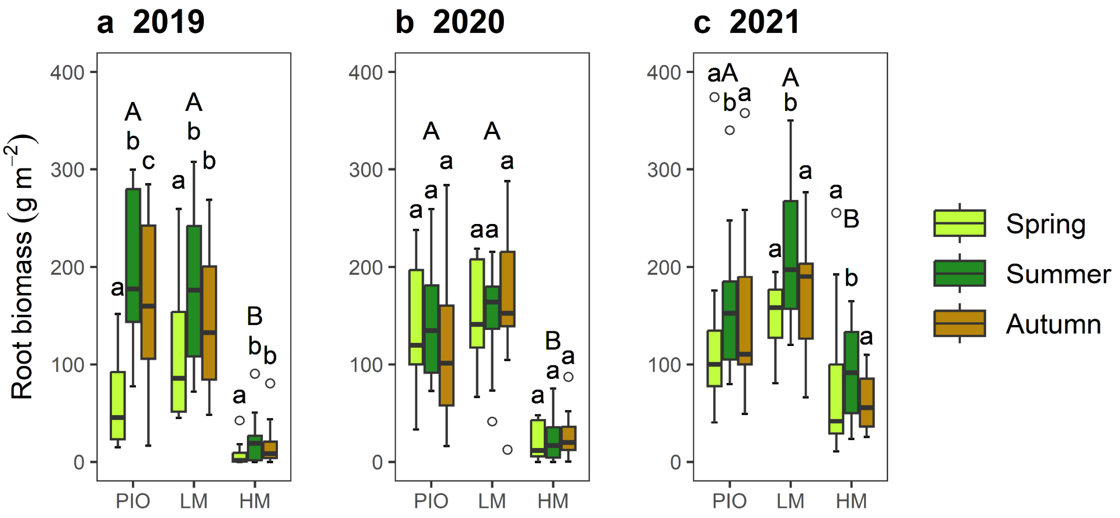

Fig. 2.2: Seasonal differences of root biomass in the upper depth (8.6±1.09 cm) for three salt marsh zones for (**a**) 2019, (**b**) 2020 and (**c**) 2021. PIO = pioneer zone, LM = low marsh, HM = high marsh. For better visualization of the high marsh values, one outlier in summer 2019 in the pioneer zone is not displayed (53 g m^{-}2). Different lowercase letters denote significant differences from Tukey HSD ($p<0.05$) between seasons within a zone. Significant differences (Tukey HSD, $p<0.05$) between zones are displayed by different uppercase letters. Boxes represent 25% and 75% quartiles from the median, whiskers represent maximum and minimum values (excluding outliers), and circles represent outliers. A temperature treatment×season×zone interaction plot for all years can be found in the Appendix (Fig. S2.2).

Influencing factors on root biomass in the lower depth

Similar to the root biomass in the upper depth, we also saw a significant interaction of zone and temperature treatment in the lower depth in 2019 (Table 2.2). While the root biomass showed a decreasing trend in the pioneer zone and the low marsh under warming, we found a significant increase of root biomass in the high marsh under warming in the lower depth (Fig. 2.3a). This contrasted with the decrease of root biomass in the upper depth in the high marsh under warming in 2019. The decrease in root biomass in the +3 °C treatments in the low marsh became more pronounced in summer and autumn, but less pronounced over the years (Appendix, Fig. S2.3). In 2020, the treatments did not significantly differ (Table 2.2), but in 2021 we found a significant interaction of zone, treatment, and season (Fig. 2.4, Table 2.2). In spring 2021, the medians in the ambient and +3 °C treatments were similar in the pioneer zone and high marsh, while in the low marsh there was a slight decrease in the +3 °C treatment (Fig. 2.4). In summer, root biomass increased in the pioneer zone under warming, while it slightly decreased in the low marsh and high marsh. The same trend was observed in autumn 2021 (Fig. 2.4). The difference between treatments was close to significance in the pioneer zone in autumn, as post-hoc pairwise comparisons showed (Tukey HSD; p=0.054), while all other pairwise comparisons of treatment within seasons were not significantly different from each other. Pooled across all treatments and seasons, we saw a significantly higher (Tukey HSD) root biomass in the low marsh than the other two zones in all years in the lower depth (Fig. 2.3, Table 2.2).

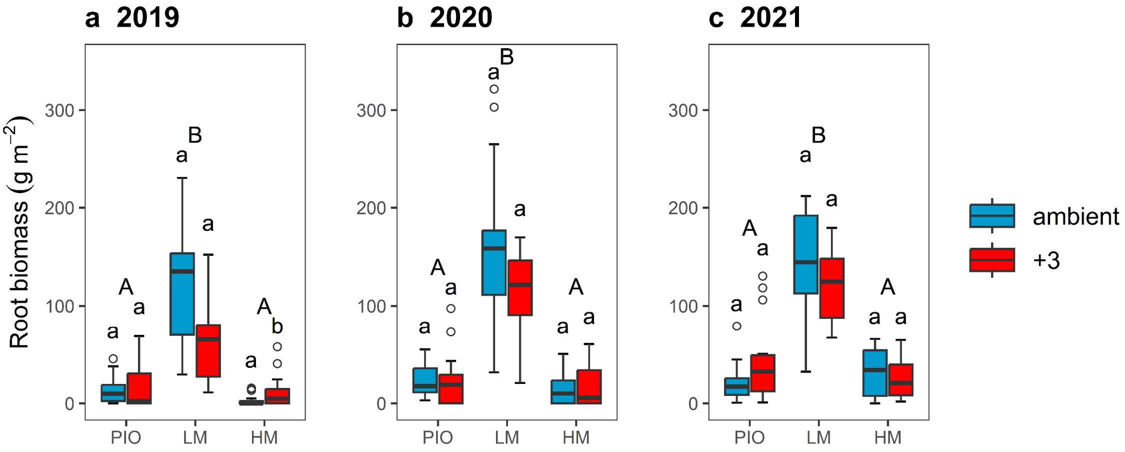

Fig. 2.3: Temperature treatment effect on root biomass in the lower depth (19-21 cm) in three salt marsh zones for (**a**) 2019, (**b**) 2020 and (**c**) 2021. PIO = pioneer zone, LM = low marsh, HM = high marsh. Different lowercase letters denote significant differences from Tukey HSD (p<0.05) between treatments. Different uppercase letters denote significant differences from Tukey HSD (p<0.05) between zones. Boxes represent 25% and 75% quartiles from the median, whiskers represent maximum and minimum values (excluding outliers), and circles represent outliers.

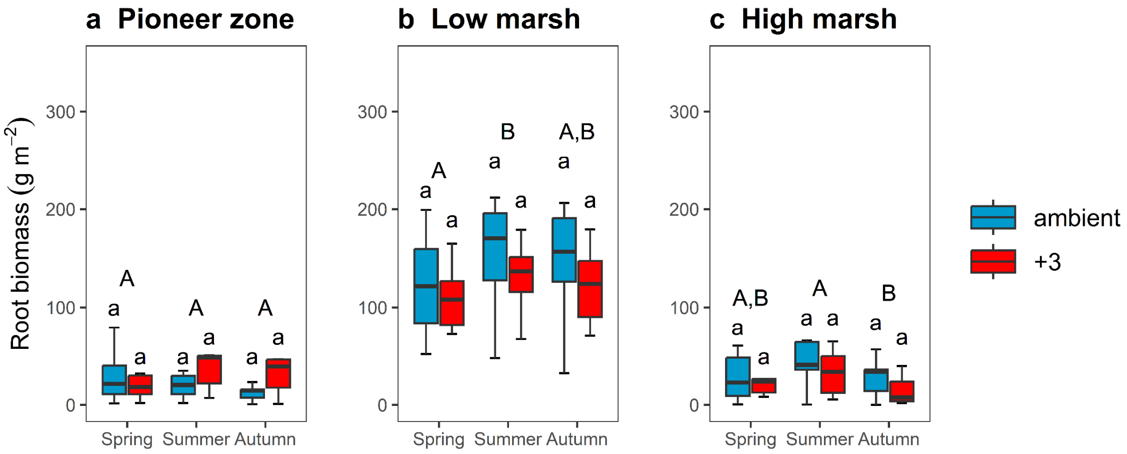

Fig. 2.4: Temperature treatment effect on root biomass in the lower depth (19-21 cm) in three salt marsh zones and three seasons in **2021**. (**a**) pioneer zone, (**b**) low marsh, (**c**) high marsh. Different lowercase letters denote significant differences from Tukey HSD ($p<0.05$) between treatments within zones. Different uppercase letters denote significant differences from Tukey HSD ($p<0.05$) between seasons within zones. The interaction plot (temperature treatment×season×zone) for all three years (2019-2021), can be found in the Appendix (Fig. S2.3). Boxes represent 25% and 75% quartiles from the median, whiskers represent maximum and minimum values (excluding outliers), and circles represent outliers.

Discussion

Compared to plant responses above the surface, the knowledge of belowground processes of plants with respect to climate change is a challenging task which has long been neglected (Bardgett et al. 2014). However, soil processes are a key component for nutrient cycling and uptake, C sequestration (Mcleod et al. 2011), root respiration (Atkin et al. 2000), soil aggregate formation, and plant-microbe interactions in the rhizosphere (Deegan et al. 2012; de Battisti et al. 2019; Noyce et al. 2019). The results of our study do not show a gradually enhancing effect of warming on root biomass production with increasing elevation in salt marshes as hypothesized. The influence of experimental warming on the root biomass was highly dependent on the study year, zone, and soil depth; and showed increases, decreases, or absolutely no responses to warming treatments. Except for the study year 2021 in the lower depth, we did not find a season-mediated warming effect. Furthermore, we found a seasonal variability of root biomass in salt marshes regardless of treatment, with the highest root biomass during summer.

Salt marsh plants of the higher elevations show drought response

Along the elevational gradient; from the pioneer zone with highly anoxic and saline soil conditions; to the low marsh where less anoxic but still quite saline soil conditions occur; to the high marsh which is flooded only during storm surges and plants during summer and early autumn may suffer from drought stress; we expected the influence of experimental warming on root biomass to become increasingly evident. We expected that soil moisture (driven by inundation frequency) would be the main changing factor along this gradient to mediate the influence of warming. Our findings partially reflect these expectations. We did indeed find evidence that root biomass responded to experimental warming in the high marsh, and there was less response in the pioneer zone and low marsh, which partly confirms our first hypothesis. However, the influence was depth- and year-dependent. Nevertheless, our results support our expectation that warming influences root biomass via the soil water content. Particularly in 2019, we found a shift of root biomass from upper depth to lower depth in the high marsh warming treatments (Figs. 2.2 and 2.4; Appendix, Table S2.2), and to a lesser extent also in the low marsh warming treatments (Appendix, Table S2.2). These findings may indicate that salt marsh plants of higher elevations suffer from drought stress due to warming and therefore expand their roots into lower depths to reach for water (Matsui and Singh 2003; Wang and Yamauchi 2006). Tang et al. (2023) suggested that the stronger effect of warming on the organic matter decomposition rate in 2019 (compared to 2018) in the MERIT experiment was due to a warming-stimulated plant growth and therefore higher substrate input. In the first year of the MERIT experiment (2018), the organic matter decomposition rate in the high marsh +3 °C treatments were comparable to the ambient treatment. But in 2019, organic matter decomposition increased in all soil depths compared to the ambient treatment. This fits with our results of the increasing rooting depth under warming in 2019, suggesting a higher input of plant material (roots) in deeper soil depths.

In the other study years (2020 and 2021), experimental warming seems to play a less important role for root biomass production of salt marsh plants, which may have two possible explanations. Either plants may have acclimated to warmer temperatures over time by increasing their physiological temperature optimum (Mawson et al. 1986; Atkin et al. 2006a), or the difference between the study years is mainly affected by climatic differences (Appendix, Fig. S2.4). In 2019, there was low precipitation during the vegetation season (Appendix, Fig. S2.4), and no flooding of the high marsh occurred between March and September (Appendix, Fig. S2.5). We thus anticipate that the increase in root biomass in the lower depth under warming in the higher marsh zones was a response to severe drought stress. This indicates that especially *E. athericus* shows a plastic response to changing environmental conditions regarding rooting depth. A recent study supporting our observation found that *E. athericus* is indeed capable of responding to changing environmental conditions and e.g. responds to increased flooding frequency with reduced root biomass (Reents et al. 2021). This plasticity may strengthen its competitive advantage, and we thus predict that under future warming (and especially under drought conditions), *E. athericus* will most likely remain dominant. In 2019, we detected a trend of decreasing belowground biomass in the lower soil depth under warming in the low marsh. This indicates that one species, which under ambient temperatures occupies this belowground space (probably *H. portulacoides* or *Limonium vulgare*; Chapman 1950; Boorman 1967), may have changed rooting depth due to warming-induced changes in soil properties. Interestingly, this trend became less pronounced in 2020 and 2021. This could be either due to gradual acclimation of these species to warming (Quan et al. 2020) or climatic differences (e.g. precipitation, ambient temperatures) between years may have affected rooting depth, like in the high marsh. There is also the possible explanation that the soil depth was occupied by other species. Therefore, warming may lead to alterations of belowground interspecific competition for space.

The overall small response of root biomass to experimental warming can also be a result of the non-linear response of belowground biomass under increasing temperatures: Noyce et al. (2019) report belowground biomass to increase in an intermediate treatment temperature of +1.7 °C, but to decline with further warming. Since we did not consider the intermediate treatment (+1.5 °C) in our study, we might have missed this non-linear effect. In another study, salt marsh and mangrove species decreased their root biomass due to warming, with a more pronounced effect in deeper soil layers (40-60 cm; Coldren et al. 2016). However, when comparing our results with other experimental warming studies conducted in salt marshes, it becomes clear that there are major differences in either the experimental set up or the environmental conditions of the marshes themselves. For example, the brackish salt marshes at the US coast studied by Noyce et al. (2019) have highly organic soils (up to 90 %) and face microtidal conditions, and therefore differ significantly from the salt marshes at the Wadden Sea. Wadden sea salt marshes are largely composed of minerogenic sediments and face meso- to macrotidal flooding (Yando et al. 2023). Other warming experiments do not actively warm the soil (Charles and Dukes 2009; Gedan and Bertness 2009; Coldren et al. 2016), like the MERIT experiment, and therefore do not simulate changing soil properties due to increasing temperatures. The open-top chambers used in these other studies do not warm the soil to the same extent as the air (Carey et al. 2018). In addition, the different plant species growing in the three zones of the salt marshes might respond differently to changing (environmental) conditions, like warming-induced droughts (Charles and Dukes 2009), shifting redox potential (Tang et al. 2023), nutrient availability (Noyce et al. 2019), and root-microbe interactions (Tang et al. 2023). Since we did not differentiate the roots on a species level, we cannot exclude shifts of root biomass on an interspecific level (mainly in the low marsh), which may be an indicator for shifts in competitive interactions. With those differences in mind, our results show that belowground biomass may be less affected by warming than anticipated. Therefore, the coastal protection function of salt marshes may not be threatened under the aspect of belowground biomass as a sediment stabilizing factor (Ford et al. 2016; de Battisti et al. 2019) with an increase of +3 °C compared to ambient temperatures.

Root biomass differs between zones, regardless of warming

We confirm results of previous studies (Redelstein et al. 2018a; Valiela et al. 1976) that the overall root biomass differs between salt marsh zones, and this is mainly dependent on the differences in species composition and in soil properties. In the upper depth, we found significantly less root biomass in the high marsh compared to the pioneer zone and low marsh, and significantly higher root biomass in the lower depth of the low marsh compared to the other two zones in all years. These results underline the complex interplay of abiotic and biotic variations along the elevational gradient in salt marshes at the Wadden Sea, which lead to the necessity of plastic rooting strategies and belowground space partitioning (Redelstein et al. 2018a, 2018b). We observed that the environmental stressors of the low marsh and pioneer zone seem to enhance the necessity of the plants to invest more into the belowground root systems. Particularly in the pioneer zone, where plants face the inundation and hydrodynamic forcing due to the regular tidal influence (Bouma et al. 2005; Schoutens et al. 2021), dense root stands are essential for anchoring in the soils. However, there seems to be a trade-off between safe anchorage and avoidance of waterlogging stress (Bouma et al. 2001; de Battisti et al. 2019). We assume this is a driver of the higher investment in root biomass in the upper soil depths in the pioneer zone. In the low marsh, interspecific competition for space, water, and nutrients probably plays an important role in root biomass distribution, as up to 20 plant species compete for space and resources here. In addition, it has also been shown that root biomass of salt marshes is affected by species diversity (Ford et al. 2016), probably due to different rooting strategies of salt marsh plants (see for example: Chapman 1950; Boorman 1967; Gray and Scott 1977). Therefore, a high root biomass in both depths was not surprising. Together with the lower root biomass in the high marsh, which is only flooded during storm tides and mainly dominated by *E. athericus*, our results support the positive correlation of root biomass and diversity, which has previously been reported for salt marshes and other ecosystems (Hooper et al. 2005; Redelstein et al. 2018a).

Seasonal influences are stronger than warming effects

Contrary to our second hypothesis, we did not detect overall changing responses of root biomass to warming between the three seasons in the upper depth. While we found a significant interaction between season, treatment, and zone in the lower depth in 2021, there were only marginal differences between the warming treatments in different seasons. Thus, there seems to be only minor differences in the influence of experimental warming on root biomass between different seasons. Nevertheless, and in agreement with part of our second hypothesis, we observed differences of root biomass between the seasons in the salt marsh zones irrespective of warming. The highest root biomass in the upper depth was indeed in summer, during the reproductive growing season. This pattern is likely enforced by a decreasing soil water content in the higher zones and the general greater need for nutrients and water to maintain or even enhance the metabolism in the reproductive growth phase (Eziz et al. 2017). Valiela et al. (1976) found the highest belowground biomass during the phase with the fastest aboveground growth in salt marshes, which was at the same time the most nutrient-limited. Thus, the high biomass in summer may be a result of the investment in the organ which traps the limiting factors of water and nutrients (Bloom et al. 1985). There was less root biomass in spring, when the plants initially started to grow, and in autumn, when plants faced the end of the growing season and roots started to decay.

Methodological considerations

The standard unit to report belowground biomass in literature is ‘g per soil surface area’. Thus, we estimated the root biomass from the root surface area of the mini-rhizotron pictures combined with soil cores. To achieve this, we took soil cores and pooled roots from all zones, depths, and treatments for our allometric measurements. However, it is common to pool together the roots to calculate the root mass per unit length (RML) in mini-rhizotron studies as the root diameter relationship did generally not change within the experiments and between treatments (Iversen et al. 2008; Cordeiro et al. 2020; Yaffar et al. 2021). Additionally, we presented the results of the root surface area from the mini-rhizotron pictures to ensure the difference between these two response variables was negligible (Appendix, Table S2.1).

The mini-rhizotron tubes were placed into the MERIT plots in 2018 at a 45°C angle, and according to Iversen et al. (2012) and references therein, it’s more optimal than inserting the tubes vertically into the soil, though we cannot exclude artificial root growth along the surface of the tubes and the influences of water that runs down along the surface of the tubes.

Conclusions

Warming-induced alterations of the root-soil interactions in salt marshes play an important role in several ecosystem services. However, our results indicate that an increase of +3 °C to ambient temperatures may not affect the coastal stabilization function, as the root biomass did not significantly change under experimental warming (with exception of the high marsh). As we observed a shift of root biomass to deeper soil layers under warming paired with drought conditions, sediment stability may be affected under future climate change. The formation and the decomposition of roots are also essential in the context of C sequestration and nutrient mineralization by microbes, and the uptake of nutrients via the roots are of great importance for the functioning and maintenance of plants and thus ecosystem function. Future studies on salt marsh belowground root processes should therefore additionally consider nutrient availability, root respirations processes, and root turnover under warming conditions.

Acknowledgements

We would like to thank Mónica Salazar-Ortiz, Sebastian Wilfinger, Alston D´Souza, Sara Rezaie, Beke Eichert, and Ella Logemann for technical assistance. We also thank Roy Rich for his expertise and knowledge and for building the MERIT experiment, Simon Thomsen, Benjamin Branoff and Daniela Yaffar for helpful advice on mini-rhizotron methodology. We thank Heather Shupe for proofreading.

Appendix

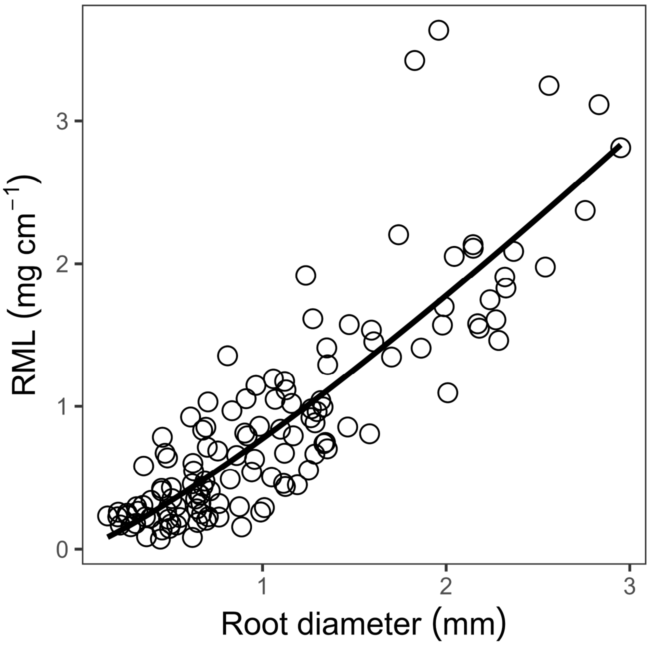

Fig. S2.1: Relationship between root diameter and RML from soil cores close to the tubes within the plots in 2021 (n=128). Data are pooled across depths and zones.

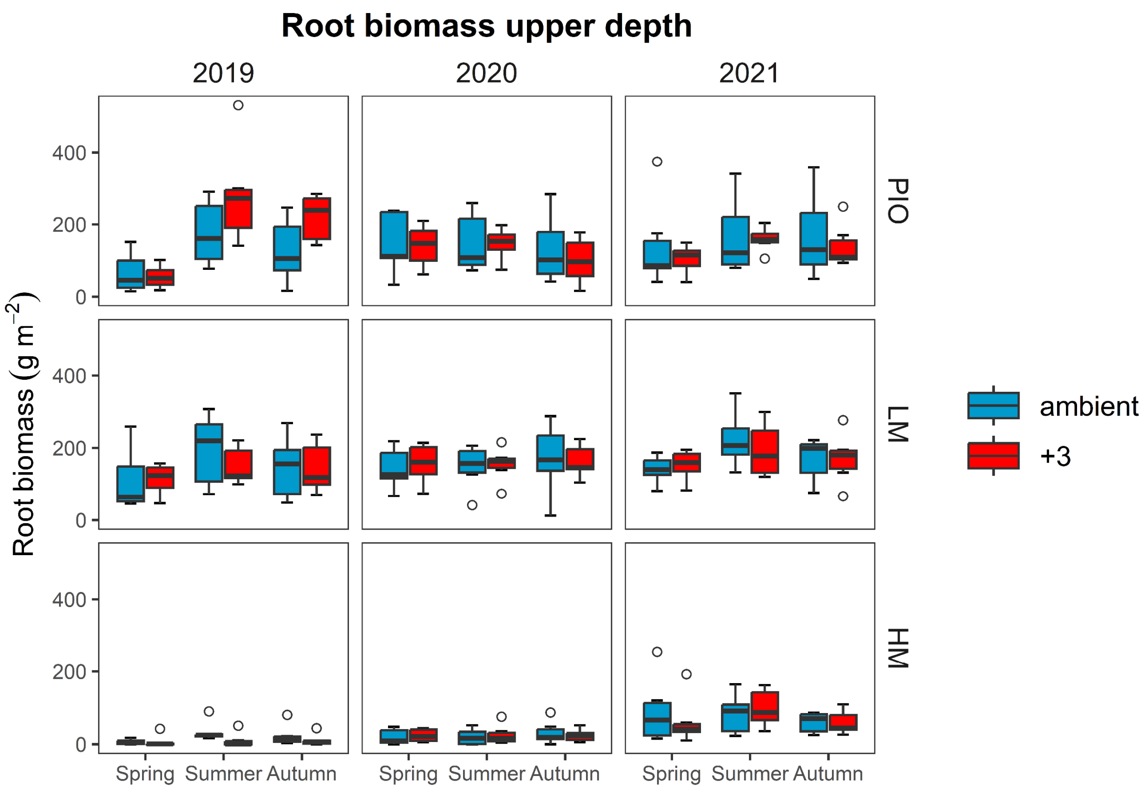

Fig. S2.2: Root biomass in the upper depth (7-9 cm) according to temperature treatment (ambient, + 3°C), season (spring, summer, autumn), and zone (PIO: pioneer zone; LM: low marsh; HM: high marsh). Boxes represent 25% and 75% quartiles from the median, whiskers represent maximum and minimum values, excluding outliers which are represented as circles.

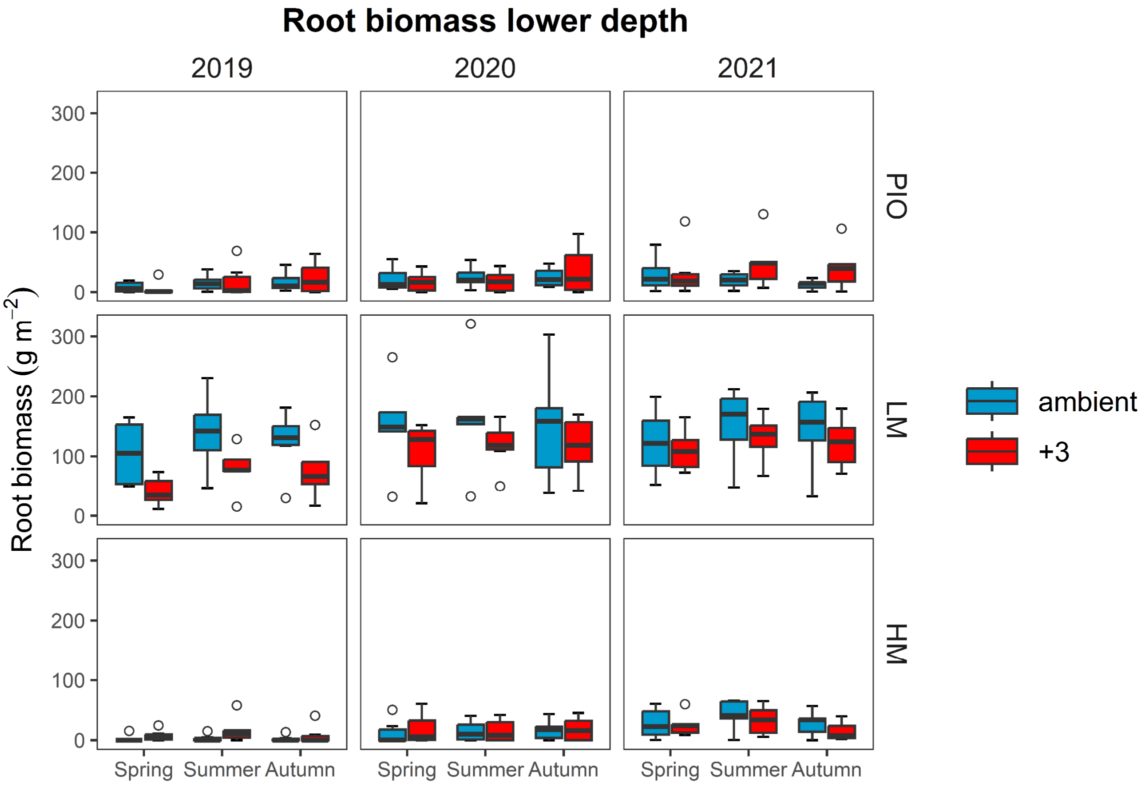

Fig. S2.3: Root biomass in the lower depth (19-21 cm) according to temperature treatment (ambient, + 3°C), season (spring, summer, autumn), and zone (PIO: pioneer zone; LM: low marsh; HM: high marsh). Boxes represent 25% and 75% quartiles from the median, whiskers represent maximum and minimum values, excluding outliers which are represented as circles.

Table S2.1: Comparison between GLMM models of root biomass (g m^{-2}) and root surface area (mm^{2}) in two temperature treatments (ambient, +3 °C), three seasons (spring, summer, autumn). Separate models were implemented each for two depths (7-9 cm,19-21 cm) in three years (2019, 2020, 2021). Models were implemented with a Tweedie distribution (link=log).Only significant outcome variables are displayed (backwards model selection based on AIC).

	Biomass				Surface Area			
	Upper depth							
2019		Chisq	df	p		Chisq	df	p
	(Intercept)	1016.18	1	<0.0001	(Intercept)	854.79	1	<0.0001
	zone	85.35	2	<0.0001	zone	81.42	2	<0.0001
	treatment	0.85	1	0.3561	treatment	1.07	1	0.3014
	season	54.01	2	0.0000	season	52.15	2	0.0000
	zone:treatment	6.73	2	0.0346	zone:treatment	7.66	2	0.0217
	zone:season	14.95	4	0.0048	zone:season	17.18	4	0.0018
2020	(Intercept)	1849.22	1	<0.0001	(Intercept)	1196.37	1	<0.0001
	zone	75.24	2	<0.0001	zone	54.10	2	<0.0001
2021	(Intercept)	3443.39	1	<0.0001	(Intercept)	534.61	1	<0.0001
	zone	25.10	2	<0.0001	zone	20.80	2	<0.0001
	season	17.82	2	0.0001	season	15.62	2	0.0004
	Lower depth							
2019	(Intercept)	133.12	1	<0.0001	(Intercept)	88.92	1	<0.0001
	zone	47.23	2	<0.0001	zone	34.51	2	<0.0001
	treatment	0.37	1	0.5443	season	6.92	2	0.0314
	season	15.05	2	0.0005	zone:season	10.25	4	0.0364
	zone:treatment	6.71	2	0.0349				
2020	(Intercept)	184.71	1	<0.0001	Intercept)	115.40	1	<0.0001
	zone	27.70	2	<0.0001	zone	21.54	2	<0.0001
2021	(Intercept)	557.32	1	<0.0001	(Intercept)	387.25	1	<0.0001
	zone	35.49	2	<0.0001	zone	32.60	2	<0.0001
	treatment	0.24	1	0.6215	treatment	0.25	1	0.6179
	season	22.40	2	<0.0001	season	29.02	2	<0.0001
	zone:treatment	1.21	2	0.5455	zone:treatment	1.18	2	0.5550
	zone:season	14.32	4	0.0063	zone:season	15.50	4	0.0038
	treatment:season	3.81	2	0.1485	treatment:season	2.00	2	0.3679
	zone:treatment: season	16.07	4	0.0029	zone:treatment: season	19.01	4	0.0008

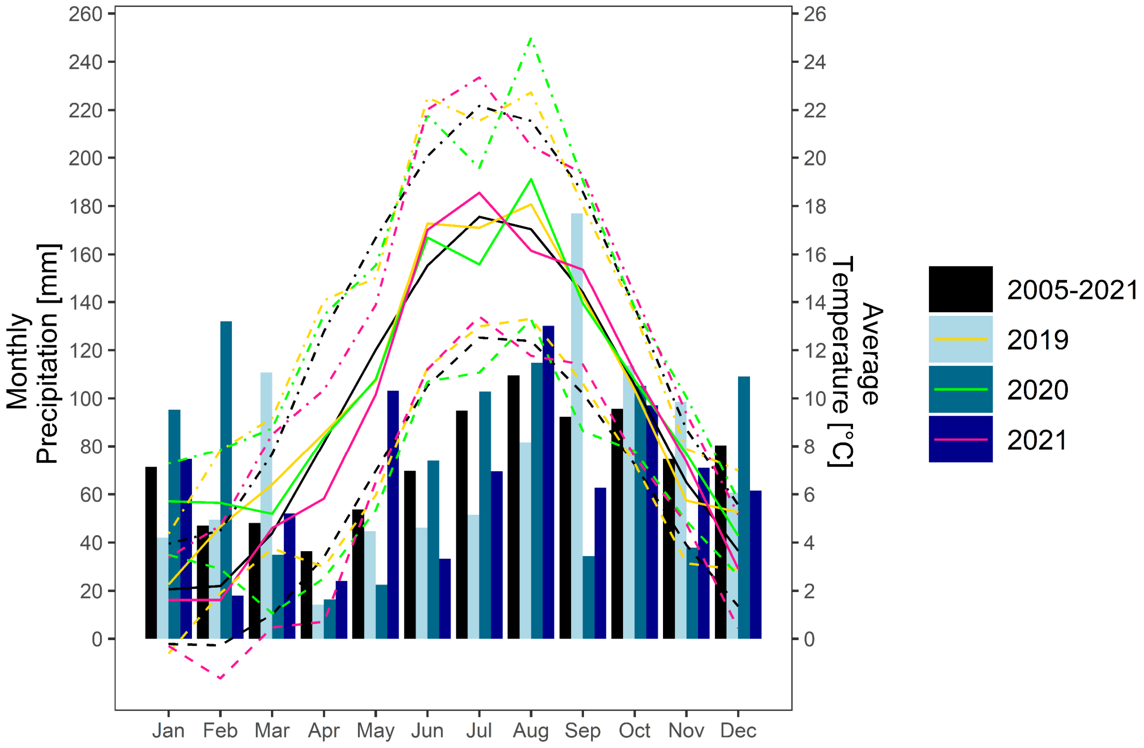

Fig. S2.4: Climate differences between study years (2019-2021) for Hattstedt (15 km away). The mean of 2005 to 2021 is indicated in black as a reference level. Monthly average temperatures are indicated by solid lines. Average minimum and maximum temperatures are indicated by dashed and dash-dotted lines respectively. Bars display the cumulative monthly precipitation. Data was provided by the German weather service (Deutscher Wetterdienst, DWD).

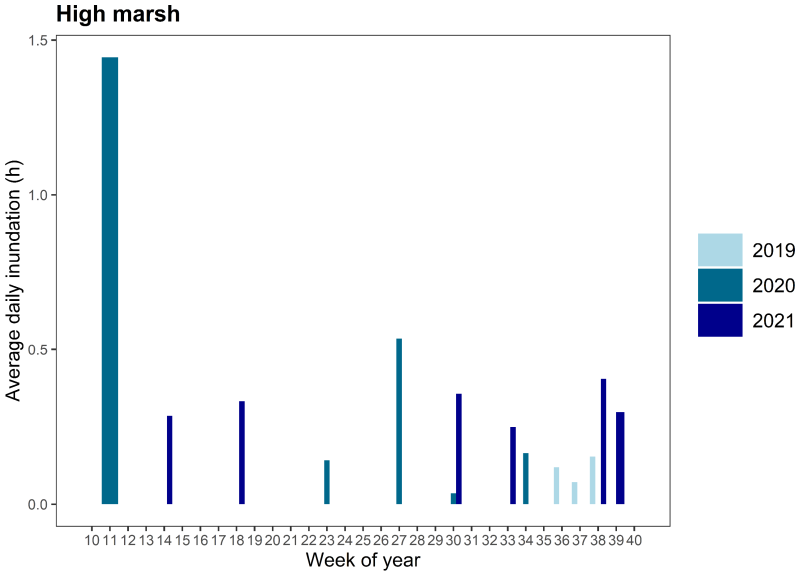

Fig. S2.5: Average flooding hours per week of year in the high marsh.

Table S2.2: Mean biomass per temperture treatment (ambient, +3 °C), soil depth (upper: 7-9 cm; lower: 19-21 cm) and zone (Pio: poineer zone, LM: low marsh; HM: high marsh). Biomass values are obtained from the allometric relationship between root mass per unit length and root diameter (see methods).

Soil depth	Zone	Treatment	Mean weight ($g\ m^2$)	sd	Year
upper	HM	+3	149.53	202.00	2019
lower	HM	+3	206.39	207.16	2019
upper	HM	ambient	296.41	176.06	2019
lower	HM	ambient	65.44	128.82	2019
upper	LM	+3	262.57	187.99	2019
lower	LM	+3	360.13	181.39	2019
upper	LM	ambient	355.11	122.93	2019
lower	LM	ambient	225.50	142.98	2019
upper	PIO	+3	274.33	128.45	2019
lower	PIO	+3	197.00	195.16	2019
upper	PIO	ambient	297.56	167.28	2019
lower	PIO	ambient	224.12	183.56	2019
upper	HM	+3	401.28	121.17	2020
lower	HM	+3	168.28	195.17	2020
upper	HM	ambient	264.39	190.23	2020
lower	HM	ambient	175.39	180.85	2020
upper	LM	+3	218.56	128.97	2020
lower	LM	+3	202.56	159.55	2020
upper	LM	ambient	211.67	122.58	2020
lower	LM	ambient	270.27	120.50	2020
upper	PIO	+3	264.22	159.59	2020
lower	PIO	+3	260.11	215.95	2020
upper	PIO	ambient	318.94	170.31	2020
lower	PIO	ambient	324.76	153.65	2020
upper	HM	+3	323.11	167.63	2021
lower	HM	+3	343.41	161.91	2021
upper	HM	ambient	346.76	170.47	2021
lower	HM	ambient	340.94	182.31	2021
upper	LM	+3	238.28	127.90	2021
lower	LM	+3	260.00	197.60	2021
upper	LM	ambient	255.06	133.27	2021
lower	LM	ambient	237.17	134.73	2021
upper	PIO	+3	193.94	161.44	2021
lower	PIO	+3	235.39	187.59	2021
upper	PIO	ambient	353.89	176.63	2021
lower	PIO	ambient	284.88	184.40	2021

3

Ecophysiological responses of plants to warming are mediated by abiotic conditions in salt marshes

Abstract

Salt marshes provide important ecosystem services such as coastal protection and carbon sequestration, but these services may be affected by climate change. Specifically, the predicted rise in global temperature and extreme weather events may have large effects on the ecosystem functions and services of salt marshes. To understand ecosystem functions and processes under changing environmental conditions, it is necessary to study the response of vegetation. In this study, we investigated the ecophysiological response of salt marsh plants to warming (+1.5 and +3.0 °C) along an elevational gradient in three salt marsh zones (pioneer zone, low marsh, high marsh) within an in-situ warming experiment at the Wadden Sea coast. We collected leaf samples of the most common plant species in each zone in different seasons (spring, summer, autumn) in 2019, 2020 and 2021 to analyse the effect of warming on the stable carbon isotopic composition ($\delta^{13}C$) and specific leaf area (SLA). $\delta^{13}C$ was affected by warming in all zones, but the direction of response differed. In the low marsh species *H. portulaciodes* and the high marsh species *E. athericus*, we found a general increase of $\delta^{13}C$ due to warming, which indicates an increasing water use efficiency (WUE). In the pioneer zone species *S. anglica*, $\delta^{13}C$ decreased due to warming. SLA was less affected by warming than by seasonal shifts. We conclude that ecophysiological responses to warming are mediated by abiotic and climatic conditions.

Introduction

The benefits of coastal ecosystems like salt marshes for humans are becoming more recognized in these times of global change (Barbier et al. 2011). Protection against storms (Möller et al. 2014) and a high amount of carbon sequestration (Chmura et al. 2003; Mcleod et al. 2011) are but two of the numerous ecosystem services provided by salt marshes (Barbier et al. 2011). However, increasing global temperatures and extreme weather events are major issues of global change (IPCC 2023) that might affect salt marshes and their ability to provide these ecosystem services.

Salt marshes form the ecotone between land and sea and are unique habitats for highly adapted species (Adam 1993; Allen 2000). The Wadden Sea salt marsh vegetation consists of many halophytes, which occur along an elevational gradient according to their competitive ability and stress tolerance (Silvestri et al. 2005; Suchrow and Jensen 2010). Species inhabiting the low end of the elevational gradient (pioneer zone) are highly stress tolerant and must withstand daily flooding and high salinity levels. Species at the high end of the gradient (high marsh) are less stress tolerant but are highly competitive and form monoclonal stands (Bockelmann and Neuhaus 1999). The high marsh is usually only flooded during storm surges in the winter months. In between the pioneer zone and the high marsh lies an intermediate zone (low marsh) which is flooded during spring tides and is more species-rich than the other zones (Suchrow and Jensen 2010; Esselink et al. 2017). The low marsh vegetation consists of a mixture of up to 20 grasses, small shrubs, and herbaceous plants. Since salt marsh species are highly adapted to their environment, the niche of each species is limited to a certain elevational range, which is typically well defined (Suchrow and Jensen 2010).

One challenge of global warming that salt marshes are confronted with is keeping pace with sea level rise through sediment accretion (Kirwan and Megonigal 2013). Accretion of salt marshes has been found to be directly influenced by biomass through accumulation of peat (Kirwan and Megonigal 2013; Morris et al. 2016) and indirectly through the increased sediment trapping by dense vegetation (Fagherazzi et al. 2012). Therefore, it is important to study the effects of increasing temperatures and their influence on the adaptability, stress tolerance, and competitive ability of salt marsh plants.

The effects of increased temperature on the physiology of plants can be both direct and indirect. In this study, direct effects are physiological adjustments due to higher temperatures within the plants, while indirect effects concern physiological adjustments which arise from changes of the physical environment (such as soil (moisture) conditions). However, the effects of higher temperatures on physiological processes at plant organ level are difficult to separate from effects of ecological processes at the site level (Sombroek and Gommes 1996). For example, higher temperatures can result in a decrease of stomatal conductance by both a decrease of the leaf water potential itself and a decrease in soil moisture or higher transpiration (Tuzet et al. 2003). It is important to note that warming can change soil water availability (Rustad et al. 2001) by both increasing the transpiration demand of plants, but also by increasing the evapotranspiration of the soil (Wang et al. 2022). Therefore, warming may indirectly induce drought conditions and drought stress. Overall, it may be difficult to distinguish if physiological changes are a result of increased temperature on the plant organ itself or due to other changing environmental conditions. Here, we will focus more on how environmental conditions impact the plant species' physiological responses to drought stress.

In salt marshes, one of the most important environmental conditions is the hydrological gradient, since soil moisture and other soil properties are influenced by the tidal inundation. In the pioneer zone, the frequent inundation and permanent waterlogging lead to anoxic conditions, and thus warming may have no influence on soil moisture conditions. But with increasing elevation and less frequent flooding, the soil moisture decreases (Bakker et al. 1985; Suchrow and Jensen 2010). Combined with warming, the further decreases in soil moisture may result in drought stress in plants at higher elevations. Therefore, we expect an increasing impact of warming on plant physiological responses through drought stress from pioneer zone to low marsh to high marsh. As a gradient has no clear boundaries, differences in elevation within zones are also important to consider. Within one salt marsh zone, elevation can still differ by several centimeters, leading to large differences in flooding frequencies and soil moisture conditions.

There are two ecophysiological indicators that may be used for detecting drought stress in plants: the discrimination of the stable carbon isotope $\delta^{13}C$ ([‰] with respect to PeeDee Belemnite) and the specific leaf area (SLA [$m^2 \cdot kg^{-1}$]; the ratio of leaf area and leaf dry mass). The carbon isotopic composition $\delta^{13}C$ can be used as a proxy for the water use efficiency (WUE) of C3 plants (Farquhar and Richards 1984; Farquhar et al. 1989; Stokes et al. 2010) because Ribulose-1,5-bisphosphate-carboxylase/oxygenase (Rubisco) reacts more easily with $^{12}CO_2$, and discriminates against $^{13}CO_2$, which diffuses back to the atmosphere (Farquhar et al. 1989; Lambers and Oliveira 2019). Drought stress leads to a decrease in stomatal conductance, which results in the use of ^{13}C in photosynthesis, and thereby the carbon discrimination is affected (Lavergne et al. 2020). The response of $\delta^{13}C$ to drought stress in C4 plants does not show the same unilateral direction. The effect of change is dependent on the leakiness of the bundle sheath cells (Cernusak et al. 2013; von Caemmerer et al. 2014), which is affected by the degree of the stress (Bowman et al. 1989). In C4 plants, the phosphoenolpyruvatcarboxylase (PEPC) fixes carbon by carboxylation in the bundle sheath cells, and the discrimination is much smaller than from Rubisco (Farquhar et al.

1989). However, linking the $\delta^{13}C$ signature of C4 plants to their photosynthetic carbon isotope discrimination has been challenging, and it may depend on the subtypes of C4 plants which also exhibit different biochemical compositions (von Caemmerer et al. 2014), as well as the environment the plant grows in. Due to the differences in discrimination of Rubisco and PEPC, the typical range of $\delta^{13}C$ in C4 plants is -10‰ to -15‰, while in C3 plants it ranges from -22‰ to -33‰ (Bender 1971).

Warming-induced drought stress typically leads to increased WUE in C3 plants (Farquhar et al. 1989). However, warming decreased the $\delta^{13}C$ values of two salt marsh species in US salt marshes, resulting in less efficient WUEs (Gedan and Bertness 2009). This was explained by an increased transpiration under warming. Because the soils were waterlogged, there was no drought effect, and thus no increase in WUE but a decrease. This study was conducted in a microtidal salt marsh in the US Atlantic coast, which has very different conditions to the NW European salt marshes in our study. To our knowledge, there are no studies on the influence of warming on $\delta^{13}C$ values of meso- or macrotidal salt marsh plants.

The second proxy that is commonly used to understand the ecophysiological response of plants to drought stress is the SLA. Due to changes in resource allocation strategies, the general response of terrestrial plants to drought stress is a is a decrease in SLA (Castro-Diez et al. 1997; Poorter et al. 2009; Gong et al. 2011). It is unknown if this response can also be observed in salt marsh species experiencing drought stress. Along the elevational gradient in salt marshes, the SLA seems to be mainly affected by salinity, waterlogging, and inundation (Minden et al. 2012). In general, the SLA increases with decreasing elevation on an interspecific level, because species at lower elevations are fast-growing and have less nutrient limitations, hence higher tissue nutrient content in salt marshes (Lavorel et al. 2007; Minden and Kleyer 2011). To our knowledge, there is only one study which looked at the effect of experimental warming on the SLA of a salt marsh species (*Phragmites australis*), which increased the SLA under warming (Zhong et al. 2014). However, it is not established yet how the SLA of common European salt marsh species is influenced by experimental warming.

In addition, seasonal changes in the ecophysiological responses of salt marsh plants may arise due to temperature differences over the course of the year. For example, drought stress might be more pronounced during summer due to precipitation patterns and increased water demand by the vegetation. Additionally, the hydrological conditions change over the course of the year depending on the marsh zone, because inundation frequencies vary seasonally.

Therefore, in less frequently inundated elevations of the low and the high marsh, soils desiccate over the course of the summer, which results in increasing drought stress. However, which and how plants' physiological processes are affected depends also on plant age, life history traits, and on the performed photosynthetic pathway (Schlichting 1986). In C3 plants, seasonal differences of δ^{13}C can mainly be attributed to climatic conditions because stomatal conductance is influenced by changes in temperature and soil moisture (Farquhar and Sharkey 1982). With warmer temperatures, C3 plants regulate their stomatal conductance, resulting in an enrichment of δ^{13}C and increased WUE (Farquhar et al. 1989). In C4 plants, seasonal differences mainly arise from allocation patterns of different organic compounds, which show different depletions in δ^{13}C and change during leaf age (von Caemmerer et al. 2014). To a lesser degree, seasonal differences are explained by climatic impacts (Sage and Kubien 2007). Similarly, seasonal differences of the SLA in plants mainly arise due to a change in resource allocation during growth (Kleyer et al. 2019), but this may also depend on drought conditions (Poorter et al. 2009). During vegetative growth, higher SLAs indicate a more efficient carbon use (Lambers and Poorter 1992). Additionally, salt marsh plants in the high marsh face increasing salt and water stress with increasing soil desiccation over the course of the year, to which they respond by increasing the leaf dry matter content or through lignification of the cell walls (Rozema et al. 1985; Vendramini et al. 2002; Minden et al. 2012). Thus, SLA may be higher in spring than in summer/autumn.

To study the effects of warming and seasonality on δ^{13}C and SLA in salt marshes, we chose five species with the highest abundance in the different salt marsh zones. In the pioneer zone, we chose two highly salt-tolerant species, namely *Spartina anglica,* the only C4 species present, and *Salicornia europaea,* a succulent. In the low marsh, we studied the dwarf shrub *Halimione portulacoides* and the grass *Puccinellia maritima*. In the high marsh, we chose the dominant grass *Elymus athericus,* which spreads clonally and forms dense stands. The study was conducted in a whole ecosystem warming experiment on the coast of the Wadden Sea. The experiment consists of a total of 27 plots in three salt marsh zones (pioneer zone, low marsh, and high marsh), with two warming treatments and one control (ambient, +1.5 °C and, +3.0 °C). Warming is achieved by microprocessor feedback-controlled active belowground warming and passive aboveground warming with domes covered with foil (Rich et al. in press).

We use the δ^{13}C signature and SLA to assess possible drought stress responses of salt marsh plants under experimental warming in spring, summer, and autumn over the course of three years (2019-2021). We hypothesize that (1) warming leads to less depleted $\delta^{13}C$ values and a decrease of SLA, and (2) the effect of warming is more pronounced in species of the high marsh, followed by low marsh and least pronounced in the pioneer zone species. For both $\delta^{13}C$ and SLA we furthermore hypothesize (3) a strong seasonal effect, which is also mediating the effect of experimental warming and (4), this seasonal effect is influenced by elevation within zones, with higher elevations leading to less depleted $\delta^{13}C$ and lower SLA and a more pronounced effect of elevation in summer and autumn.

Methods

Study area

In 2018, the MERIT (‘Marsh Ecosystem Response to Increased Temperatures’) experiment was set up on the Hamburger Hallig (54° 35′ 58″ N, 8° 49′ 8″ E). The site is part of the Schleswig-Holstein Wadden Sea National Park and a UNESCO world heritage site. The mean annual temperature between 1991 and 2021 was 9.6 °C and the mean annual precipitation was 859 mm (Deutscher Wetterdienst, DWD). The study site is a typical semi-natural salt marsh on the Wadden Sea coast with three distinct zones. The tidal range at the site is approximately 3.0 m (Stock 2011). Due to the average surface elevation of the pioneer zone being below mean high tide (MHT), it is typically flooded twice daily. *Spartina anglica* Hubb. and *Salicornia europaea* agg. (L.) are the dominant vegetation. Lying directly above MHT, the low marsh is only flooded during spring tides (Essink et al. 2005). The vegetation is more species-rich, with up to 20 species, with higher abundances of *Halimione portulacoides* (L.) Aell., *Limonium vulgare* Mill. and *Puccinellia maritima* (Huts.) Parl. In the high marsh (flooded only during storm tides), the vegetation is dominated by dense stands of *Elymus athericus* (Lk) Kerg.

Experimental design

There are 27 plots in the MERIT experiment, which is set up in a full factorial design. Within each zone (pioneer zone, low marsh, and high marsh), the temperature treatments (ambient temperature, +1.5 °C, and +3.0 °C) are replicated three times. The active belowground warming approach is achieved by 31 vertical resistance pins (GX 088L3100, 9.8Ω m^{-1}, Danfoss, Denmark), reaching 1 m deep into the ground and resistance cables (GX 088L3100, 9.8Ω m^{-1}, Danfoss, Denmark) on the soil surface. The aboveground warming treatment is achieved by domes covered with different amounts of clear plastic foil (Tang et al. 2023; Rich et al. in press). The foils are deployed from late spring to autumn (Table 3.1). The electrical heating system is generally switched on during the vegetation period (Table 3.1) and switched off in autumn/winter, as sensors and heating system are vulnerable to damage by heavy storm tides. The heating system is controlled and monitored continuously by sensors deployed at different depths (Rich et al. in press).

Elevation and zones

We estimated the absolute elevation of each plot with respect to the German vertical datum (NHN) by measuring the four corners of each plot with a laser levelling device (Spectra precision laser LL500, Spectra-Physics, USA) and taking the mean. We included this within zone elevation gradient, to account for possible differences of hydrology and other soil parameters.

Table 3.1: Sampling dates and warming treatment deployment for 2019-2021.

Year	2019	2020	2021
Foil deployment	Mar 1st*- Sep 25th	Mar 10th- Sep 14th	Apr 9th- Oct 4th
Belowground warming	Mar 14th - Sep 22nd	Mar 10th- Sep 27th	Mar 23rd- Oct 4th
Spring sampling	May 8th- 10th	May 25th-27th	May 11th -14th
Summer sampling	July 15th- 16th	July 16th-17th	July 20th-23rd
Autumn sampling	Sep 18th-21st	Sep 14th-16th	Sep 14th- 16th

*due to storm damage in the pioneer zone in 2019, foils were removed from March 19th to April 17th.

Sample collection

The δ^{13}C values and SLA of leaves were used to assess the WUE and drought stress of five common salt marsh species. Each species was only sampled within the zone where they were highly abundant. We sampled *S. europaea* and *S. anglica* in the pioneer zone, *H. portulacoides* and *P. maritima* in the low marsh, and *E. athericus* in the high marsh. At each sampling date (Table 3.1), three individuals per species per plot were sampled. We collected the youngest leaf or leaves according to the following protocol: for grasses (*S. anglica, P. maritima, E. athericus*), we sampled the blade of the uppermost leaf; for *S. europaea,* we collected the entire individual; and for *H. portulacoides*, we sampled three leaves of one individual at the tips of a branch. Individuals were chosen randomly, but only individuals with intact leaves were sampled. After cutting, the leaves were immediately put on a picture frame with a white background and a reference square of 1*1 cm², a glass plate was pressed on top, and a picture was taken from above (Panasonic Lumix FT25). Then the leaves were put in paper bags and transported back to the laboratory, where they were oven-dried at 70 °C for at least 48 hours.

Sample processing and analysis

SLA

We weighed each sample after oven drying. To calculate the specific leaf area, the area of each leaf in each picture was measured using GIMP (The GIMP Development Team 2019).

$\delta^{13}C$

In 2019, a small piece from each leaf was taken for analysis. In 2020 and 2021, dry leaf samples were ground and homogenized in a ball mill (Retsch, Hahn, Germany). δ^{13}C values were determined using an isotope ratio mass spectrometer (Nu Horizon, Nu Instruments, Wrexham, UK). Samples were analysed alongside the laboratory standard commonly known as BBOT (2,5-Bis-(5-tert-butyl-2-benzo-oxazol-2-yl) thiophene). The isotopic compositions of all samples are reported compared to PeeDee Belemnite (PDB) as parts per thousand (‰) using the standard δ-notation. The measurement precision of the isotopic analysis was <0.1‰.

Statistical analyses

All statistical analyses were performed with R version 4.3.1 (R Core Team 2023). We used linear mixed effects models (LME) to analyse the effect of temperature treatment (ambient, +1.5 °C, and +3 °C), season (spring, summer, and autumn), and within zone elevation (continuous) on δ^{13}C and SLA of each species separately. Plot ID was used as a random effect to account for repeated measures. The baseline factor levels were set to ‘ambient’ and ‘spring’ respectively. Both the δ^{13}C and SLA were averaged per plot for each sampling date, to avoid pseudo-replication. We analysed each year separately, as we were not primarily interested in differences between years. Each model was implemented with an interaction term between temperature treatment and season and another interaction term between elevation and season. Backwards model selection (Crawley 2007) based on AIC was performed. Model assumptions were validated visually and with statistical tests if the graphs did not give clear results. If normality of the residuals and homoscedasticity was not shown, we tried log or BoxCox transformations of the response variable to meet these requirements. In all but one case, model requirements were achieved. We tried different transformations for the δ^{13}C values of *H. portulacoides* to achieve normality of the residuals, but none of the transformations improved it. Therefore, we did a sensitivity analysis, since there was only one major outlier driving the heavy-tailed distribution of the residuals. We implemented the model with and without the outlier and compared the results. To display the results of all models, we used ANOVA-like tables using the ‘Anova’ function of the car package (Fox and Weisberg 2019), with a type III error structure. Post-hoc pairwise comparisons were done by using estimated marginal means (EMM) and Tukey HSD. We used the additional R packages ggplot2 (Wickham 2016), emmeans (Lenth 2023), nlme (Pinheiro et al. 2023), MASS (Venables and Ripley 2002), car (Fox and Weisberg 2019), and patchwork (Pedersen 2023).

Results

Influence of temperature treatment, season, and elevation on $\delta^{13}C$

Spartina anglica

The δ^{13}C values of *S. anglica* were influenced by temperature treatment and season or their interaction in all three years (Table 3.2). In 2019, the δ^{13}C values became more depleted with increasing temperature. This trend was also detectable in summer and autumn 2020 and 2021 (Fig. 3.1). Post-hoc pairwise comparisons (Tukey HSD) showed a significant difference between ambient and +3 temperature treatments ($p=0.0268$) in summer 2020 with less depleted δ^{13}C values in the ambient treatments (Fig. 3.1b), this trend was also visible in autumn. However, if we looked at the influence of temperature treatment on seasonal differences (Appendix, Fig. S3.1), we found a significant difference between seasons in the ambient temperature treatment, while in the other two temperature treatments, the seasons did not differ (Appendix, Fig. S3.1). In 2021, the differences between seasons were significant in the ambient and +1.5 temperature treatment (Appendix, Fig. S3.1).

There was a significant influence of the interaction between elevation and season on δ^{13}C in 2019 (Table 3.2). The δ^{13}C values were most depleted in spring compared to summer and autumn. The seasonal trends of the influence of elevation became more negative from spring to summer, but in autumn, the influence of elevation was only marginal (Appendix, Fig. S3.2). In 2020, elevation had a significant influence on δ^{13}C as well. With increasing elevation, the δ^{13}C became more negative (Appendix, Fig. S3.3).

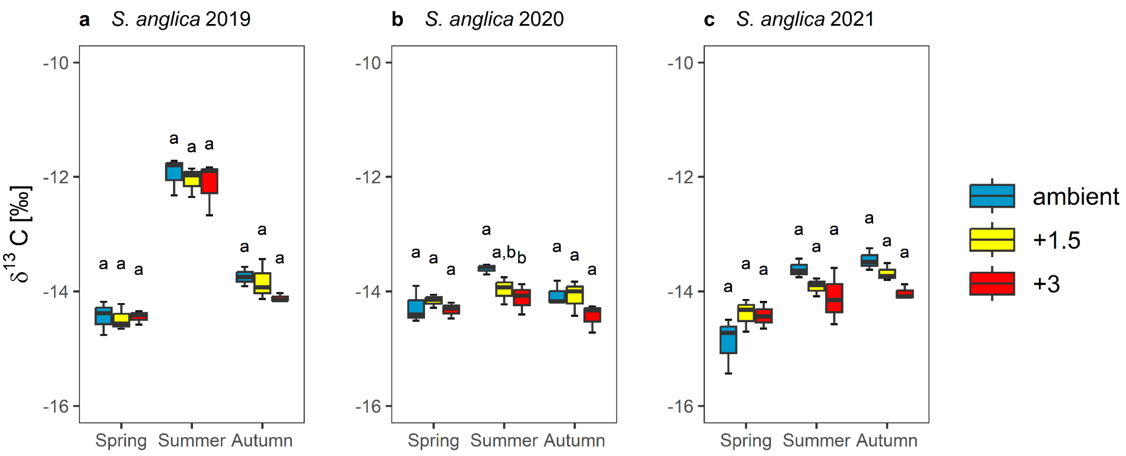

Fig. 3.1: Temperature treatment effects on δ^{13}C of *Spartina anglica* in different seasons in (**a**) 2019 (n=27), (**b**) 2020 (n=27) and (**c**) 2021 (n=27). Note that the data are plotted in original scale for a) 2019, but the statistical analyses were performed with a log (x+a) transformation. Different lowercase letters denote significant differences of post-hoc pairwise comparisons (Tukey HSD, p<0.05) between temperature treatments within one season. Boxes represent 25% and 75% quartiles from the median, whiskers represent maximum and minimum values.

Salicornia europaea

There was a significant interaction effect of temperature treatment and season for *S. europaea* in 2019 (Table 3.2). We saw significantly more depleted δ^{13}C values in summer compared to autumn in all temperature treatments, but the depletion became less pronounced with increasing temperature (ambient: -30.6‰ ± 0.4‰ vs. -26.6‰ ± 0.4‰, Tukey HSD: p=0.0002; +1.5: -30.2‰ ± 0.4‰ vs. -27.2‰ ± 0.4‰, Tukey HSD :p=0.0012 and +3: -29.3‰ ± 0.4‰ vs. -26.7‰ ± 0.4‰, Tukey HSD: p=0.0024). In 2020, season was the only factor which had a significant influence on δ^{13}C (Table 3.2). The δ^{13}C values were significantly less depleted in spring (-28.4‰ ± 0.28‰) compared to summer (-29.4‰ ± 0.28‰, Tukey HSD: p=0.0321), and also significantly lower in summer (-29.4‰ ± 0.28‰) compared to autumn (-28.2‰ ± 0.28‰, Tukey HSD: p=0.0106). In 2021, there were no significant effects of any variable on the δ^{13}C value of *S. europaea* (Table 3.2).

Halimione portulacoides

The sensitivity analysis for *H. portulacoides* in 2019 showed that there was a strong influence of one outlier (temperature treatment +1.5, -29.61‰) on the model results of δ^{13}C.

The model which included all the values (model 1 for 2019), had a significant effect of season on the δ^{13}C value, while the model excluding the outlier (model 2 for 2019), and the models in 2020 and 2021 had a significant effect of temperature treatment and season and their interaction as well (Table 3.2). In general, there was an increase in the δ^{13}C values over the course of the year, leading to the least depleted values in autumn and an additional increase due to warming treatments (Fig. 3.2). However, post-hoc pairwise comparisons revealed no significant differences between temperature treatments in any year (Fig. 3.2).

There was also a significant interaction between season and elevation in 2020. In spring and autumn, the log δ^{13}C decreased with increasing elevation (-0.014, 95% CI: -0.0604 to 0.0325; -0.0235, 95% CI: -0.0699 to 0.0230) while in summer it increased (0.0218, 95% CI: -0.0247 to 0.0682).

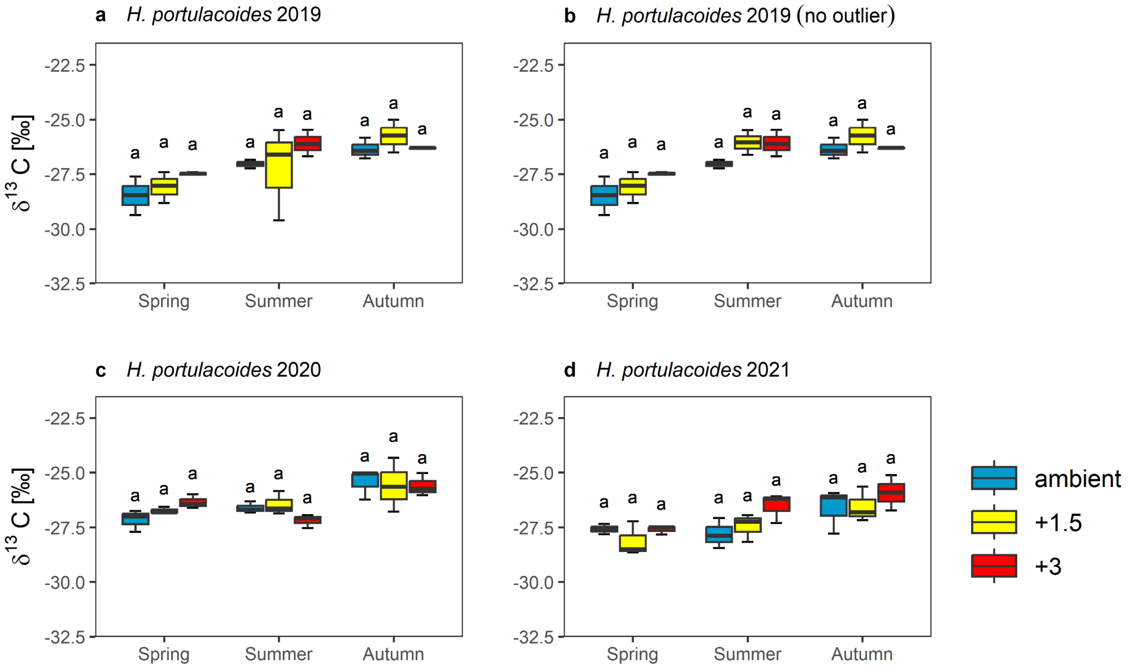

Fig. 3.2: Temperature treatment effects on δ^{13}C of *Halimione portulaciodes* in different seasons for (**a**) 2019 (n=25), (**b**) 2019 excluding one outlier (n=24), (**c**) 2020 (n=27), and (**d**) 2021 (n=26). Note that the data are plotted in original scale for c) 2020 and d) 2021, but the statistical analyses were performed with a log (x+a) transformation. Different lowercase letters denote significant differences of post-hoc pairwise comparisons (Tukey HSD, p<0.05) between temperature treatments within one season. Boxes represent 25% and 75% quartiles from the median, whiskers represent maximum and minimum values.

Table 3.2: Summary statistics of the linear mixed effect models.

		treatment			season			elevation			treatment:season			elevation:season		
	distribution	χ^2	df	p	χ^2	df	p	χ^2	df	p	χ^2	df	p	χ^2	df	p
2019																
$\delta^{13}C$																
S. anglica	*Gaussian*	14.19	2	0.00083	13.93	2	0.0009	8.27	1	0.004	-		-	9.25	2	0.0098
S. europaea	*Gaussian*	8.8	2	0.012	91.54	1	2.00E-16	-		-	6.25	2	0.044	-		-
H. portulacoides	*Gaussian*	-		-	36.29	2	1.32E-08	-		-	-		-	-		
**H. portulacoides*	*Gaussian*	6.38	2	0.041	72.1	2	<2E-16	-			12.19	4	0.016	-		-
P. maritima	*Gaussian (log)*	-		-	28.32	2	7.07E-07	5.77	1	0.02	-		-	-		-
E. athericus	*Gaussian (log)*	9.16	2	0.01	38.82	2	3.72E-09	-		-	-		-	-		-
SLA																
S. anglica	*Gaussian*	7.15	2	0.028	23.86	2	6.59E-06	-		-	14.87	4	0.0049	-		-
H. portulacoides	*Gaussian*	-		-	7.25	2	2.70E-02	0.0001	1	n.s.	-		-	6.04	2	0.049
P. maritima	*Gaussian (BoxCox)*	-		-	38.61	2	4.14E-09	9.58	1	2.00E-03	-		-	-		-
E. athericus	*Gaussian*	5.7	2	0.058**	84.24	2	2.00E-16	-		-	-		-	-		-
2020																
$\delta^{13}C$																
S. anglica	*Gaussian*	2.36	2	n.s.	29.28	2	4.38E-07	12.99	1	0.0003	10.91	4	0.028	-		-
S. europaea	*Gaussian*	-		-	14.59	2	0.0007	-		-	-		-	-		-
H. portulacoides	*Gaussian (log)*	5.51	2	n.s.	9.64	2	0.008	1.07	1	n.s.	18.34	4	0.001	9.31	2	0.001
P. maritima	*Gaussian*	0.51	2	n.s.	9.85	2	0.007	0.0006	1	n.s.	26.12	4	2.99E-05	10.27	2	0.006
E. athericus	*Gaussian*	6.16	2	0.045	6.55	2	0.038	-		-	-		-	-		-

<u>SLA</u>																
S. anglica	*Gaussian*	1.63	2	n.s.	0.59	2	n.s.	-		-	9.9	4	0.042	-		-
H. portulacoides	*Gaussian*	-		-	44.95	2	1.74E-10	-		-	-		-	-		-
P. maritima	*Gaussian (BoxCox)*	-		-	-		-	-		-	-		-	-		-
E. athericus	*Gaussian*	-		-	29.89	2	3.23E-07	3.35	1	0.067**	-		-	-		-
2021																
$\delta^{13}C$																
S. anglica	*Gaussian*	8.3	2	0.0158	71.76	2	2.62E-16	-		-	21.39	4	0.0003	-		-
S. europaea	*Gaussian*	-		-	-		-	-		-	-		-	-		-
H. portulacoides	*Gaussian (log)*	7.37	2	0.025	12.94	2	0.002	-		-	15.82	4	0.003	-		-
P. maritima	*Gaussian (log)*	-		-	191.61	2	2.20E-16	6.29	1	0.012	-		-	-		-
E. athericus	*Gaussian*	4.84	2	n.s.	5.75	2	n.s.	2.01	1	n.s.	12.58	4	0.013	6.5	2	0.039
<u>SLA</u>																
S. anglica	*Gaussian*	-		-	56.68	2	4.91E-13	-		-	-		-	-		-
H. portulacoides	*Gaussian (cube root)*	20.79	2	3.06E-05	17.13	2	0.0002	5.12	1	0.024	18.62	4	0.0009	-		-
P. maritima	*Gaussian*	-		-	-		-	-		-	-		-	-		-
E. athericus	*Gaussian*	-		-	148.08	2	2.00E-16	-		-	-		-	-		-

*Sensitivity analysis, one strong outlier with much higher residuals was removed (temperature treatment +1.5, -29.61‰). **Minimal models based on lowest AIC, included non-significant variable.

Puccinellia maritima

The log δ^{13}C value of *P. maritima* was significantly influenced by season and elevation in 2019 and 2021 (Fig. 3.3a, c; Table 3.2). In 2020, there was a significant influence of the interactions between season and elevation and season and temperature treatment on δ^{13}C (Table 3.2). With increasing elevation, there was a negative trend of log δ^{13}C in 2019 (Fig. 3.3a) and we saw a significantly higher depletion of log δ^{13}C in spring compared to summer (p=0.0178) and spring compared to autumn (p=0.0009).

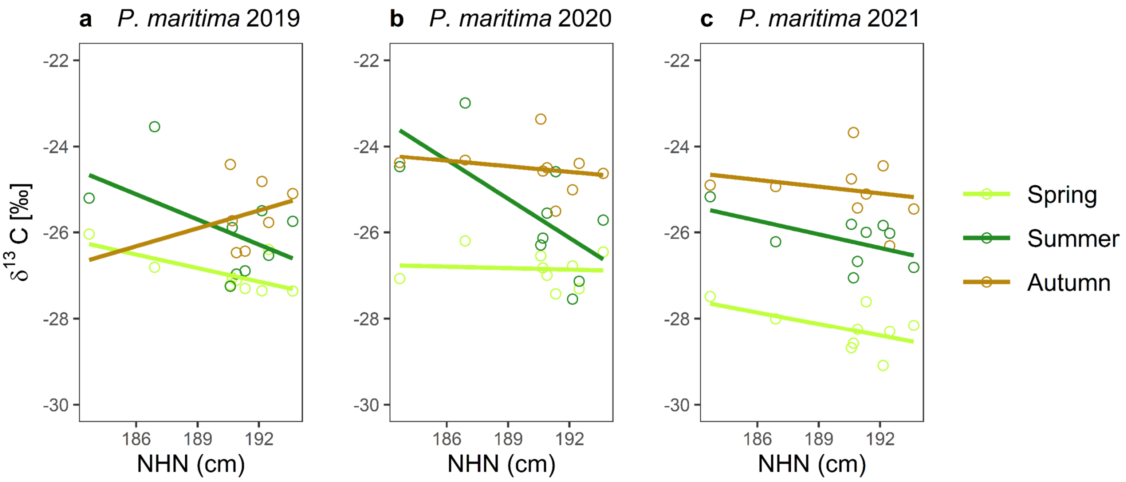

Fig. 3.3: Influence of elevation and season on δ^{13}C of *Puccinellia maritima* for (**a**) 2019 (n=25), (**b**) 2020 (n=27) and (**c**) 2021 (n=27). Elevations is expressed with reference to the German vertical datum (NHN). Note that the data are plotted in original scale, not log (x+a) transformed as the statistical analyses were performed for (a) 2019, and (c) 2021.

In 2020, we saw a significant difference in post hoc pairwise comparisons (Tukey HSD) in summer between ambient and +1.5 temperature treatment (-26.6‰ ± 0.38‰ vs. -24.5‰ ± 0.37‰, Tukey HSD: P=0.026). The differences of δ^{13}C between seasons had different patterns, depending on the temperature treatment (Table 3.3). There was a significant difference in the trends of elevation in 2020 (Fig. 3.3b). In summer, δ^{13}C strongly decreased with increasing elevation, while in autumn, δ^{13}C decreased with increasing elevation. In spring, there was a marginal positive trend. In 2021, there was a significant difference (Tukey HSD) between all seasons (Fig. 3.3c) on log δ^{13}C of *P. maritima* and log δ^{13}C slightly decreased with increasing elevation.

Table 3.3: $\delta^{13}C$ (estimated marginal means) for *Puccinellia maritima* in relation to different temperature treatment × season combinations in 2020. CL=confidence limit. SE=standard error. Df=degrees of freedom.

Treatment	Season	$\delta^{13}C$ [‰]	SE	df	lower.CL	upper.CL
ambient	Spring	-26.97	0.38	5	-27.95	-25.99
+1.5	Spring	-26.87	0.37	5	-27.81	-25.93
+3	Spring	-26.69	0.37	5	-27.64	-25.74
ambient	Summer	-26.60	0.38	5	-27.58	-25.62
+1.5	Summer	-24.50	0.37	5	-25.44	-23.56
+3	Summer	-25.71	0.37	5	-26.66	-24.75
ambient	Autumn	-24.62	0.38	5	-25.60	-23.64
+1.5	Autumn	-24.79	0.37	5	-25.73	-23.85
+3	Autumn	-24.15	0.37	5	-25.11	-23.20

Elymus athericus

The (log) $\delta^{13}C$ of *E. athericus* was mainly affected by temperature treatment and season in all years. However, in 2021 there was a significant interaction effect between both variables, and no significant main effects (Table 3.2, Fig. 3.4). In 2021, we also found a significant effect of the interaction between elevation and season on $\delta^{13}C$. Post-hoc pairwise comparisons (Tukey HSD) showed no significant difference between the temperature treatments in 2019 (Fig. 3.4a), but there was a positive trend of the $\delta^{13}C$ values from ambient to +3 in all seasons. In spring, there was not a difference detectable in summer and autumn, and there were less depleted $\delta^{13}C$ values in 2019 in the warming treatments (Fig. 3.4a; see Table 3.4 for back transformed EMM $\delta^{13}C$ values of *E. athericus*). In 2020, $\delta^{13}C$ became less negative with increasing treatment temperature in spring and summer, but those trends were not significant (Fig. 3.4b). In 2021, $\delta^{13}C$ values became significantly more depleted with increasing elevation in spring (-0.048‰, 95% CI: -1.7‰ to 0.07‰), but in summer and autumn they increased with increasing elevation (0.075‰, 95% CI: -0.07‰ to 0.22‰; 0.047‰, 95% CI: -0.07‰ to 0.17‰).

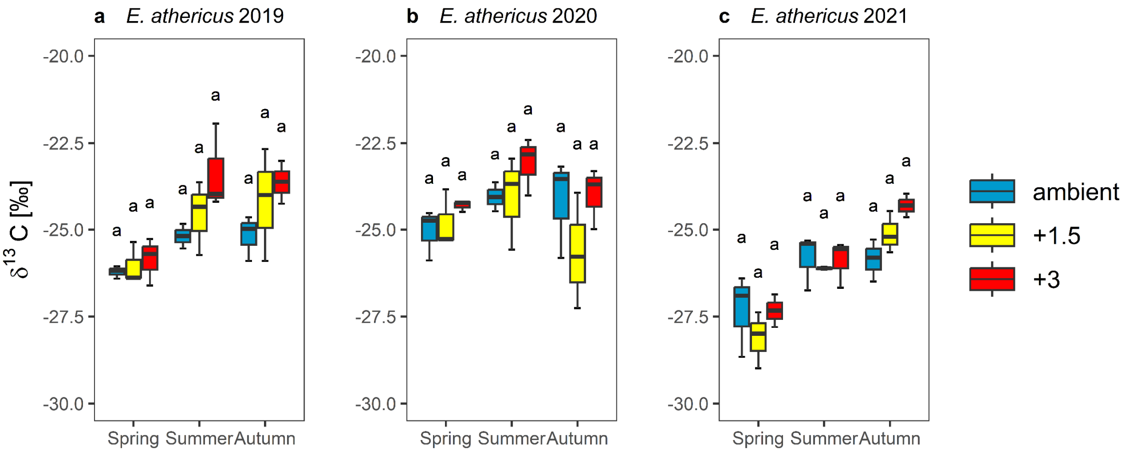

Fig. 3.4: Treatment effects on δ^{13}C of *Elymus athericus* in different seasons in (**a**) 2019 (n=27), (**b**) 2020 (n=27) and (**c**) 2021 (n=25). Note that the data are plotted in original scale for a) 2019, but the statistical analyses were performed with a log (x+a) transformation. Different lowercase letters denote significant differences of post-hoc pairwise comparisons (Tukey HSD, p<0.05) between temperature treatments within one season. Boxes represent 25% and 75% quartiles from the median, and whiskers represent maximum and minimum values.

Table 3.4: Back-transformed δ^{13}C values (estimated marginal means) of significant effects in 2019 for *E. athericus*. CL= confidence limit.

Variable	factor levels	δ^{13}C [‰]	lower CL	upper CL
Treatment	ambient	-25.56	-26.10	-24.95
	+1.5	-25.10	-25.74	-24.36
	+3	-24.47	-25.19	-23.64
Season	spring	-26.06	-26.54	-25.52
	summer	-24.47	-25.15	-23.70
	autumn	-24.42	-25.10	-23.70

Influence of temperature treatment, season, and elevation on the specific leaf area

Spartina anglica

We found seasonal influences on the SLA of *S. anglica* in all years (Table 3.2). In the ambient temperature treatment, SLA decreased from spring to autumn (Fig. 3.5a, blue boxes), with a significant difference between spring and autumn in 2019. In the warming treatments (+1.5 and +3), there was no difference between seasons (Fig. 3.5a; yellow and red boxes). In 2020, SLA showed different patterns of temperature treatment within seasons. There was a decrease in spring, an increase in summer, and no trend in autumn with increasing temperatures (Fig. 3.5b). However, there were no significant differences. The SLA values in 2021 differed significantly between spring and summer and spring and autumn (Fig. 3.5c).

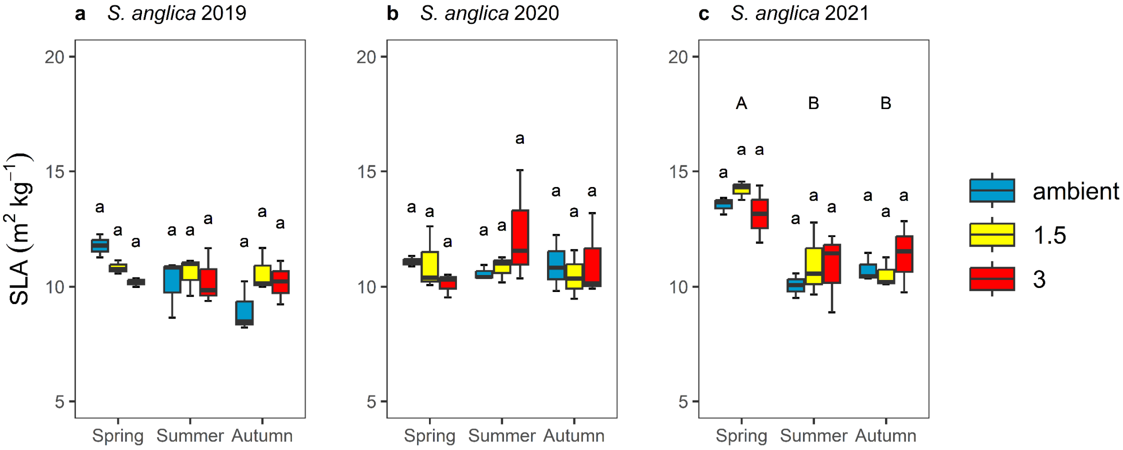

Fig. 3.5: Treatment effects on the SLA of *Spartina anglica* in different seasons (**a**) 2019 (n=27), (**b**) 2020 (n=27), and (**c**) 2021 (n=27). Different lowercase letters denote significant differences of post-hoc pairwise comparisons (Tukey HSD, $p<0.05$) between temperature treatments within one season. Different uppercase letters denote significant differences between seasons for significant main effect. Boxes represent 25% and 75% quartiles from the median, whiskers represent maximum and minimum values (excluding outliers), and circles represent outliers.

Halimione portulacoides

The SLA of *H. portulacoides* was strongly influenced by season (or an interaction with season) in all years (Table 3.2). In 2019, SLA slightly decreased with increasing elevation in spring (-0.06, 95 % CI: -0.39 to 0.28), but increased with increasing elevation in summer (0.24, 95% CI: -0.09 to 0.58) and autumn (0.013 95% CI: -0.20 to 0.47). In 2020, SLA was higher in spring (8.9 $m^2 \cdot kg^{-1}$± 0.38 $m^2 \cdot kg^{-1}$) and summer (9.9 $m^2 \cdot kg^{-1}$±0.38 $m^2 \cdot kg^{-1}$) than in autumn (6.5 $m^2 \cdot kg^{-1}$± 0.38 $m^2 \cdot kg^{-1}$). In 2021, SLA had different seasonal patterns within temperature treatments. SLA decreased with warming in spring, decreased in summer, and all temperature treatments were comparable but there was a slight increase in the +1.5 °C treatment in autumn (Appendix, Fig. S3.4).

Puccinellia maritima

In 2019, we saw a significant influence of season and elevation on the BoxCox transformed SLA values of *P. maritima*. With increasing elevation, SLA increased ($p=0.0002$) and there was a significant decrease from spring to summer and autumn (Fig. 3.6). In the other years, we found no influence of any factors on SLA (Table 3.2).

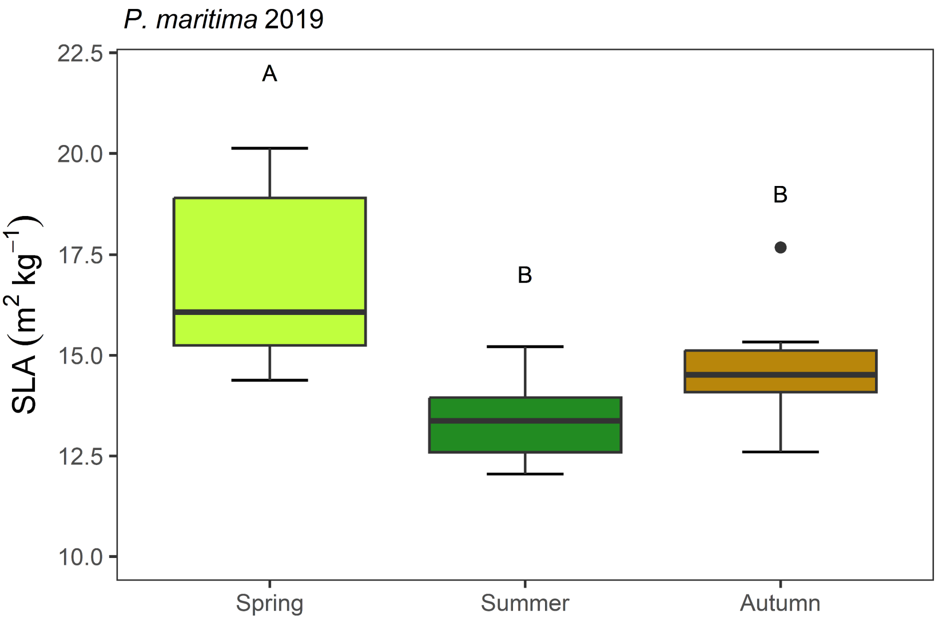

Fig. 3.6: Seasonal differences between the SLA (specific leaf area) of *Puccinellia maritima* in 2019. ($n=27$). Different uppercase letters denote significant differences of post-hoc pairwise comparisons (Tukey HSD, $p<0.05$) between seasons. Response variable was BoxCox transformed, but data are plotted in original scale. Boxes represent 25% and 75% quartiles from the median, whiskers represent maximum and minimum values (excluding outliers), and circles represent outliers.

Elymus athericus

The SLA of *E. athericus* was influenced by season in all years (Table 3.2). The SLA decreased from spring to summer and autumn in 2019 (Fig. 3.7a). The ambient temperature treatment had slightly higher SLA values than both warming treatments (12.2 $m^2 \cdot kg^{-1}$ ± 0.32 $m^2 \cdot kg^{-1}$ vs. 11.4 $m^2 \cdot kg^{-1}$ ± 0.32 $m^2 \cdot kg^{-1}$, respectively). In 2020, SLA was highest in spring and lowest in summer, while in autumn it increased again (Fig. 3.7b). In 2021, all seasons differed significantly from each other (Fig. 3.7c). The value was highest in spring, lowest in summer, and slightly higher than summer in autumn.

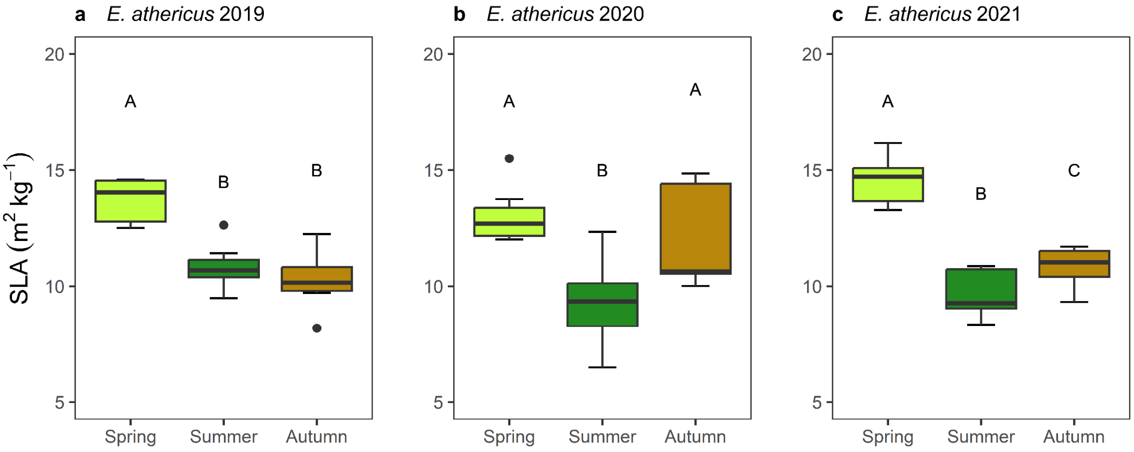

Fig. 3.7: Seasonal changes of SLA (specific leaf area) of *Elymus athericus* in (**a**) 2019 (n=27), (**b**) 2020 (n=27), and (**c**) 2021 (n=27). Different uppercase letters denote significant differences of post-hoc pairwise comparisons (Tukey HSD, $p<0.05$) between seasons. Boxes represent 25% and 75% quartiles from the median, whiskers represent maximum and minimum values (excluding outliers), and circles represent outliers.

Discussion

With the present study, we assessed ecophysiological responses of European salt marsh species under experimental warming in different seasons along an elevational gradient. In contrast to our first hypothesis, we observed no unilateral positive response of $\delta^{13}C$ and no unilateral negative response of SLA to warming treatments. However, in some species we saw a clear effect of warming on $\delta^{13}C$. In the following sections, we will discuss the effects of warming on both ecophysiological traits with respect to zone, elevation, season, and climatic differences between years.

Influence of salt marsh zone on ecophysiological responses to warming

The two grasses from the pioneer zone and the high marsh were most affected by the warming treatments. $\delta^{13}C$ of *S. anglica* and *E. athericus* was significantly influenced by temperature treatment (or an interaction with temperature treatment) in all three years. Additionally, the low marsh species *H. portulacoides* was also significantly affected by the interaction of warming and season. Therefore, our second hypothesis, that the warming effect is most pronounced in the high marsh, could not be confirmed. The less negative $\delta^{13}C$ values suggest that WUE of *E. athericus* increased with experimental warming. We saw the same trend for *H. portulacoides* in the low marsh. This indicates that the marsh species at higher elevations increase their WUE under warming. However, the WUE of the succulent pioneer species *S. europaea* was higher in ambient plots in autumn than in +1.5 and + 3, which is in line with the results of Gedan and Bertness (2009), who found C3 plants under permanent waterlogging (no drought stress) showed a reduced WUE under warming due to increased transpiration. In contrast to the unilateral response of C3 plants to drought, the $\delta^{13}C$ values of C4 plants can increase, decrease, or stay constant, depending on the leakiness of the bundle sheath cells (Cernusak et al. 2013). Thus, the interpretation of the warming effect on *S. anglica* is more difficult. We observed the same trend of more negative $\delta^{13}C$ values in the warmed plots, which would also support the reduced WUE of salt marsh species under warming at waterlogged conditions (Gedan and Bertness 2009). However, in a study by Ghannoum et al. (2002), drought stress led to decreased $\delta^{13}C$ values in C4 grasses, which is the opposite direction as for C3 plants. This was related to the control of water loss in relation to carbon gain on the leaf level (Ghannoum et al. 2002). Therefore, we could also argue that there was at least a trend of increased WUE in *S. anglica* under experimental warming, especially during summer and autumn (Fig. 3.1). Another explanation could be that the change of $\delta^{13}C$ due to experimental warming in *S. anglica* may arise from direct physiological responses at the plant organ level (like changing compound allocation; Sage and Kubien 2007; von Caemmerer et al. 2014), rather than indirect environmental induced changes due to warming. Furthermore, increasing temperatures may increase salt stress during the non-flooded periods in the pioneer zone, by enhancing evapotranspiration of water on the soil surface. Salt stress, like drought stress, may reduce the stomatal conductance in leaves (Munns 2011), which would result in the similar effect for the CO_2 discrimination. Salt marsh plants experiencing increased salt stress often invest into N-rich osmoprotectants (Flowers and Colmer 2008), which would be reflected in higher N concentrations in the leaves. For *S. anglica*, we saw a decreasing trend in C/N (not statistically analysed) with warming in summer and autumn in 2020 and 2021 (Appendix, Fig. S3.5), which supports the conclusion that even regularly inundated species seem to experience increased drought/salt stress due to experimental warming.

Seasonal change of ecophysiological responses to warming

In line with hypothesis three, the general trend of increasing WUE of the low marsh and high marsh species over the course of the vegetation period might arise from the continuous desiccation of the marsh soils during summer. This is most pronounced in the higher parts of the salt marsh, due to low flooding frequencies in the summer months. This was especially important in the high marsh, where the effect of warming was less pronounced in spring, which indicates that there is an additive effect of continuing desiccation and higher air temperatures (Charles and Dukes 2009). During summer and autumn, *E. athericus* showed increased WUEs which were even higher under warming. In case of *H. portulacoides*, we found a strong seasonal effect resulting in an increase of the WUE over the course of the entire vegetation period and an additional increase in WUE due to warming.

However, we found the strongest differences between seasons in the pioneer zone in 2019, with a difference of ~2 ‰ in $\delta^{13}C$ of *S. anglica* between spring and summer. This indicates that this impact was either pioneer zone or species specific. Unfortunately, we did not sample the other pioneer species (*S. europaea*) in spring 2019, thus we cannot compare both species in their seasonal response between spring and summer. If we compare the effect of season on $\delta^{13}C$ in *S. anglica* to the other years, this effect is also unexpected and might be due to climatic differences between years (see below). Changing isotopic discriminations between seasons may also be an indicator for a change in resource allocation throughout the year. These differences can be detected through $\delta^{13}C$ signatures of different compounds compared to the bulk leaf signature in both C3 and C4 plants. Typically, lipids, proteins, lignin, and organic acids are depleted compared to bulk $\delta^{13}C$, while starch, sucrose and cellulose are isotopically enriched (Hobbie and Werner 2004; Tcherkez et al. 2011; von Caemmerer et al. 2014). The results for the SLA support that there was a change in resource allocation, since the SLA of *E. athericus, H. portulacoides, and P. maritima* showed a significant decrease between spring and summer/autumn in 2019 and in *E. athericus* and *H. portulacoides* also in 2021. Lower SLA means higher tissue densities, which may for example be achieved by lignification of cell walls, because of physiological water deficits due to osmotic stress

(Rozema et al. 1985; Reents et al. 2022) or due to drought stress, which in the higher parts of the marsh increases during the vegetation period.

Variation in elevation gradient as a mediator for seasonal change

The variation in elevation within a zone was an important factor for $\delta^{13}C$ of the grass species of the pioneer zone and low marsh. Our fourth hypothesis could be partly confirmed, since the $\delta^{13}C$ of *P. maritima* and *S. anglica* decreased with increasing elevation in two of the study years. We assume the opposite direction of the elevation effect in autumn 2019 was due to missing values at lower elevations in this year (Fig. 3.3a). We would not expect a decrease of the $\delta^{13}C$ values with increasing elevation if the response was only dependent on soil moisture. We conclude that soil properties like salt content and decreasing redox potential with decreasing elevation (Davy et al. 2011) may also have an influence on the $\delta^{13}C$ values of *P. maritima* and *S. anglica*. For example, Guy et al. (1986) found that increasing salinity led to increasing $\delta^{13}C$ values in *Puccinellia*. Typically, the salinity decreases with increasing elevation in Wadden Sea salt marshes (Bockelmann and Neuhaus 1999; Suchrow and Jensen 2010). Our results show increasing $\delta^{13}C$ with decreasing elevation in salt marsh plants. This indicates that the WUE increases with salt stress. However, since we did not measure soil salinity, we cannot make a clear statement. In 2019, we also saw decreasing SLAs with decreasing elevation, which was also found for increasing salinity for the SLA of *P. maritima* (Lenssen et al. 1995). Our results indicate that *P. maritima* has a high plasticity and may adjust traits like SLA and WUE according to environmental stressors.

Climatic impacts on ecophysiological responses

Over the course of our three-year study period, we found a couple of interesting interannual differences in the results, which we were not able to explain with the analyzed factors. We assume that the annual climate variability seems to have an impact on the effect of warming and seasonality on $\delta^{13}C$. An increasing WUE due to seasonal differences of plants can be a result of both temperature and water availability (Zhang et al. 2022), which may differ due to precipitation patterns and inundation frequencies throughout the year and between years. For example, we could not explain the extreme difference between the $\delta^{13}C$ values of *S. anglica* in 2019, by seasonal or warming effects. We saw seasonal changes in all years, but the difference of ~2 ‰ between spring and summer was unique. The study years differed in their precipitation pattern and had the highest air temperatures at different periods during summer (Appendix, Fig. S3.6). 2019 was the year with the lowest average precipitation during the vegetation period. Low precipitation may have increased salt stress, however, positive changes in salinity levels usually last only shortly after a rainfall event in inundated marsh zones (Noe and Zedler 2001). Nevertheless, chronic salt stress (due to continuously low precipitation amounts) may have a strong effect on the bundle sheath leakiness (Farquhar et al. 1989; von Caemmerer et al. 2014), which can result in this detectable influence on the $\delta^{13}C$ values (Bowman et al. 1989). Since the C/N of *S. anglica* was lower in summer 2019 (Appendix, Fig. S3.5) compared to the other summers, this supports the possible effect of salt stress and the investment into N-rich osmoprotectants (Flowers and Colmer 2008), which would be reflected in higher N concentrations in the leaves.

E. athericus had much lower $\delta^{13}C$ values in spring (and the other seasons) in 2021 compared to other years. The possible explanation for this observation is higher soil water potentials due to precipitation and flooding. Firstly, there was a flooding event in the high marsh in the same week as the sampling campaign (Appendix, Fig. S3.7), while in the other two years, the high marsh was not flooded from March to July (2020) and from March to September (2019), respectively. Secondly, there was also a high amount of precipitation in May 2021 (Appendix, Fig. S3.6). The low WUEs in spring 2021 are probably a direct result of the soil moisture conditions after these flooding and precipitation events.

Methodological considerations

The change of the sample processing (using aliquots of the leaf in 2019 vs. powder of the ground leaf in 2020/2021) may be somewhat reflected in our results. However, since we found also major differences between the two latter years in terms of climatic impacts and not an overall pattern between 2019 vs. 2020/2021, we think the slightly differing methodology did not have a great effect on the results. It may be worth noting that most of the low marsh plots are scattered around an elevation of 190 cm (NHN, German vertical datum), but two plots are positioned lower (10-15 cm). It is very likely that those plots drove the significance we observed for elevation.

Conclusions

Our study gives novel insights on possible ecophysiological adjustments of common European salt marsh species under experimental warming. While the warming effect was not universal across all species and zones, our findings demonstrate that salt marsh species are affected by rising temperatures on a physiological level. Especially species at higher elevations such as *Elymus athericus* or *Halimione portulacoides* with lower flooding frequencies are prone to drought stress. However, under the applied warming conditions, these drought-stressed species adjust their WUE accordingly. The effect of warming on the pioneer zone species was more striking and more difficult to interpret. Under frequent inundation, temperature change may lead to direct physiological alterations of the plant organ as well as mediate indirect processes like enhanced transpiration and a resulting decrease in WUE. This study demonstrated that the WUE of salt marsh plants may be influenced by experimental warming depending on the location within the marsh. With increasing elevation, the influence of drought stress became more apparent, but climatic influences like precipitation or storm floods may ameliorate the effects of warming-induced drought stress. It was also shown that some species are more affected than others, which leads to the conclusion that warming-induced ecophysiological responses are an important aspect to consider for interspecific competition and future salt marsh plant community development. Future studies should focus on disentangling the influence of salinity, nutrients, redox and water content of salt marsh soils and include elevated CO_2 levels, because the influence of warming and elevated CO_2 is especially important for the performance of and competition between C3 and C4 plants (Noyce et al. 2019; Smith et al. 2022).

Acknowledgments

We would like to thank Mónica Salazar Ortiz for conducting the measurements during E.O.´s parental leave. Thank you to Sebastian Wilfinger, Ella Logemann, Niclas Krauss, Beke Eichert and Thomas Ostertag for technical assistance. Furthermore, we thank Christoph Reisdorff and Peter Mueller for helpful advice regarding the measurements. We thank Heather Shupe for proofreading. We also thank all project partners of MERIT and the technical team. A special thanks to Roy Rich for his knowledge and expertise in building the MERIT experiment.

Appendix

1. Warming related trends of δ^{13}C in salt marsh species

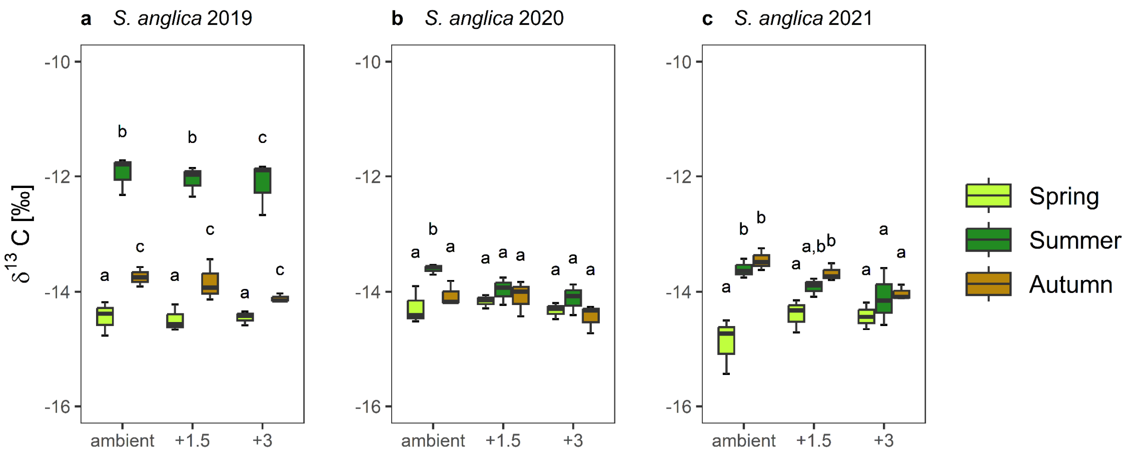

Fig. S3.1: Seasonal effects on δ^{13}C in the different warming treatments for *Spartina anglica* in (**a**) 2019 (n=27), (**b**) 2020 (n=27) and (**c**) 2021 (n=27). Different lowercase letters denote significant differences of post-hoc pairwise comparisons (Tukey HSD, p<0.05) between seasons within one treatment. Different uppercase letters denote significant differences of post-hoc pairwise comparisons (Tukey HSD, p<0.05) between treatments. Boxes represent 25% and 75% quartiles from the median, whiskers represent maximum and minimum values.

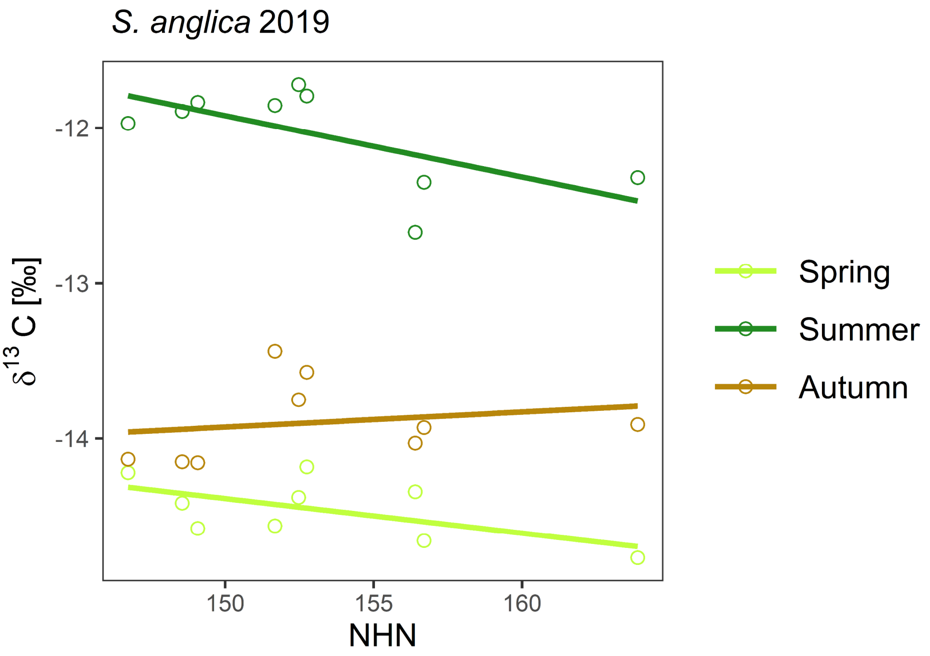

Fig. S3.2: Elevation × treatment influence on δ^{13}C of *Spartina anglica* in 2019 (n=27).

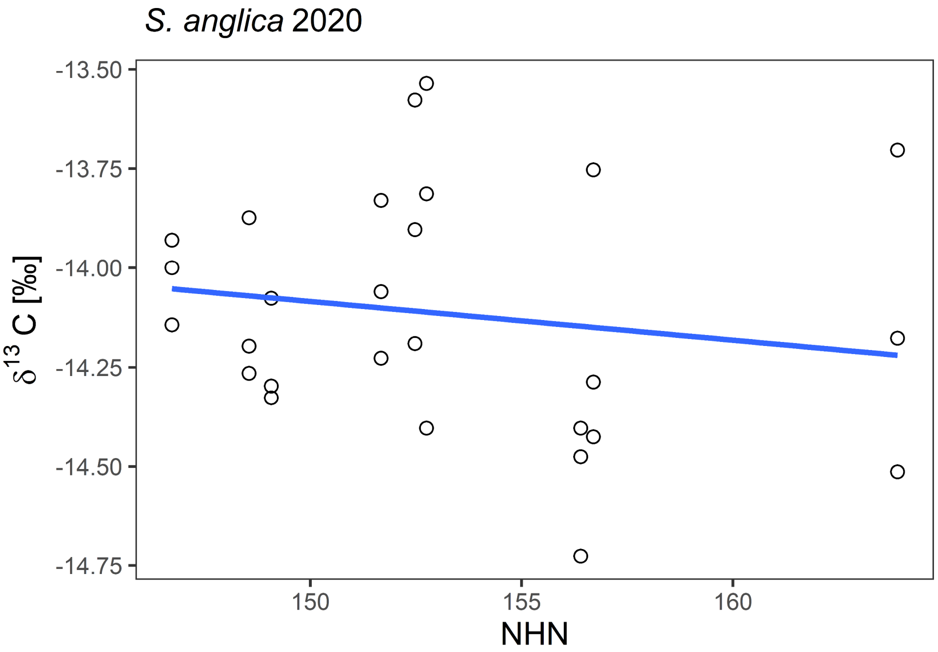

Fig. S3.3: Elevation influence on $\delta^{13}C$ of *Spartina anglica* in 2020 (n=27).

Table S3.1: Contrasts table of season within treatments (Tukey HSD, $p<0.05$) for $\delta^{13}C$ of *Halimione portulacoides* in 2019 excluding one outlier (treatment +1.5, -29.61‰).

contrast	treatment	estimate	SE	df	t ratio	p
Spring - Summer	ambient	-1.44	0.323	9	-4.47	0.004
Spring - Autumn	ambient	-2.12	0.323	9	-6.57	<0.001
Summer - Autumn	ambient	-0.68	0.323	9	-2.10	0.144
Spring - Summer	+1.5	-1.76	0.375	9	-4.68	0.003
Spring - Autumn	+1.5	-2.29	0.375	9	-6.09	<0.001
Summer - Autumn	+1.5	-0.53	0.438	9	-1.20	0.481
Spring - Summer	+3	-1.38	0.323	9	-4.26	0.005
Spring - Autumn	+3	-1.05	0.374	9	-2.80	0.050
Summer - Autumn	+3	0.33	0.374	9	0.88	0.664

2. Warming related trends of SLA in salt marsh species

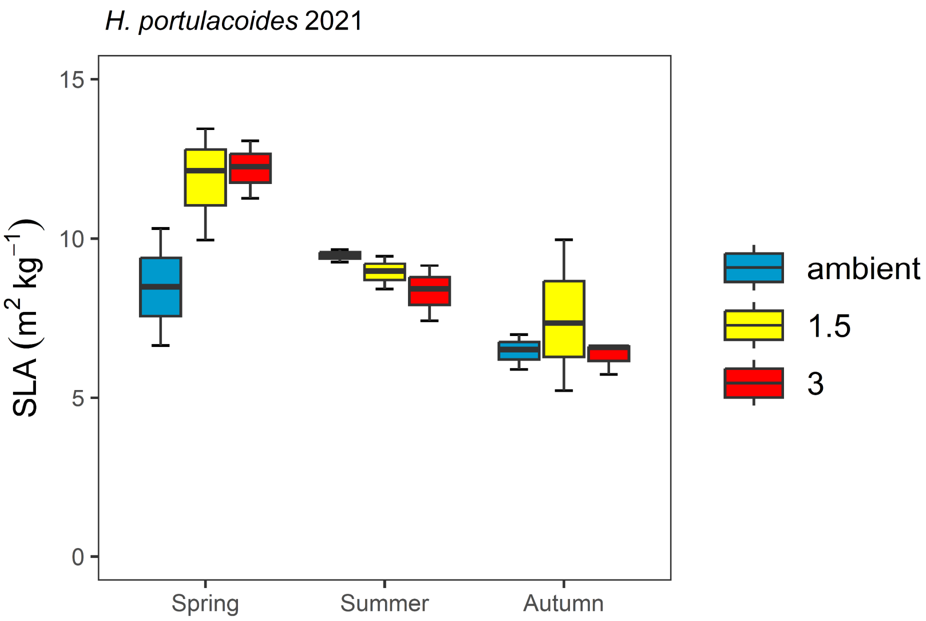

Fig. S3.4: Seasonal change of temperature treatment effect on SLA of *Halimione portulacoides* in 2021. Boxes represent 25% and 75% quartiles from the median, whiskers represent maximum and minimum values.

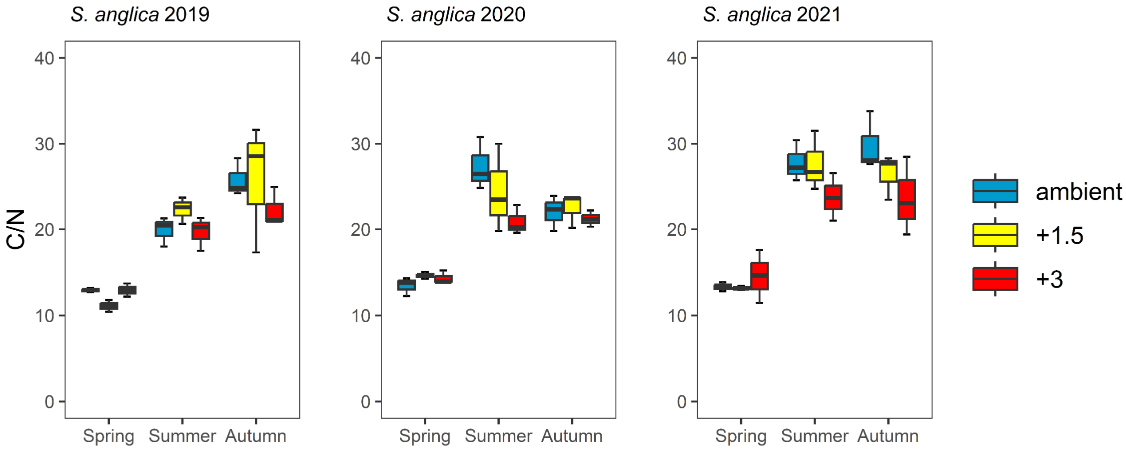

Fig. S3.5: Leaf carbon:nitrogen (C/N) ratio of *Spartina anglica* in different temperature treatments for 2019-2021. Boxes represent 25% and 75% quartiles from the median, whiskers represent maximum and minimum values.

3. Climatic differences between study years

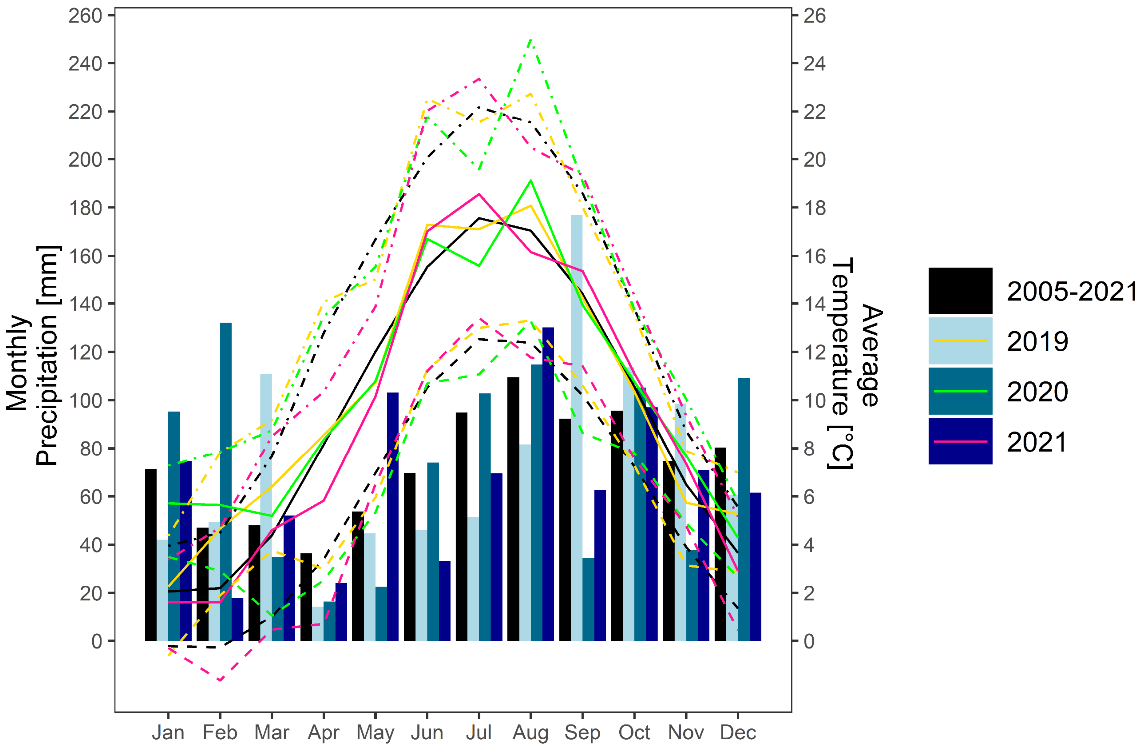

Fig. S3.6: Climate differences between study years (2019-2021) for Hattstedt (15 km away). The mean of 2005 to 2021 is indicated in black as a reference level. Monthly average temperatures are indicated by solid lines. Average minimum and maximum temperatures are indicated by dashed and dash-dotted lines respectively. Bars represent monthly cumulative precipitation. Data was provided by the German weather service (Deutscher Wetterdienst, DWD).

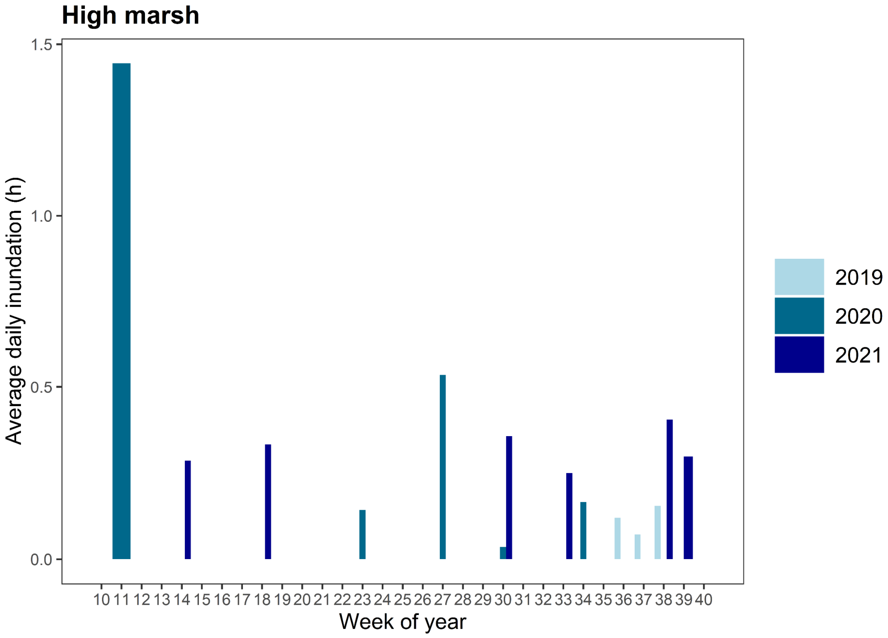

Fig. S3.7: Average daily inundation of the high marsh over the course of the vegetation period. Values are averaged over one week (week of year). Week 10 starts on March 4th, 2nd, 8th in 2019, 2020 and 2021, respectively.

4

Warming experiment indicates that increasing global temperatures may not affect windows of opportunity for salt marsh seedlings

Abstract

With increasing global temperatures, it remains unclear, how seedling emergence and survival will be affected in highly dynamic ecosystems like salt marshes. Our study combines passive and active warming treatments with the natural inundation dynamics of salt marshes. We studied the effects of active soil and passive air warming (ambient temperature, + 1.5 °C and + 3.0 °C) on seedling numbers of different species in three salt marsh zones (pioneer zone, low marsh, and high marsh) and on seedling survival (lifespan), seedling diversity and species richness in two salt marsh zones (low marsh and high marsh) in situ. We found a significant influence of warming on Shannon diversity at two dates, but we found no significant effects of warming on seedling number and survival. However, we found trends, which differed between zones. In the pioneer zone, seedling numbers were slightly higher in the warming treatments than in the ambient treatment from April to June. Whereas, in the high marsh, seedling numbers decreased more in the warming treatments during the same period. The median lifespan was slightly reduced under warming treatments. We conclude warming might have increased drought stress for seedlings in the high marsh, which led to the trend of lower seedling numbers and shorter survival times. Seedling number decreased with elevation, which could be attributed to both species specific strategies and differences in available space. Median survival differed significantly between species, which we assume is due to differing life-history traits like seed size and if the species are annual or perennial.

Introduction

Salt marshes play an important role for climate change mitigation and adaptation. They protect the coastline against storms (Shepard et al. 2011; Möller et al. 2014) and sequester large amounts of carbon (Mcleod et al. 2011). With increasing mean annual temperatures, it is important to understand how salt marshes and the ecosystem services they provide will be affected. While it is known that warming can increase productivity and growth of salt marsh species (Charles and Dukes 2009; Gedan and Bertness 2010; Coldren et al. 2016) and lead to a loss of biodiversity (Gedan and Bertness 2009), the influence of warming on seedling establishment and survival in salt marshes is understudied.

In temperate salt marshes, seedling establishment in regularly flooded low elevations is strongly influenced by stressors like sediment disturbance and sediment dynamics during flooding (Balke et al. 2013; Cao et al. 2018), and drought in higher-elevated, less frequently flooded marsh zones (Uyeda et al. 2019; van Regteren et al. 2020). The absence or low magnitude of the mentioned stressors and other unfavorable conditions, like competition or unsuitable substrates, provide a “window of opportunity” for seedling establishment and recruitment (Jelinski and Cheliak 1992; Eriksson and Fröborg 1996; Balke et al. 2011). While windows of opportunity for recruitment can be temporally and spatially unpredictable (Eriksson and Fröborg 1996), the conditions needed for such windows to open or close can be predicted based on both the intrinsic timescales of the seedling and the variability of the stressor (see van Belzen et al. 2022). For dynamic coastal ecosystems, Balke et al. (2011) defined disturbance-free periods of a defined minimum duration as “windows of opportunity” for seedling establishment on regularly flooded bare patches (see also Hu et al. 2015; Bouma et al. 2016). For seedlings to become anchored, they need time without flooding (2–3 d) to develop a minimal root length (Balke et al. 2011). A time with low magnitude of disturbance (bed shear stress), needs to follow to allow seedlings to withstand sediment dynamics like resuspension/erosion in regularly flooded salt marshes (Hu et al. 2015). The magnitude of physical disturbance on seedlings depends on salt marsh elevation as it defines the flooding frequency and duration (Noe and Zedler 2000; Silvestri et al. 2005). With increasing elevation and less flooding, physical disturbance becomes less severe. Here, especially at the highest elevation with no flooding during summer, soil moisture is a critical environmental factor influencing the windows of opportunity for seedling recruitment (Noe and Zedler 2001; Uyeda et al. 2019; van Regteren et al. 2020). In semi-arid grasslands, it has been shown that the window of opportunity for seedling survival opens when high moisture levels are present in the shallow soil layers during and after germination and when competition with adult grasses is reduced (de Dios et al. 2012). Conditions for seedling establishment in the high elevated salt marshes of the Wadden Sea are very similar to those conditions due to low soil moisture levels during the summer in combination with competition by the perennial grass *Elymus athericus* dominating the vegetation (Bockelmann and Neuhaus 1999). Overall, the windows of opportunity for seedling establishment in salt marshes are thus mainly defined by a time without or with little disturbances by flooding and bed sheer stress at the low end, but by a time without drought and/or competition at the high end of the elevation gradient. As different species inhabit different elevations (Silvestri et al. 2005; Esselink et al. 2017), seed availability differs between zones (Bakker et al. 1985; Rand 2000). Establishment is therefore also affected by life-history strategies in combination with open space (Bakker et al. 1985; Bakker and de Vries 1992; Lõhmus et al. 2020), distance to seed source (Rand 2000; Kaminsky et al. 2015), and dispersal patterns (Bakker et al. 1985; Rand 2000).

Another abiotic factor that is especially relevant for the initiation of germination in spring is temperature. A handful of greenhouse studies have examined the effect of temperature as a factor on germination of salt marsh species (e.g., Ungar 1977; Egan and Ungar 1999; Noe and Zedler 2000). Germination rates of salt marsh species depend on temperature regimes (Egan and Ungar 1999; Noe and Zedler 2000), meaning that both day and night temperatures affect successful germination. The results of Egan and Ungar (1999) show how temperature optima play an important role in germination processes. As these temperature requirements may be species specific, warming could alter plant community structure. For example, some marsh species (*Salicornia europaea* and *Phragmites australis*) had increased germination rates with warming, others (*Spartina patens*) saw a reduction in germination rate (Ungar 1977; Martin 2017). Furthermore, warming can rapidly reduce species diversity and richness in salt marshes (Gedan and Bertness 2009), which might already be detectable on the seedling level. Increased salinity (a potential side effect of warming) had a negative effect on Shannon diversity and abundance of salt marsh seedlings (Kottler and Gedan 2020). It also remains unclear how seedling survival in salt marshes will be affected by increased temperatures. It has been shown that in-situ seedling survival in mangroves was positively affected by elevated temperatures, that is, in more Southern latitudes (Pickens et al. 2019), but in a warming experiment mangrove seedling survival was not affected by warming (Coldren et al. 2016). All of the mentioned studies on warming effects on germination of salt marsh species were conducted in controlled environments and thus lack the combination of stressors (i.e., flooding frequency, sediment dynamics, low soil moisture, and competition) that may affect seedling survival in situ. Hence, to improve our understanding of the salt marsh ecosystem with regards to global warming it is important to understand how germination and seedling survival is affected by warmer temperatures in combination with other natural processes.

To analyze the interplay of warming with the natural dynamics along an elevational gradient in salt marshes, in-situ studies are necessary. Previous in-situ warming experiments in salt marshes used passive aboveground warming to study effects on plant community assembly (Gedan and Bertness 2009, 2010), biomass allocation (Gedan and Bertness 2010) and growth (Coldren et al. 2016). However, these passive open top chambers do not warm the salt marsh soil effectively in contrast to the air (Carey et al. 2018), and warming the soil is necessary for creating a soil temperature profile that would have ecological significance. We therefore established the experiment MERIT ("Marsh Ecosystem Response to Increased Temperature"), which combines active belowground heating (via electrical heating pins and cables) and passive aboveground warming (domes covered with foil) while allowing for all other processes like tidal inundation to be unchanged. We monitored the number of seedlings at specific time steps in three salt marsh zones along the elevation gradient (pioneer zone, low marsh, high marsh) under three different temperature scenarios (ambient temperature, + 1.5 °C, and + 3.0 °C) during spring and summer 2019. From the data, we calculated the proportion of survived seedlings at the end of the study (based on maximum seedling numbers per plot). Additionally, we monitored emergence and survival (lifespan) of individual seedlings in the low marsh and high marsh under the three different temperature scenarios. From this dataset we also calculated the species richness and Shannon diversity of the seedlings. We hypothesized that (1) the effect of warming on seedling number and seedling survival (recruitment) is zone specific. We expected warming to increase seedling number and proportion of surviving seedlings in the pioneer zone because the germination rate of the pioneer species *S. europaea* was positively affected by warming (Ungar 1977). In the low marsh and high marsh, we expected seedling number and surviving seedlings as well as lifespan to decrease under warming due to increasing drought stress. We also hypothesized that (2) warming leads to a decrease in Shannon diversity and species richness. Furthermore, we hypothesized (3) seedling number, diversity and richness and seedling survival to be zone specific. We expected a decreasing number of seedlings from pioneer zone to high marsh due to differences in life-history and dispersal strategies. Whereas we expected the highest species richness and diversity in the low marsh. Due to a less dense canopy structure hence a higher light availability, we expected a higher seedling survival in the low marsh than in the high marsh. In addition, we hypothesized (4) seedling number and survival (lifespan) to be species specific. We expected a higher seedling number of annual species but a higher survival of perennial species.

Methods

Study area and experimental design

The “Marsh Ecosystem Response to Increased Temperature” (MERIT) experiment was set up in 2018 on the Hamburger Hallig in the German Wadden Sea (54°35′58″N, 8°49′8″E). The site is part of the Schleswig-Holstein Wadden Sea National Park and listed by UNESCO as a World Heritage site. The tidal range at the site is ~ 3.0 m (Stock 2011). The mean annual temperature between 1989 and 2019 was 9.54 °C and the mean annual precipitation was 862.3 mm [data from the German Meteorological Service (DWD, https://opendata.dwd.de/climate_environment/CDC/observations_germany/climate/daily/kl/historical/) last accessed 20 October 2022 for Sankt Peter-Ording, which is 37 km away from the study site]. The distinct marsh zones are defined by their elevation and flooding regime and are vegetated by the typical plant communities of the Wadden Sea salt marshes (Esselink et al. 2017). The pioneer zone is dominated by *Spartina anglica* Hubb. with high frequencies of *S. europaea* agg. (L.). The low marsh is vegetated by a mix of 15–20 halophytes, with higher abundances of *Halimione portulacoides* (L.) Aell., *Limonium vulgare* Mill., and *Puccinellia maritima* (Huts.) Parl. The high marsh is dominated by *E. athericus* (Lk) Kerg. Species names follow Oberdorfer (2001).

In each of the three zones, nine plots were set up leading to 27 plots in total. Each plot has a diameter of 3.1 m. There are three temperature treatment levels, namely + 1.5 °C, + 3.0 °C, and control (ambient temperature), hereafter referred to as ambient, + 1.5, and + 3 treatment, respectively. Treatments are replicated three times per zone (n = 3). Aboveground, the warming treatment is passive via domes covered with different amounts of plastic foil, which results in warming efficiency similar to other open top chambers (R. Rich et al. unpubl.). Belowground, an active warming approach is achieved by a combination of vertical resistance pins, reaching 1 m deep into the ground, and horizontally deployed resistance cables on the soil surface. The belowground warming treatments are monitored and regulated by microprocessor-based feedback control. The soil warming is generally switched on from March to September and switched off in autumn/winter to protect the sensors and heating system from heavy storm tides. Domes for aboveground warming are covered with foil from

March to September. More details on the experimental design can be found in Tang et al. (2023) and will be described by R. Rich et al. (unpubl.). In 2019, soil warming was switched-on on March 14th, treatment levels were overall reached on March 22nd after some initial technical issues. Foils were installed at the domes between February 28th and March 5th. Due to storm damage, the foils were removed from March 19th to April 17th in the pioneer zone.

Seedling monitoring

To assess seedling emergence and survival in each of the 27 plots, we marked a 10 × 10 cm^2 subplot in each plot with wooden sticks on March 7, 2019. In the pioneer zone and high marsh, the subplots were placed at the same position in each plot, because the vegetation composition is very homogenous. In the low marsh, the subplots were chosen based on similar vegetation structure to assure comparability. Within these subplots, all seedlings visible to the bare eye were recorded weekly from mid-March (8 d prior to the onset of the belowground warming treatments) to June and biweekly from June until the end of September 2019. Individuals in the low and high marsh were marked with numbered toothpicks as soon as they emerged. Seedlings of the pioneer zone were only counted and not marked individually, due to the high numbers of *S. europaea* agg. individuals. Besides marked single *S. europaea* agg. individuals, there were also *S. europaea* agg. cohorts in the low marsh in one ambient (one *S. europaea* agg. cohort) plot and in one + 3 treatment plot (two *S. europaea* agg. cohorts), which were not individually marked due to their high numbers. The cohort individual numbers were considered in the models with total seedling numbers per plot, but not in the survival analyses. Species were in general identified as soon as detectable. However, some of these species are hard to distinguish during early development, therefore we combined them into taxa (*Spergularia marina* and *Spergularia media* = *Spergularia* sp., *Atriplex prostrata*, and *Atriplex littoralis* = *Atriplex* sp.).

Statistical analyses

Warming effects on seedling numbers over the course of the vegetation period

To assess the influence of warming on seedling numbers during the experiment, we chose dates of ecological interest and performed generalized linear models (GLMs). We first chose the dates with the peak seedling numbers per zone. Since the peaks were at different dates, we performed single GLMs for each zone and date. In the low marsh, we performed two separate GLMs for both peaks (Fig. 4.1). Total seedling number was entered as a response variable and temperature treatment (ambient, + 1.5, and + 3) was entered as the predictor. Furthermore, we tested the influence of temperature treatments on seedling numbers 1 week after full warming capacity was reached (March 28th) and at the peak of the vegetation period in mid-July (July 17th). For these dates, we performed one GLM each, with total seedling number as response variable and temperature treatment (ambient, + 1.5, and + 3) and zone (pioneer zone, low marsh, and high marsh) as predictors. We used ambient treatment and pioneer zone as reference levels respectively. We implemented these models with an interaction term and performed a backwards selection for the minimal adequate models after Crawley (2007). We chose a negative binomial error distribution for all models but one (high marsh at peak seedling numbers, where we used a Quasi-Poisson distribution) because the Poisson models were over-dispersed. Post-hoc pairwise comparisons were performed for all factors using estimated marginal means. The model assumptions were validated visually.

Influence of warming on seedling species richness and Shannon diversity

Species richness and Shannon diversity were additionally considered as response variables to detect possible effects of warming on a seedling community level. We implemented the same model structures and variables for the specific dates as for the seedling number models and tested for the same dates. The species richness models were implemented with a Poisson error distribution, but some of them were underdispersed. We then choose a Conway-Maxwell-Poisson error distribution to account for that. We implemented two-way ANOVAS for the Shannon diversity. For the high marsh at the peak seedling number, we used a log-rank test, since the data did not meet the assumptions of an ANOVA. Since we recorded species numbers only in the low marsh and high marsh, the analyses do not include the pioneer zone. Post-hoc pairwise comparisons, reference levels, implementation of an interaction term, and validation of model assumptions were performed as described above.

Maximum seedling numbers

To analyze the effect of temperature treatment (ambient, + 1.5, and + 3) and zone (pioneer zone, low marsh, and high marsh) on maximum number of seedlings per plot, we used a GLM. A negative binomial error distribution was used because the Poisson model was over-dispersed. In contrast to the previous analysis, we used the single highest seedling number of each plot to analyze the effects of factors. Therefore, the dates, when these maximum numbers were reached, differ also within zones. Post-hoc pairwise comparisons, reference levels, implementation of an interaction term, and validation of model assumptions were performed as described above.

Survival analysis

We used Kaplan–Meier survival curves to estimate the survival of individuals of the low marsh and the high marsh, as seedlings in the pioneer zone were not marked as individuals due to their high number. The analysis is based on the survivor function, which is the probability to survive up to time T (Kleinbaum and Klein 2004). At time 0, the survival probability equals 1. The survival probability of 0.5 equals the median survival time, which is also the median lifespan. Individuals, which survived up to the end of the study, became censored. Censoring means that we can only say the lifespan is at least as long as the time from emergence to the end of the study. There is no information past that time (Kleinbaum and Klein 2004). We analyzed the survival of pooled seedlings with separate Kaplan–Meier survival curves for temperature treatment (ambient, + 1.5, and + 3), zone (low vs. high marsh) and taxon. We choose taxa with a minimum number of 15 individuals to assess possible taxon effects on survival: *S. europaea* agg., *H. portulacoides*, *Atriplex* sp. and *Spergularia* sp. (Table 4.1). The survival probability at 28 weeks (length of the study period) was used to estimate the percentage of individuals, which survived of each species. Due to different emergence dates, the lifespan of surviving individuals can be different at the end of the study. Individuals, which reached the end of the study but emerged later in the study, are indicated by the crosses (censored) in the survival curves. In addition, we tested the species-specific warming effects on *S. europaea* agg., because it was the only species with a high number of seedlings (> 100) in the low and high marsh, where we had marked them individually. For all separate Kaplan–Meier survival curves we used log-rank tests to compare group differences. We did post-hoc pairwise comparisons with Bonferroni correction for significant results.

Percentage of surviving seedlings

We calculated the percentage of surviving seedlings at the end of the study for all zones as a proportion. To get a proportion for each plot, the maximum seedling number per plot was divided by the number of individuals from the last sampling date of this plot. This proportion is only based on total numbers at the date with the maximum numbers and at the end of the study and does not consider that some individuals might have died before the date of the maximum or emerged after it. It therefore only gives an estimation of the likelihood to survive (recruitment) until the end of the vegetation period in September. For the proportion of seedlings that survived, a GLM with a quasibinomial error distribution was used, because the binomial model was over-dispersed. We entered the proportion of seedlings that survived as response and temperature treatment (ambient, + 1.5, and + 3) and zone (pioneer zone, low marsh, and high marsh) as explanatory variables. Post-hoc pairwise comparisons, reference levels, implementation of an interaction term, and validation of model assumptions were performed as described above. All statistical analyses were performed with R version 4.1.2 (R Core Team 2021) applying a significance level of $p < 0.05$ for all tests. The additional R packages used in the statistical analyses are listed in the Supporting Information Fig. S4.1 section 5.

Results

Germination started between March 7th and 14th in nearly all plots. In some plots there were already a few seedlings prior to the time when we marked the plots. We detected seedlings from at least 10 different taxa (Table 4.1). *S. europaea* agg. had the highest number of individuals in total. In the pioneer zone, *S. europaea* agg. contributed to more than 95% of the individuals. Other seedlings in the pioneer zone included *Spergularia* sp. and *Suaeda maritima*. In the low marsh, the seedling diversity was highest with nine taxa, while we found seedlings from five taxa in the high marsh. The mean overall species richness of the seedlings was 3.3 ± 0.9 in the low marsh and 2.4 ± 1.1 in the high marsh. The overall Shannon diversity in the low marsh was 0.79 ± 0.27 and in the high marsh it was 0.69 ± 0.48.

Temporal patterns of seedling numbers

Overall, the mean seedling numbers increased until early April and afterwards declined in the pioneer zone and high marsh. The low marsh showed a second peak, where the highest seedling numbers were observed in May (Fig. 4.1). Between April and June, the seedling numbers showed fluctuations in all zones. The highest seedling numbers were reached in early April in the pioneer zone and high marsh (11th and 4th, respectively) and early May

(2nd) in the low marsh. At these dates, we counted a total of 1440 seedlings in the pioneer zone, 219 seedlings in the low marsh and 48 seedlings in the high marsh.

Table 4.1: Number of seedlings from the identified taxa of the low marsh and the high marsh.

Taxon	total	Low marsh	High marsh	Life history strategy
Artemisia maritima	4	4	0	perennial
Atriplex sp.	18	0	18	annual
Elymus athericus	4	0	4	perennial
Glaux maritima	1	1	0	perennial
Halimione portulacoides	19	13	6	perennial
Puccinellia maritima	1	1	0	perennial
Salicornia europaea agg.	146*	138*	8	annual
Spergularia sp.	54	38	16	annual/perennial
Suaeda maritima	10	10	0	annual
Triglochin maritima	3	3	0	perennial
Unknown	30	9	21	
Total	290	217	73	

*Excluding seedlings from three additional cohorts of *S. europaea agg*. in the low marsh, which were followed only as total number per observation and not marked individually (see methods).

At these peaks, there was no significant difference between temperature treatments in any of the zones. The effect of zone on seedling number was significant both 1 week after warming capacity was fully reached and at the peak of the vegetation period. However, there was no difference in seedling numbers between temperature treatments at these dates. The interaction was also not significant for both dates (Table 4.2, Fig. 4.1). Although there were no significant warming effects on seedling number at the tested times (Table 4.2), we did observe some trends (Fig. 4.1). In the pioneer zone, mean seedling numbers were slightly higher in both warming treatments than in the ambient treatment in early spring (March to mid-April), whereas afterwards only the + 3 treatment continued to have higher mean seedling numbers compared to ambient plots. In the low marsh (Fig. 4.1), the ambient and + 3 treatment mean seedling numbers increased and decreased with a steeper slope, while the + 1.5 numbers remained lower and stayed stable from early April until June. The high marsh seedling numbers showed a similar development in all treatments until the first peak in early April (4th). Afterwards, the seedling numbers stayed higher in the ambient treatment, while seedling numbers steadily decreased in the + 3 treatment. From July on, the seedling numbers in the high marsh stayed constant until the end of the study period (Fig. 4.1). There was a mass die-off in late April in the pioneer zone, while the numbers increased in the low marsh and stayed constant in the high marsh. After that die-off, mean seedling numbers stayed constant in the pioneer zone in all temperature treatments, but from the end of May on seedling numbers declined steadily until the end of the study. Mean seedling numbers in the low marsh declined steadily from early May on until mid of July and afterwards stayed constant. Until the end of July, there were more individuals in the low marsh than in the high marsh. Afterwards, the seedling numbers in the low marsh were as low as in the high marsh (Fig. 4.1).

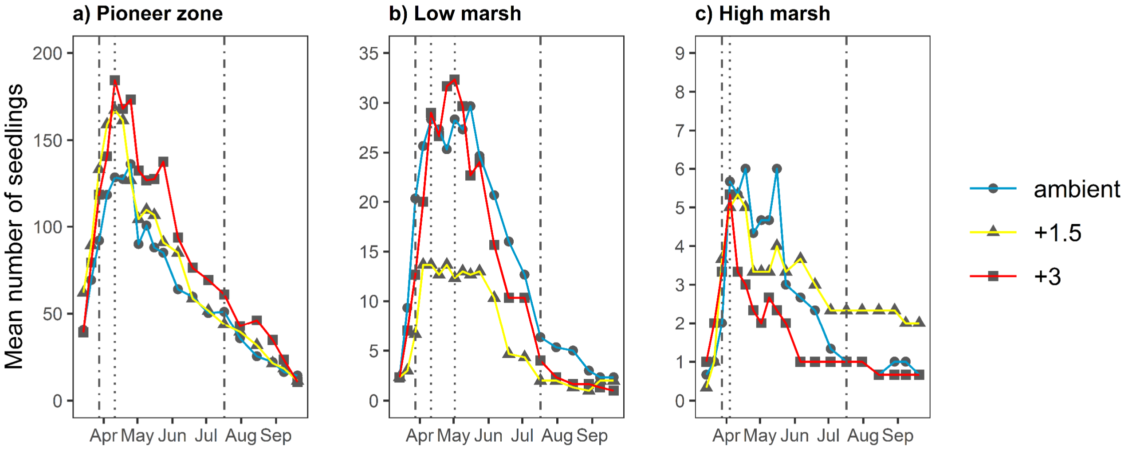

Fig. 4.1: Mean number of seedlings per temperature treatment (n = 3) over the course of the vegetation period 2019. Seedling numbers are displayed for (**a**) pioneer zone, (**b**) low marsh and (**c**) high marsh. Temperature treatment levels are ambient temperature, +1.5 °C and +3.0 °C. Dash-dotted lines display dates at which GLM over all zones were calculated (one week after warming capacity was fully reached and at the peak of the vegetation period), dotted lines display dates where single GLMs were conducted for maximum seedling numbers per zone. Note that the y-axis scales differ. For better visualization, error bars are not displayed. Barplots with error bars of the analysed dates can be found in the Supplementary Information of the online version of this article (Supplementary Information Figs. S4.1-S4.3).

Table 4.2: Model variables and results of all GLMs and two-way ANOVAs.

		Treatment		Zone		Zone:treatment	
	Distribution	$LR_{df = 2}$	p	$LR_{df = 2}$	p	$LR_{df = 4}$	p
Maximum no. of seedlings[*]	Negative binomial	1.4	n.s.	52.5	< 0.0001	3.2	n.s.
Survived seedlings	Quasi-binomial	-5.3	n.s.	3.5	n.s.	-8	n.s.
Seedling no. at warming at full capacity	Negative binomial	0.1	n.s.	48.2	< 0.0001	5.3	n.s.
Seedling no. at peak of vegetation season	Negative binomial	0.5	n.s.	42.1	< 0.0001	4.7	n.s.
Seedling no. at peak total no. pioneer[†]	Negative binomial	0.7	n.s.	-	-	-	-
Seedling no. at peak 1 total no. LM[‡]	Negative binomial	2.2	n.s.	-	-	-	-
Seedling no. at peak 2 total no. LM[†]	Negative binomial	2.6	n.s.	-	-	-	-
Seedling no. at peak total no. HM[†]	Quasi-Poisson	0.1	n.s.	-	-	-	-
		$LR_{df=2}$		$LR_{df=1}$		$LR_{df=2}$	
Overall species richness	COMpoisson	0.02	n.s.	3.8	n.s.	0.007	n.s.
Species richness at warming at full capacity	COMpoisson	0.1	n.s.	0.05	n.s.	0.06	n.s.
Species richness peak of vegetation season	Poisson	0.08	n.s.	3.3	n.s.	1.7	n.s.
Species richness peak 1 total no. LM[‡]	COMpoisson	4.1	n.s.	-	-	-	-
Species richness peak 2 total no. LM[†]	COMpoisson	7.8	0.001	-	-	-	-
Species richness peak total no. HM	Poisson	1.5	n.s.	-	-	-	-
Shannon diversity peak total no. HM	non-parametric[§]	2.6	n.s.	-	-	-	-

		$F_{df=2}$		$F_{df=1}$		$F_{df=2}$	
Overall Shannon diversity	Gaussian	1.2	n.s.	0.4	n.s.	0.4	n.s.
Shannon diversity at warming at full capacity	Gaussian	4.6	0.03	2.6	n.s.	0.1	n.s.
Shannon diversity peak of vegetation season	Gaussian	0.02	n.s.	0.05	n.s.	0.3	n.s.
Shannon diversity peak 1 total no. LM[‡]	Gaussian	2.8	n.s.	-	-	-	-
Shannon diversity peak 2 total no. LM[†]	Gaussian	6.2	0.003	-	-	-	-

---

The levels of zone are pioneer, low marsh, and high marsh. The temperature treatments are ambient temperature, +1.5 °C and +3.0 °C. LR are likelihood ratio tests (Chi-square distribution) to estimate significance of each factor included in the model. Corresponding p values are given for significant outcome ($p < 0.05$), n.s. stands for not significant. Interaction between factors is indicated by “:”. The final models included factors with significant influence based on backwards selection via LR and F tests.

*Maximum seedling number of each single plot over the entire course of the experiment.

†Highest number of seedlings in total per zone.

‡First peak in the low marsh.

[§] A comparison with a Kruskal-Wallis log-rank test was implemented.

Species richness and Shannon diversity

The pooled species richness and Shannon diversity of all seedlings emerging during the study period was neither significantly different between the temperature treatments nor the zones and there was no interaction of the variables (Table 4.2, Supporting Information Fig. S4.4). There was a significant effect of warming on the Shannon diversity soon after warming was at full capacity (Table 4.2, Supporting Information Fig. S4.5). However, post-hoc paired comparisons revealed that the Shannon diversity of the ambient treatment and the + 3 treatment of the pooled seedlings from the low marsh and high marsh did not differ significantly (0.05), and the + 1.5 treatment and the + 3 treatment just were significantly different close to non-significance (0.047). There was a significant influence of warming on the Shannon diversity and species richness (Table 4.2, Supporting Information Fig. S4.6) in the low marsh at the second peak of the total seedling number. At the other dates there was no significant influence of the temperature treatments on Shannon diversity or species richness in both zones (Table 4.2, Supporting Information Figs. S4.7, S4.8).

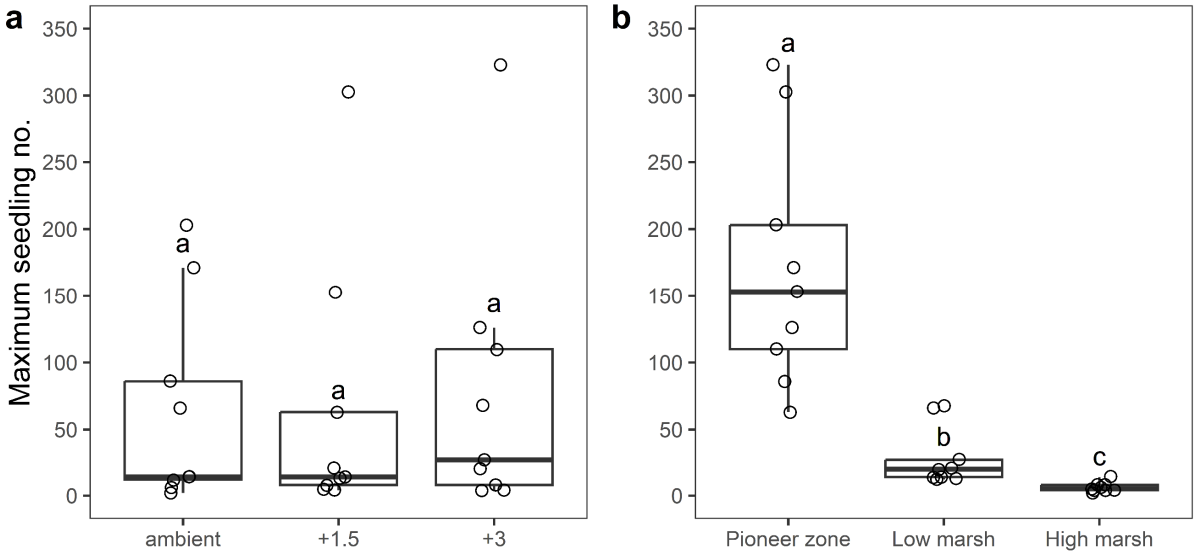

Fig. 4.2: Maximum seedling numbers in 27 subplots of (**a**) temperature treatments and (**b**) marsh zones. Boxes represent 25 % and 75 % quartiles from the median (thick line), whiskers represent maximum and minimum values, excluding outliers. Data points are shown as circles. Different letters denote significant differences from post-hoc pairwise comparisons (Tukey HSD, p < 0.05) for (a) and (b) separately.

Maximum seedling numbers

The temperature treatments had no significant effect on maximum seedling numbers per plot and there was no interaction of temperature treatment and zone (Fig. 4.2a, Table 4.2). Maximum seedling numbers differed significantly between marsh zones (Fig. 4.2b, Table 4.2). The highest numbers occurred in the pioneer zone, with an average of 171 ± 30 individuals per subplot. The maximum numbers of seedlings in the low marsh were on average 28 ± 7 individuals per subplot. The lowest numbers of seedlings occurred in the high marsh, with 6 ± 1 individuals.

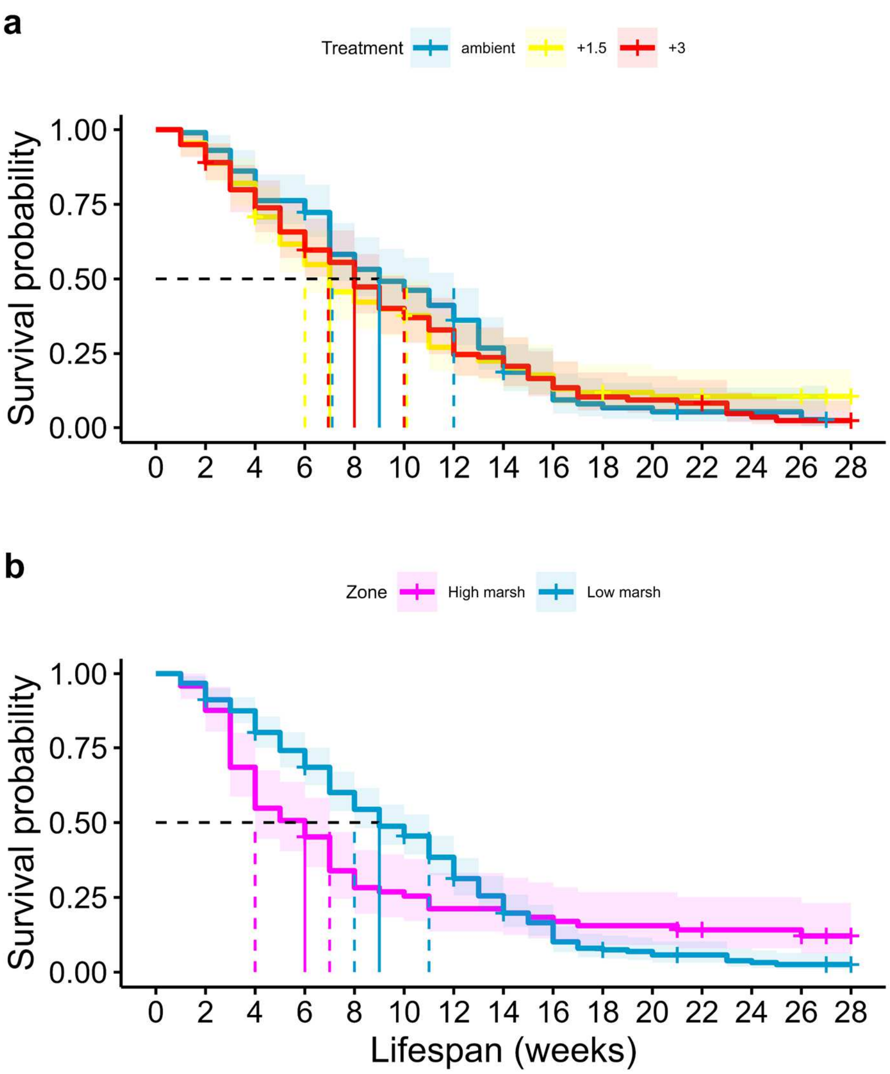

Fig. 4.3: Kaplan-Meier survival curves of pooled seedlings with estimates of 95 % confidence intervals for (**a**) temperature treatments and (**b**) salt marsh zones. A survival probability of 1 is equivalent to 100 %. The median survival time (corresponding to 0.5 survival probability) and confidence intervals are indicated by solid and dashed lines, respectively. Crosses indicate censored individuals. Note that lifespan and not continuous time is plotted on the x-axis.

Influence of warming on survival

In all temperature treatments, survival probabilities decreased with increasing lifespan. In the ambient treatment plots, the median survival time was 9 weeks. Warming decreased median survival of pooled seedlings to 7 and 8 weeks in the + 1.5 and + 3 treatment, respectively (Fig. 4.3a). However, these differences were non-significant according to the log-rank test (Chi^2 = 0.6; p = 0.7).

Survival in marsh zones

The median lifespan of seedlings did not differ significantly (Chi^2 = 0.7; p = 0.4) between marsh zones. The median survival time of individuals in the low marsh (9 weeks) was 3 weeks longer than in the high marsh (6 weeks; Fig. 4.3b). After a survival time of 15 weeks, the survival probability was slightly higher in the high marsh than in the low marsh. However, the confidence intervals (CI) overlap and the difference between zones was not significant.

Species-specific differences in survival

The log-rank test of the median survival times (median lifespan) was significant for the four analyzed taxa (Chi^2 = 16.9; p = 0.0008; Fig. 4.4a). Post-hoc pairwise comparisons resulted in a significant difference between the survival curves of *H. portulacoides* and *S. europaea* agg. (p = 0.0013) and *H. portulacoides* and *Spergularia* sp. (p = 0.0006). The other curves did not significantly differ from each other. *H. portulacoides* had the longest median survival time with 15 weeks. It had also the highest final survival probability with 31% of the seedlings reaching an age of 28 weeks. Although the median survival time of *Atriplex* sp. was the lowest of all species (5.5 weeks), *Atriplex* sp. seedlings also had a 22% chance of surviving 28 weeks. Of the *S. europaea* agg. seedlings, the chance of surviving 28 weeks was 5% and median lifespan was 9 weeks. The survival curve for *Spergularia* sp. seedlings showed a median survival time of 6.5 weeks. *Spergularia* sp. seedlings had a shorter total lifespan of 23 weeks compared to the other species, meaning after 23 weeks the survival probability was 0 (Fig. 4.4a).

Influence of warming on survival of S. europaea

For *S. europaea* agg. seedlings, the temperature treatment effect was not significant either (Chi^2 = 2.6; p = 0.3). But a trend toward shorter median survival times of seedlings in warming treatments was also detectable (Fig. 4.4b). In ambient treatments, median lifespan was 3 weeks longer than in + 3 treatments and 4 weeks longer than in + 1.5 treatments. However, the total survival probability (lifespan) was lower in ambient treatments. After a lifespan of 18 weeks, the survival probability became 0, while in warming treatments the survival probability stayed slightly above 0 for the + 1.5 treatment and for the + 3 treatment the survival probability became 0 after a lifespan of 24 weeks.

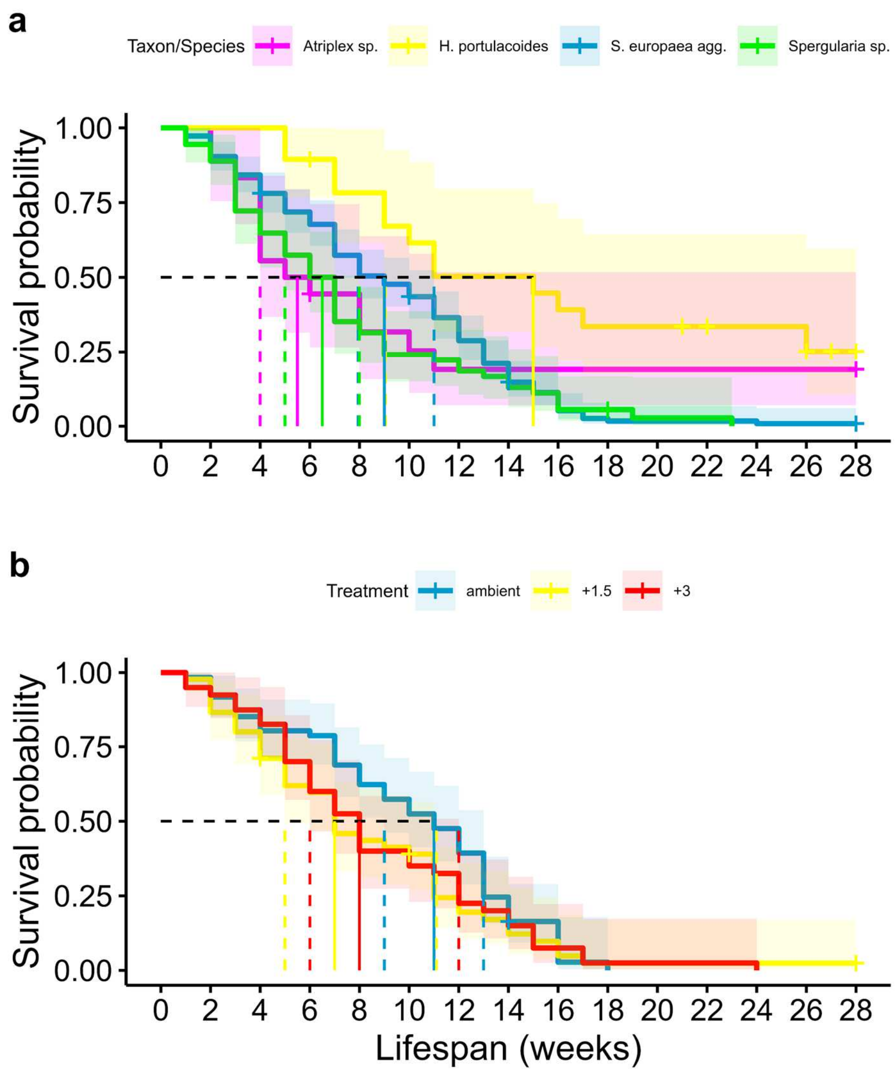

Fig. 4.4: Kaplan-Meier survival curves with estimates of 95 % confidence intervals (CI) for (**a**) four taxa with n > 15. *Atriplex* sp.: n = 18; *Halimione portulacoides*: n = 19; *Salicornia europaea* agg.: n = 146 and *Spergularia* sp.: n = 54 and for (**b**) *Salicornia europaea* agg. (n = 146) grouped by temperature treatments in the low and high marsh. A survival probability of 1 is equivalent to 100 %. The median survival time (corresponding to 0.5 survival probability) and confidence intervals are indicated by solid and dashed lines, respectively. Due to low number of events, there are no upper limits for the CI of *Atriplex* sp. and *H. portulacoides*. Crosses indicate censored individuals. Note that lifespan and not continuous time is plotted on the x-axis.

Proportion of surviving seedlings

The proportion of seedlings, which survived until the end of the observation period, did not significantly differ between temperature treatments and zones (Fig. 4.5, Table 4.2). The median proportion of surviving seedlings of the three temperature treatments was 8.3%, 15.3%, and 3.1% for ambient, + 1.5 and + 3, respectively (Fig. 4.5a). In the pioneer zone, a median of 6.3% survived, in the low marsh 3.7% and in the high marsh 25% per subplot (Fig. 4.5b).

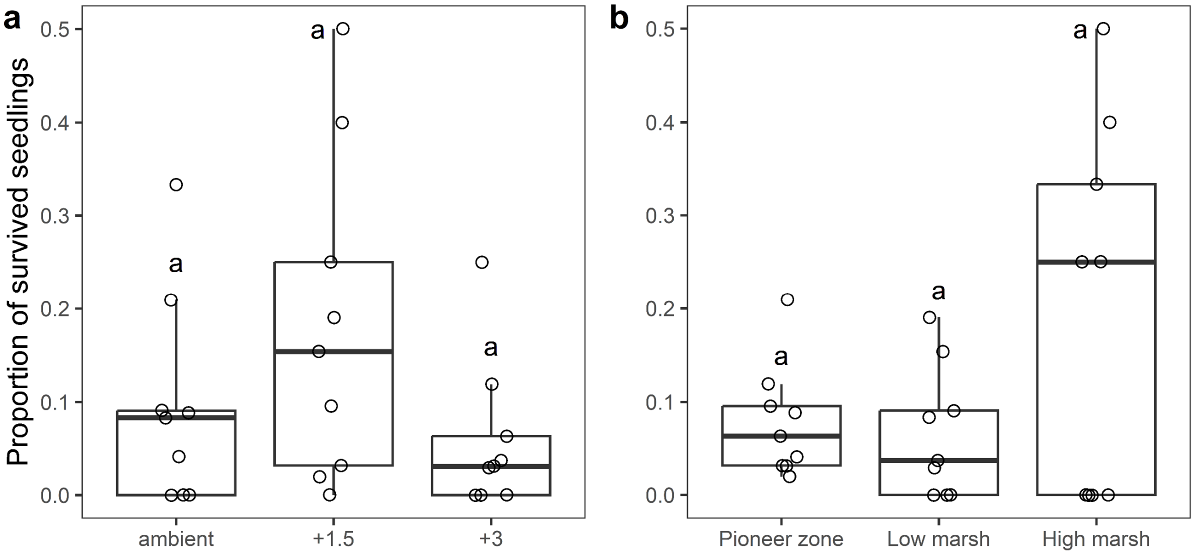

Fig. 4.5: End of season seedling survival in 27 subplots. The proportion of seedlings which survived until the end of the study in relation to maximum seedling numbers. A proportion of 0.5 corresponds to 50 %. (**a**) proportion of surviving seedlings for the temperature treatments (ambient temperature, +1.5 °C, +3.0 °C). (**b**) proportion of surviving seedlings in the different marsh zones (pioneer zone, low marsh, and high marsh). Boxes represent 25 % and 75 % quartiles from the median (thick line), whiskers represent maximum and minimum values, excluding outliers. Data points are shown as circles. Different letters denote significant differences from post-hoc pairwise comparisons (Tukey HSD, $p < 0.05$) for (a) and (b) separately.

Discussion

Influence of warming on seedling numbers, emergence, diversity, and survival

Our first hypothesis, that warming will have a zone-specific influence on seedling number, was not supported, as we saw no effects of warming on seedling number, nor any interactions between warming and salt marsh zone. The species temperature optima might be within a range and therefore the applied warming of up to + 3.0 °C does not affect germination of the studied salt marsh species directly. However, there are trends in the pioneer zone, which suggest that warmer temperatures may lead to increased seedling emergence (Ungar 1977) soon after warming was at full capacity on March 22nd. This coincided with a neap tide period (around March 28th) with less flooding and shorter flooding durations during that week (Supporting Information Figs. S4.9, S4.10). Therefore, another possible explanation would be a faster growth and reaching of a tolerance threshold (van Belzen et al. 2022) during the window of opportunity with benign physical conditions (Hu et al. 2015; Cao et al. 2018) resulting in higher survival probabilities during the spring tides around April 5th, which may also be reflected in the higher counts of the next sampling date (April 11th). In the low marsh we saw high seedling numbers of *Salicornia europea* agg., in two of the plots (ambient and + 3), which is most likely caused by a high adult plant abundance from the previous year (Supporting Information Fig. S4.11; Ungar and Woodell 1993; Rand 2000). Additionally, seeds from the pioneer zone might be transported there more easily (Wolters and Bakker 2002; Wolters et al. 2005), because of the position and elevation of these two plots within the experiment. Between the end of March and the end of April, the precipitation was very low at the study site [data from the German Meteorological Service, (DWD, https://opendata.dwd.de/climate_environment/CDC/observations_germany/climate/monthly/kl/historical/) last accessed 07 October 2022 for Hattstedt, which is 15 km from the study site] and there were no floodings in the high marsh. Drought conditions may impact seedling establishment in salt marshes, depending on the species (Kuhn and Zedler 1997; Noe and Zedler 2000; Uyeda et al. 2019). As increased temperatures can lead to increased evapotranspiration, seedlings in the high marsh warming treatments might have suffered more from drought stress (Egan and Ungar 1999; van Regteren et al. 2020) than those in the ambient treatments, explaining the trend of a faster decline of mean seedling numbers. As clonal propagation of perennial species (Shumway and Bertness 1992; Nieva et al. 2001; Mateos-Naranjo et al. 2008) is considered as the more common strategy, this might only have a minor influence on vegetation cover of perennial species such as the dominant grass *E. athericus*. However, annual species like *Atriplex littoralis*, *Atriplex prostrata* and *Spergularia marina* could become negatively affected with future warming.

Contrary to the expectation of our first hypothesis - that warming will reduce the lifespan of the low and high marsh seedlings - we found no significant differences between the survival curves of the temperature treatments in general. In mangroves, seedling survival was not affected in a warming experiment either (Coldren et al. 2016). However, we did observe a (non-significant) reduction of median lifespan of pooled seedlings and of *S. europaea* agg. seedlings in the warming treatments, which may have been caused by increased drought stress with warmer temperatures. This might have shortened the time (window of opportunity) for seedlings to establish as soil moisture levels may strongly influence germination processes like germination speed and seedling emergence in salt marshes (Noe and Zedler 2000, 2001). While most early emerging *S. europaea* agg. seedlings died, we saw a few of those seedlings in the warming treatments survive until the end of the study period, which may be an indication that they used the window of opportunity by reaching a tolerance threshold toward drought conditions (van Belzen et al. 2022). Nevertheless, the chance of establishment was equal in all treatments, therefore warming does not significantly alter seedling establishment with the applied temperature treatments. However, the windows of opportunity for seedling emergence and especially survival in the higher marsh zones might change in the future due to exposure to prolonged and/or more intense temperature increases, as was found for seedlings exposed to drought conditions in different habitats (de Dios et al. 2012; Davis et al. 2016; van Regteren et al. 2020). The increase of species richness and Shannon diversity at the peak of the seedling numbers in the low marsh and Shannon diversity in the week after warming capacity was fully reached, was contrary to our second hypothesis. This was also contrary to the results for salt marsh communities by Gedan and Bertness (2009), but the significant positive effect of warming on seedling Shannon diversity in our experiment needs to be interpreted with care, since it could also be an artifact of seed abundance caused by adult plant abundance in the plots (Ungar and Woodell 1993; Rand 2000).

Zonation effects on seedlings

The decline in maximal seedling numbers from pioneer to low marsh to high marsh zones supports part of our third hypothesis. The generally higher seedling numbers in the pioneer zone (mainly *S. europaea* agg.) compared to the other zones, are probably related to a high individual seed production, even at high population densities (Jefferies et al. 1981) and high seed numbers in the soil seed bank (Wolters and Bakker 2002). We found no significant difference between the Shannon diversities and species richnesses between zones, which did not confirm our expectation. The absence of seedlings from other species in the pioneer zone like *Spartina anglica*, which makes half of the vegetation coverage, may be explained by an only episodically occurrence of seedling establishment of *S. anglica* (Hu et al. 2021). The large variation of maximum seedling numbers within the pioneer zone is probably caused by adult plant abundance and therefore distance to seed source (Ungar and Woodell 1993; Rand 2000; Wolters et al. 2005). This assumption is supported by the positive linear relationship between abundance of *S. europaea* agg. in 2018 and seedling numbers in 2019 (Supporting Information Fig. S4.11). The die-off in the pioneer zone in late April/early May was probably caused by strong sedimentation (van Regteren et al. 2020) corresponding with the spring tides around the 19th of April. While flooding times and flooding durations were similar for both the week before and the week of the main die-off, there was a change to landwards winds in the week of the main die-off. This may have increased wind waves and sediment deposition (see McManus and Alizai 1987; Maynard et al. 2011), by increasing suspended sediment concentrations (SSC) in the flooding water due to resuspension on the mudflats (Weir and McManus 1987) as well as influenced current velocities and turbulence (Bouma et al. 2005). Sediment deposition and increased turbulence increase the risk of seedlings to become buried (van Regteren et al. 2020) or dislodged due to scouring (Bouma et al. 2009a; Friess et al. 2012; Schoutens et al. 2021) and bed erosion, which exceeds the critical erosion depth of seedlings (Hu et al. 2021).

There was no significant difference between the proportion of surviving seedlings between marsh zones, which did not confirm the expectation of our third hypothesis for seedling survival. Seedling survival increased with higher elevations within the pioneer zone (Cao et al. 2021), but we saw a nonsignificant trend of the proportion of surviving individuals being greater in the high marsh. In our study, this could indicate that *E. athericus* also provides shelter for seedlings and alleviates stress, and not only outcompetes them for space (Bertness and Leonard 1997) and light (Bakker et al. 1985; Bakker and de Vries 1992).

Species specific reactions of seedling survival

Median seedling lifespan differed significantly between some of the species, which partly confirmed our fourth hypothesis. The low survival probabilities of *S. europaea* agg. and *Spergularia* sp., were in line with our fourth hypothesis (assuming that most of the *Spergularia* sp. seedlings were from the annual species). We did not expect the high survival probability for *Atriplex* sp., as both species in the study area are annuals. However, the recorded *Salicornia*, *Spergularia* and *Atriplex* species differ in their seed size (Sterk 1969; Ungar 1979; Bakker and de Vries 1992). Species with small seeds produce much more seeds than large-seeded species (Henery and Westoby 2001), which gives them an initial advantage in seedling numbers. Species with large seeds provide more food for the seedling and were found to have an advantage to establish under a closed canopy (Gross and Werner 1982; Goldberg and Werner 1983). Thus, the high survival probabilities of *Atriplex* sp. individuals can probably be attributed to their large (heavy) seeds (Bakker and de Vries 1992; Moles and Westoby 2004). Therefore, the interspecific differences in seedling survival might simply be differences due to life-history traits (Davy and Smith 1985; Kaminsky et al. 2015).

Conclusions

This study provides first insights of germination and seedling survival patterns under experimental warming along the elevation gradient in a temperate salt marsh. The advantage of this field experiment is the possibility to study warming effects in combination with other abiotic stressors like inundation and drought. Salt marsh seedling emergence and survival was not significantly affected by a temperature increase of up to + 3 °C. However, due to the trends we detected in warmed plots and the significant influence on Shannon diversity at some dates, we conclude there may be future effects under increasing global temperatures in the high marsh. We mainly expect an earlier onset of drought conditions due to increased evapotranspiration and rare floodings in the summer months, whereas the pioneer zone will probably be affected in a different way. One possible scenario in the pioneer zone could be a faster growth of the seedlings and therefore a shorter window of opportunity for establishment under warming may be needed. Due to practical reasons, we were unfortunately not able to measure seedling size and mark pioneer zone individuals. Additional studies, which focus on the different abiotic and biotic interactions of the distinct zones more specifically and include size measurements of seedlings, are necessary to disentangle the zone-specific reactions of seedling establishment under global warming.

Acknowledgments

We would like to thank all project partners of the Wadden Sea National Park for their cooperation. Kristin Ludewig provided advice on the study design. We thank Roy Rich for providing his knowledge and expertise to build the MERIT warming experiment. We also thank the team, which kept the experiment running and helped with the deployment, especially Simon

Thomsen, Stefan Knaak, Jörn Ehlers, and Tom Kamin. We would also like to thank three anonymous reviewers for their valuable feedback, which enabled us to greatly improve this article. E. O. was funded by the Bauer-Hollmann Stiftung and Pro Exzellenzia plus. Open Access funding enabled and organized by Projekt DEAL.

Supplementary Information:

1. Mean seedling numbers for specific time steps: peak of seedling numbers per zone, one week after full warming capacity was reached and at the peak of the vegetation period.

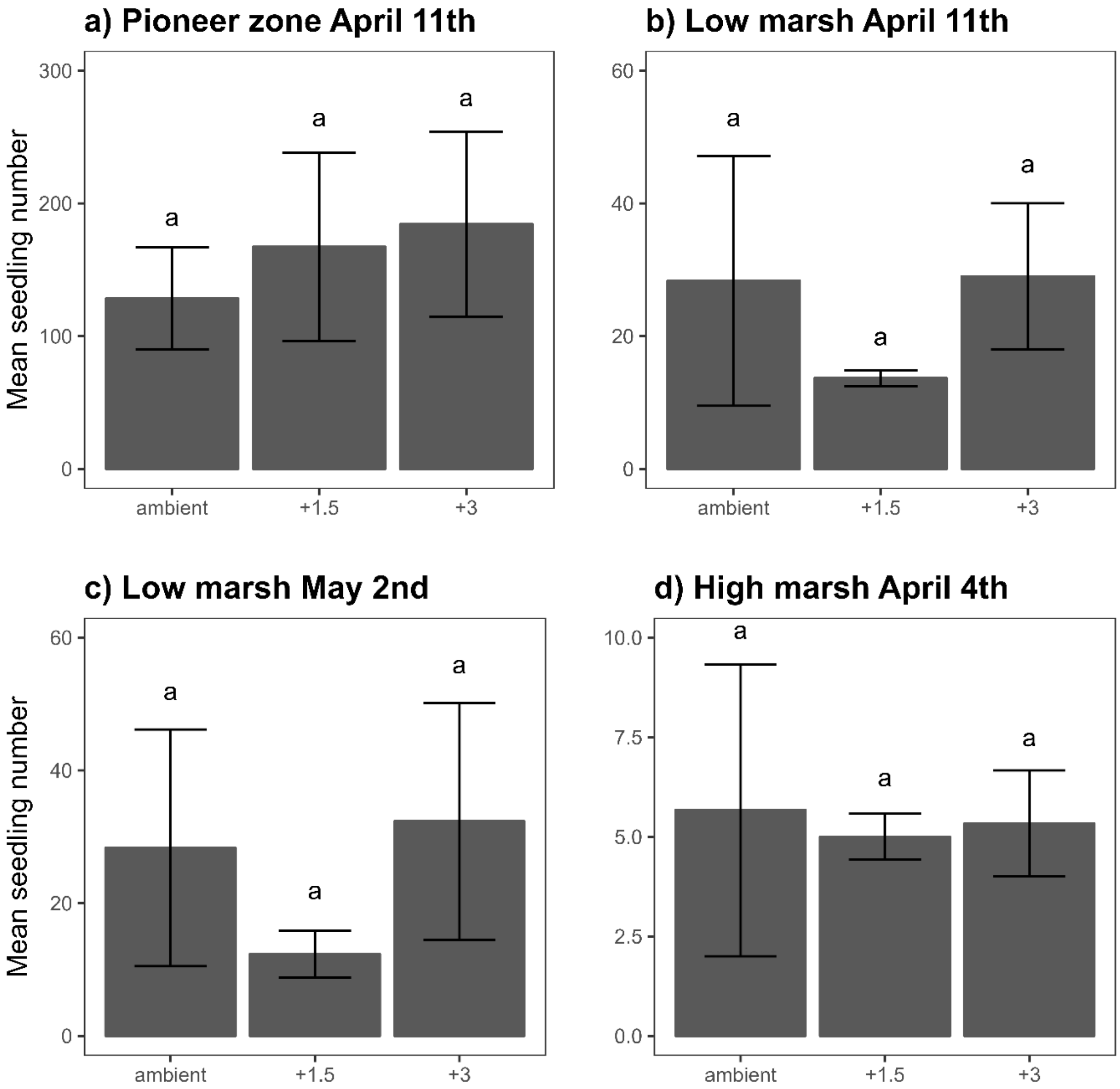

Fig. S4.1: Peak seedling numbers per zone a) pioneer zone, b) low marsh at the first peak c) low marsh at the second peak and d) high marsh. Boxes represent 25 % and 75 % quartiles from the median (thick line), whiskers represent maximum and minimum values. Data points are shown as circles. Mean values are shown as crosses. Different letters denote significant differences from post-hoc pairwise comparisons (Tukey HSD, p<0.05) for a), b), c) and d) separately.

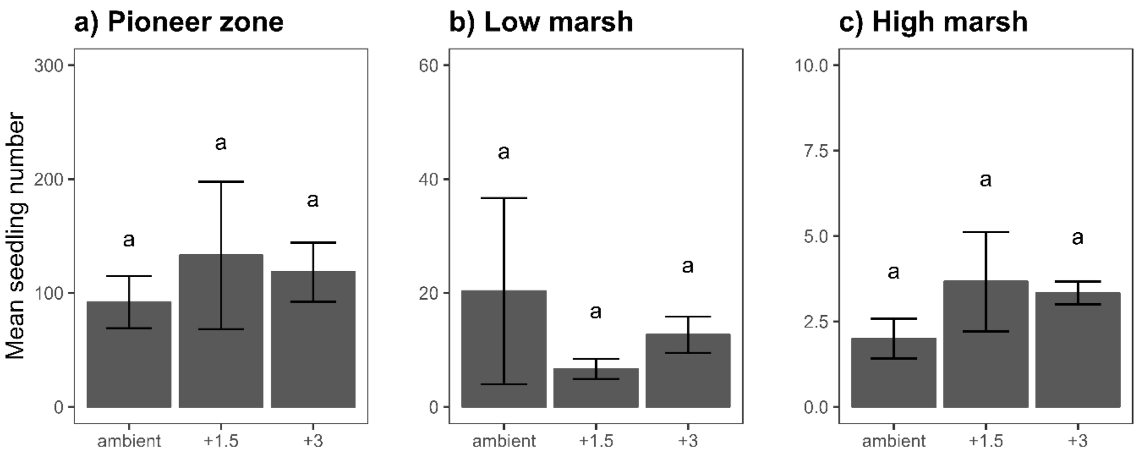

Fig. S4.2: Seedling numbers one week after full warming capacity was reached (March 28th). a) pioneer zone, b) low marsh and c) high marsh. Boxes represent 25 % and 75 % quartiles from the median (thick line), whiskers represent maximum and minimum values. Data points are shown as circles. Mean values are shown as crosses. Different letters denote significant differences from post-hoc pairwise comparisons (Tukey HSD, p<0.05).

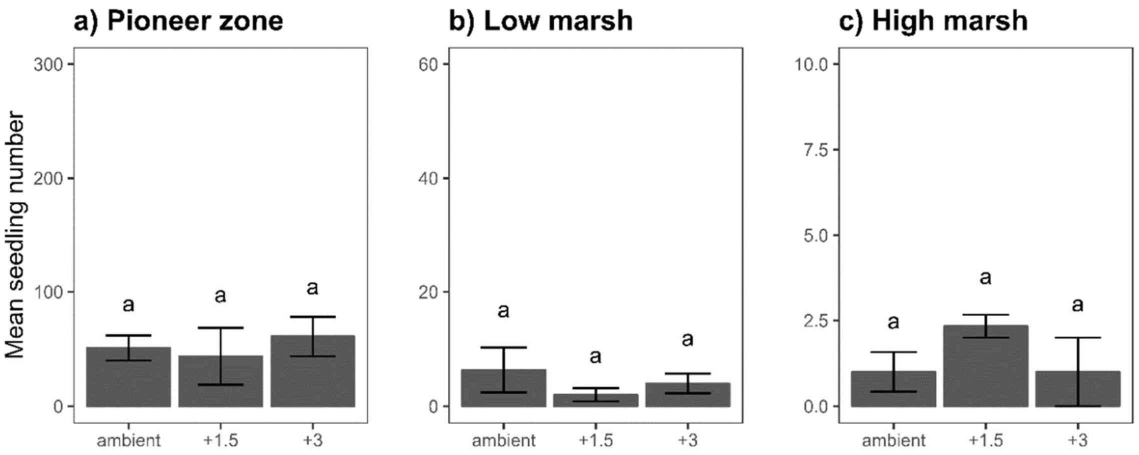

Fig. S4.3: Seedling numbers at the peak of the vegetation season (July 17th). a) pioneer zone, b) low marsh) and high marsh. Boxes represent 25 % and 75 % quartiles from the median (thick line), whiskers represent maximum and minimum values. Data points are shown as circles. Mean values are shown as crosses. Different letters denote significant differences from post-hoc pairwise comparisons (Tukey HSD, p<0.05).

2. Shannon diversity and species richness of seedlings in the low marsh and high marsh

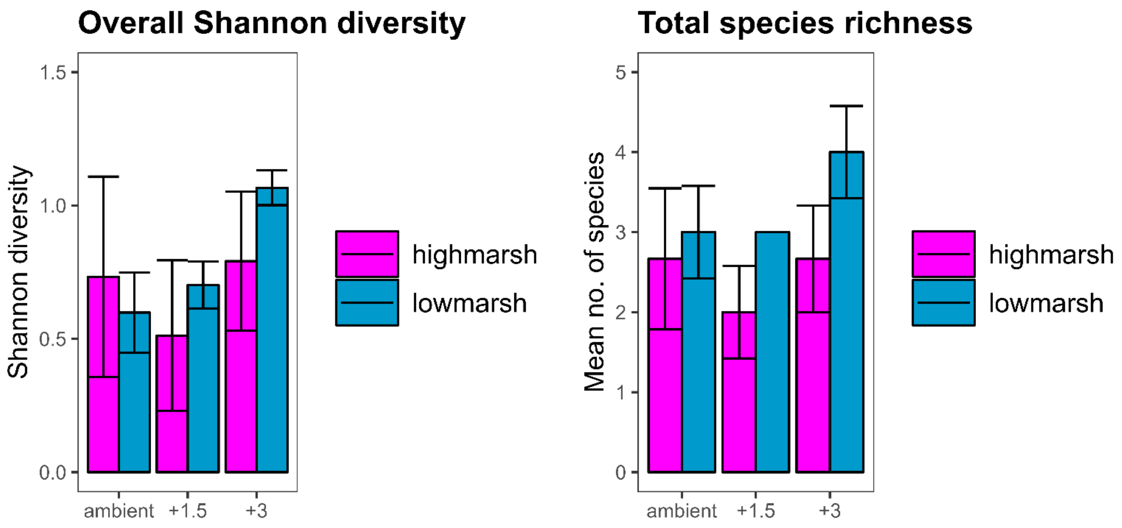

Fig. S4.4: Shannon diversity and species richness over all sampling dates.

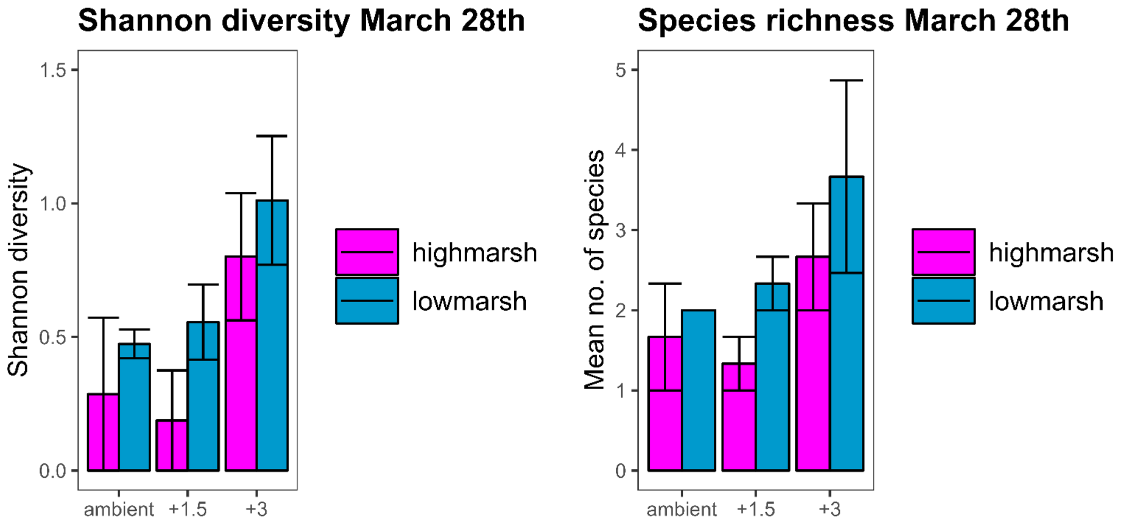

Fig. S4.5: Shannon diversity and species richness one week after warming capacity was reached.

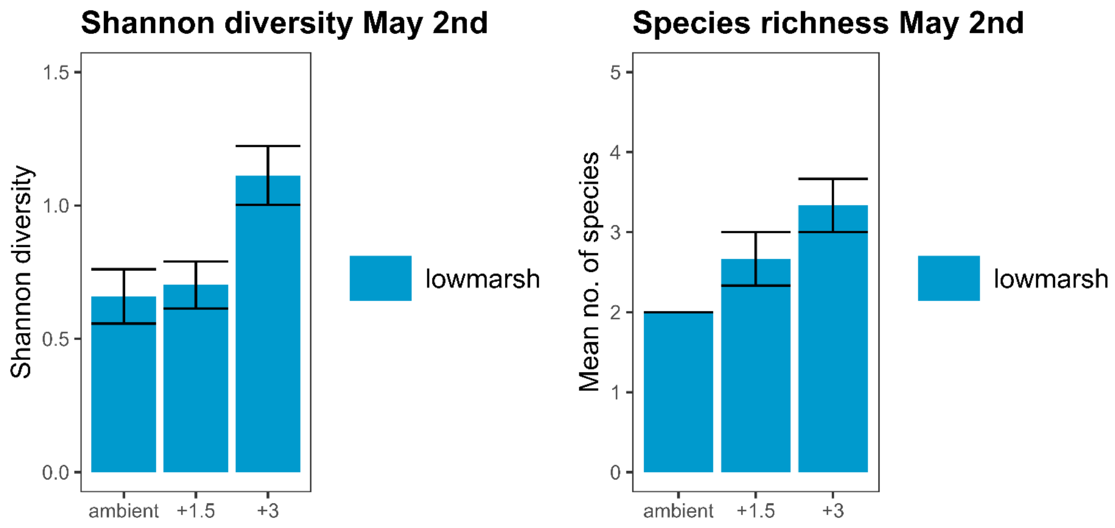

Fig. S4.6: Shannon diversity and species richness at the second peak seedling no. in the low marsh.

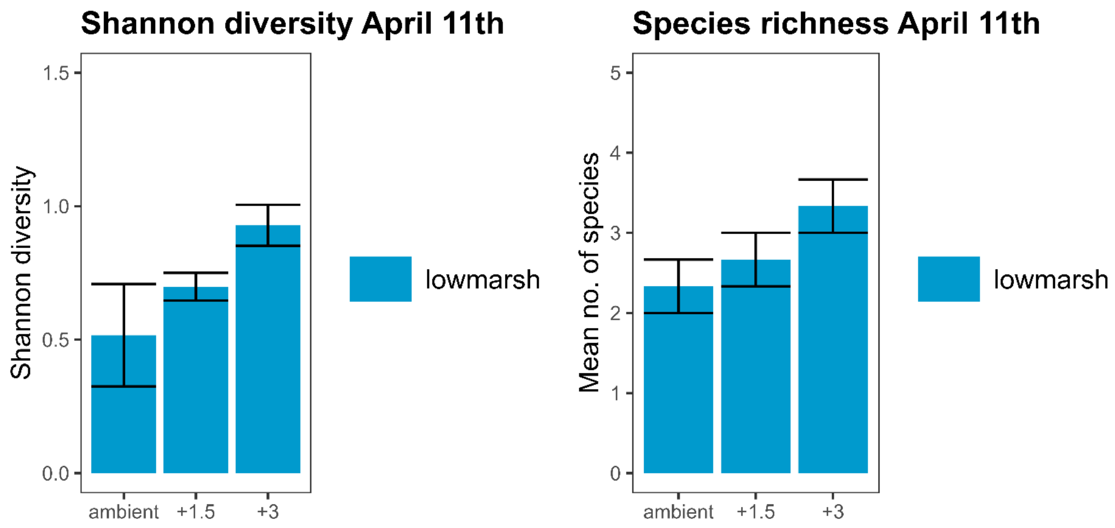

Fig. S4.7: Shannon diversity and species richness at the first peak seedling no. in the low marsh.

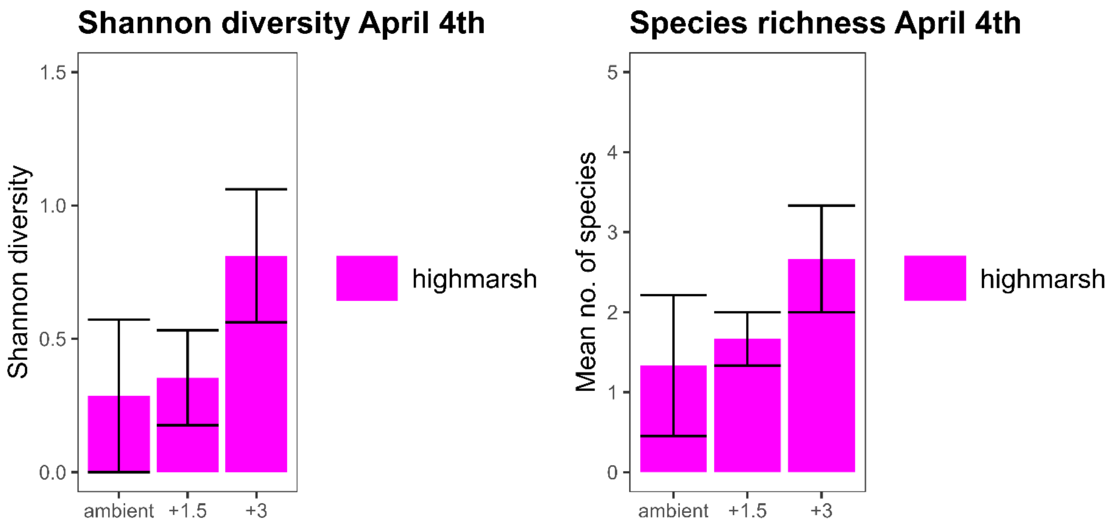

Fig. S4.8: Shannon diversity and species richness at the peak seedling no. in the high marsh.

3. Flooding of the pioneer zone

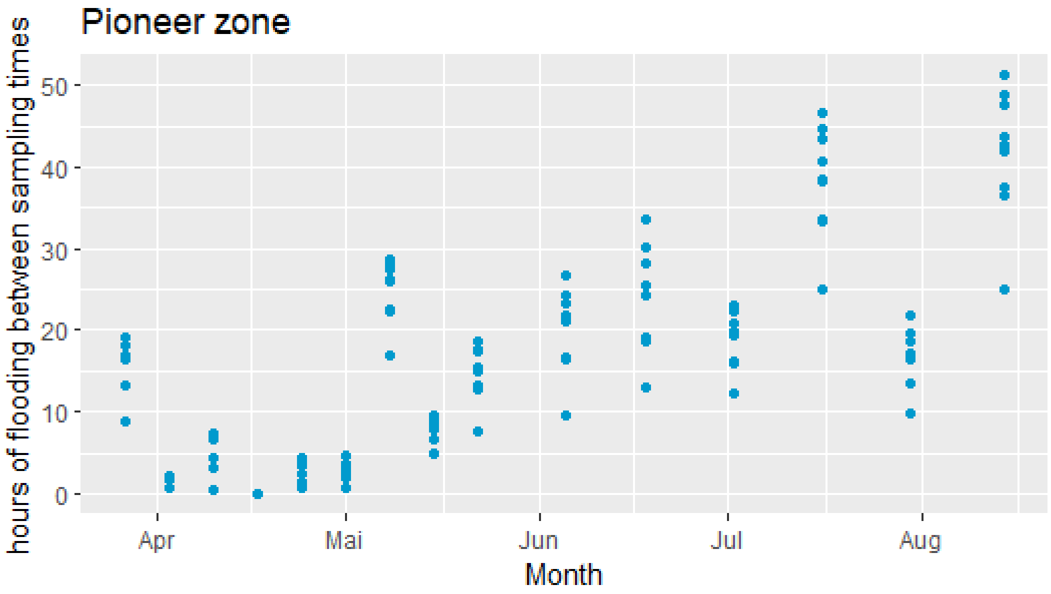

Fig. S4.9: Hours of flooding between two consecutive sampling dates in the pioneer zone. Hours are cumulative and displayed at the end of the period. From June on the sampling frequency was two weeks, therefore the number of hours of flood is higher.

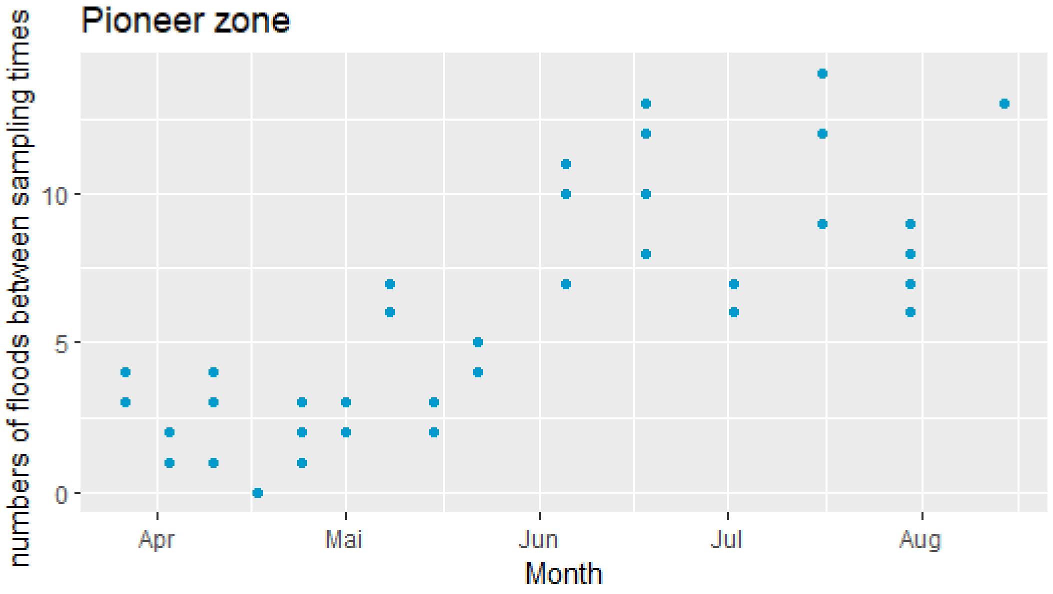

Fig. S4.10: Number of floods between two sampling times in the pioneer zone. From June on the sampling frequency was two weeks, therefore the number of floods is higher.

4. Adult plant abundance and seedling numbers

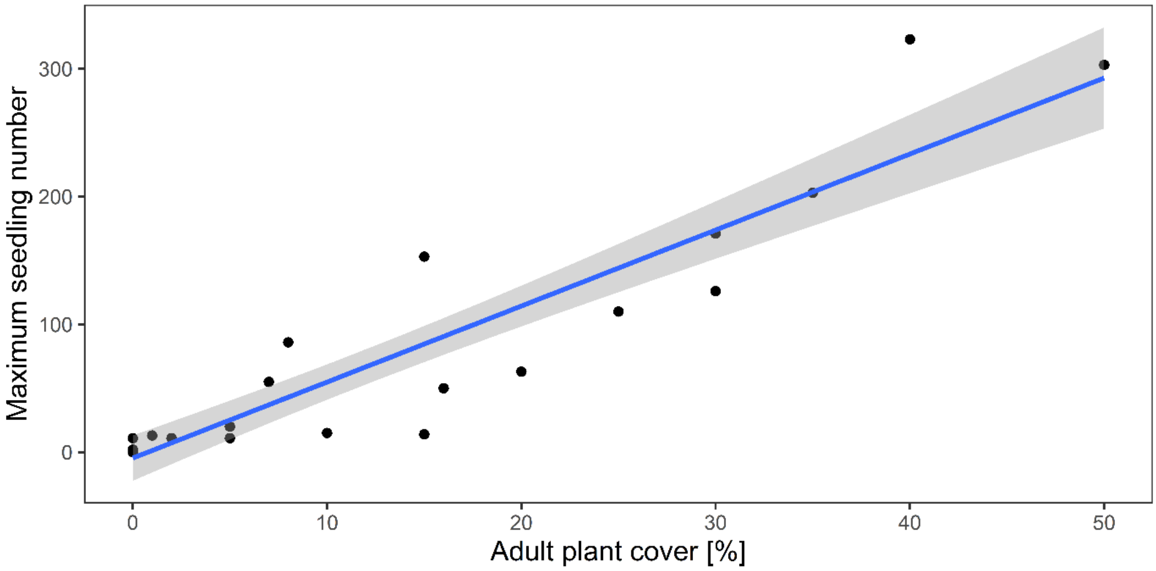

Fig. S4.11: Relationship of *Salicornia europaea* agg. seedling numbers 2019 and adult plant cover of the previous year (July 2018). For the pioneer zone maximum seedling numbers were used, for the other zones total number of seedlings. Adult plant cover was recorded as percentage of total cover of plants in the subsection of the subplot for seedling counts.

5. Additional R packages used for statistical analyses:

We used the additional packages car (Fox and Weisberg 2019), DHARMa (Hartig 2021), dplyr (Wickham et al. 2021), emmeans (Lenth 2022), ggplot2 (Wickham 2016), ggpubr (Kassambara 2020), lubridate (Grolemund and Wickham, 2011), MASS (Venables and Ripley 2002), multcomp (Hothorn et al. 2008), multcompView (Graves et al. 2019), performance (Lüdecke et al. 2021), spaMM (Rousset and Ferdy 2014), survival (Therneau 2021), survminer (Kassambara et al. 2017), vcd (Meyer et al. 2006) and vegan (Oksanen et al. 2016).

5

General discussion

The aim of this thesis was to examine how global warming affects the performance of salt marsh plants. This general discussion of the results comprises three main sections. First, I will summarize the key findings of each study including short conclusions. Second, I will establish connections between the key findings, discuss, and synthesize them to a comprehensive overarching result. Third, I will close the synthesis with implications of the results, the identification of knowledge gaps for future research, and the overall conclusion of this thesis.

5.1 Key findings

Root biomass dynamics under warming (Fig. 5.1; chapter 2)

1. In general, there was little effect of warming on total root biomass across years and zones. This indicates that the soil stabilization by roots against erosion may not be affected under future warming.
2. Root biomass in the high marsh shifted from the upper soil depth to the lower soil depth in the warming treatments under the continuous drought conditions of 2019. This indicates that the high marsh plants show belowground phenotypic plasticity in order to access water in deeper soil layers and that the reaction to warming is strongly mediated by soil moisture conditions, which in turn are mediated by precipitation and elevation.
3. The root biomass was zone-dependent. In the upper soil depth, root biomass was higher in the pioneer zone and low marsh than in the high marsh. Contrastingly, in the lower depth, the root biomass was highest in the low marsh, whereas it was comparably low in the pioneer zone and the high marsh. These differences of root biomass between depths indicate that the rooting depth is also zone-specific and probably influenced by soil factors (moisture, redox, nutrients, and salinity) and interspecific competition for space.

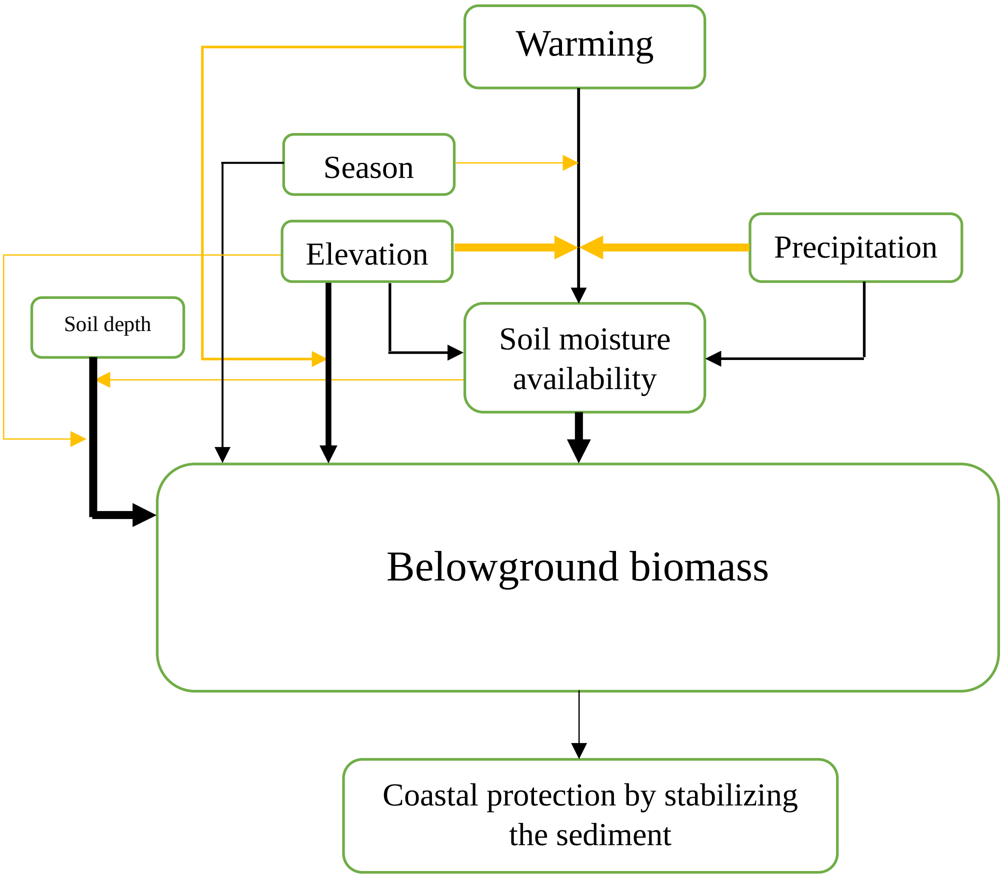

Fig. 5.1: How does warming alter belowground productivity? Bolder arrows indicate more important effects. Direct effects are indicated by black arrows, mediating (indirect) effects are displayed in yellow. The effect of warming on soil moisture is strongly mediated by precipitation patterns and elevation (zone, flooding), and less strongly mediated by season. The elevation is a proxy for flooding frequency and duration, species composition, and gradually changing soil factors (nutrients, redox potential, and salinity). The species composition and soil factors have a direct influence on root biomass and are mediated by warming. The effect of soil moisture availability becomes more important with increasing elevation. The influence of soil depth is very strong and mediated by soil moisture and by elevation (species and redox) as it differs between zones. The coastal protection function may not change due to warming.

Response of ecophysiological traits of plants to warming (Fig. 5.2; chapter 3)

1. Warming had significant effects on ecophysiological traits of salt marsh plants in all zones. However, the responses of traits showed interannual variations and were species-specific. In general, WUE of salt marsh species was more affected by temperature treatment than SLA. This indicates that the response to increasing temperatures may lead to shifts in species composition, depending on the plasticity and adaptability of single species within the community.
2. Generally, the low marsh species (*H. portulacoides*) and the high marsh species (*E. athericus*) showed drought responses under warming. Both increased their WUE under experimental warming in all years, as indicated by increasing $\delta^{13}C$ values, which was often mediated by seasonal effects. The pioneer zone species (*S. anglica*) showed a general decrease of $\delta^{13}C$ under experimental warming. Thus, the soil water availability seems to play an important role for the direction and intensity of the warming effect.
3. In the low marsh, the within-zone elevational gradient was an important factor for plant responses, as *P. maritima* showed a decreasing WUE with increasing elevation. Furthermore, the SLA of *H. portulacoides* changed along the elevational gradient in two years. This indicates that warming is not the main stressor for some marsh species, but rather soil properties connected with elevation. Additionally, these species show a high phenotypic plasticity, as they adapted their ecophysiological traits according to the environmental stressors.
4. Both WUE and SLA were strongly affected by seasonal changes. Season was a mediator of warming effects, but irrespective of warming, the ecophysiological traits were affected by seasonal changes and climatic factors as well. Therefore, the influence of up to +3 °C warming on physiological and metabolic processes during life cycle stages seems to be only marginal when compared to the adaptation to seasonal influence.

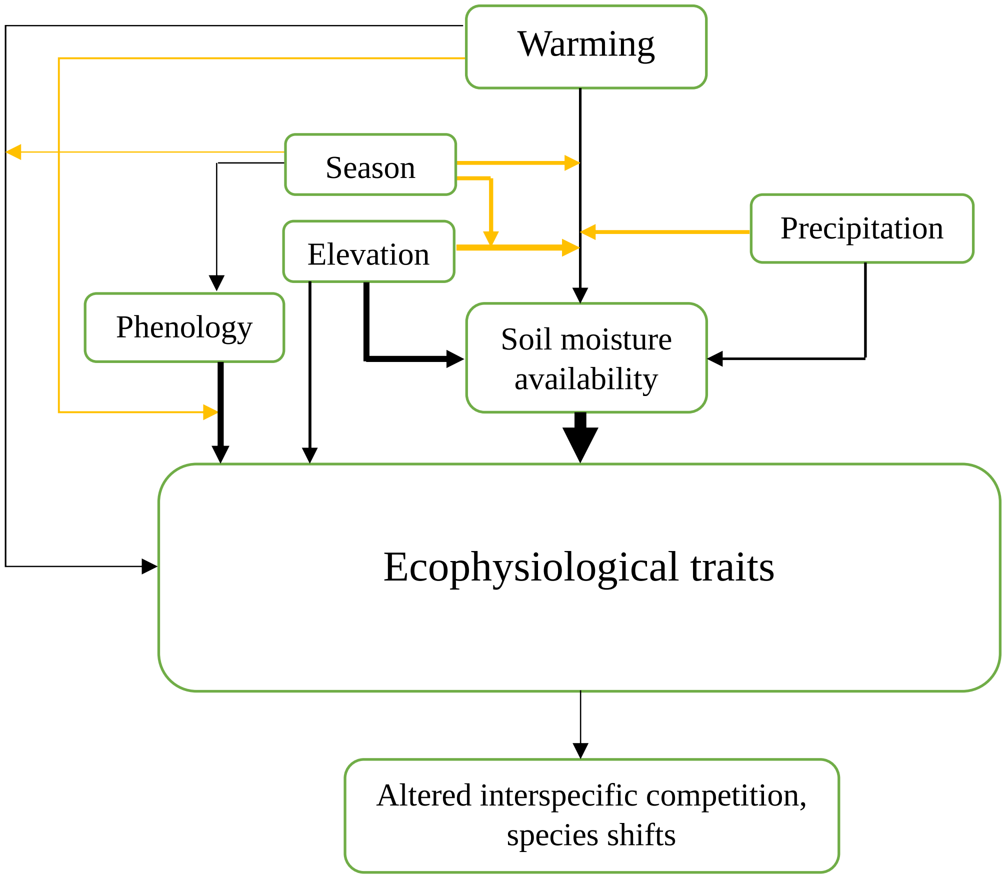

Fig. 5.2: Does warming lead to drought responses of ecophysiological traits as indicated by WUE and SLA? Bolder arrows indicate more important effects. Direct effects are indicated by black arrows, mediating (indirect) effects are displayed in yellow. Soil moisture availability has a strong influence on ecophysiological drought responses. The elevation has an important effect on soil moisture availability and mediates the direction of the ecophysiological response of plant traits to warming in different salt marsh zones. The elevation is a proxy for flooding frequency and duration, species composition, and gradually changing soil factors (nutrients, redox potential, salinity). As different species show different reactions, there is also a direct influence of elevation on responses of ecophysiological traits. The seasonal effect on the influence of warming is also a strong mediator on soil moisture availability. Season mediates both, the general change between seasons across zones, and the influence of elevation on the warming effect on soil moisture availability. The seasonal change of temperature also mediates the direct effect of warming on ecophysiological traits. Phenological processes affect ecophysiological traits of salt marsh species and are mediated by warming. Warming may alter interspecific competition, due to differing responses between species.

Seedling survival and recruitment under warming (Fig. 5.3; chapter 4)

1. Seedling emergence slightly increased under warming in the pioneer zone. In the high marsh, the decrease after peak seedling emergence was slightly faster due to warming. This indicates that warming has different impacts on seedlings depending on the zone.
2. The seedling Shannon diversity and species richness was significantly higher under warming at peak seedling numbers in the low marsh. This may either be a result of increasing abiotic stress (drought) leading to a decrease in interspecific competition, or it may be an artifact of the seed abundance in the plots.
3. The percentage of recruited seedlings did not change due to temperature treatment and seedling survival was not significantly affected by experimental warming. There were trends which showed a decreased median survival probability under warming by one to two weeks under +3 °C and +1.5 °C, respectively. Therefore, warming may lead to a decrease in seedling survival due to an increase in drought conditions at higher elevations under increasing temperatures.
4. The survival probability was significantly affected by plant species irrespective of warming, and seedling numbers differed significantly between zones. Thus, the influence of changing soil conditions along the elevational gradient may be of great importance for seedling establishment. However, interspecific differences in recruitment may mainly arise due to differences in life-history traits.

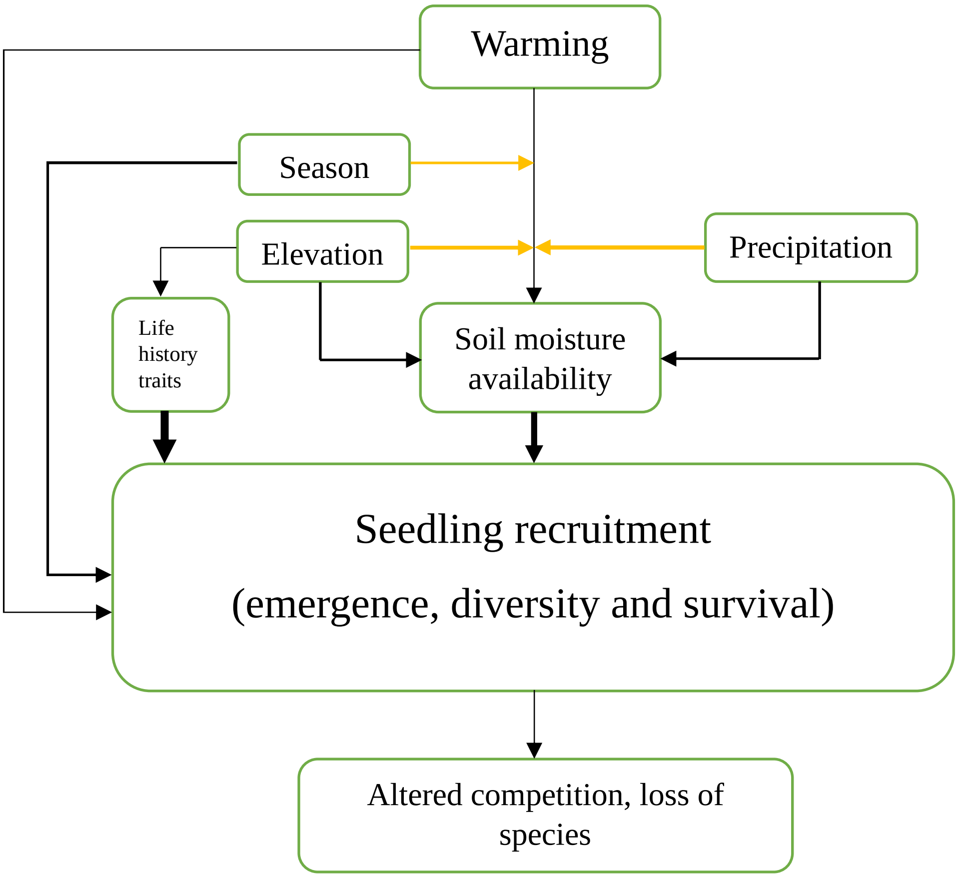

Fig. 5.3: How is seedling recruitment affected by warming? Bolder arrows indicate more important effects. Direct effects are indicated by black arrows, mediating (indirect) effects are displayed in yellow. The elevation is a proxy for flooding frequency and duration, species composition, seed availability, and gradually changing soil factors (nutrients, redox potential, salinity). The seedling emergence (numbers) is highly dependent on life history traits which are different between zones (elevation), and which also affect survival probability. Flooding frequency (elevation) and precipitation mediate the influence of warming on soil moisture conditions. Season has a mediating effect on the warming effect on soil moisture, indicated by a change in species richness and Shannon diversity of seedlings in early development and at peak vegetation season which may indicate shift in species competition. The direct effect of season is a decreasing number of emerging seedlings with increasing time. Warming may alter competition and lead to a loss of annual species mainly in the higher marsh zones.

5.2 The impact of warming on the performance of salt marsh plants

Plants are a key element of the salt marsh ecosystem. The performance of the plant affects vegetation performance, which determines how the ecosystem functions and which services it will provide. In this thesis, the influence of warming on plant response concerning the aspects of belowground biomass production, ecophysiological (drought) traits, and seedling recruitment was studied. These three aspects of plant performance show differences and similarities in their reaction to warming. In general, the influence of warming was strongest on ecophysiological traits of the salt marsh plants. In contrast, both seedling recruitment and root biomass were almost unaffected by warming. However, the effect of warming became more important when it affected or interacted with soil water availability. In general, warming may decrease soil moisture availability (Rustad et al. 2001) by directly increasing evapotranspiration (Wang et al. 2022), and by increasing the water demand of plants due to increased transpiration rates (Gates 1968). This indirect effect of warming on plant performance via soil water availability is one important factor which I will elaborate on. I will discuss it with respect to differences between salt marsh zones, species, and seasons. Furthermore, I will elaborate on why warming independent factors may have had a greater influence on salt marsh plant performance in some cases.

Plant performance differs between salt marsh zones due to changing soil moisture conditions mediated by warming

Based on the differences in inundation frequency and duration between Wadden Sea salt marsh zones, the influence of warming on soil moisture conditions is mediated by elevation. Although I did not measure soil moisture in my thesis, I assume that there is a decreasing soil water content with increasing elevation in general. After an intensive literature review on ‘soil water content’ and ‘soil moisture’ in salt marshes, I did not find any studies which have explicitly measured soil water content or soil moisture along an elevational gradient in NW European salt marshes in-situ. However, Bakker et al. (1985) measured the moisture content of soils along a dune to salt marsh gradient by the weight loss of soil samples at 105 °C after 24 h and found a clear increasing moisture content gradient from dunes to low marsh. Suchrow and Jensen (2010) showed that the elevational gradient of salt marshes in the North Sea is strongly associated with moisture. In their study, moisture was indirectly quantified for 2,691 plots by using the unweighted mean of Ellenberg´s indicator values for moisture (Ellenberg et al. 1992) as a surrogate for (soil) moisture per plot (Suchrow and Jensen 2010). Therefore, my assumption is based on 1) the direct correlation between elevation and flooding frequency, which is also confirmed by the species composition within each zone (Bockelmann et al. 2002; Silvestri et al. 2005; Suchrow and Jensen 2010), and the correlation between species composition and moisture availability (Suchrow and Jensen 2010). The species composition can be used as proxy for soil moisture because the species growing in each zone have different flooding tolerances (Yando et al. 2023). 2) I personally observed that the topsoil became increasingly dry during the year with increasing elevation.

We found a drought response of species in higher elevated marsh zones. The increased drought response was especially detectable in the response of ecophysiological traits and root biomass distribution in the high marsh. The high marsh plant *Elymus athericus* showed drought responses by an increased WUE due to warming in all years (chapter 3). I would assume that this response enables *E. athericus* (in most circumstances) to cope with warming-induced decreases in soil water availability without any negative effect, as there was no general increase in root biomass due to warming in the high marsh. However, warming paired with severe drought conditions, as we observed in the high marsh in 2019 (chapter 2), also lead to a shift in rooting depth. This was evident by a decrease of root biomass in the upper depth, with a simultaneous increase of root biomass in the lower depth. Therefore, an increase in WUE and deeper rooting to reach water in deeper soil layers underline the high potential of *Elymus athericus* to cope with (warming-induced) drought conditions and indicate a high phenotypic plasticity. It has been shown before that *Elymus athericus* is highly susceptible to hydrological changes, and that there are two genotypes (low marsh- and high marsh genotype) adapted to different flooding conditions (Mueller et al. 2021; Reents et al. 2021). Furthermore, the impact of flooding on biomass responses differs between the genotypes: while both show a similar reduction in belowground biomass with increased flooding frequency, the decrease in aboveground biomass of the high marsh genotype is greater (Reents et al. 2021). Our results show that low flooding frequency also impacts *Elymus athericus*. However, in case of drought conditions, root biomass does not increase in general (as we may expect), but shifts to lower soil depths. Additionally, the end-of-season aboveground biomass in the high marsh showed an increasing trend under warming in 2019 (Appendix, Fig. 5.1), which may indicate that warming stimulated high marsh aboveground productivity even under drought conditions. This result confirms Reents et al. (2022), who reported that *Elymus athericus* individuals were slightly taller after applying a drought treatment, suggesting an increased aboveground biomass.

In the low marsh, we also observed warming-induced responses of plant performance. WUE of *H. portulacoides* generally increased under warming (chapter 3), which was more pronounced in summer. Interestingly, in summer 2020, WUE decreased in this species due to warming. That summer had high precipitation amounts (Appendix chapter 3; Fig. S3.6), and the low marsh was flooded for a longer duration during the weeks before the measurements (Appendix, Fig. S5.2). Therefore, the response of ecophysiological traits of salt marsh plants to warming seems highly dependent on soil water availability (Fig. 5.2). Gedan and Bertness (2009) studied the effect of warming on WUE via $\delta^{13}C$ of two forb panne species in an open-top chambers experiment in a New England salt marsh. Both species showed a decreased $\delta^{13}C$ under warming (Gedan and Bertness 2009), which indicates a decreased WUE (Farquhar et al. 1982, 1989). As the forb panne habitat is consistently waterlogged, there is no water limitation, hence they explained their results by a less efficient water use (where WUE decreases) due to increasing transpiration under warming (Gedan and Bertness 2009). Our results for *H. portulacoides* support this finding, as warming paired with potentially high soil water availability led to a decrease in WUE.

In contrast to the high marsh, we found a slight decrease of root biomass in both depths of the low marsh in 2019, but it was more pronounced in the lower depth. As low marsh flooding occurred occasionally during the vegetation period, plants may not have experienced drought stress to the same extent. Nevertheless, rooting depth may have changed due to warming-induced changes in soil properties (Charles and Dukes 2009; Noyce et al. 2019; Tang et al. 2023). Interestingly, this trend diminished in the following years. One explanation could be gradual acclimation of these species to warming (Quan et al. 2020). Another possibility may be, that climatic differences (e.g. precipitation, ambient temperature) between years could have affected rooting depth, as seen in the high marsh. Since we did not distinguish between species, this may also be an indication of a shift in rooting depth by one species, followed by the occupation of this soil depth by another. Therefore, warming may lead to alterations of belowground interspecific competition for space.

In the pioneer zone, we found responses of plant performance as well. There was a trend of increased root biomass under warming in all study years in the upper depth (7-9 cm) and $\delta^{13}C$ of *S. anglica* generally decreased with warming. This may be interpreted as a direct result of higher transpiration in warmed plots, leading to reduced WUE under waterlogging (Gedan and Bertness 2009). However, translating $\delta^{13}C$ into WUE of C4 plants like *S. anglica* is not as straightforward as with C3 plants (von Caemmerer et al. 2014), because the discrimination of PEPC is much smaller as from Rubisco (Farquhar et al. 1989; von Caemmerer et al. 2014). Furthermore, the biochemical composition and allocation of compounds is thought to be of higher importance for the isotopic signature of C4 plants, as the different organic compounds show different isotopic enrichments or depletions compared to bulk leaf signature (von Caemmerer et al. 2014). Therefore, and in contrast to the results of the high- and low marsh, the responses in the pioneer zone may not be affected by low soil water availability, but may indicate a decrease of waterlogging stress under warming (Gedan and Bertness 2009), or may also be affected by stress related to other soil factors like salinity or nutrient availability (Bowman et al. 1989; Farquhar et al. 1989; von Caemmerer et al. 2014; Tang et al. 2023; Noyce et al. 2019).

The overall lack of significant changes in root biomass due to warming across zones, seasons, and years was unexpected (chapter 2). One possible explanation for this unexpected result is that the response might be non-linear. A warming experiment on the US Atlantic coast, which also warms the soil (Noyce et al. 2019; Smith et al. 2022), showed that root biomass response to warming was non-linear and showed similar values between ambient temperatures and +3.4 °C warming (Noyce et al. 2019). However, in an intermediate treatment (+1.7 °C), there was an increase in root-to-shoot ratio. Since we did not measure the intermediate treatment response in our experiment (+1.5 °C) for root biomass, we may have missed the identification of similar effects. Noyce et al. (2019) also reported that between +1.7 and +3.4 °C warming increases N mineralization, providing the plants with sufficient N supply to shift their biomass allocation aboveground. The aboveground biomass data of the low marsh (Appendix, Fig. S5.1) indicates similar biomass values in ambient +3 °C treatments, but in the +1.5 °C treatment, the biomass is lower. Which may indicate an increased belowground biomass allocation under +1.5 °C, as in the study of Noyce et al. (2019). However, these results remain to be statistically tested. Furthermore, it is important to consider that the salt marshes of the US Atlantic coast and the Wadden Sea are different in terms of soil conditions. The soils at the US Atlantic coast contain more organic carbon and are wetter (Niering 1997; Yando et al 2023), which may lead to differences in N mineralization alterations due to warming. However, the study of Tang et al. (2023) suggests that the organic matter decomposition rate increased due to warming in Wadden Sea salt marshes, which may also indicate an increased N mineralization.

To synthesize the findings regarding root biomass and ecophysiologal traits with respect to zone-warming interactions, salt marsh plants of the higher marsh zones seem to cope with the effect of warming on decreasing soil water availability by first adjusting their WUE, and then only showing other responses like increasing the rooting depth under serious drought conditions. The main influence of warming in the pioneer zone was a decrease in $\delta^{13}C$, which either indicates a decreasing WUE under warming and waterlogged soils (Gedan and Bertness 2009), or it may be an implication for a change in biochemical composition of leaves (von Caemmerer et al. 2014) as a direct reaction to warming or induced by other stressors like salinity.

Zone-specific plant performance

In general, and irrespective of warming, we found differences between salt marsh zones which most likely originate from both abiotic conditions and biotic interactions. The root biomass, for example, was mainly affected by salt marsh zone and did not significantly change under warming conditions, except in combination with drought (see above). Seedling emergence was also strongly affected by salt marsh zone (chapter 4).

Root biomass pooled over depths was highest in the low marsh compared to pioneer zone or high marsh, which is in line with (Redelstein et al. 2018a), who found a higher fine root biomass in the low marsh than in both pioneer zone and high marsh. In the upper depth, however, root biomass was similar between pioneer zone and low marsh. A higher belowground biomass in the upper soil depth may be important for the pioneer zone species to ensure safe anchorage in the soil and to avoid the permanent stress of anoxia in deeper layers. In the low marsh, species show belowground space partitioning, as indicated by a higher root biomass in the lower depth. This can be interpreted as both competitive and facilitative behavior because the partitioning of space leads to the use of more resources (also known as the complementary effect) and is regarded as one major mechanism of positive biodiversity-ecosystem functioning relations (Loreau and Hector 2001). In the high marsh, roots are mainly limited to the topsoil, which probably helps to efficiently use water supplied by precipitation or occasional flooding, but under drought conditions, they can shift to deeper soil layers to reach for water (chapter 2). Therefore, the differences between salt marsh root biomass are highly affected by the different adaptations to soil (moisture) conditions of the species within each zone (Bouma et al. 2001; Redelstein et al. 2018a).

Differences in seedling emergence are mainly affected by differences in life history traits between zones and the dominant species (Davy and Smith 1985). In the pioneer zone, 95 % of seedlings originated from *Salicornia europaea* agg., which is an annual and thus needs to complete its life cycle within one vegetation period. The dispersal strategy of *S. europaea* agg. is to produce many small seeds (Jefferies et al. 1981), which was reflected in the high emergence, and maximum seedling numbers we found in the pioneer zone (chapter 4). In the low marsh, we found seedlings originated from different species, but maximum seedling numbers were much lower than in the pioneer zone. Here, competition for space and light (Bakker et al 1985; Bakker and de Vries 1992) might prevent higher seedling emergence. In the high marsh, we only found the lowest seedling emergence in general, but the percentage of seedlings that survived was higher (~25 %) than in the other zones (low marsh 3.7 %; pioneer zone 6.3 %; not significant). The dominant species *E. athericus* is a clonal perennial with pronounced vegetative growth (Bockelmann and Neuhaus 1999). By this life-history and the associated lower dependence on regeneration by seed germination, the species avoids drought conditions in the seedling stage. The main emerging and surviving seedlings in the high marsh were of *Atriplex* sp., which produce large heavy seeds (Bakker and de Vries 1992; Moles and Westoby 2004) that are advantageous for seedling survival by providing more resources and increase survival probability under closed canopy (Gross and Werner 1982; Goldberg and Werner 1983).

Seasonal impacts of warming effects on plant performance

Temperature thresholds are important for all physiological and metabolic processes, especially for species growing in temperate regions (Baskin and Baskin 1988; Körner 2006). Here, seasonal temperature variations determine the initiation or inhibition of processes like germination, growth, and senescence. An increase in temperature in spring or autumn may change the length of the vegetation period, and thereby phenology. This may have major influences on the performance of plants, as it may affect reproduction and survival. Reents (2022) found a prolonged vegetation period in the MERIT experiment under warming by using NDVI sensors. This was most pronounced in the high marsh, but also an earlier green up in the pioneer zone was detected (Reents 2022). In chapter 4 of my thesis, we also observed a steeper increase in seedling numbers under warming in spring, as soon as the treatments were at full capacity. Unfortunately, the initial seedling emergence was not observed under warming treatments due to technical difficulties. I assume that the effect we detected would be much more pronounced if warming had started earlier in 2019. It has been shown before that germination of salt marsh species is highly temperature dependent, and that there are species which thrive better under warming (Ungar 1977; Egan and Ungar 1999; Martin 2017). However, an earlier germination may increase the risk of pioneer zone seedlings to experience storms and extreme flooding, which occur in early spring, and thus could alter recruitment success. Overall, with warming starting after initiation of germination, the survival of seedlings was not significantly affected by warming in both the low marsh and the high marsh (chapter 4). We found a slight reduction in survival probability under warming, but general seedling survival was low in all treatments, and seedling numbers peaked between April and May. This implies that soil water availability is an important factor for seedling survival, as the soils are not yet desiccated in spring. Germination and seedling establishment of salt marsh species depend on soil moisture and salinity conditions in the topsoil (Shumway and Bertness 1992; Noe and Zedler 2000). In contrast to mature plants, salt marsh seedlings probably cannot cope with the general increasing topsoil desiccation over the course of the season irrespective of warming, because of their shallow root systems.

Contrary to seedlings, mature salt marsh plants (species-dependent) seem to change their performance in response to the interacting effect of soil water availability and warming, depending on the season. The interacting effect of season and temperature treatment was most pronounced in the response of ecophysiological traits of salt marsh plants (chapter 3). Especially the plants of the higher marsh zones (*Elymus athericus* and *H. portulacoides*) showed seasonal variations regarding the influence of warming on their ecophysiological traits. But we also found a seasonal effect of warming on root biomass production under dry conditions in 2019 (chapter 2; Appendix chapter 2, Figs. S2.2, S2.4). In the high marsh, we found root biomass in the warming treatments did not decrease in spring, but decreased in summer and autumn in the upper depth in 2019. The opposite pattern was visible in the pioneer zone. While in spring, the root biomass in the upper depth was comparable to ambient treatments, in summer and autumn, it increased in the warming treatments in the pioneer zone. I already discussed the influence of zone in this matter, but this also shows the clear interacting effect of zone and season on root biomass in the upper depth in 2019 (chapter 2). And even though not statistically significant, it seems that warming also slightly interacted with the seasonal effects in different zones. I assume this contrasting effect of warming in these two zones is not strong in spring, when all zones have been flooded more regularly. However, in summer the soils desiccate under warming, which for the pioneer zone may lead to less anoxic conditions and hence an increase in root biomass. I speculate that warming minimizes the trade-off between rooting depth and soil anoxia in the pioneer zone. Continuous warming may lead to drought conditions in the high marsh, where the plants shift their rooting depth to deeper soil layers. Therefore, I want to emphasize that seasonal and climatic factors are also strong mediators of the soil water availability and thus of the warming response of salt marsh plants (Figs. 5.1 and 5.2).

To synthesize, the results of my thesis suggest that the adaptation to seasonality is stronger than the influence of +1.5 °C and +3.0 °C warming. While warming leads to plastic responses in salt marsh plants by increasing WUE and altering SLA in some species, general patterns of salt marsh plant performance were mostly affected by phenology. In general, root biomass was lower in spring and autumn and increased in summer. SLA was strongly affected by season, with generally higher SLA in spring and a decrease in summer and autumn, and warming did not change this general trend. Therefore, plant performance was less affected by warming than expected. However, with the expected increase of both temperatures and extreme weather events (IPCC 2023), the performance of salt marsh plants may change, as was indicated by an interacting effect of warming and drought conditions (chapters 2 and 3).

Salinity effects with respect to warming

Salinity of salt marsh soils is one important factor, which I did not discuss at length since I focused on soil moisture. However, I will shortly elaborate on how salinity might have influenced some of my results. In general, salinity is high in the pioneer zone, where soils are inundated daily, and decreases with elevation (Bockelmann and Neuhaus 1999; Suchrow and Jensen 2010). Warming might have increased salt accumulation in the less flooded soils (low marsh and high marsh), due to increased evapotranspiration. The effect of salt stress is important for ecophysiological responses (Rozema et al. 1985; Duarte et al. 2013) and plants react similarly to those under drought stress (Mahajan and Tuteja 2005). Therefore, the effect of warming and potential increases in soil salinity on salt marsh plants is difficult to disentangle without experimentally testing it. With respect to the results of my thesis, warming may have increased soil salinity, and therefore some of the plant responses may also partly be a reaction to increased salt stress. The responses of ecophysiological traits in the pioneer zone may be one example. Salt stress leads to similar mechanisms in plants as drought stress (Munns 2011). For example, the pioneer zone species *S. anglica* responded to possibly changing soil salinities under increased evapotranspiration with warming in summer and autumn (2020, 2021), by a decrease in SLA (and lower C/N), which may be an indication for lignification of cell walls due to osmotic stress (Rozema et al. 1985), and an investment in N-rich osmoprotectants (Flowers and Colmer 2008) to cope with salt stress.

5.3 Implications

Further knowledge gaps

The aspects of plant performance under warming, which were captured by the studies conducted for this thesis, imply that, in general, salt marsh plants in the Wadden Sea area may cope with warming of up to +3 °C. The colonisation of salt marshes by new individuals seems not to be affected by warming, as the survival and thus recruitment of seedlings was not significantly changed due to warming. However, adult individuals of different species showed different ecophysiological responses, indicating a possible advantage under warming for species with a higher plasticity. While these results suggest that salt marsh seedlings may not be affected by warming, it remains to be verified how and if warming may alter competition of mature salt marsh plants and thereby seed production or dispersal patterns. Warming lead to a rapid decline of species diversity in a US salt marsh (Gedan and Bertness 2009), which ultimately also alters dispersal, because dispersal is highly affected by adult plant abundance (Appendix chapter 4; Fig. S4.11; Rand 2000). Additionally, less individuals of a certain species produce less seeds in total. Furthermore, the viability of seeds may be impaired by stratification requirements (Walck et al. 2011), which should be studied by implementing winter warming treatments. Therefore, studies which capture Wadden Sea salt marsh species composition responses under warming are necessary to predict if some species may become outcompeted in the future. These studies should also aim to answer if biodiversity affects ecosystem services, such as the coastal protection function of salt marshes. A possible effect of species diversity on sediment stability was reported by Ford et al. (2016). Even though the results of my thesis showed that root biomass mostly did not change under warming, and thus sediment stability may also not change (de Battisti et al. 2019), the effect of biodiversity remains to be studied.

Accretion in salt marshes remains one of the most important aspects under global change scenarios in relation to sea-level rise. While the results of my thesis suggest that warming alone did not alter belowground biomass, I also presented preliminary evidence for an increased aboveground biomass due to warming, especially in the high marsh (Appendix,

Fig. S5.1). An increased aboveground biomass may alter accretion rates by sediment trapping (Fagherazzi et al. 2012; see also Schulze et al. 2022 for Hallig salt marshes close to the MERIT site), but also increase vulnerability to wave damage (Schoutens et al. 2021). Under current temperature conditions, dislodgement of salt-marsh plants has not been observed, but rather stem breakage was reported (Rupprecht et al. 2017; Schoutens et al. 2021; Reents et al. 2022). I recommend investigating the possible effect of higher aboveground biomass accumulation due to warming, with respect to possible changes of plant resistance (Charles and Dukes 2009; de Battisti et al. 2019, Schoutens et al. 2021; Reents et al. 2022) on sediment dynamics for a deeper understanding of future marsh development. Additionally, it is necessary to study root biomass along a continuous depth gradient and distinguish roots between species (especially in the low marsh). Because this would disentangle possible alterations of belowground interspecific competition due to warming by detecting patterns of root biomass under warming across a soil profile.

The response of single ecophysiological traits to warming, as investigated in this thesis ($\delta^{13}C$), indicates high- and low marsh species may perform well under higher temperatures because they adjust their WUE. A knowledge gap remains for the interpretation of the pioneer zone results, because it was difficult to make a statement without data on soil salinity and moisture content in the pioneer zone under warming conditions. For future studies, regarding ecophysiological trait responses of salt marsh plants to warming, I recommend including measurements on soil moisture content and salinity levels (especially in the pioneer zone). To better understand the response of $\delta^{13}C$ in C4 salt marsh species like *Spartina anglica,* it is also recommended to include analyses of biochemical properties under warming conditions (von Caemmerer et al. 2014). Such measurements would give insights on whether C4 salt marsh plants in waterlogged areas change their composition of organic material, and if this may be the reason why $\delta^{13}C$ decreased under warming.

Furthermore, climate warming scenarios predict a combination of increasing temperatures and elevated CO_2 (IPCC 2023), and it has been shown that plant performance responses differ between combined treatments of warming and CO_2 and single treatments (Naudts et al. 2013; Noyce et al. 2019). Experiments which combine these factors with natural processes of salt marshes are rare (Noyce et al. 2019; Smith et al. 2022), and in salt marshes with a high tidal amplitude and wave impact, like those studied in this thesis, are extremely difficult. Therefore, the research of this thesis gives an important baseline for the single warming effect on plant performance. This, combined with the results from warming × CO_2 studies in other systems or with warming × CO_2 mesocosm experiments at the Wadden Sea (Koop-Jakobsen and Dolch 2023), may help to predict future meso- to macrotidal salt marsh development under rising atmospheric CO_2 levels.

Conclusions

The work presented in my thesis is the first to report how combined aboveground and belowground warming influences plants in meso- to macrotidal salt marshes of the Wadden Sea under natural conditions. The results highlight the importance of studying different aspects of plant responses to warming, as we found minor responses of recruitment and root biomass, but demonstrated that warming affected salt marsh plants on a physiological level. Despite the different intensities of responses to warming, we observed one overarching similarity: the mediating effect of soil moisture. Thus, salt marsh plants seem to cope with warming according to their position along a hydrological gradient. It should be noted that the plastic responses of salt marsh plants, as indicated by an adjustment of WUE with warming, probably also account for interannual abiotic differences (temperature, salinity, flooding frequency) and thus may be a necessary adaptation. However, we were also able to demonstrate that warming and low water availability resulted in further plastic responses, indicated by root biomass shifts. Especially with respect to warming-induced drought conditions, the higher marsh zones were more affected than the pioneer zone. Climate change scenarios predict both accelerated sea-level rise and highly unpredictable precipitation patterns. Therefore, it remains unknown whether a potentially increasing aboveground biomass under warming, with the potential to trap more sediments and accelerate accretion, will enable salt marshes to keep pace with sea-level rise. Nevertheless, warming-induced drought conditions may increase at higher elevations, especially with low precipitation. The results of this thesis indicate a high phenotypic plasticity of certain studied salt marsh plants, which may enable them to cope with future warming of up to +3 °C. However, it remains unknown whether a possible competitive replacement of less well performing species under warming will lead to alterations of ecosystem functions and thus affect ecosystem services in the future.

Appendix

1. Aboveground biomass sampling

The aboveground biomass was harvested mid-September of each year (2019-2021). We harvested all standing biomass, by directly clipping above soil surface in three squares in the centre of the plots. In 2019 and 2020 the squares were 10×10 cm² in 2021 they were 20×20 cm². We took the samples to the laboratory and dried them at 70 °C for 48 h. Aboveground biomass (g m^{-2}) was estimated by taking the mean of the three samples per plot and extrapolating them to 1 m². Due to initial methodological conception, the aboveground biomass was not harvested in the low marsh in 2019.

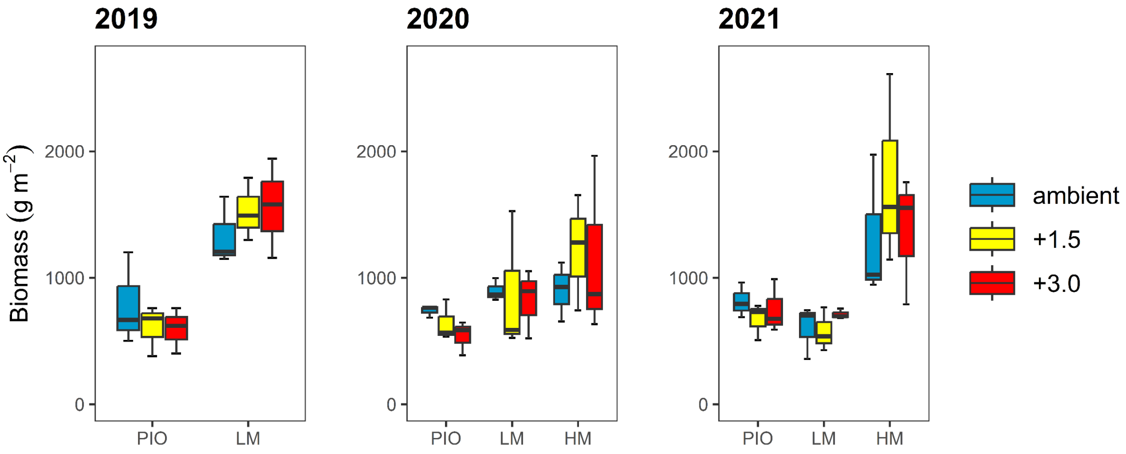

Fig. S5.1: End-of-season aboveground biomass for 2019-2021.

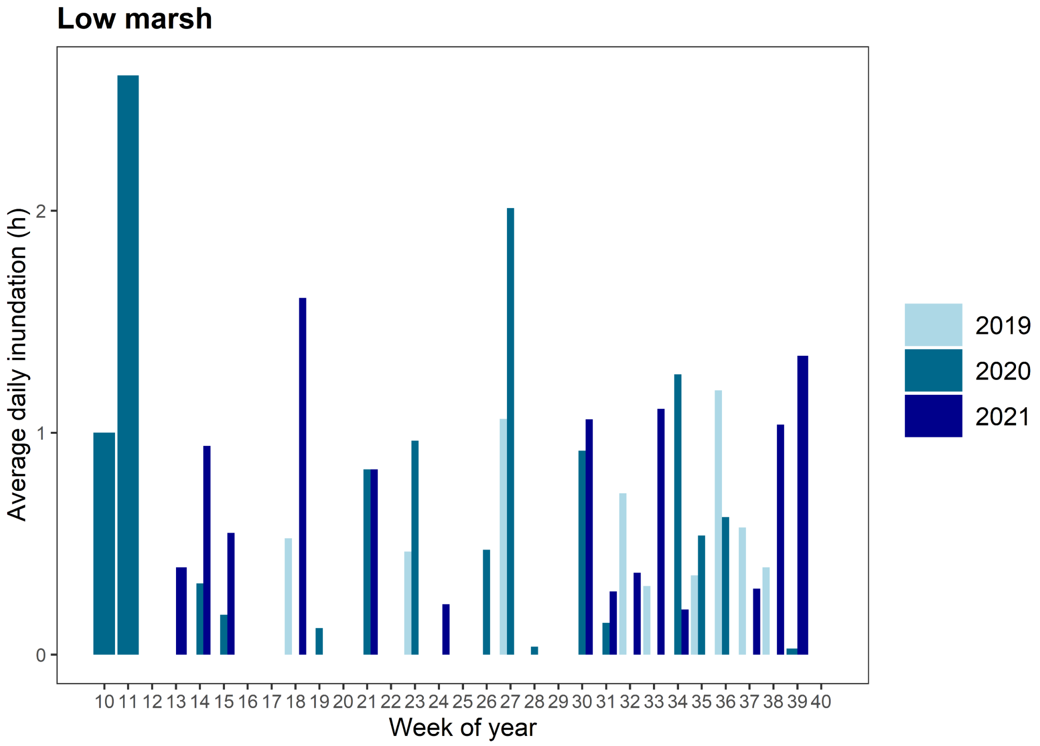

Fig. S5.2: Average daily inundation of the low marsh over the course of the vegetation period. Values are averaged over one week (week of year). Week 10 starts on March 4th, 2nd, 8th in 2019, 2020 and 2021, respectively.

References

Ackerly, D. D. (2004). Functional traits of chaparral shrubs in relation to seasonal water deficit and disturbance. *Ecological Monographs*, 74, 25–44.

Adam, P. (1993). *Saltmarsh ecology*. Cambridge University Press.

Adam, P. (2002). Saltmarshes in a time of change. *Environmental Conservation*, *29*(1), 39-61.

Adler, P. B., Leiker, J., & Levine, J. M. (2009). Direct and indirect effects of climate change on a prairie plant community. *PLoS One*, *4*(9), e6887.

Allen, J. R. (2000). Morphodynamics of Holocene salt marshes: a review sketch from the Atlantic and Southern North Sea coasts of Europe. *Quaternary Science Reviews, 19*(12), 1155-1231.

Armitage, A. R., Highfield, W. E., Brody, S. D., & Louchouarn, P. (2015). The contribution of mangrove expansion to salt marsh loss on the Texas Gulf Coast. *PloS one*, *10*(5), e0125404.

Atkin, O. K., Edwards, E. J., & Loveys, B. R. (2000). Response of root respiration to changes in temperature and its relevance to global warming. *New Phytologist*, *147*(1), 141-154.

Atkin, O. K., Loveys, B. R., Atkinson, L. J., & Pons, T. L. (2006a). Phenotypic plasticity and growth temperature: understanding interspecific variability. *Journal of Experimental Botany, 57*(2), 267-281.

Atkin, O. K., Scheurwater, I., & Pons, T. L. (2006b). High thermal acclimation potential of both photosynthesis and respiration in two lowland Plantago species in contrast to an alpine congeneric. *Global Change Biology*, *12*(3), 500-515.

Bai, W. M., Xia, J. Y., Wan, S. Q., Zhang, W. H., & Li, L. H. (2012). Day and night warming have different effect on root lifespan. *Biogeosciences, 9*(1), 375-384.

Bakker, J. P., Dijkstra, M., & Russchen, P. T. (1985). Dispersal, germination and early establishment of halophytes and glycophytes on a grazed and abandoned salt-marsh gradient. *New Phytologist*, *101*(2), 291-308.

Bakker, J. P., & De Vries, Y. (1992). Germination and early establishment of lower salt-marsh species in grazed and mown salt marsh. *Journal of Vegetation Science, 3*(2), 247-252.

Bakker, J. P., Bos, D., & De Vries, Y. (2003). To graze or not to graze: that is the question. In *Proceedings of the 10th International Scientific Wadden Sea Symposium* (Vol. 67). Ministry of Agriculture, Nature Management and Fisheries.

Baldwin, A. H., Jensen, K., & Schönfeldt, M. (2014). Warming increases plant biomass and reduces diversity across continents, latitudes, and species migration scenarios in experimental wetland communities. *Global Change Biology*, *20*(3), 835-850.

Balke, T., Bouma, T. J., Horstman, E. M., Webb, E. L., Erftemeijer, P. L., & Herman, P. M. (2011). Windows of opportunity: thresholds to mangrove seedling establishment on tidal flats. *Marine Ecology Progress Series, 440*, 1-9.

Balke, T., Webb, E. L., van den Elzen, E., Galli, D., Herman, P. M., & Bouma, T. J. (2013). Seedling establishment in a dynamic sedimentary environment: a conceptual framework using mangroves. *Journal of Applied Ecology*, *50*(3), 740-747.

Barbier, E. B., Hacker, S. D., Kennedy, C., Koch, E. W., Stier, A. C., & Silliman, B. R. (2011). The value of estuarine and coastal ecosystem services. *Ecological Monographs*, *81*(2), 169-193.

Bardgett, R. D., Mommer, L., & de Vries, F. T. (2014). Going underground: root traits as drivers of ecosystem processes. *Trends in Ecology & Evolution*, *29*(12), 692-699.

Baskin, C. C., & Baskin, J. M. (1988). Germination ecophysiology of herbaceous plant species in a temperate region. *American Journal of Botany*, *75*(2), 286-305.

Bender, M. M. (1971). Variations in the 13C/12C ratios of plants in relation to the pathway of photosynthetic carbon dioxide fixation. *Phytochemistry*, *10*(6), 1239-1244.

Bertness, M. D., & Leonard, G. H. (1997). The role of positive interactions in communities: Lessons from intertidal habitats. *Ecology*, 78(7), 1976–1989.

Bloom, A. J., Chapin III, F. S., & Mooney, H. A. (1985). Resource limitation in plants-an economic analogy. *Annual review of Ecology and Systematics*, *16*(1), 363-392.

Bockelmann, A. C., Bakker, J. P., Neuhaus, R., & Lage, J. (2002). The relation between vegetation zonation, elevation and inundation frequency in a Wadden Sea salt marsh. *Aquatic Botany*, *73*(3), 211-221.

Bockelmann, A. C., & Neuhaus, R. (1999). Competitive exclusion of Elymus athericus from a high-stress habitat in a European salt marsh. *Journal of Ecology*, 87(3), 503–513.

Bolle, L. J., Neudecker, T., Vorberg, R., Damm, U., Diederichs, B., Jager, Z. et al. (2009). Trends in Wadden Sea Fish Fauna. Part I: Trilateral Cooperation. With assistance of Wageningen IMARES. Dutch Ministry of Agriculture, Nature and Food Quality (LNV) (C108/08).

Boorman, L. A. (1967). Limonium Vulgare Mill. and L. Humile Mill. *Journal of Ecology*, 55(1), 221-232.

Boschker, H. T. S., De Brouwer, J. F. C., & Cappenberg, T. E. (1999). The contribution of macrophyte-derived organic matter to microbial biomass in salt-marsh sediments: Stable carbon isotope analysis of microbial biomarkers. *Limnology and Oceanography*, *44*(2), 309-319.

Bouma, T. J., de Vries, M., Low, E., Kusters, L., Herman, P. M. J., Tanczos, I. C., ... & Regenmortel, S. V. (2005). Flow hydrodynamics on a mudflat and in salt marsh vegetation: identifying general relationships for habitat characterisations. *Hydrobiologia*, *540*, 259-274.

Bouma, T. J., de Vries, M., & Herman, P. M. (2010). Comparing ecosystem engineering efficiency of two plant species with contrasting growth strategies. *Ecology*, *91*(9), 2696-2704.

Bouma, T. J., Friedrichs, M., Klaassen, P., Van Wesenbeeck, B. K., Brun, F. G., Temmerman, S., ... & Herman, P. M. J. (2009a). Effects of shoot stiffness, shoot size and current velocity on scouring sediment from around seedlings and propagules. *Marine Ecology Progress Series*, *388*, 293-297.

Bouma, T. J., Friedrichs, M., Van Wesenbeeck, B. K., Temmerman, S., Graf, G., & Herman, P. M. J. (2009b). Density-dependent linkage of scale-dependent feedbacks: A flume study on the intertidal macrophyte *Spartina anglica*. *Oikos*, *118*(2), 260-268.

Bouma, T. J., Nielsen, K. L., Van Hal, J., & Koutstaal, B. (2001). Root system topology and diameter distribution of species from habitats differing in inundation frequency. *Functional Ecology*, *15*(3), 360-369.

Bouma, T. J., van Belzen, J., Balke, T., Van Dalen, J., Klaassen, P., Hartog, A. M., ... & Herman, P. M. J. (2016). Short-term mudflat dynamics drive long-term cyclic salt marsh dynamics. *Limnology and Oceanography*, *61*(6), 2261-2275.

Bowman, W. D., Hubick, K. T., von Caemmerer, S., & Farquhar, G. D. (1989). Short-term changes in leaf carbon isotope discrimination in salt-and water-stressed C4 grasses. *Plant Physiology*, *90*(1), 162-166.

Brooks, A., & Farquhar, G. D. (1985). Effect of temperature on the CO 2/O 2 specificity of ribulose-1, 5-bisphosphate carboxylase/oxygenase and the rate of respiration in the light: Estimates from gas-exchange measurements on spinach. *Planta*, *165*, 397-406.

Brooks, M. E., Kristensen, K. van Benthem, K. J., Magnusson, A. Berg, C. W., Nielsen, A., Skaug, H. J., Mächler, M., & Bolker, B. M. (2017). glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modelling. *The R Journal*, *9*(2), 378-400.

Cahoon, D. R., McKee, K. L., & Morris, J. T. (2021). How plants influence resilience of salt marsh and mangrove wetlands to sea-level rise. *Estuaries and Coasts*, *44*(4), 883-898.

Camill, P. (2010). Global change. *Nature Education Knowledge*, *3*(10).

Cao, H., Zhu, Z., Balke, T., Zhang, L., & Bouma, T. J. (2018). Effects of sediment disturbance regimes on Spartina seedling establishment: Implications for salt marsh creation and restoration. Limnology and Oceanography, 63(2), 647–659.

Cao, H., Zhu, Z., van Belzen, J., Gourgue, O., van de Koppel, J., Temmerman, O. S., ... & Bouma, T. J. (2021). Salt marsh establishment in poorly consolidated muddy systems: effects of surface drainage, elevation, and plant age. *Ecosphere*, *12*(9), e03755.

Carey, J. C., Kroeger, K. D., Zafari, B., & Tang, J. (2018). Passive experimental warming decouples air and sediment temperatures in a salt marsh. *Limnology and Oceanography: Methods, 16*(10), 640–648.

Castro-Diez, P., Villar-Salvador, P., Pérez-Rontomé, C., Maestro-Martínez, M., & Montserrat-Martí, G. (1997). Leaf morphology and leaf chemical composition in three Quercus (Fagaceae) species along a rainfall gradient in NE Spain. *Trees*, 11, 127-134.

Cernusak, L. A., Ubierna, N., Winter, K., Holtum, J. A., Marshall, J. D., & Farquhar, G. D. (2013). Environmental and physiological determinants of carbon isotope discrimination in terrestrial plants. *New Phytologist*, *200*(4), 950-965.

Chapin III, F. S., Shaver, G. R., Giblin, A. E., Nadelhoffer, K. J., & Laundre, J. A. (1995). Responses of arctic tundra to experimental and observed changes in climate. *Ecology, 76*(3), 694-711.

Chapman, V. J. (1950). Halimione Portulacoides (L.) Aell. *Journal of Ecology, 38*(1), 214-222.

Charles, H., & Dukes, J. S. (2009). Effects of warming and altered precipitation on plant and nutrient dynamics of a New England salt marsh. *Ecological Applications*, 19(7), 1758–1773.

Chen, X., Zhang, X., Church, J. A., Watson, C. S., King, M. A., Monselesan, D., ... & Harig, C. (2017). The increasing rate of global mean sea-level rise during 1993–2014. *Nature Climate Change*, *7*(7), 492-495.

Chmura, G. L. (2013). What do we need to assess the sustainability of the tidal salt marsh carbon sink? *Ocean & Coastal Management*, *83*, 25-31.

Chmura, G. L., Anisfeld, S. C., Cahoon, D. R., & Lynch, J. C. (2003). Global carbon sequestration in tidal, saline wetland soils. *Global Biogeochemical Cycles*, *17*(4).

Coldren, G. A., Barreto, C. R., Wykoff, D. D., Morrissey, E. M., Langley, J. A., Feller, I. C., & Chapman, S. K. (2016). Chronic warming stimulates growth of marsh grasses more than mangroves in a coastal wetland ecotone. *Ecology,* 97(11), 3167–3175.

Cordeiro, A. L., Norby, R. J., Andersen, K. M., Valverde-Barrantes, O., Fuchslueger, L., Oblitas, E., ... & Quesada, C. A. (2020). Fine-root dynamics vary with soil depth and precipitation in a low-nutrient tropical forest in the Central Amazonia. *Plant-Environment Interactions*, *1*(1), 3.

Crawley, M. J. (2007). The R Book. John Wiley.

Crosby, S. C., Sax, D. F., Palmer, M. E., Booth, H. S., Deegan, L. A., Bertness, M. D., & Leslie, H. M. (2016). Salt marsh persistence is threatened by predicted sea-level rise. *Estuarine, Coastal and Shelf Science*, *181*, 93-99.

Davidson, N. C., & Finlayson, C. M. (2018). Extent, regional distribution and changes in area of different classes of wetland. *Marine and Freshwater Research*, *69*(10), 1525-1533.

Davidson, N. C., & Finlayson, C. M. (2019). Updating global coastal wetland areas presented in Davidson and Finlayson (2018). *Marine and Freshwater Research*, *70*(8), 1195-1200.

Davis, F. W., & others. (2016). Shrinking windows of opportunity for oak seedling establishment in southern California mountains. *Ecosphere*, 7(11), e01573.

Davy, A. J., Brown, M. J., Mossman, H. L., & Grant, A. (2011). Colonization of a newly developing salt marsh: disentangling independent effects of elevation and redox potential on halophytes. *Journal of Ecology*, *99*(6), 1350-1357.

Davy, A. J., & Smith, H. (1985). Population differentiation in the life-history characteristics of salt-marsh annuals. *Vegetatio*, 61(1/3), 117–125.

de Battisti, D., Fowler, M. S., Jenkins, S. R., Skov, M. W., Rossi, M., Bouma, T. J., ... & Griffin, J. N. (2019). Intraspecific root trait variability along environmental gradients affects salt marsh resistance to lateral erosion. *Frontiers in Ecology and Evolution*, *7*, 150.

de Dios, V. R., Weltzin, J. F., Sun, W., Huxman, T. E., & Williams, D. G. (2012). Windows of opportunity for Prosopis velutina seedling establishment and encroachment in a semiarid grassland. Perspectives in Plant Ecology, Evolution and Systematics, 14(4), 275-282.

Deegan, L. A., Johnson, D. S., Warren, R. S., Peterson, B. J., Fleeger, J. W., Fagherazzi, S. & Wollheim, W. M. (2012). Coastal eutrophication as a driver of salt marsh loss. *Nature*, *490*(7420), 388-392.

de Leeuw, J., de Munck, W., Olff, H., & P Bakker, J. (1993). Does zonation reflect the succession of salt-marsh vegetation? A comparison of an estuarine and a coastal bar island marsh in The Netherlands. *Acta Botanica Neerlandica*, *42*(4), 435-445.

de Leeuw, J., Olff, H., & Bakker, J. P. (1990). Year-to-year variation in peak above-ground biomass of six salt-marsh angiosperm communities as related to rainfall deficit and inundation frequency. *Aquatic Botany*, *36*(2), 139-151.

Donat, M. G., Renggli, D., Wild, S., Alexander, L. V., Leckebusch, G. C., & Ulbrich, U. (2011). Reanalysis suggests long-term upward trends in European storminess since 1871. *Geophysical Research Letters*, *38*(14).

Doody J.P. (2008). Saltmarsh conservation, management and restoration. Springer. 217 pp.

Doughty, C. L., Langley, J. A., Walker, W. S., Feller, I. C., Schaub, R., & Chapman, S. K. (2016). Mangrove range expansion rapidly increases coastal wetland carbon storage. *Estuaries and Coasts*, *39*, 385-396.

Duarte, C. M. (2014). Global change and the future ocean: a grand challenge for marine sciences. *Frontiers in Marine Science*, *1*, 63.

Duarte, C. M., Middelburg, J. J., & Caraco, N. (2005). Major role of marine vegetation on the oceanic carbon cycle. *Biogeosciences*, *2*(1), 1-8.

Duarte, B., Santos, D., Marques, J. C., & Caçador, I. (2013). Ecophysiological adaptations of two halophytes to salt stress: photosynthesis, PS II photochemistry and anti-oxidant feedback–implications for resilience in climate change. *Plant Physiology and Biochemistry, 67*, 178-188.

Dukes, J. S., & Mooney, H. A. (1999). Does global change increase the success of biological invaders? *Trends in Ecology & Evolution, 14*(4), 135-139.

Dukes, J. S., Pontius, J., Orwig, D., Garnas, J. R., Rodgers, V. L., Brazee, N., ... & Ayres, M. (2009). Responses of insect pests, pathogens, and invasive plant species to climate change in the forests of northeastern North America: what can we predict? *Canadian Journal of Forest Research, 39*(2), 231-248.

Egan, T. P., & Ungar, I. A. (1999). The effects of temperature and seasonal change on the germination of two salt marsh species, Atriplex prostrata and Salicornia europaea, along a salinity gradient. *International Journal of Plant Sciences*, 160(5), 861–867.

Ellenberg, H., Weber, H.E., Düll, R., Wirth, V., Werner, W., Paulissen, D. (1992). Zeigerwerte von Pflanzen in Mitteleuropa, 2nd edn. *Scripta Geobotanica* 18:1–258. Verlag Erich Goltze, Göttingen

Elschot, K., Bakker, J. P., Temmerman, S., van de Koppel, J., & Bouma, T. J. (2015). Ecosystem engineering by large grazers enhances carbon stocks in a tidal salt marsh. *Marine Ecology Progress Series, 537*, 9-21.

Erchinger, H. F. (1985). Dünen, Watt und Salzwiesen. *Das Niedersächsische Ministerium für Ernährung, Landwirtschaft und Forsten, Hannover, DE.*

Eriksson, O., & Fröborg, H. (1996). "Windows of opportunity" for recruitment in long-lived clonal plants: Experimental studies of seedling establishment in Vaccinium shrubs. *Canadian Journal of Botany*, 74(9), 1369-1374.

Esselink P., van Duin W.E., Bunje J., Cremer J., Folmer E.O., Frikke J., Glahn M., de Groot A.V., Hecker N., Hellwig U., Jensen K., Körber P., Petersen J. & Stock M. (2017) *Salt marshes*. In: Wadden Sea Quality Status Report. Eds.: Kloepper S. et al., Common Wadden Sea Secretariat, Wilhelmshaven, Germany. Last updated 21.12.2017. Downloaded *15.07.2023* qsr.waddensea-worldheritage.org/reports/saltmarshes

Esselink, P., Zijlstra, W., Dijkema, K. S., & van Diggelen, R. (2000). The effects of decreased management on plant-species distribution patterns in a salt marsh nature reserve in the Wadden Sea. *Biological Conservation, 93*(1), 61-76.

Essink, K., Dettmann, C., Frake, H., Laursen, K., Lüerßen, G., & Wiersinga, W. A. (2005). *Wadden Sea quality status report 2004* (No. 19). Common Wadden Sea Secretariat.

Eziz, A., Yan, Z., Tian, D., Han, W., Tang, Z., & Fang, J. (2017). Drought effect on plant biomass allocation: A meta-analysis. *Ecology and Evolution, 7*(24), 11002-11010.

Fagherazzi, S., Kirwan, M. L., Mudd, S. M., Guntenspergen, G. R., Temmerman, S., D'Alpaos, A., ... & Clough, J. (2012). Numerical models of salt marsh evolution: Ecological, geomorphic, and climatic factors. *Reviews of Geophysics*, *50*(1).

Farquhar, G. D., Ball, M. C., Von Caemmerer, S., & Roksandic, Z. (1982). Effect of salinity and humidity on δ 13 C value of halophytes—evidence for diffusional isotope fractionation determined by the ratio of intercellular/atmospheric partial pressure of CO 2 under different environmental conditions. *Oecologia*, *52*, 121-124.

Farquhar, G. D., Ehleringer, J. R., & Hubick, K. T. (1989). Carbon isotope discrimination and photosynthesis. *Annual Review of Plant Biology*, *40*(1), 503-537.

Farquhar, G. D., & Richards, R. A. (1984). Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes. *Functional Plant Biology*, *11*(6), 539-552.

Farquhar, G. D., & Sharkey, T. D. (1982). Stomatal conductance and photosynthesis. *Annual Review of Plant Physiology*, *33*(1), 317-345.

Flowers, T. J., & Colmer, T. D. (2008). Salinity tolerance in halophytes. *New Phytologist*, 945-963.

Ford, H., Garbutt, A., Ladd, C., Malarkey, J., & Skov, M. W. (2016). Soil stabilization linked to plant diversity and environmental context in coastal wetlands. *Journal of Vegetation Science*, *27*(2), 259-268.

Fox, J., & Weisberg, S. (2019). An {R} Companion to Applied Regression, Third Edition. Thousand Oaks CA: Sage. URL:https://socialsciences.mcmaster.ca/jfox/Books/Companion/

Friess, D. A., Krauss, K. W., Horstman, E. M., Balke, T., Bouma, T. J., Galli, D., & Webb, E. L. (2012). Are all intertidal wetlands naturally created equal? Bottlenecks, thresholds and knowledge gaps to mangrove and saltmarsh ecosystems. *Biological Reviews*, *87*(2), 346-366.

Friess, D. A., Yando, E. S., Alemu, J. B., Wong, L. W., Soto, S. D., & Bhatia, N. (2020). Ecosystem services and disservices of mangrove forests and salt marshes. *Oceanography and Marine Biology*.

Gates, D. M. (1968). Transpiration and leaf temperature. *Annual Review of Plant Physiology*, *19*(1), 211-238.

Gedan, K. B., Altieri, A. H., & Bertness, M. D. (2011). Uncertain future of New England salt marshes. *Marine Ecology Progress Series*, *434*, 229-237.

Gedan, K. B., & Bertness, M. D. (2009). Experimental warming causes rapid loss of plant diversity in New England salt marshes. *Ecology Letters*, 12(8), 842–848.

Gedan, K. B., & Bertness, M. D. (2010). How will warming affect the salt marsh foundation species Spartina patens and its ecological role? *Oecologia*, 164(2), 479–487.

Ghannoum, O., Von Caemmerer, S., & Conroy, J. P. (2002). The effect of drought on plant water use efficiency of nine NAD–ME and nine NADP–ME Australian C4 grasses. *Functional Plant Biology*, *29*(11), 1337-1348.

Goldberg, D. E., & Werner, P. A. (1983). The effects of size of opening in vegetation and litter cover on seedling establishment of goldenrods (Solidago spp.). *Oecologia*, 60, 149-155.

Gong, X. Y., Chen, Q., Lin, S., Brueck, H., Dittert, K., Taube, F., & Schnyder, H. (2011). Tradeoffs between nitrogen-and water-use efficiency in dominant species of the semiarid steppe of Inner Mongolia. *Plant and Soil*, 340, 227-238.

Granse, D., Suchrow, S., & Jensen, K. (2021). Long-term invasion dynamics of Spartina increase vegetation diversity and geomorphological resistance of salt marshes against sea level rise. *Biological Invasions*, *23*(3), 871-883.

Granse, D., Titschack, J., Ainouche, M., Jensen, K., & Koop-Jakobsen, K. (2022). Subsurface aeration of tidal wetland soils: Root-system structure and aerenchyma connectivity in Spartina (Poaceae). *Science of The Total Environment*, *802,* 149771.

Graves, S., Piepho, H. P., & Selzer, L. (2019). With help from Sundar Dorai-Raj. Multcompview: Visualizations of Paired Comparisons. R Package Version 0.1-8.

Gray, A. J., & Scott, R. (1977). Puccinellia maritima (Huds.) Parl.: (Poa maritima Huds.; Glyceria maritima (Huds.) Wahlb.). *Journal of Ecology*, *65*(2), 699-716.

Grey, A., Costeira, R., Lorenzo, E., O’Kane, S., McCaul, M. V., McCarthy, T., ... & Kelleher, B. P. (2023). Geochemical properties of blue carbon sediments through an elevation gradient: study of an anthropogenically impacted coastal lagoon. *Biogeochemistry*, *162*(3), 381-408.

Grime, J. P. (1977). Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *The American Naturalist*, *111*(982), 1169-1194.

Grolemund, G., & Wickham, H. (2011). Dates and times made easy with lubridate. *Journal of Statistical Software*, 40, 1-25.

Gross, K. L., & Werner, P. A. (1982). Colonizing abilities of 'biennial' plant species in relation to ground cover: Implications for their distributions in a successional sere. *Ecology*, 63(4), 921-931.

Gunderson, C. A., O'hara, K. H., Campion, C. M., Walker, A. V., & Edwards, N. T. (2010). Thermal plasticity of photosynthesis: the role of acclimation in forest responses to a warming climate. *Global Change Biology*, *16*(8), 2272-2286.

Guo, H., Zhang, Y., Lan, Z., & Pennings, S. C. (2013). Biotic interactions mediate the expansion of black mangrove (*Avicennia germinans*) into salt marshes under climate change. *Global Change Biology*, *19*(9), 2765-2774.

Guy, R. D., Reid, D. M., & Krouse, H. R. (1986). Factors affecting 13C/12C ratios of inland halophytes. I. Controlled studies on growth and isotopic composition of Puccinellia nuttalliana. *Canadian Journal of Botany, 64*(11), 2693-2699.

Hagedorn, F., Joseph, J., Peter, M., Luster, J., Pritsch, K., Geppert, U., ... & Arend, M. (2016). Recovery of trees from drought depends on belowground sink control. *Nature Plants*, *2*(8), 1-5.

Hartig, F. (2021). DHARMa: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models. R package version 0.4.4. https://CRAN.R-project.org/package=DHARMa

Henery, M. L., & Westoby, M. (2001). Seed mass and seed nutrient content as predictors of seed output variation between species. *Oikos*, *92*(3), 479-490.

Hobbie, E., & Werner, R. A. (2004). Intramolecular, compound-specific, and bulk carbon isotope patterns in C3 and C4 plants: a review and synthesis. *New Phytologist*, *161*(2), 371-385.

Hooper, D. U., Chapin III, F. S., Ewel, J. J., Hector, A., Inchausti, P., Lavorel, S., ... & Wardle, D. A. (2005). Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecological Monographs*, *75*(1), 3-35.

Hopple, A. M., Wilson, R. M., Kolton, M., Zalman, C. A., Chanton, J. P., Kostka, J., ... & Bridgham, S. D. (2020). Massive peatland carbon banks vulnerable to rising temperatures. *Nature Communications*, *11*(1), 2373.

Hothorn, T., Bretz, F., & Westfall, P. (2008). Simultaneous inference in general parametric models. *Biometrical Journal: Journal of Mathematical Methods in Biosciences*, 50(3), 346-363.

Howison, R. A., Olff, H., Steever, R., & Smit, C. (2015). Large herbivores change the direction of interactions within plant communities along a salt marsh stress gradient. *Journal of Vegetation Science*, *26*(6), 1159-1170.

Hu, Z., van Belzen, J., van der Wal, D., Balke, T., Wang, Z. B., Stive, M., & Bouma, T. J. (2015). Windows of opportunity for salt marsh vegetation establishment on bare tidal flats: The importance of temporal and spatial variability in hydrodynamic forcing. *Journal of Geophysical Research: Biogeosciences, 120*(7), 1450-1469.

Hu, Z., & others. (2021). Mechanistic modeling of marsh seedling establishment provides a positive outlook for coastal wetland restoration under global climate change. *Geophysical Research Letters, 48*(22), e2021GL095596.

IPCC. (2023). *Climate Change 2023: Synthesis Report.* Contribution of Working Groups I, II and III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, H. Lee and J. Romero (eds.)]. IPCC, Geneva, Switzerland, pp. 35-115.

Iversen, C. M., Ledford, J., & Norby, R. J. (2008). CO2 enrichment increases carbon and nitrogen input from fine roots in a deciduous forest. *New Phytologist*, *179*(3), 837-847.

Iversen, C. M. Murphy, M. T. Allen, M. F. Childs, J. Eissenstat, D. M. Lilleskov, E. A. Sarjala, T. M. Sloan, V. L. & Sullivan, P. F. (2012). Advancing the use of minirhizotrons in wetlands. *Plant and Soil*, *352*(1), 23-39.

Ives, A. R. (1995). Measuring competition in a spatially heterogeneous environment. *The American Naturalist*, *146*(6), 911-936.

Jackson, R. B., Canadell, J., Ehleringer, J. R., Mooney, H. A., Sala, O. E., & Schulze, E. D. (1996). A global analysis of root distributions for terrestrial biomes. *Oecologia*, *108*, 389-411.

Jarvi, M. P., & Burton, A. J. (2020). Root respiration and biomass responses to experimental soil warming vary with root diameter and soil depth. *Plant and Soil*, *451*, 435-446.

Jefferies, R. L., Davy, A. J., & Rudmik, T. (1981). Population Biology of the Salt Marsh Annual Salicornia Europaea agg. *The Journal of Ecology*, *69*(1), 17.

Jelinski, D. E., & Cheliak, W. M. (1992). Genetic diversity and spatial subdivision of Populus tremuloides (Salicaceae) in a heterogeneous landscape. *American Journal of Botany*, *79*(7), 728-736.

Kaminsky, J., Alberti, J., Aguiar, M., & Iribarne, O. (2015). Biological and physical factors affecting the colonization of vegetation-free patches in a SW Atlantic salt marsh. *Marine Ecology Progress Series*, *531*, 33-41.

Kassambara, A., Kosinski, M., Biecek, P., & Fabian, S. (2017). survminer: Drawing Survival Curves using ‘ggplot2’. R package version 0.3,1. https://CRAN.Rproject.org/package=survminer

Kassambara, A. (2020). ggpubr:'ggplot2'Based Publication Ready Plots. R package version 0.4. 0. Computer software]. https://cran-r-project. org/web/packages/ggpubr/indes. html.

Kirwan, M. L., Guntenspergen, G. R., & Morris, J. T. (2009). Latitudinal trends in Spartina alterniflora productivity and the response of coastal marshes to global change. *Global Change Biology*, *15*(8), 1982-1989.

Kirwan, M. L., & Megonigal, J. P. (2013). Tidal wetland stability in the face of human impacts and sea-level rise. *Nature*, *504*(7478), 53-60.

Kirwan, M. L., Temmerman, S., Skeehan, E. E., Guntenspergen, G. R., & Fagherazzi, S. (2016). Overestimation of marsh vulnerability to sea level rise. *Nature Climate Change*, *6*(3), 253-260.

Kleinbaum, D. G., & Klein, M. (2004). *Survival analysis*. New York: Springer.

Kleyer, M., Trinogga, J., Cebrián-Piqueras, M. A., Trenkamp, A., Fløjgaard, C., Ejrnæs, R., ... & Blasius, B. (2019). Trait correlation network analysis identifies biomass allocation traits and stem specific length as hub traits in herbaceous perennial plants. *Journal of Ecology, 107*(2), 829-842.

Koffijberg, K., Frikke, J., Hälterlein, B., Reichert, G., & Andretzke, H. (2016). Breeding birds in trouble: a framework for an action plan in the Wadden Sea. *CWSS, Wilhelmshaven*.

Koop-Jakobsen, K., & Dolch, T. (2023). Future climate conditions alter biomass of salt marsh plants in the Wadden Sea. *Marine Biodiversity*, *53*(3), 41.

Koop-Jakobsen, K., & Wenzhöfer, F. (2015). The dynamics of plant-mediated sediment oxygenation in Spartina anglica rhizospheres—a planar optode study. *Estuaries and Coasts*, *38*, 951-963.

Körner, C. (2006). Significance of temperature in plant life. *Plant Growth and Climate Change,* 48-69.

Kottler, E. J., & Gedan, K. (2020). Seeds of change: characterizing the soil seed bank of a migrating salt marsh. *Annals of Botany, 125*(2), 335-344.

Kuhn, N. L., & Zedler, J. B. (1997). Differential effects of salinity and soil saturation on native and exotic plants of a coastal salt marsh. *Estuaries, 20*(2), 391–403.

Lambers, H., Oliveira, R. S. (2019). Plant Physiological Ecology. third edit. New York: Springer.

Lambers, H., & Poorter, H. (1992). Inherent variation in growth rate between higher plants: a search for physiological causes and ecological consequences. In *Advances in Ecological Research* (Vol. 23, pp. 187-261). Academic Press.

Lavergne, A., Sandoval, D., Hare, V. J., Graven, H., & Prentice, I. C. (2020). Impacts of soil water stress on the acclimated stomatal limitation of photosynthesis: insights from stable carbon isotope data. *Global Change Biology*, *26*(12), 7158-7172.

Lavorel, S., Díaz, S., Cornelissen, J. H. C., Garnier, E., Harrison, S. P., McIntyre, S., ... & Urcelay, C. (2007). Plant functional types: are we getting any closer to the Holy Grail? *Terrestrial Ecosystems in a Changing World*, 149-164.

Lenssen, G. M., Van Duin, W. E., Jak, P., & Rozema, J. (1995). The response of Aster tripolium and Puccinellia maritima to atmospheric carbon dioxide enrichment and their interactions with flooding and salinity. *Aquatic Botany, 50*(2), 181-192.

Lenth, R. (2022). emmeans: estimated marginal means, aka least-squares means. R package version 1.4. 7. 2020.

Leuschner, C., Gebel, S., & Rose, L. (2013). Root trait responses of six temperate grassland species to intensive mowing and NPK fertilisation: a field study in a temperate grassland. *Plant and Soil, 373*, 687-698.

Lin, D., Xia, J., & Wan, S. (2010). Climate warming and biomass accumulation of terrestrial plants: a meta-analysis. *New Phytologist*, *188*(1), 187-198.

Loebl, M., van Beusekom, J. E., & Reise, K. (2006). Is spread of the neophyte Spartina anglica recently enhanced by increasing temperatures? *Aquatic Ecology*, *40*(3), 315-324.

Lõhmus, K., Balke, T., & Kleyer, M. (2020). Spatial and temporal patterns of initial plant establishment in salt marsh communities. *Journal of Vegetation Science, 31*(6), 1124–1134.

Loreau, M., & Hector, A. (2001). Partitioning selection and complementarity in biodiversity experiments. *Nature, 412*(6842), 72-76.

Lüdecke, D., Ben-Shachar, M. S., Patil, I., Waggoner, P., & D. Makowski, D. (2021). performance: An R package for assessment, comparison and testing of statistical models. *Journal of Open Source Software*, 6(60).

Mahajan, S., & Tuteja, N. (2005). Cold, salinity and drought stresses: an overview. *Archives of Biochemistry and Biophysics*, *444*(2), 139-158.

Martin, R. M. (2017). Effects of Warming on Invasive Phragmites australis and Native Spartina patens Seed Germination Rates and Implications for Response to Climate Change. *Northeastern Naturalist, 24*(3), 235–238.

Mateos-Naranjo, E., Redondo-Gómez, S., Luque, C. J., Castellanos, E. M., Davy, A. J., & Figueroa, M. E. (2008). Environmental limitations on recruitment from seed in invasive Spartina densiflora on a southern European salt marsh. *Estuarine, Coastal and Shelf Science, 79*(4), 727–732.

Matsui, T., & Singh, B. B. (2003). Root characteristics in cowpea related to drought tolerance at the seedling stage. *Experimental Agriculture*, *39*(1), 29-38.

Mawson, B. T., Svoboda, J., & Cummins, R. W. (1986). Thermal acclimation of photosynthesis by the arctic plant Saxifraga cernua. *Canadian Journal of Botany*, *64*(1), 71-76.

Maximov, N.A. (1929). The plant in relation to water. a study of the physiological basis of drought resistance. London, UK: Allen & Unwin.

Maynard, C., McManus, J., Crawford, R. M. M., & Paterson, D. (2011). A comparison of short-term sediment deposition between natural and transplanted saltmarsh after saltmarsh restoration in the Eden Estuary (Scotland). *Plant Ecology and Diversity, 4*(1), 103–113.

Mcleod, E., & others. (2011). A blueprint for blue carbon: Toward an improved understanding of the role of vegetated coastal habitats in sequestering CO2. *Frontiers in Ecology and the Environment, 9*(10), 552–560.

McManus, J., & Alizai, S. A. K. (1987). Variations in marsh surface levels in the upper Tay Estuary. *Proceedings of the Royal Society of Edinburgh, Section B: Biological Sciences, 92*(3-4), 345-358.

McOwen, C., Weatherdon L.V., Bochove, J., Sullivan, E., Blyth, S., Zockler, C., Stanwell-Smith, D., Kingston, N., Martin, C.S., Spalding, M., & Fletcher S (2017). A global map of saltmarshes (v6.1). *Biodiversity Data Journal* 5: e11764.

Meyer, D., Zeileis, A., & Hornik, K. (2006). The Strucplot Framework: Visualizing Multi-Way Contingency Tables with vcd. *Journal of Statistical Software*, 17(3), 1-48.

Minden, V., Andratschke, S., Spalke, J., Timmermann, H., & Kleyer, M. (2012). Plant trait–environment relationships in salt marshes: Deviations from predictions by ecological concepts. *Perspectives in Plant Ecology, Evolution and Systematics*, *14*(3), 183-192.

Minden, V., & Kleyer, M. (2011). Testing the effect–response framework: key response and effect traits determining above-ground biomass of salt marshes. *Journal of Vegetation Science*, *22*(3), 387-401.

Mitchell, M. L., & Adam, P. (1989). The relationship between mangrove and saltmarsh communities in the Sydney region. *Wetlands (Australia)*, *8*(2), 37-46.

Moles, A. T., & Westoby, M. (2004). Seedling survival and seed size: a synthesis of the literature. *Journal of Ecology, 92*(3), 372-383.

Möller, B., Chen, H., Schmidt, T., Zieschank, A., Patzak, R., Türke, M., ... & Posch, S. (2019). rhizoTrak: a flexible open source Fiji plugin for user-friendly manual annotation of time-series images from minirhizotrons. *Plant and Soil*, *444*, 519-534.

Möller, I., Kudella, M., Rupprecht, F., Spencer, T., Paul, M., Van Wesenbeeck, B. K., ... & Schimmels, S. (2014). Wave attenuation over coastal salt marshes under storm surge conditions. *Nature Geoscience, 7*(10), 727-731.

Möller, I., & Spencer, T. (2002). Wave dissipation over macro-tidal saltmarshes: Effects of marsh edge typology and vegetation change. *Journal of Coastal Research*, (36), 506-521.

Morris, J. T., Barber, D. C., Callaway, J. C., Chambers, R., Hagen, S. C., Hopkinson, C. S., ... & Wigand, C. (2016). Contributions of organic and inorganic matter to sediment volume and accretion in tidal wetlands at steady state. *Earth's future*, *4*(4), 110-121.

Morris, J. T., Langley, J. A., Vervaeke, W. C., Dix, N., Feller, I. C., Marcum, P., & Chapman, S. K. (2023). Mangrove Trees Outperform Saltmarsh Grasses in Building Elevation but Collapse Rapidly Under High Rates of Sea-Level Rise. *Earth's Future*, *11*(4), e2022EF003202.

Morris, J. T., Sundareshwar, P. V., Nietch, C. T., Kjerfve, B., & Cahoon, D. R. (2002). Responses of coastal wetlands to rising sea level. *Ecology*, *83*(10), 2869-2877.

Mueller, P., Do, H. T., Smit, C., Reisdorff, C., Jensen, K., & Nolte, S. (2021). With a little help from my friends: physiological integration facilitates invasion of wetland grass Elymus athericus into flooded soils. *Oikos*, *130*(3), 431-439.

Mueller, P., Granse, D., Nolte, S., Do, H. T., Weingartner, M., Hoth, S., & Jensen, K. (2017). Top-down control of carbon sequestration: grazing affects microbial structure and function in salt marsh soils. *Ecological Applications, 27*(5), 1435-1450.

Mueller, P., Ladiges, N., Jack, A., Schmiedl, G., Kutzbach, L., Jensen, K., & Nolte, S. (2019). Assessing the long-term carbon-sequestration potential of the semi-natural salt marshes in the European Wadden Sea. *Ecosphere, 10*(1), e02556.

Mueller, P., Granse, D., Nolte, S., Weingartner, M., Hoth, S., & Jensen, K. (2020). Unrecognized controls on microbial functioning in Blue Carbon ecosystems: The role of mineral enzyme stabilization and allochthonous substrate supply. *Ecology and Evolution, 10*(2), 998-1011.

Mueller, P., Kutzbach, L., Mozdzer, T. J., Jespersen, E., Barber, D. C., & Eller, F. (2023). Minerogenic salt marshes can function as important inorganic carbon stores. *Limnology and Oceanography, 68*(4), 942-952.

Munns, R. (2011). Plant adaptations to salt and water stress: differences and commonalities. *Advances in Botanical Research, 57,* 1-32.

Naudts, K. V., van den Berge, J., Janssens, I. A., Nijs, I., & Ceulemans, R. (2013). Combined effects of warming and elevated CO_2 on the impact of drought in grassland species. *Plant and Soil, 369,* 497-507.

Nehring, S., & Hesse, K. J. (2008). Invasive alien plants in marine protected areas: the Spartina anglica affair in the European Wadden Sea. *Biological Invasions, 10,* 937-950.

Nicholls, R.J., Wong P.P., Burkett VR, Codignotto, J.O., Hay, J.E., McLean, R.F., Ragoonaden, S., Woodroffe, C.D. (2007). Coastal systems and low-lying areas. In: Parry ML, Canziani OF, Palutikof JP, van der Linden PJ, Hanson CE (eds) Climate change 2007: Impacts, adaptation and vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. *Cambridge University Press,* Cambridge, UK, pp 315–357.

Niering, W.A. (1997). Tidal wetland restoration and creation along the east coast of North America. In: Urbanska KM, Webb NR, Edwards PS (eds) Restoration ecology and sustainable development. *Cambridge University Press*, Cambridge, pp 259–285.

Nieva, F. J. J., Díaz-Espejo, A., Castellanos, E. M., & Figueroa, M. E. (2001). Field variability of invading populations of Spartina densiflora Brong. in different habitats of the odiel marshes (SW Spain). *Estuarine, Coastal and Shelf Science, 52*(4), 515–527.

Noe, G. B., & Zedler, J. B. (2000). Differential effects of four abiotic factors on the germination of salt marsh annuals. *American Journal of Botany, 87*(11), 1679–1692.

Noe, G. B., & Zedler, J. B. (2001). Variable rainfall limits the germination of upper intertidal marsh plants in Southern California. *Estuaries, 24*(1), 30–40.

Nolte, S., Müller, F., Schuerch, M., Wanner, A., Esselink, P., Bakker, J. P., & Jensen, K. (2013a). Does livestock grazing affect sediment deposition and accretion rates in salt marshes? *Estuarine, Coastal and Shelf Science, 135,* 296-305.

Nolte, S., Koppenaal, E. C., Esselink, P., Dijkema, K. S., Schuerch, M., De Groot, A. V., ... & Temmerman, S. (2013b). Measuring sedimentation in tidal marshes: a review on methods and their applicability in biogeomorphological studies. *Journal of Coastal Conservation, 17,* 301-325.

Noyce, G. L., Kirwan, M. L., Rich, R. L., & Megonigal, J. P. (2019). Asynchronous nitrogen supply and demand produce nonlinear plant allocation responses to warming and elevated CO2. *Proceedings of the National Academy of Sciences*, *116*(43), 21623-21628.

Oberdorfer, E. (2001). *Pflanzensoziologische Exkursionsflora für Deutschland und angrenzende Gebiete*. Achte, stark überarbeitete und ergänzte Aufl. Eugen Ulmer, Stuttgart (Hohenheim).[1].

Olff, H. D., De Leeuw, J., Bakker, J. P., Platerink, R. J., & Van Wijnen, H. J. (1997). Vegetation succession and herbivory in a salt marsh: changes induced by sea level rise and silt deposition along an elevational gradient. *Journal of Ecology*, 799-814.

Oksanen, J., & others (2016). vegan: Community Ecology Package. R package version 2.4-0.

Patterson, C. S., & Mendelssohn, I. A. (1991). A comparison of physicochemical variables across plant zones in a mangal/salt marsh community in Louisiana. *Wetlands*, *11*, 139-161.

Pedersen, T. (2023). patchwork: The Composer of Plots_. R package version 1.1.3, <https://CRAN.R-project.org/package=patchwork>.

Pennings, S. C., & Callaway, R. M. (1992). Salt marsh plant zonation: the relative importance of competition and physical factors. *Ecology*, *73*(2), 681-690.

Peñuelas, J., Sardans, J., Estiarte, M., Ogaya, R., Carnicer, J., Coll, M., ... & Jump, A. S. (2013). Evidence of current impact of climate change on life: a walk from genes to the biosphere. *Global Change Biology*, *19*(8), 2303-2338.

Pickens, C. N., Sloey, T. M., & Hester, M. W. (2019). Influence of salt marsh canopy on black mangrove (Avicennia germinans) survival and establishment at its northern latitudinal limit. *Hydrobiologia, 826*(1), 195–208.

Pinheiro, J., Bates, D., R Core Team. (2023). _nlme: Linear and Nonlinear Mixed Effects Models_. R package version 3.1-162,<https://CRAN.R-project.org/package=nlme>.

Poorter, H., Niklas, K. J., Reich, P. B., Oleksyn, J., Poot, P., & Mommer, L. (2012). Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. *New Phytologist*, *193*(1), 30-50.

Poorter, H., Niinemets, Ü., Poorter, L., Wright, I. J., & Villar, R. (2009). Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. *New Phytologist*, *182*(3), 565-588.

Post, E., & Pedersen, C. (2008). Opposing plant community responses to warming with and without herbivores. *Proceedings of the National Academy of Sciences*, *105*(34), 12353-12358.

Quan, Q., Zhang, F., Meng, C., Ma, F., Zhou, Q., Sun, F., & Niu, S. (2020). Shifting biomass allocation determines community water use efficiency under climate warming. *Environmental Research Letters*, *15*(9), 094041.

R Core Team. (2021). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. URL: https://www.R-project.org/.

R Core Team. (2023). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. URL: https://www.R-project.org/.

RStudio Team (2023). RStudio: Integrated Development for R. RStudio, PBC, Boston, MA URL http://www.rstudio.com/.

Rand, T. A. (2000). Seed dispersal, habitat suitability and the distribution of halophytes across a salt marsh tidal gradient. *Journal of Ecology, 88*, 608–621.

Redelstein, R., Coners, H., Knohl, A., & Leuschner, C. (2018b). Water sources of plant uptake along a salt marsh flooding gradient. *Oecologia*, *188*, 607-622.

Redelstein, R., Dinter, T., Hertel, D., & Leuschner, C. (2018a). Effects of inundation, nutrient availability and plant species diversity on fine root mass and morphology across a saltmarsh flooding gradient. *Frontiers in Plant Science, 9*, 98.

Reents, S. (2022). *Climate change in salt marshes-Effects of hydrodynamic forcing, sea level rise and higher temperatures on coastal vegetation* (Doctoral dissertation, Staats-und Universitätsbibliothek Hamburg Carl von Ossietzky).

Reents, S., Möller, I., Evans, B. R., Schoutens, K., Jensen, K., Paul, M., ... & Nolte, S. (2022). Species-specific and seasonal differences in the resistance of salt-marsh vegetation to wave impact. *Frontiers in Marine Science, 9*, 898080.

Reents, S., Mueller, P., Tang, H., Jensen, K., & Nolte, S. (2021). Plant genotype determines biomass response to flooding frequency in tidal wetlands. *Biogeosciences*, *18*(2), 403-411.

Rich, R., Mueller, P., Fuss, M., Goncalves, S., Ostertag, E., Reents, S., Tang, H., Tashjian, A., Thomsen, S., Jensen, K., & Nolte, S. (in press). Design and assessment of a novel approach for ecosystem warming experiments in high-energy tidal wetlands. *Journal of Geophysical Research: Biogeosciences.*

Rousset, F., & Ferdy, J. B. (2014). Testing environmental and genetic effects in the presence of spatial autocorrelation. *Ecography*, 37(8), 781-790.

Rozema, J., Bijwaard, P., Prast, G., & Broekman, R. (1985). Ecophysiological adaptations of coastal halophytes from foredunes and salt marshes. *Vegetatio, 62*, 499-521.

Rupprecht, F., Wanner, A., Stock, M., & Jensen, K. (2015). Succession in salt marshes–large-scale and long-term patterns after abandonment of grazing and drainage. *Applied Vegetation Science*, *18*(1), 86-98.

Rupprecht, F., Möller, I., Paul, M., Kudella, M., Spencer, T., Van Wesenbeeck, B. K., ... & Schimmels, S. (2017). Vegetation-wave interactions in salt marshes under storm surge conditions. *Ecological Engineering*, *100*, 301-315.

Rustad, L. E. J. L., Campbell, J., Marion, G., Norby, R., Mitchell, M., Hartley, A., ... & Gcte-News. (2001). A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. *Oecologia*, *126*, 543-562.

Sage, R. F., & Kubien, D. S. (2007). The temperature response of C3 and C4 photosynthesis. *Plant, Cell & Environment*, *30*(9), 1086-1106.

Sage R.F., Sharkey T.D. & Pearcy R.W. (1990). The effect of leaf nitrogen and temperature on the CO2 response of photosynthesis in the C3 dicot Chenopodium album L. *Australian Journal of Plant Physiology* 17, 135–148.

Schepers, L. (2017). *Spatial patterns and bio-geomorphological effects of vegetation loss in a submerging coastal marsh* (Doctoral dissertation, University of Antwerp).

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C. Saalfeld, S., Schmid, B., Tinevez, J.-Y., White, D. J., Hartenstein, V., Eliceiri, K., Tomancak, P. & Cardona, A. (2012). Fiji: an open-source platform for biological-image analysis. *Nature Methods*, *9*(7), 676-682.

Schlichting, C. D. (1986). The evolution of phenotypic plasticity in plants. *Annual Review of Ecology and Systematics*, *17*(1), 667-693.

Schoutens, K., Reents, S., Nolte, S., Evans, B., Paul, M., Kudella, M., ... & Temmerman, S. (2021). Survival of the thickest? Impacts of extreme wave-forcing on marsh seedlings are mediated by species morphology. *Limnology and Oceanography*, *66*(7), 2936-2951.

Schulte, P., Alegret, L., Arenillas, I., Arz, J. A., Barton, P. J., Bown, P. R., et al. (2010). The Chicxulub asteroid impact and mass extinction at the Cretaceous-Paleogene boundary. *Science* 327, 1214–1218.

Schulze, D., Jensen, K., & Nolte, S. (2022). Effects of small-scale patterns of vegetation structure on suspended sediment concentration and sediment deposition in a salt marsh. *Estuarine, Coastal and Shelf Science*, *278*, 108125.

Sharkey T.D. (1988). Estimating the rate of photorespiration in leaves. *Physiologia Plantarum* 73, 147–152.

Shepard, C. C., Crain, C. M., & Beck, M. W. (2011). The protective role of coastal marshes: A systematic review and meta-analysis. *PLoS ONE*, *6*(11).

Shi, Z., Sherry, R., Xu, X., Hararuk, O., Souza, L., Jiang, L., ... & Luo, Y. (2015). Evidence for long-term shift in plant community composition under decadal experimental warming. *Journal of Ecology, 103*(5), 1131-1140.

Shields, L. M. (1950). Leaf xeromorphy as related to physiological and structural influences. *The Botanical Review, 16,* 399-447.

Shumway, S. W., & Bertness, M. D. (1992). Salt stress limitation of seedling recruitment in a salt marsh plant community. *Oecologia, 92*(4), 490–497.

Silvestri, S., Defina, A., & Marani, M. (2005). Tidal regime, salinity, and salt marsh plant zonation. *Estuarine, Coastal and Shelf Science, 62*(1–2), 119–130.

Smith, A. J., Noyce, G. L., Megonigal, J. P., Guntenspergen, G. R., & Kirwan, M. L. (2022). Temperature optimum for marsh resilience and carbon accumulation revealed in a whole-ecosystem warming experiment. *Global Change Biology, 28*(10), 3236-3245.

Snow, A. A., & Vince, S. W. (1984). Plant zonation in an Alaskan salt marsh: II. An experimental study of the role of edaphic conditions. *The Journal of Ecology,* 669-684.

Sombroek & Gommes in: Bazzaz, F. A., & Sombroek, W. G. (Eds.). (1996). Global climate change and agricultural production: direct and indirect effects of changing hydrological, pedological, and plant physiological processes. *Food & Agriculture Org.*

Sterk, A. A. (1969). Biosystematic studies on Spergularia media and S. marina in the Netherlands. III. The variability of S. media and S. marina in relation to the environment. *Acta Botanica Neerlandica, 18*(4), 561-577.

Stock, M. (2011). Patterns in surface elevation change across a temperate salt marsh platform in relation to sea-level rise. *Coastline Reports, 17*(3), 33-48.

Stokes, V. J., Morecroft, M. D., & Morison, J. I. (2010). Comparison of leaf water use efficiency of oak and sycamore in the canopy over two growing seasons. *Trees, 24,* 297-306.

Suchrow, S., & Jensen, K. (2010). Plant species responses to an elevational gradient in German North Sea salt marshes. *Wetlands, 30*(4), 735-746.

Tang, H., Nolte, S., Jensen, K., Rich, R., Mittmann-Goetsch, J., & Mueller, P. (2023). Warming accelerates belowground litter turnover in salt marshes–insights from a Tea Bag Index study. *Biogeosciences, 20*(10), 1925-1935

Tcherkez, G., Mahé, A., & Hodges, M. (2011). 12C/13C fractionations in plant primary metabolism. *Trends in Plant Science, 16*(9), 499-506.

The GIMP Development Team. (2019). *GIMP*. Retrieved from https://www.gimp.org

Therneau, T. M. (2021). A Package for Survival Analysis in R_. R package version 3.2-13, URL: https://CRAN.R-project.org/package=survival>.

Tomlinson, P. B. (1986). The Botany of Mangroves. *Cambridge University Press* London.

Tripathee, R., & Schäfer, K. V. R. (2015). Above-and belowground biomass allocation in four dominant salt marsh species of the eastern United States. *Wetlands*, *35*, 21-30.

Turner, R. E., Swenson, E. M., Milan, C. S., Lee, J. M., & Oswald, T. A. (2004). Below-ground biomass in healthy and impaired salt marshes. *Ecological Research*, *19,* 29-35.

Tuzet, A., Perrier, A., & Leuning, R. (2003). A coupled model of stomatal conductance, photosynthesis and transpiration. *Plant, Cell & Environment*, *26*(7), 1097-1116.

Ungar, I. A. (1977). Salinity, temperature, and growth regulator effects on seed germination of Salicornia europaea L. *Aquatic Botany, 3*(C), 329–335.

Ungar, I. A. (1979). Seed dimorphism in Salicornia europaea L. *Botanical Gazette, 140*(1), 102-108.

Ungar, I. A., & Woodell, S. R. J. (1993). The relationship between the seed bank and species composition of plant communities in two British salt marshes. *Journal of Vegetation Science*, *4*(4), 531–536.

Ursino, N., Silvestri, S., & Marani, M. (2004). Subsurface flow and vegetation patterns in tidal environments. *Water Resources Research*, *40*(5).

Uyeda, K. A., Deutschman, D. H., & Crooks, J. A. (2019). Effects of Salt and Drought Stress on Germination of Non-native Plants in the Salt Marsh to Upland Transition Zone. *Estuaries and Coasts*, *42*(6), 1686–1694.

Valiela, I., Teal, J. M., & Persson, N. Y. (1976). Production and dynamics of experimentally enriched salt marsh vegetation: Belowground biomass 1. *Limnology and Oceanography, 21*(2), 245-252.

van Belzen, J., Fivash, G. S., Hu, Z., Bouma, T. J., & Herman, P. M. (2022). A probabilistic framework for windows of opportunity: the role of temporal variability in critical transitions. *Journal of the Royal Society Interface, 19*(190), 20220041.

van Klink, R., Nolte, S., Mandema, F. S., Lagendijk, D. G., WallisDeVries, M. F., Bakker, J. P., ... & Smit, C. (2016). Effects of grazing management on biodiversity across trophic levels–The importance of livestock species and stocking density in salt marshes. *Agriculture, Ecosystems & Environment*, *235*, 329-339.

van Regteren, M., Meesters, E. H., Baptist, M. J., de Groot, A. V., Bouma, T. J., & Elschot, K. (2020). Multiple Environmental Variables Affect Germination and Mortality of an Annual Salt Marsh Pioneer: Salicornia procumbens. *Estuaries and Coasts, 43*(6), 1489–1501.

Venables, W. N., & Ripley, B. D. (2002). Modern Applied Statistics with S. Fourth Edition. Springer, New York. ISBN 0-387-95457-0

Vendramini, F., Díaz, S., Gurvich, D. E., Wilson, P. J., Thompson, K., & Hodgson, J. G. (2002). Leaf traits as indicators of resource-use strategy in floras with succulent species. *New Phytologist*, *154*(1), 147-157.

von Caemmerer, S., Ghannoum, O., Pengelly, J. J., & Cousins, A. B. (2014). Carbon isotope discrimination as a tool to explore C4 photosynthesis. *Journal of Experimental Botany, 65*(13), 3459-3470.

Wahl, T., Haigh, I. D., Woodworth, P. L., Albrecht, F., Dillingh, D., Jensen, J., ... & Wöppelmann, G. (2013). Observed mean sea level changes around the North Sea coastline from 1800 to present. *Earth-Science Reviews*, *124*, 51-67.

Walck, J. L., Hidayati, S. N., Dixon, K. W., Thompson, K. E. N., & Poschlod, P. (2011). Climate change and plant regeneration from seed. *Global Change Biology*, *17*(6), 2145-2161.

Wang, Z., Ding, L., Wang, J., Zuo, X., Yao, S., & Feng, J. (2016). Effects of root diameter, branch order, root depth, season and warming on root longevity in an alpine meadow. *Ecological Research*, *31*, 739-747.

Wang, R., Li, L., Gentine, P., Zhang, Y., Chen, J., Chen, X., ... & Lü, G. (2022). Recent increase in the observation-derived land evapotranspiration due to global warming. *Environmental Research Letters*, *17*(2), 024020.

Wang, H., van der Wal, D., Li, X., Van Belzen, J., Herman, P. M., Hu, Z., ... & Bouma, T. J. (2017). Zooming in and out: Scale dependence of extrinsic and intrinsic factors affecting salt marsh erosion. *Journal of Geophysical Research: Earth Surface*, *122*(7), 1455-1470.

Wang, H. & Yamauchi, A. (2006). Growth and functions of roots under abiotic stress in soil. In B. Huang (Ed.), *Plant-Environment Interaction*s (3rd ed. pp. 271-320). CRC Press, New York.

Wanner, A., Suchrow, S., Kiehl, K., Meyer, W., Pohlmann, N., Stock, M., & Jensen, K. (2014). Scale matters: Impact of management regime on plant species richness and vegetation type diversity in Wadden Sea salt marshes. *Agriculture, Ecosystems & Environment*, *182*, 69-79.

Wellstein, C., Poschlod, P., Gohlke, A., Chelli, S., Campetella, G., Rosbakh, S., ... & Beierkuhnlein, C. (2017). Effects of extreme drought on specific leaf area of grassland species: A meta-analysis of experimental studies in temperate and sub-Mediterranean systems. *Global Change Biology*, *23*(6), 2473-2481.

Weir, D. J., & McManus, J. (1987). The role of wind in generating turbidity maxima in the Tay Estuary. *Continental Shelf Research, 7*(11–12), 1315–1318.

Wickham, H. (2016). Data analysis. In ggplot2 (pp. 189-201). Springer, Cham.

Wickham, H., François, R., Henry, L., & Mueller, K. (2021). dplyr: A Grammar of Data Manipulation. R package version 1.0.7. https://CRAN.R-project.org/package=dplyr

Wilson, R. M., Hopple, A. M., Tfaily, M. M., Sebestyen, S. D., Schadt, C. W., Pfeifer-Meister, L., ... & Hanson, P. J. (2016). Stability of peatland carbon to rising temperatures. *Nature Communications*, *7*(1), 13723.

Wolters, M., & Bakker, J. P. (2002). Soil seed bank and driftline composition along a successional gradient on a temperate salt marsh. *Applied Vegetation Science*, *5*(1), 55–62.

Wolters, M., Garbutt, A., & Bakker, J. P. (2005). Plant colonization after managed realignment: The relative importance of diaspore dispersal. *Journal of Applied Ecology*, *42*(4), 770–777.

Wright, I. J., Reich, P. B., & Westoby, M. (2001). Strategy shifts in leaf physiology, structure and nutrient content between species of high- and low-rainfall and high- and low-nutrient habitats. *Functional Ecology*, 15, 423–434.

Yaffar, D., Wood, T. E., Reed, S. C., Branoff, B. L., Cavaleri, M. A., & Norby, R. J. (2021). Experimental warming and its legacy effects on root dynamics following two hurricane disturbances in a wet tropical forest. *Global Change Biology*, *27*(24), 6423-6435.

Yando, E. S., Jones, S. F., James, W. R., Colombano, D. D., Montemayor, D. I., Nolte, S., ... & Sergienko, L. (2023). An integrative salt marsh conceptual framework for global comparisons. *Limnology and Oceanography Letters*.

Young, I. R., Zieger, S., & Babanin, A. V. (2011). Global trends in wind speed and wave height. *Science*, *332*(6028), 451-455.

Zhang, L., Lan, S., Zhao, L., Yi, H., & Han, G. (2022). Temporal variation of water-use efficiency and water sources of Phragmites australis in the coastal wetland: Based on stable isotopic composition (δ13C and δ18O). *Ecological Indicators*, *139*, 108957.

Zhong, Q., Gong, J., Wang, K., & Zhang, C. (2014). Effects of 3-year air warming on growth of two perennial grasses (Phragmites australis and Imperata cylindrica) in a coastal salt marsh reclaimed for agriculture. *Aquatic Botany*, *117*, 18-26.

Abstract

Ecosystem services provided by salt marshes, like coastal protection or carbon sequestration, become increasingly important under future climate change scenarios. Rising global temperatures threaten the salt marsh ecosystem by accelerated rates of sea-level rise and the higher risk of extreme weather events like storms. Global warming may also alter ecosystem functions and thereby ecosystem services may be affected. The persistence of salt marshes under rising temperatures, is highly dependent on the performance of salt marsh plants. However, the influence of warming on plants in meso- to macrotidal salt marshes is still largely unknown. Thus, this thesis aims to improve the knowledge on salt marsh plant responses to warming in their natural environment. The thesis comprises of three studies, which were conducted in a whole ecosystem warming experiment on the German Wadden Sea coast. The influence of warming on salt marsh plant performance was studied under the aspects of belowground biomass (chapter 2), ecophysiological traits (chapter 3), and seedling recruitment (chapter 4).

Chapter 2 investigates the effect of warming (+3 °C) on root biomass in different soil depths (7-9 cm, 19-21 cm), seasons (spring, summer, autumn), and salt marsh zones (pioneer zone, low marsh, high marsh). We found little response of root biomass to warming in general. However, warming combined with prolonged drought conditions in 2019 led to a shift of root biomass to lower soil depths in the high marsh. Irrespective of warming, we found higher root biomass in the upper soil depth and root biomass differed between salt marsh zones. Pooled across depths, root biomass was highest in the low marsh followed by pioneer zone and lowest in the high marsh.

Chapter 3 focuses on the response of ecophysiological traits (δ^{13}C and SLA) of five salt marsh plants (*Salicornia europaea*, *Spartina anglica*, *Halimione portulacoides*, *Puccinellia maritima*, *Elymus athericus*) from different salt marsh zones (pioneer zone, low marsh, high marsh) to warming (+1.5 °C, +3 °C) in different seasons (spring, summer, autumn). The influence of warming on δ^{13}C was evident in all salt marsh zones. The direction of change was mediated by differing abiotic conditions along the elevational gradient. In the higher marsh zones, increasing δ^{13}C values under warming indicated a higher water use efficiency of plants, while in the pioneer zone the response was the opposite, more complex, and difficult to interpret. SLA was mainly affected by season, which was evident by generally higher SLAs in spring and lower SLA in summer and autumn.

Chapter 4 studies the influence of warming (+1.5 °C, +3 °C) on seedling emergence in all three salt marsh zones (pioneer zone, low marsh, high marsh) and survival and seedling diversity in two salt marsh zones (low marsh, high marsh). Warming slightly increased seedling numbers in spring in the pioneer zone and slightly accelerated the mortality of seedlings in the high marsh. Seedling survival, and thus recruitment, was not significantly affected by warming, but the median lifespan was reduced. However, seedling survival curves differed significantly between different species. We found significant differences between the median survival time of *Halimione. portulacoides* (15 weeks) and the median survival times of *Salicornia europaea* agg. (9 weeks) and *Spergularia* sp. (6.5 weeks).

In general, salt marsh plants seem to cope with warmer temperatures by adjusting their water use efficiency according to their position along the elevational gradient. With drought conditions, which may occur in the high marsh during summer, the plants also show additional plastic responses such as adjusting their rooting depth to reach for water. The results of this thesis suggest that the studied salt marsh plants are highly plastic in their response to warming, therefore the ecosystem function may not be impaired under warming. However, the response to warming was also species-specific and thus warming may lead to a shift of salt marsh species composition in the future.

Zusammenfassung

Ökosystemdienstleistungen, wie die Küstenschutzfunktion und Kohlenstoffbindung, welche durch Salzmarschen bereitgestellt werden, gewinnen zunehmend an Bedeutung im Hinblick auf zukünftige Klimawandelszenarien. Die ansteigenden globalen Temperaturen stellen aufgrund von beschleunigtem Meeresspiegelanstieg und des höheren Risikos für Extremwetterereignisse, wie Stürmen, eine Bedrohung für das Ökosystem Salzmarsch dar. Die globale Erwärmung könnte auch Funktionen innerhalb des Ökosystems verändern und dadurch könnten auch die Ökosystemdienstleistungen verändert werden. Der Fortbestand von Salzmarschen unter steigenden Temperaturen hängt stark von der Performanz der Salzmarschpflanzen ab. Allerdings ist bisher wenig über den Einfluss von Erwärmung auf Pflanzen in meso- bis makrotidenbeeinflussten Salzmarschen bekannt. Dementsprechend ist das Ziel dieser Dissertation, den Wissensstand über den Erwärmungseinfluss auf Salzmarschpflanzen zu verbessern. Die Dissertation besteht aus drei Studien, welche in einem in situ Ökosystem Erwärmungsexperiment an der Küste des deutschen Wattenmeers durchgeführt wurden. Der Einfluss von Erwärmung auf Salzmarschpflanzen wurde unter den Aspekten unterirdische Biomasse (Kapitel 2), ökophysiologische Merkmale (Kapitel 3) und Keimungserfolg (Kapitel 4) untersucht.

In Kapitel 2 wird der Effekt von Erwärmung (+3 °C) auf die Wurzelbiomasse in verschiedenen Bodentiefen (7-9 cm, 19-21 cm), Saisons (Frühling, Sommer, Herbst) und Salzmarschzonen (Pionierzone, Untere Marsch, Obere Marsch) untersucht. Generell haben wir nur eine geringe Reaktion der Wurzelbiomasse auf Erwärmung gefunden. Allerdings führte Erwärmung kombiniert mit Dürrebedingungen in der Oberen Marsch im Jahr 2019 zu einer Verschiebung der Wurzelbiomasse in die untere Bodentiefe. Unabhängig von Erwärmung haben wir eine größere Wurzelbiomasse in der oberen Bodentiefe gefunden und die Wurzelbiomasse unterschied sich zwischen den Salzmarschzonen. Tiefenübergreifend war die Wurzelbiomasse in der Unteren Marsch am höchsten, gefolgt von der Pionierzone und der Oberen Marsch.

Kapitel 3 fokussiert sich auf die Reaktion von ökophysiologischen Merkmalen ($\delta^{13}C$ und SLA) von fünf Salzmarschpflanzen (*Salicornia europaea, Spartina anglica, Halimione portulacoides, Puccinellia maritima, Elymus athericus)* aus unterschiedlichen Salzmarschzonen (Pionierzone, Untere Marsch, Obere Marsch) auf Erwärmung (+1.5 °C, +3 °C) in unterschiedlichen Saisons (Frühling, Sommer, Herbst). Der Einfluss von Erwärmung auf das Isotopenverhältnis der Kohlenstoff-Isotope ($\delta^{13}C$) war in allen

Salzmarschzonen ersichtlich. Die Richtung der Änderung wurde durch sich ändernde abiotische Bedingungen entlang des Höhengradienten vermittelt. In den höhergelegenen Marschzonen deuteten zunehmende $\delta^{13}C$ Werte eine erhöhte Wassernutzungseffizienz der Pflanzen an. In der Pionierzone dagegen, war die Reaktion in die entgegengesetzte Richtung, komplexer und schwer zu interpretieren. Die Spezifische Blattfläche (SLA) wurde hauptsächlich durch die Saison beeinflusst. Dies konnte man an den generell höheren SLA-Werten im Frühling und den niedrigeren SLA-Werten im Sommer und Herbst erkennen.

Kapitel 4 untersucht den Erwärmungseinfluss (+1.5 °C, +3 °C) auf die Keimung von Samen in allen drei Salzmarschzonen (Pionierzone, Untere Marsch, Obere Marsch) und das Überleben der Keimlinge, sowie die Diversität in zwei Salzmarschzonen (Untere Marsch, Obere Marsch). Erwärmung führte zu einem leichten Anstieg der Keimlingsanzahl im Frühling in der Pionierzone und zu einer leicht beschleunigten Mortalität in der Oberen Marsch. Das Überleben und daher die Rekrutierung wurde nicht signifikant durch Erwärmung beeinflusst, aber die mediane Lebensspanne wurde reduziert. Das Überleben der Keimlinge zwischen verschiedenen Arten, war allerdings signifikant unterschiedlich. Wir fanden signifikante Unterschiede zwischen der medianen Lebensspanne von *Halimione portulaciodes* (15 Wochen) und den medianen Lebensspannen von *Salicornia europaea* agg. (9 Wochen) und *Atriplex* sp. (6,5 Wochen).

Grundsätzlich scheinen Salzmarschpflanzen mit wärmeren Temperaturen zurechtkommen, indem sie ihre Wassernutzungseffizienz gemäß ihrer Position entlang des hydrologischen Gradienten anpassen. Mit Dürrebedingungen, welche in der Oberen Marsch im Sommer vorkommen können, zeigen die Pflanzen auch zusätzliche plastische Reaktionen wie die Anpassung der Wurzeltiefe, um an Wasser zu gelangen. Die Ergebnisse dieser Dissertation lassen annehmen, dass die untersuchten Salzmarschpflanzen eine hohe Plastizität in ihrer Reaktion auf Erwärmung zeigen. Deswegen könnte die Funktion des Ökosystems durch Erwärmung unbeeinträchtigt bleiben. Allerdings sind die die Reaktionen auf Erwärmung artenabhängig und daher könnte Erwärmung in Zukunft zu einer Artenverschiebung in Salzmarschen führen.

Danksagung

Ich möchte mich herzlich bei Stefanie Nolte und Kai Jensen für die Betreuung meiner Arbeit bedanken. Kai danke ich besonders für die fachliche Unterstützung, welche ich immer bekam, wenn ich sie brauchte und auch für die Freiheiten, die ich genossen habe. Steffi möchte ich für ihre motivierende Art, ihr großes persönliches Engagement und die fachliche Unterstützung danken. Außerdem für die moralische Unterstützung, welche mir sehr geholfen hat diese Arbeit abzuschließen.

Ein großer Dank geht auch an das gesamte MERIT Team. Besonders die Zusammenarbeit mit der ersten „Kohorte“ Miriam Fuß, Salomé Goncalves Huq, Svenja Reents und Hao Tang hat mir viel Freude bereitet. Danken möchte ich außerdem Roy Rich, Simon Thomsen, Peter Müller und Viktoria Unger für die große Hilfe bei allen Fragen rund um das Experiment.
Mónica Salazar Ortiz danke ich für die hervorragende technische Assistenz, besonders für die Durchführung der Messungen während meiner Elternzeit. Außerdem Sebastian Wilfinger, Beke Eichert, Alston D´Souza, Niclas Krauss, Ben Kohnert, Ella Logemann und Monica Wilson für die Unterstützung bei Feld- und Laborarbeit. Andrea-Carolin Menzel danke ich für die entspannte und produktive Zusammenarbeit.

Der AG Pflanzenökologie danke ich für die gemeinsame Zeit. Besonders Claudia Mählmann für die Beantwortung aller bürokratischen Fragen und auch für die Organisation von vielen Kleinigkeiten, welche nicht unbemerkt bleiben sollen.
Ein besonderer Dank geht auch an meine Freundin Heather Shupe fürs Korrekturlesen und für die wunderbaren Erinnerungen und gemeinsamen Erlebnisse in Hamburg.

Natürlich danke ich auch meinem Vater und meiner Mutter, ohne die ich nicht dort wäre, wo ich heute bin.

Zuletzt möchte ich meiner kleinen Familie danken: das Wunderbarste, was ich während meiner Doktorarbeit erleben durfte, war die Ankunft und das Wachsen unseres Sohns Benjamin! Mein größter Dank geht an meinen Mann Thomas, welchen ich in unserer AG kennenlernen durfte und der mir immer zur Seite stand. Danke für deine Liebe, deinen Zuspruch und deine Geduld!

Heather Shupe
Tungenesveien 118
4071, Randaberg
Norway

October 16, 2023

To whom it may concern,

As a native English speaker, I hereby declare that the Ph.D. thesis: “Warming effects on plants in Wadden Sea salt marshes” has been written in concise and correct English (US).

Sincerely,

Heather Shupe

Author contributions

Chapter 1: E. Ostertag wrote this chapter.

Chapter 2: E. Ostertag and A.-C. Menzel contributed **equally** to the conceptualization and draft of the manuscript. E. Ostertag and S. Nolte contributed to the design and implementation of the belowground biomass study. E. Ostertag planned and conducted the measurements in 2019 and supervised the measurements in 2020 and 2021. E. Ostertag did the statistical analyses for the allometric root biomass conversion and created the figures. E. Ostertag wrote the first draft of the introduction, results, and the methods (except for the statistical analyses section) and edited the discussion. A.C. Menzel did the spring measurements in 2021, conducted or supervised the root picture processing and did the statistical analyses except for the allometric root biomass conversion, wrote the initial draft of the discussion and edited introduction, results, and methods. S. Nolte and K. Jensen established the warming experiment MERIT, developed the initial research idea of the study, supervised E. Ostertag and A.-C. Menzel, and edited and commented on earlier drafts of the manuscript.

Chapter 3: E. Ostertag conceptualized, planned, and conducted or supervised the measurements and lab work. E. Ostertag did the statistical analyses and wrote the initial draft of the manuscript. S. Nolte and K. Jensen established the warming experiment MERIT, developed the initial research idea of the study, supervised E. Ostertag, and edited and commented on earlier drafts of the manuscript.

Chapter 4: E. Ostertag, S. Nolte and K. Jensen conceptualized the study. E. Ostertag planned and conducted the field work, did the statistical analyses, and wrote the initial draft of the manuscript. V. Unger, S. Nolte, and K. Jensen commented on and edited earlier drafts of the manuscript. S. Nolte, and K. Jensen established the warming experiment MERIT, and supervised E. Ostertag

Chapter 5: E. Ostertag wrote this chapter based on the findings of chapters 2-4. Additional data on aboveground biomass was sampled in collaboration with Svenja Reents.

Co-Author Affiliations

Kai Jensen
Applied Plant Ecology, Universität Hamburg, Hamburg, Germany

Andrea-Carolin Menzel
Applied Plant Ecology, Universität Hamburg, Hamburg, Germany

Stefanie Nolte
School of Environmental Sciences, University of East Anglia, Norwich, UK
Centre for Environment, Fisheries and Aquaculture Science, Lowestoft, UK

Viktoria Unger
Applied Plant Ecology, Universität Hamburg, Hamburg, Germany

Published chapters information

Chapter 4

Warming experiment indicates that increasing global temperatures may not affect windows of opportunity for salt marsh seedlings

Published in: *Limnology & Oceanography*
Publisher: Wiley
Year: 2023

Doi: 10.1002/lno.12419
Authors: **Eva Julia Marie Ostertag,** Kai Jensen, Viktoria Unger, Stefanie Nolte

E. Ostertag contributed substantially to the design, conception, and implementation of the germination study. E. Ostertag planned and conducted the field work, did the statistical analyses, and wrote the initial draft of the manuscript.

Prof. Dr. Kai Jensen

Eidesstattliche Versicherung

Hiermit erkläre ich an Eides statt, dass ich die vorliegende Dissertationsschrift selbst verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt habe.

Hamburg, den 19.10.2023 Unterschrift: Eva Ostertag