

# Global Change Effects on Decomposition Processes in Salt Marshes

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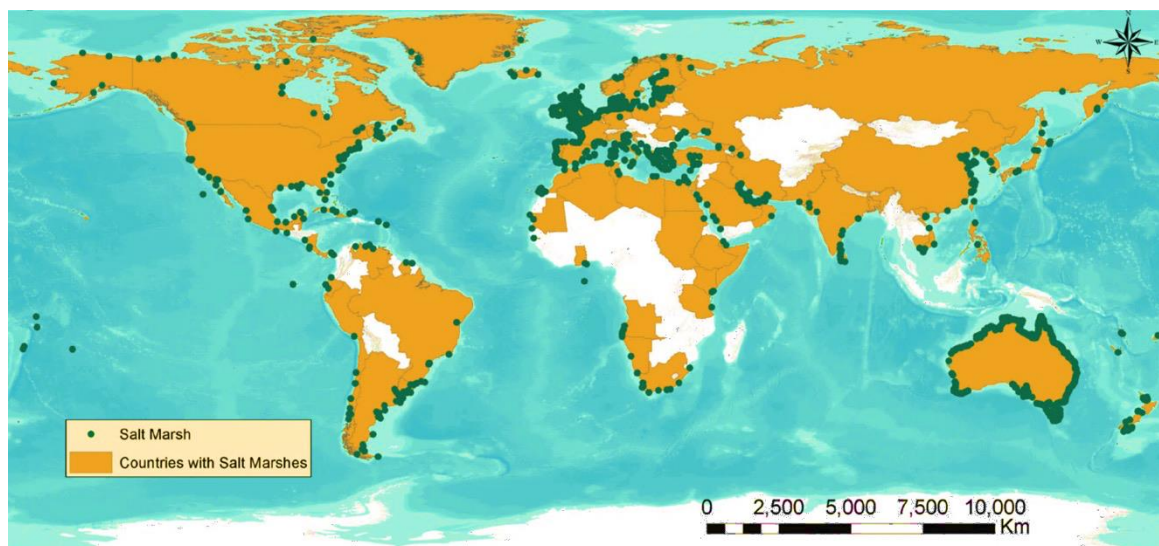
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# 1 General introduction

## 1.1 Ecosystem services of salt marshes

Salt marshes are located in the intertidal zone between terrestrial and marine environments, and are regularly flooded by tides (Himes-Cornell et al., 2018; Sousa et al., 2017). Globally, the area of salt marshes is estimated to be appr. 0.5 Million hectares, distributed among more than 40 countries (Mcowen et al., 2017). Salt marshes mainly develop on low-energy shorelines in temperate and high-latitudes, such as parts of Europe, America, Australia, and East Asia (Figure 1.1). The vegetation of salt marshes is dominated by many different types of salt-tolerant plants, such as herbs, grasses, and low shrubs (Greenberg et al., 2014; van Dobben and Slim, 2012), and these plant species need to be adapted to increased salinity. In addition, the vegetation of salt marshes is essential to their stability and facilitates sedimentation (Morris et al., 2013).



**Figure 1.1** Global distribution of salt marshes from Murray et al., (2011).

**Table 1.1** Examples of ecosystem services provided by salt marshes (from Russi et al., 2013 and Barbier et al., 2011).

Ecosystem service	Role of wetland structure
Erosion control	Capture of sediments and soil retention
Flood protection	Regulation of the flow of water; water storage capacity
Water provision	Regular supply of water due to the ability to store water in a reservoir; groundwater recharge
Water purification	Natural filtration through nutrient uptake; retention of particles and pollutants
Food	Habitat for fish, mollusks, plants, and other animal species used for food
Raw materials	Habitat for grasses, and other plants used for fiber and fuel
Cultural values	Many cultures have spiritual values and religious practices associated with wetlands
Tourism	Aesthetic features of wetlands; open water; habitat for biodiversity
Carbon sequestration	Vegetation and soils capture carbon dioxide and other greenhouse gases from the atmosphere, soil sediments can contribute to the long-term storage of organic matter
Climate regulation	Water bodies can stabilize local temperatures.

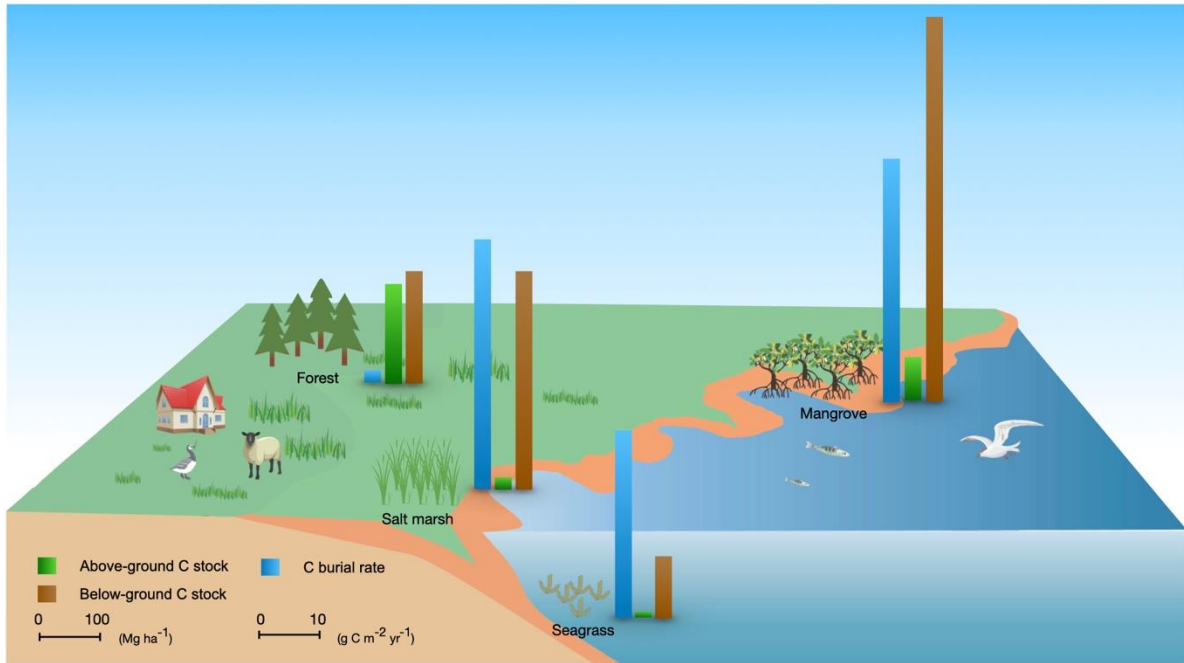
Salt marshes provide many ecosystem services (Table 1.1), which can be categorized into provisioning, cultural, and supporting services (Davidson et al., 2017). The provisioning services include ground for livestock grazing, but also habitat for wild foods, such as plants, birds, fish, and crustaceans (Choi et al., 2001; Davidson et al., 2017; Green and ElMBERG, 2014). The cultural services of salt marshes are considered valuable, as salt marshes supply a variety of landscapes to attract tourists (i.e. birdwatchers and walkers), and offer artistic inspiration as well as educational opportunities (Davidson et al., 2017). Salt marshes further provide supporting services, like plant primary production, nutrient cycling, soil formation, and biodiversity (Bellingham et al., 2005; Cattrijsse and Hampel, 2006; Himes-Cornell et al., 2018). Indeed, salt marshes are also known as the “ecological guardians of the coast”, as they play important roles in flood protection, and for the improvement of water quality for adjacent marine and estuarine environments (Duarte et al., 2013; Himes-Cornell et al.,



2018). Since the start of the 21<sup>st</sup> century, the long-term carbon storage and high carbon sequestration rate of salt marshes have gained much attention (Chmura et al., 2003; Drake et al., 2015; Duarte et al., 2013; Kirwan and Mudd, 2012; McLeod et al., 2011; Mueller et al., 2019; Spivak et al., 2019). This ecosystem service is now also frequently referred to as ‘blue carbon’ (Macreadie et al., 2019; McLeod et al., 2011).

## **1.2 Carbon sequestration in salt marshes**

Salt marshes, together with mangrove forests and seagrass beds, belong to the “blue carbon” ecosystems, which sequester approximately 4.8-87.2 Tg carbon per year and represent a major part of the soil carbon sink (McLeod et al., 2011) (Figure 1.2). Salt marshes can store large quantities of carbon due to two reasons: First, tidal environments have high primary productivity meaning that plants sequester comparatively large amounts of carbon dioxide into their tissue (Fornara and Tilman, 2008; Keppler et al., 2006). The primary production in salt marshes ranges from 1.50 to 6.75 kg·m<sup>-2</sup>·year<sup>-1</sup> (Madrid et al., 2012) and is thus higher than net primary production in many other terrestrial ecosystems (i.e. forests in North America range from 1.12 to 2.55 kg·m<sup>-2</sup>·year<sup>-1</sup> and forests of the East Asian tropical region are estimated to have a net primary production of 0.85 kg·m<sup>-2</sup>·year<sup>-1</sup>) (Byun et al., 2019). Second, regular flooding leads to low oxygen availability and high salinity in marsh soils (Marani et al., 2004; Sousa et al., 2017) which slows down the organic matter decomposition of dead plant material and results in a high accumulation of organic matter in sediments (Orwin et al., 2010; Wang et al., 2019). In addition, a part of the organic matter stored in salt marshes can also be ascribed to sea-level rise, because of the allochthonous organic matter input by flooding (Kirwan and Megonigal, 2013; Wright et al., 2013).



**Figure 1.2** Comparison among plant (above-ground and below-ground) carbon stocks and soil mean long-term carbon sequestration rates (C burial rate) of coastal wetlands and other ecosystems (adapted from Adame et al., 2013 and McLeod et al., 2011).

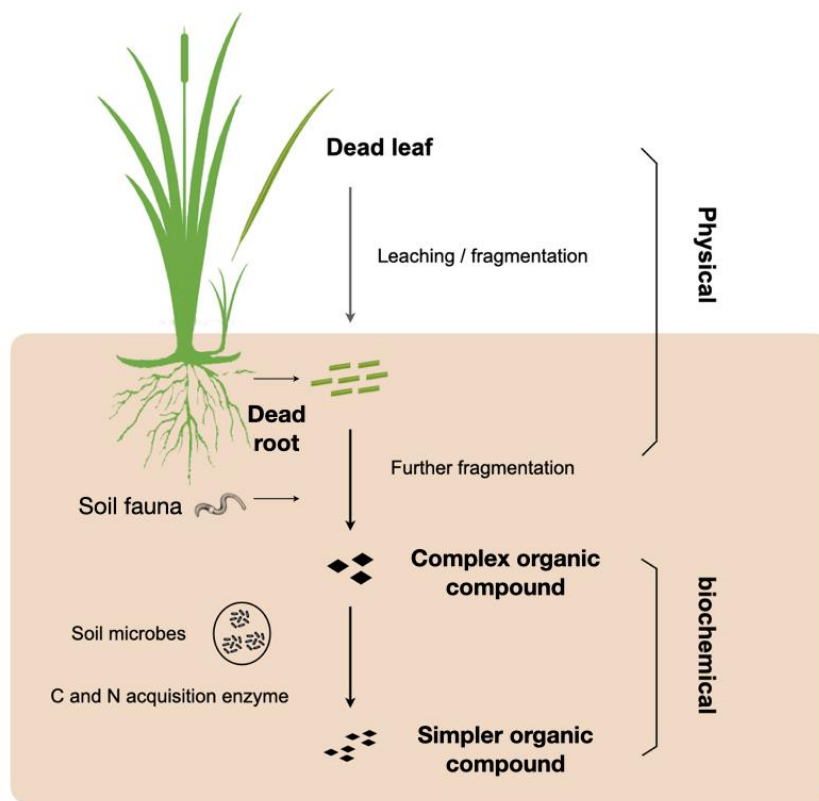
### 1.3 Organic matter decomposition in salt marshes

The decomposition of organic matter is a central factor regulating carbon sequestration of ecosystems (Post and Kwon, 2000; Russell et al., 2015). In salt marshes, organic matter decomposition begins with fresh plant litter and ultimately ends with its transfer to the different forms of soil organic matter (Chomel et al., 2016; Djukic et al., 2018; Singh and Gupta, 1977). During the decomposition process, two general stages can be identified, namely the physical breakdown and the biochemical transformation (Prescott, 2010; Schimel and Schaeffer, 2012).

The process of physical breakdown includes leaching and fragmentation, and is the initial stage of decomposition, which helps to break down plant litter into smaller pieces (Schimel and Schaeffer, 2012; Yan et al., 2019). Leaching is the abiotic removal of soluble

compounds by water, resulting in a rapid initial loss of compounds from the fresh litter (Marley et al., 2019). Litter fragmentation is the physical breakdown of plant litter into smaller particles by detritivores like soil fauna, and it plays a particularly vital role in retention, breakdown, and nutrient cycling (Pavao-Zuckerman, 2018; Sauvadet et al., 2017; Yang et al., 2012). These fragmented particles may contain an abundance of water-soluble nutrients which get dissolved in the water and seep into soils or sediments (Ferreira et al., 2006; Smuda and Glomb, 2013).

Biochemical transformation is the process by which the organic substances (i.e. labile or recalcitrant substrates) break down into simpler compounds by the action of soil microbial activity (Gutknecht et al., 2006; Prescott, 2010). Soil enzymes are mainly derived from root exudates, plant litter, and microorganisms (Hendriksen et al., 2016; Sinsabaugh and Shah, 2011). They have been identified as the rate-limiting step of organic matter decomposition (Moorhead et al., 2012; Sinsabaugh et al., 2009; Sinsabaugh and Shah, 2011). For example, the  $\beta$ -glucosidase is considered as the major enzyme in microbial carbon acquisition (Mueller et al., 2017; Sihi et al., 2019), and its activity is typically used to assess the organic matter decomposition in both marine and terrestrial ecosystems (Mueller et al., 2017; Sinsabaugh et al., 2009). Leucine-aminopeptidase and chitinase are the most commonly measured indicators of microbial nitrogen acquisition. They are closely associated with soil nitrogen mineralization and microbial nitrogen demand (Cenini et al., 2016; Sinsabaugh et al., 2012, 2008). Therefore, knowledge regarding the regulation of the activities of these enzymes by abiotic and biotic factors is crucial for understanding the mechanisms of soil organic matter decomposition in salt marsh ecosystems (Sinsabaugh et al., 2009).



**Figure 1.3** General scheme describing organic matter decomposition with the two major processes of physical and biochemical breakdown.

## Effects of abiotic factors on organic matter decomposition

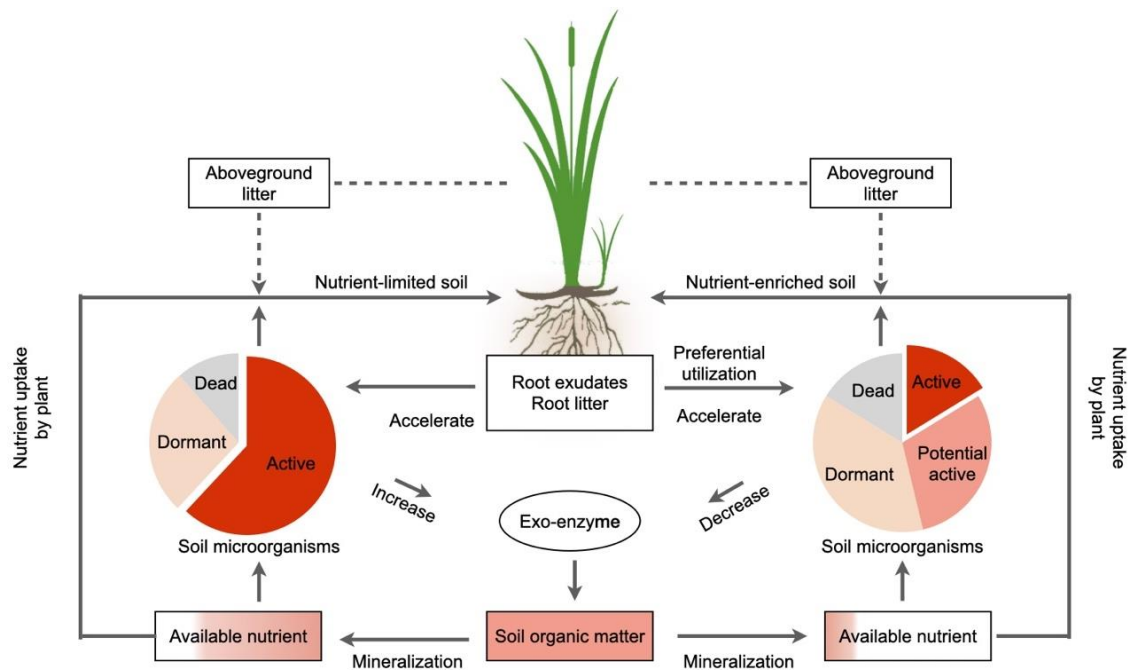
Abiotic factors exert strong control on soil organic matter dynamics by modulating the rates of biological activity of the microbial community and thereby also the rate of organic matter decomposition (Alberti et al., 2010; Noe and Zedler, 2000). Among different abiotic factors, temperature, oxygen availability, moisture, and pH in soils exert a strong influence on organic matter decomposition (Davidson and Janssens, 2006; Gavazov et al., 2014; Wolf et al., 2007). Particularly temperature can be considered as the main factor in determining organic matter decomposition. For instance, in wetland ecosystems, even small increases in temperature can significantly stimulate soil microbial activity and therefore have a positive effect on the rates of organic matter decomposition (Conant et al., 2011; Davidson

and Janssens, 2006). Besides, soil moisture is another main factor, acting in two contrasting ways as a modulator of organic matter decomposition. On the one hand, soil water solubilizes substrates and increases their availability in active microbial sites through diffusion (Kirschbaum, 1995). As soil moisture increases, it also reduces the physiological stress on microbes by reducing soil matric potential (Sierra et al., 2015). On the other hand, an increase in soil moisture fills up available pore spaces and thus reduces oxygen availability for the microbial community (Ghezzehei et al., 2019; Sierra et al., 2017). Yet, soil oxygen is a highly preferred terminal electron acceptor and critical for the deconstruction of complex organic compounds. More importantly, oxygen availability exerts an important control on the speed of organic matter decomposition in salt marsh soils. Hence, an optimum curve describes the relationship between soil moisture and organic matter decomposition (Kirwan and Guntenspergen, 2012).

## **Effects of biotic factors on organic matter decomposition**

Plant growth is considered an important biotic factor that can affect the organic matter decomposition in salt marshes, both directly through plant primary production and indirectly through root exudation (Mueller et al., 2016), a phenomenon referred to as the “priming effect” (Figure 1.4). However, both positive and negative rhizosphere priming effects on soil organic matter decomposition have been discussed in different ecosystems (Blagodatskaya and Kuzyakov, 2008; Fu and Cheng, 2002; Yue et al., 2014; Zhu et al., 2014). For instance, carbon allocation belowground for root growth and exudation of labile organic compounds may stimulate soil microbial activity (Blagodatskaya and Kuzyakov, 2008; Chen et al., 2014), which leads to an increase in organic matter decomposition (Fu and Cheng, 2002). Alternatively, plants acquire nutrients from the soil and this might

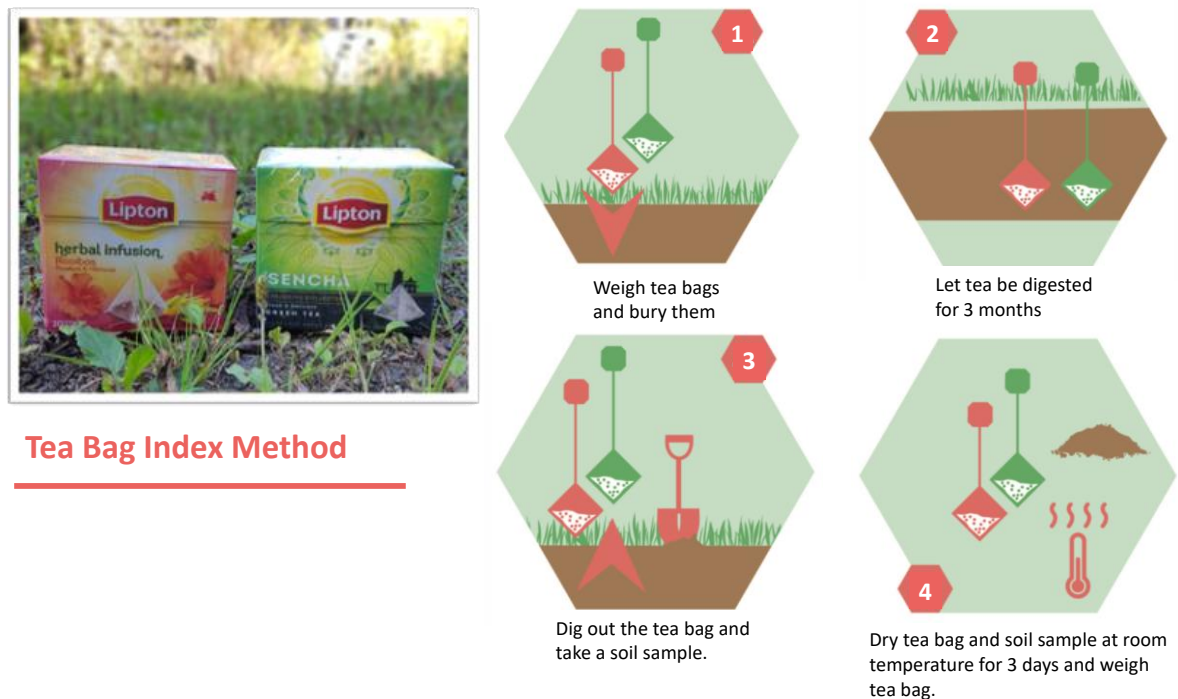
inhibit the activity of soil microbes, which ultimately suppress organic matter decomposition (Larsen et al., 2015).



**Figure 1.4** Conceptual diagram about the mechanism of rhizosphere priming effects. In nutrient-limited soils (left), plants allocate more photosynthates belowground and support soil microorganisms with carbon and energy. Thus, microbial biomass and activities increase and stimulate production of exo-enzymes by the rhizosphere microorganisms to decompose soil organic matter and release nutrients, referred to as the positive priming effect. In contrast, in nutrient-enriched soils (right), microorganisms have less demand for nutrients and thus preferentially utilize root exudates, leading to reduced production of extracellular enzymes. Furthermore, as plants provide less photosynthates belowground, microbial biomass and activities will decrease as well. Consequently, the decomposition of organic matter slows down, which is considered a negative priming effect. The pink color of the available nutrient pool indicates mineral nutrients derived from mineralization of organic matter. The area of the pink color represents the amount of mineral nutrients derived from mineralization of organic matter. (figure modified from Yue et al., 2014)

Furthermore, both inter- and intraspecific variation in plants can affect organic matter decomposition. Both quantity and quality of plant litter vary between plant species. The amount of litter and also its chemical constitution (e.g. content of lignin, cellulose, and hemicellulose) affect organic matter decomposition (Córdova et al., 2018; Prescott, 2010; Yan et al., 2018). Thus, it is difficult to compare litter decomposition rates across

ecosystems or sites. To facilitate such comparisons, Keuskamp et al. (2013) developed an efficient method for assessing litter decomposition and organic matter transformation, using commercially available tea as standardized material, a method known as the “Tea Bag Index” (Figure 1.5). This approach allows for the determination of the decomposition rate constant ( $k$ ) and the stabilization factor ( $S$ ), which describes the fraction of labile and rapidly decomposable organic matter that becomes stabilized during deployment.



**Figure 1.5** The Tea Bag Index method at a glance (modified from teatime4schools). Briefly, two different types of tea bags are weighed and deployed at 5 cm soil depth. Tea bags are retrieved after an incubation period of three months, carefully separated from roots and soil, dried at 70 °C, and weighed again. The parameters determined by the Tea Bag Index are the decomposition rate constant and the stabilization factor, which are calculated following Keuskamp et al., (2013) and Mueller et al., (2018).

During the last decade, evidence has accumulated that intraspecific plant variation or plant genotypes can play a critical role in regulating litter decomposition (Hines et al., 2014; Madritch and Lindroth, 2011; Wang et al., 2014). Different plant genotypes of the same species can induce important changes in soil microbial activity and plant litter quality due

to their differences in physiology and growth (Bandau et al., 2017; Madritch et al., 2006; Semchenko et al., 2017). These effects may potentially lead to differences in organic matter decomposition. However, the mechanisms linking intraspecific genetic variation with organic matter decomposition is still poorly understood in salt marshes, even though significant genetic differentiation within salt marsh species has been identified (Bustos-Korts et al., 2018; Crutsinger et al., 2006; Fischer et al., 2014; Madritch et al., 2006; Schweitzer et al., 2008b; Semchenko et al., 2017).

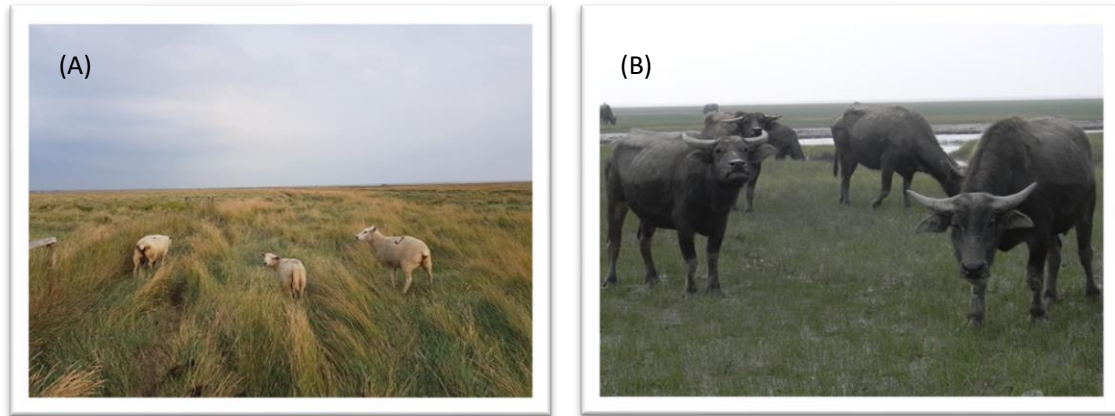
## **1.4 Effects of global change on organic matter decomposition**

Global change refers to planetary-scale changes affecting the earth system as a whole. These changes include climate, biogeochemical cycles, biodiversity, sea-level, and land-use (Camill, 2010). Currently, the major drivers of global change can be attributed to anthropogenic impacts. For instance, ongoing global warming and biodiversity loss are mainly driven by human activities and their related land-use change, including e.g. agriculture and urbanization (Hautier et al., 2015; Nelson et al., 2006). Current research in global change factors mainly tries to understand how drivers of change impact ecological systems across different scales (Bardgett et al., 2008; Burns et al., 2013; Mueller et al., 2018; Nelson et al., 2006; Zhang et al., 2015). In salt marshes, recent investigations on global change mainly focused on the effects of accelerated sea-level rise (Kathilankal et al., 2008; Kirwan and Guntenspergen, 2010; Kirwan and Megonigal, 2013), of climate change (Duarte et al., 2013), and of land-use change (Mueller et al., 2017; Nolte et al., 2015, 2013) on the ecosystem. All these global change drivers are also expected to have important consequences for carbon sequestration of salt marshes.



## **Land-use change**

Salt marshes and other tidal marshes have been subjected to different types of land use, such as mowing and livestock grazing for a long time, especially in Europe and in East Asia. Livestock grazing is known to change vegetation and soil properties (Davidson et al., 2017; Di Bella et al., 2015; Keshta et al., 2020), which can in turn alter the carbon and nutrient cycle. The most frequently hypothesized mechanism by which grazing affects organic matter decomposition in salt marshes is soil compaction by trampling (Mueller et al., 2017). Livestock treading the salt marshes leads to an increase in soil bulk density and a decrease in soil oxygen availability (Elschot et al., 2015; Tang et al., 2020), which is expected to inhibit soil microbial activity and ultimately slows down organic matter decomposition (Mueller et al., 2017). However, livestock grazing is also known to alter plant primary production and species composition (Bakker et al., 2020; Jones et al., 2004; Mueller et al., 2017), which in turn can influence the quality and quantity of plant litter, root exudation, and also of allochthonous organic matter input to the organic matter pool salt marsh soils (Haynes et al., 2014; Mueller et al., 2017; Olsen et al., 2011). These changes in microbial substrate supply can potentially control the microbial carbon and nutrient demand, and thus affect soil organic matter decomposition. However, the link between soil compaction by trampling and soil microbial activity has never been demonstrated in salt marshes.



**Figure 1.6** Examples of livestock grazing in salt marshes: (A) Hamburger Hallig, Schleswig-Holstein, Germany; (B) Chongming Island, Shanghai, China.

## **Accelerated sea-level rise**

Since the start of the 20<sup>th</sup> century, the average global sea-level rose by 3.3 mm per year, primarily due to human-induced global warming and associated melting of glaciers as well as the “thermal expansion” of seawater (IPCC, 2007). According to widespread perceptions, accelerated sea-level rise was until recently expected to lead to severe salt marsh loss (Horton et al., 2018; Kirwan et al., 2016b; Wang et al., 2019). Yet, this may be highly overestimated, as recent studies have recognized that the losses of salt marshes would probably be much smaller than expected due to the increased accretion under higher rates of sea-level rise. Only the most rapid scenarios of sea-level rise might be problematic for salt marshes (Kirwan et al., 2016a). Nevertheless, we still need knowledge on how sea-level rise and increased flooding frequencies affect organic matter decomposition and hence carbon sequestration in salt marsh soils. A great number of empirical studies suggest that sea-level rise affects both abiotic and biotic factors (Kirwan and Megonigal, 2013; Kirwan and Mudd, 2012; Rogers et al., 2019) which, in turn, control organic matter decomposition.

Sea-level rise may decrease the rate of organic matter decomposition via abiotic conditions, for example, due to lower soil oxygen availability and increased soil salinity (Kirwan et al., 2013; Wang et al., 2019). However, Kirwan et al. (2013) suggested that the relationship between relative sea-level and the rate of organic matter decomposition follows an optimum curve. That is, moderate sea-level rise may supply nutrients and more suitable circumstances for soil microbial activity, thus accelerating organic matter decomposition. Conversely, higher rates of sea-level rise result in a decrease in soil oxygen availability and suppress the activity of soil microbes, thereby slowing down the rate of organic matter decomposition.

Biotic factors, especially plant growth, also mediate organic matter decomposition in response to sea-level rise. Here, an indirect pathway of effects of sea-level rise on organic matter decomposition is proposed, represented through changes in plant litter quality and the input of labile organic compounds (i.e. root exudates) to the soil environments. According to the results from Mueller et al., (2016), the response of organic matter decomposition to sea-level rise in salt marshes is not directly affected by relative sea-level and soil oxygen availability, but indirectly influenced by plant primary production. This implies that plants may exert an overriding effect on the decomposition of organic matter in salt marshes. Therefore, further studies need to pay close attention to how plant species or intraspecific variation in plants mediate the effect on organic matter decomposition under sea-level rise.

## **Global warming**

The global temperature has risen about 0.9 °C since the late 19<sup>th</sup> century, a change driven largely by increased atmospheric carbon dioxide concentrations and other anthropogenic emissions into the atmosphere (IPCC, 2007). Global warming is now affecting rates of

decomposition in salt marsh ecosystems (Kirwan and Mudd, 2012; Spivak et al., 2019). The temperature-sensitivity of organic matter decomposition can be described by a common set of principles of kinetic theory (Davidson and Janssens, 2006; Kuzyakov and Xu, 2013; Razavi et al., 2015). These indicate temperature-induced increases in soil microbial activity and directly accelerate the rate of organic matter decomposition in the relatively cold, temperate zone (Davidson and Janssens, 2006). Contrastingly, in warmer climates, higher temperatures could slow down the soil microbial activity indirectly via reductions in soil water availability (Di Nardo et al., 2004; Krivtsov et al., 2006). Furthermore, Mueller et al., (2018) suggest that temperature-induced increases in the initial step of organic matter decomposition are not driven by changes in rates of litter decomposition, but by changes in the transformation of fresh and rapidly decomposable organic matter into stable organic compounds.

Yet, the impacts of climate warming on organic matter decomposition remain unclear due to contradictory results from warming experiments in various ecosystems (Allison et al., 2010; Hicks Pries et al., 2017; Kirschbaum, 1995; Kirwan and Mudd, 2012; Rubenstein et al., 2017; Schuur et al., 2008). These could have been caused by interactions between warming and other abiotic factors. Soil moisture, as the major abiotic factor in salt marshes, for example, could mediate the response of organic matter decomposition to warming. Generally, rising temperatures can result in drought conditions and thus indirectly slow down the rate of decomposition. But these interactive effects of temperature and soil moisture may be less important to organic matter decomposition in tidal marshes, due to regular flooding (Kirwan et al., 2014; Kirwan and Blum, 2011). Furthermore, warming effects on organic matter decomposition via plant growth are still poorly understood. Prior experiments found both positive and negative plant growth responses to warming in tidal marshes depending on plant species. For instance, a warming experiment conducted in the

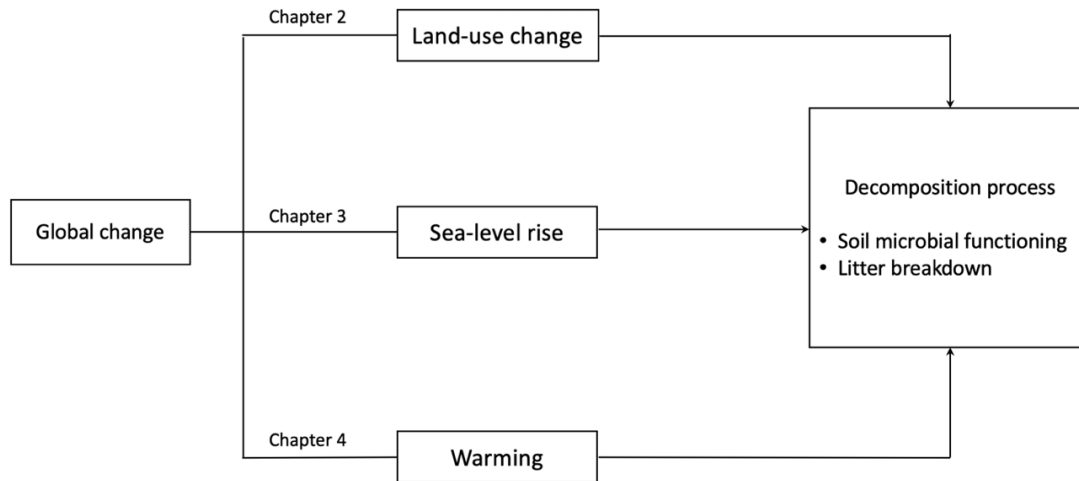
U.S Global Change Research Wetland found that plant primary production at sites dominated by C<sub>3</sub> plants was increased by higher temperatures, whereas at sites with C<sub>4</sub> plants dominating vegetation, primary production was significantly decreased with warming (Noyce et al., 2019). However, the European salt marshes differ from North American marshes. The major difference is, that European salt marshes are minerogenic marshes (Nolte et al., 2013), but salt marshes in North American are mainly organogenic (Hanson et al., 2016; Hughes, 2004). Therefore, these marshes differ in the carbon content of the soil and other soil properties. Furthermore, the plant species of both types of salt marshes are different. The dominant species in North American salt marshes are *Spartina alterniflora*, *Juncus roemerianus*, and *Distichlis spicata* (Noyce et al., 2019). In contrast, *Salicornia europaea*, *Spartina anglica*, *Elymus athericus*, *Puccinellia maritima*, *Scirpus maritimus*, and *Phragmites australis* are dominant species in European salt marshes (Garbutt et al., 2017; Suchrow et al., 2012). These differences probably lead to a different response of organic matter decomposition to global climate change in these marsh types. Thus, it is essential to develop a field warming experiment in European salt marshes and investigate the response of decomposition processes to rising temperatures, which could supply more accurate predictions for carbon cycling under global climate change.

## 1.5 Thesis outline

The main aim of this thesis is to improve the understanding of the effects of global change on soil microbial activity and thus organic matter decomposition in salt marshes (Figure 1.7). Three major factors of global change were chosen for this study, namely sea-level rise, livestock grazing, and warming. Therefore, the central research questions of this thesis are,

- 1) How does global change affect soil microbial activity and organic matter decomposition?

2) Which abiotic and/or biotic factors drive the effect of global change on soil microbial activity and organic matter decomposition?



**Figure 1.7** General structure of this thesis. Chapter 2 studies the livestock grazing effects on organic matter decomposition via soil exo-enzyme activity and litter breakdown. Chapter 3 describes the interaction effect of sea-level rise and plant-genotype on soil microbial activity, with potential implications for organic matter decomposition. Chapter 4 focuses on the effects of warming on soil microbial activity and litter breakdown in whole soil profiles.

The work presented here was conducted in the Dongtan salt marsh on Chongming Island, China (**Chapter 2**), and in the salt marshes of the European Wadden Sea (**Chapter 3** and **4**). **Chapter 2** assesses the mechanisms by which grazing affects organic matter decomposition by quantifying the activity of microbial exo-enzymes and further litter decomposition parameters. **Chapter 3** presents the interaction effect of sea-level rise and plant-genotype identity on soil microbial functioning, including the exo-enzyme activity and the composition of the microbial community, with potential implications for organic matter decomposition. **Chapter 4** explores the mechanisms by which warming may alter rates of organic matter decomposition by assessing soil microbial functioning (i.e. soil exo-enzyme activity and microbial biomass) and litter decomposition at whole-soil profiles of salt marshes.

# 2 Grazing mediates soil microbial activity and litter decomposition in salt marshes

## Abstract

Salt marshes contribute to climate change mitigation because of their great capacity to store organic matter (OM) in soils. Most of the research regarding OM turnover in salt marshes in times of global change focuses on effects of rising temperature and accelerated sea-level rise, while effects of land-use change have gained little attention. The present work investigates the mechanisms by which livestock grazing can affect OM decomposition in salt marsh soils. In a grazing exclusion experiment at the mouth of the Yangtze estuary, China, we assessed soil microbial exo-enzyme activity (EEA) to gain insight into the microbial carbon (C) and nitrogen (N) demand. Additionally, we studied the decomposition of plant litter in soil using the Tea Bag Index (TBI), a widely used standardized litter bag assay to fingerprint soil decomposition dynamics. Based on EEAs, grazing markedly reduced microbial C acquisition, whereas microbial N acquisition was strongly increased. These opposing grazing effects were also evident in the decomposition of standardized plant litter: The decomposition rate constant ( $k$ ) and the stabilization ( $S$ ) of litter were not inversely related, as would be expected, but instead both were reduced by livestock grazing. Our data suggest that grazing effects on EEAs and litter decomposition can just partly be explained by grazing-driven soil compaction and resulting lower oxygen availability, which has previously been hypothesized as a main pathway by which grazing can reduce microbial activity in wetland soils. Instead, grazing effects on microbial nutrient demand occurs to be an at least equally important control on soil decomposition processes.

**Keywords:** Livestock, Carbon sequestration, Land-use change, Blue carbon, Enzyme stoichiometry, Tea Bag Index

## **1 Introduction**

As “blue carbon” ecosystems, salt marshes store large amounts of carbon (C) in form of organic matter (OM) in their soils (Chmura et al., 2003) and are considered one of the most important long-term C sinks of the biosphere (Duarte et al., 2013; McLeod et al., 2011). Salt marshes not only play an important role in coastal C sequestration, and thus contribute to climate change mitigation, they also provide other highly valued ecosystem services, like coastline protection and biodiversity support (Barbier et al., 2011; Möller et al., 2014). However, these ecosystem services of salt marshes are affected by global change (i.e. climate and land-use change), and therefore, there is a growing interest to understand how global change factors alter the potential for ecosystem-service delivery (Kirwan and Megonigal, 2013).

Several studies evaluated how C sequestration in salt marshes is affected by climate change factors, such as rising temperatures and accelerated sea-level rise (Kirwan et al., 2013; Mueller et al., 2016; Mueller et al., 2018; Rogers et al., 2019), whereas land-use change, such as the introduction or abandonment of livestock grazing, received much less attention. The use of salt marshes for livestock grazing has a long history especially in Europe, where it dates back to pre-historic times (Barr and Bell, 2017; Nolte et al., 2015; Tessier et al., 2003), but livestock grazing in salt marshes can also be found in South America (Di Bella et al., 2015; Sica et al., 2016) and East-Asia (He et al., 2015; Ning et al., 2019; Suzuki and Suzuki, 2011). Yet, the effect of this common land-use practice on C sequestration is unclear. Recent studies, mostly from Europe, provide equivocal results concerning grazing effects on soil C stocks and sequestration rates in salt marshes (Elschot et al., 2015; Ford



et al., 2019; Harvey et al., 2019; Morris and Jensen, 1998). C sequestration in salt marshes is controlled by two primary processes, namely OM input via plant primary production and OM output via decomposition (Kirwan et al., 2013). Both of these processes are strongly affected by livestock grazing (Mueller et al., 2017), with a large number of studies outlining that livestock grazing reduces biomass (reviewed by Davidson et al., 2017)). Yet, most of these studies quantified only effects on aboveground biomass, while those also quantifying belowground biomass are scarce (Davidson et al., 2017). Among these, Elschot et al. (2015) demonstrated that livestock grazing could in fact promote higher belowground biomass and thereby increase OM input to the soil.

In comparison to grazing effects on biomass production in salt marshes, its effects on OM decomposition is far less understood (Elschot et al., 2015; Mueller et al., 2017). To understand how livestock grazing regulates OM decomposition in salt marshes, particularly two mechanisms need consideration, namely grazing effects on the microbial substrate supply (i.e. changes in OM quality and quantity) and grazing effects on soil oxygen availability (Elschot et al., 2015). Grazing can affect the microbial substrate supply via several mechanisms including changes in plant primary production and species composition, which regulate the quality and amount of plant litter, root exudates, and allochthonous OM entering the soil OM pool of salt marshes (Ford et al., 2013; Mueller et al., 2019; Olsen et al., 2011). These changes in microbial substrate supply are potentially important controls of the microbial C and nutrient demand and thus affect OM decomposition (Sinsabaugh et al., 2008).

The probably most frequently hypothesized mechanism by which grazing affects decomposition in wetland soils is trampling-driven soil compaction (i.e. reduction of pore space) and the resulting reduction of soil oxygen availability (Elschot et al., 2015; Kauffman et al., 2004; Mueller et al., 2017; Schrama et al., 2013). This, in turn, can inhibit

microbial exo-enzyme activity (EEA), metabolism, and ultimately OM decomposition (Davidson and Janssens, 2006; Freeman et al., 2001; Megonigal et al., 2004). Indeed, recent insights from salt marsh ecosystems could provide some evidence of trampling-driven soil compaction and resulting reductions in oxygen availability (Elschot et al., 2015). Yet, the link between trampling-driven soil compaction and decomposition has never been demonstrated.

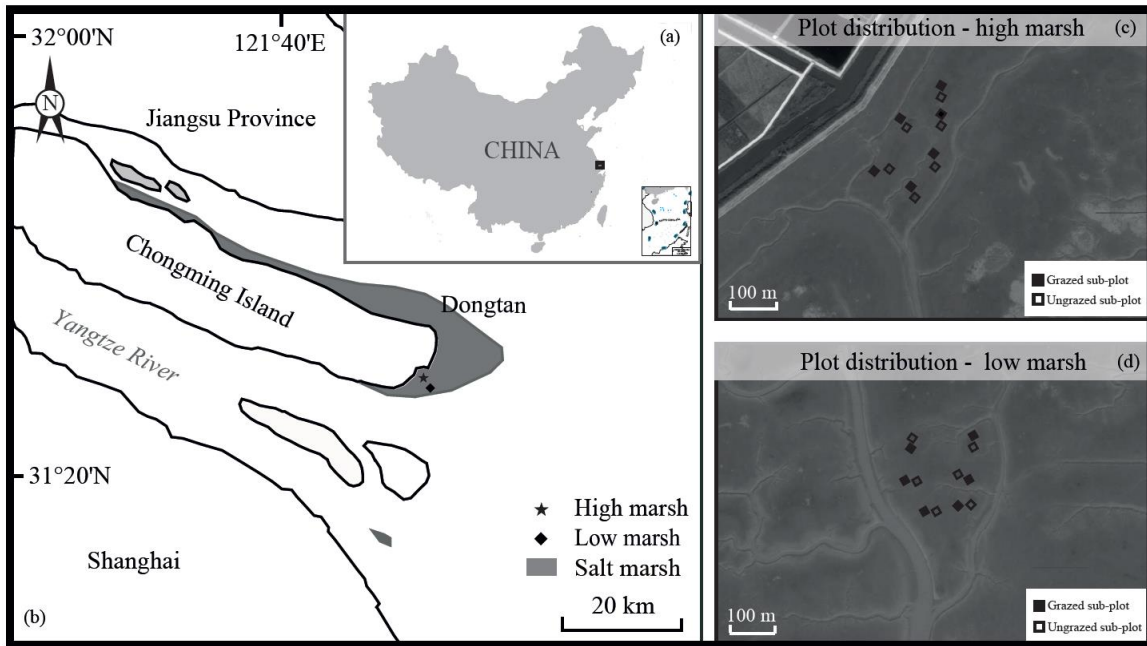
The aim of this study is to improve the mechanistic understanding of how livestock grazing affects decomposition processes in salt marsh soils. The study was conducted in a grazing-exclusion experiment in a Chinese salt marsh ecosystem of the Yangtze estuary. Estimates of the total Chinese salt marsh area highly variable and range between  $1.2 \times 10^6$  and  $3.5 \times 10^6$  ha (Hu et al., 2019; Meng et al., 2017). Livestock grazing is a relatively common land-use in Chinese salt marshes (Davidson et al., 2017); however, data on grazing frequency and intensity is not available. We assessed the mechanisms by which grazing affects OM decomposition by quantifying the activity of microbial exo-enzymes and litter decomposition parameters. EEAs are regarded as the rate-limiting step of the decomposition processes and reveal insight into microbial C and nutrient demands (Sinsabaugh et al., 2008). However, EEA dynamics have yet poorly been studied in salt marsh ecosystems. We hypothesize (1) that grazing reduces EEAs by increasing soil bulk density and thereby lowering soil oxygen availability. We hypothesize (2) that grazing induced reductions in EEAs will translate into a higher degree of litter stabilization in soil and, inversely, lower rates of litter decomposition.

## **2 Methods**

### **2.1 Site description and experimental design**

This study was carried out in the Dongtan salt marsh on Chongming island, China (31°28'N, 121°56'E), close to the city of Shanghai (Figure 2.1a, b). The Dongtan salt marsh covers an area of 4000 ha and is one of the largest tidal wetlands of the Yangtze estuary. It is a minerogenic salt marsh with high rates of sediment deposition, a typical feature of the Chinese salt-marsh landscape (Yang et al., 2008, 2001). The study area is exposed to subtropical humid monsoon climate, and the annual temperature and precipitation are 15.3 °C and 1022 mm, respectively. The average spring tide range is approximately 2.5-3.5 m. The mean elevation of high marsh is 380 cm above sea level, resulting in an average monthly inundation frequency of 17. The mean elevation of the low marsh is 330 cm above sea level, resulting in an average monthly inundation frequency of 39. The salt marsh is grazed by cattle from early April to late October at a stocking density of approximately one cattle per ha (Yang et al., 2017). Dominant plant species in the high marsh are *Phragmites australis* and *Carex scabrifolia*, while *Scirpus mariqueter* and *C. scabrifolia* are dominant in the low marsh. Both marsh zones have similar grazing levels (Yang et al., 2017).

The design of the experiment has previously been described by Yang et al. (2017). Briefly, it includes two marsh zones (low vs. high) and land-use (grazed vs. ungrazed) as factors. In 2014, six replicate plots were established in each of the marsh zones. Each plot contains two sub-plots of 15 × 15 m, a grazed (control) and an ungrazed (exclusion) sub-plot. The total number of sub-plots is N = 24 (6 replicates × 2 marsh zones × 2 grazing treatments), and distance between plots within a marsh zone was 50-100 m (Figure 2.1c, d).



**Figure 2.1** Location of the experimental site (a, b) and sub-plot distribution in low- and high-marsh zones (c, d).

## 2.2 Biomass and soil bulk density

Belowground biomass was sampled to understand potential differences in belowground OM input, and thus microbial substrate supply, between grazed and ungrazed sub-plots. Samples were collected in May of 2016 within three randomly positioned 25 cm × 25 cm quadrats using a PVC corer of 15 cm diameter into 20 cm depth in each sub-plot. Samples were washed to remove all soil with a 0.5 mm sieve, dried at 70°C for 72 h, and weighed. This work is focused on belowground C dynamics. For aboveground biomass data, we refer the reader to previous work of our group (Yang et al., 2017). Soil bulk density (BD (g/cm<sup>3</sup>) = soil dry weight (g) / soil volume (cm<sup>3</sup>) was determined as a proxy for soil compaction and thus oxygen availability (Schrama et al., 2013). One soil sample was collected in each sub-plot and separated in three depth increments (0-5 cm, 5-10 cm, and 10-15 cm) using a 3.2-cm diameter soil corer. Samples were dried at 70 °C for 72 h and weighed.

### 2.3 Exo-enzyme assays

Following Sinsabaugh et al. (2009), we measured soil  $\beta$ -glucosidase activity for the assessment of microbial C acquisition. Leucine-aminopeptidase and chitinase activity were measured for the assessment of microbial N acquisition. Soil samples were collected in May 2016 using a 3.2-cm diameter corer to sample the topsoil (0-5 cm). EEAs were determined in fluorometric assays following Mueller et al., (2017). In brief, a 20-g subsample of fresh topsoil from each sub-plot was mixed in 20 mL deionized water, and the homogeneous slurry was stored at -20 °C until further analysis. Well-plate assays were conducted to measure potential enzyme activity. Plates were incubated in the dark at 20 °C for 16 h and read on a Multi-Detection Microplate Reader (Bio-tek Synergy™ HT, Winooski, USA). Activities reported refer to normalized EEAs per unit soil OM (i.e. specific EEA), in order to obtain a measure for OM decomposition rate (Morrissey et al., 2014; Mueller et al., 2017). OM contents of subsamples were assessed using loss on ignition following the protocol of Wang et al., (2011) for marine sediments (550 °C for 5 h).

### 2.4 Decomposition of standardized plant litter

We studied the decomposition of standardized litter to control for potential differences in native plant-litter quality (i.e. microbial substrate quality) between grazed and ungrazed treatments of our field experiment. Specifically, the decomposition rate constant ( $k$ ) and the stabilization factor ( $S$ ) were assessed following the Tea Bag Index (TBI) protocol (Keuskamp et al., 2013). The decomposition rate constant ( $k$ ) describes the decomposition rate constant – i.e. the rate at which mass is lost over time – a parameter typically presented in litter-bag studies. However, in the TBI approach,  $k$  only refers to the labile (i.e. hydrolyzable) fraction of the deployed material.  $S$  describes the part of the labile,

hydrolyzable fraction that did not decompose due to soil environmental factors leading to its stabilization (Keuskamp et al., 2013). The TBI is a standardized litter-decomposition assay using commercially available tea materials as standardized plant litter, which has been widely applied to characterize and compare decomposition dynamics within and across ecosystems (Keuskamp et al., 2013; Mueller et al., 2018). In each sub-plot, two polypropylene tea bags (55 mm x 50 mm) were buried from early June to late August 2015, one containing green tea and one containing rooibos tea. Tea bags were deployed at 5 cm soil depth. The initial weight of the contents was determined by subtracting the weight of empty bags. Bags were retrieved after an incubation period of 90 days, carefully separated from roots and soil, dried for 48 h at 70 °C, and weighed. The TBI parameters  $k$  and  $S$  were calculated following the tidal-wetland-adapted TBI protocol by Mueller et al., (2018):

$$(1) W_r(t) = a_r e^{-kt} + (1-a_r),$$

$$(2) S = 1-a_g/H_g,$$

$$(3) a_r = H_r(1-S).$$

$W_r(t)$  describes the weight of the rooibos substrate after the incubation time ( $t$  in days);  $a_r$  is the labile and  $1-a_r$  is the recalcitrant part of the rooibos substrate;  $k$  is the decomposition rate constant;  $S$  is the stabilization factor;  $a_g$  is the decomposable part of green tea substrate, and  $H_g$  is the hydrolyzable fraction of the green tea substrate. The decomposable part of the rooibos substrate is calculated in Eq. (3) based on the hydrolyzable fraction ( $H_r$ ) and  $S$ . We used the  $H_g$  and  $H_r$  values published in Mueller et al., (2018), because the tea materials used for the present study are from the same batches.

## 2.5 Statistical analyses

Two-way ANOVAs were conducted to test for effects of land-use (grazed vs. ungrazed) and marsh zone (high marsh vs. low marsh) on belowground biomass, soil bulk density, EEAs ( $\beta$ -glucosidase, leucine-aminopeptidase, chitinase, and the ratio of  $\beta$ -glucosidase activity / (leucine-aminopeptidase activity + chitinase), and TBI parameters ( $k$  and  $S$ ). Tukey HSD tests were conducted for pairwise comparisons. Normal distribution of residuals was assessed visually and equal sample sizes across groups assured robustness for parametric testing (McGuinness, 2002). Linear regression was used to test for the hypothesized relationships between bulk density, EEAs, and TBI parameters. All analyses were conducted using the statistical software OriginPro 2018 (OriginLab Corp., NorthamptonCity, USA).

### **3 Results**

#### **3.1 Plant biomass and soil bulk density**

Belowground plant biomass was significantly decreased by grazing, but not affected by marsh zone and the interaction of grazing and marsh zone (Table 2.1). Grazing decreased belowground plant biomass by 31% in the high marsh and by 54% in the low marsh (Table 2.2). Bulk density was significantly higher in low-marsh vs. high-marsh plots (Table 2.2). Grazing increased bulk density, whereas this effect was only marginally significant in the topsoil ( $p = 0.051$ ) and more pronounced with soil depth (Table 2.1). Grazing increased the bulk density at 5-10 cm depth by 10% in the low marsh and by 14% in the high marsh. Similarly, grazing increased bulk density at 10-15 cm soil depth by 16% in the low marsh grazing and 7% in the high marsh (Table 2.2).

**Table 2.1** ANOVA table for two-way (marsh zone and grazing treatment) analyse. Response variables are belowground biomass (BB), bulk density (BD),  $\beta$ -glucosidase activity (GLU), leucine-aminopeptidase activity (LAP), chitinase activity (CHI), the ratio of C- vs. N-acquiring enzymes (GLU/(CHI+LAP)), stabilization factor (*S*), and decomposition rate constant (*k*). n = 6, degrees of freedom for main- and interaction-effect tests = 1.

	Marsh zone		Grazing		Interaction	
	F	p-value	F	p-value	F	p-value
BB	1.45	ns	5.10	<0.05	0.22	ns
BD (0-5 cm)	7.94	<0.05	4.31	<0.1	2.02	ns
BD (5-10 cm)	6.56	<0.05	22.63	<0.001	0.02	ns
BD (10-15 cm)	3.21	<0.1	18.13	<0.001	2.35	ns
GLU	0.28	ns	5.79	<0.05	1.18	ns
LAP	0.06	ns	11.88	<0.05	0.08	ns
CHI	0.00	ns	1.18	ns	0.43	ns
GLU/(CHI+LAP)	0.16	ns	15.45	<0.001	1.78	ns
<i>S</i>	4.22	<0.1	167.89	<0.001	2.57	ns
<i>k</i>	3.84	ns	6.38	<0.05	0.32	ns

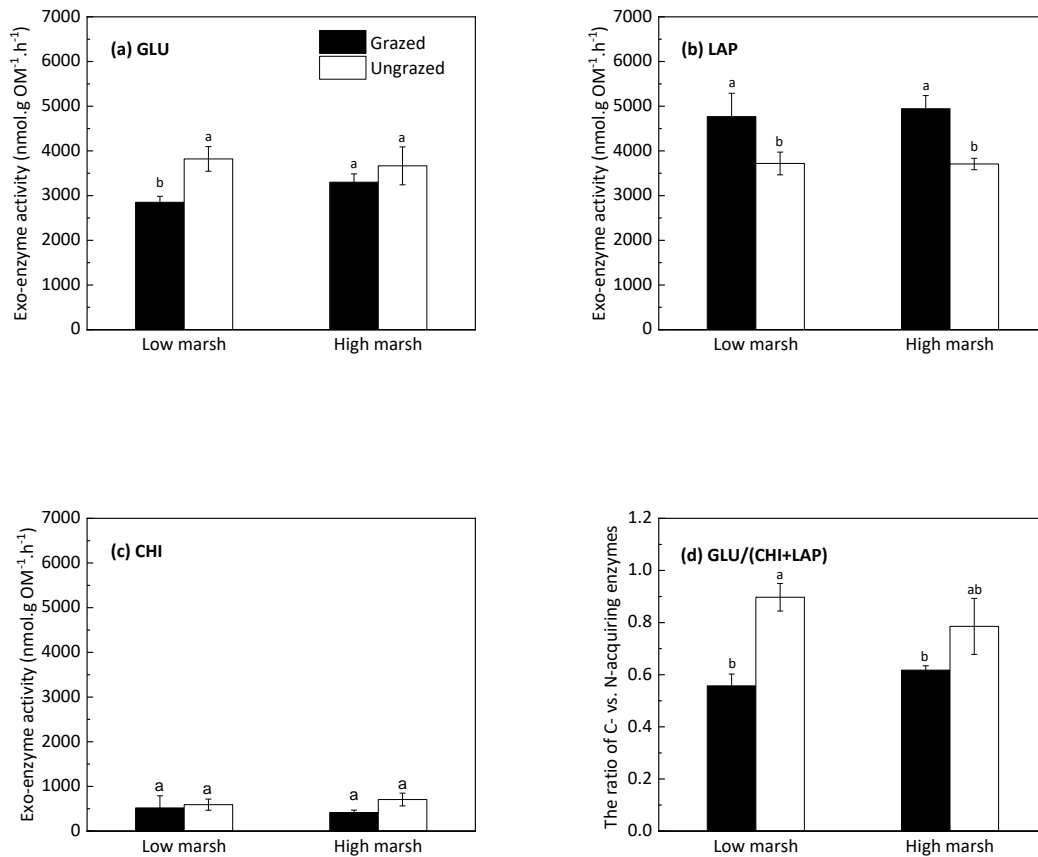
**Table 2.2** Belowground biomass (BB) and bulk density (BD) at different soil depths in grazed and ungrazed sub-plots of low and high marsh zones. Values are means  $\pm$  SE (n = 6). Letters indicate significant differences at  $p \leq 0.05$  within rows based on Tukey's HSD.

Section	Low marsh		High marsh	
	Grazed	Ungrazed	Grazed	Ungrazed
BB ( $\text{g}\cdot\text{m}^{-2}$ )	324.89 $\pm$ 56.24 a	707.90 $\pm$ 76.46 b	559.85 $\pm$ 153.91 b	810.57 $\pm$ 214.45 b
BD (0-5 cm) ( $\text{g}\cdot\text{cm}^{-3}$ )	1.29 $\pm$ 0.01 a	1.27 $\pm$ 0.02 a	1.25 $\pm$ 0.05 a	1.13 $\pm$ 0.05 a
BD (5-10 cm) ( $\text{g}\cdot\text{cm}^{-3}$ )	1.36 $\pm$ 0.03 a	1.22 $\pm$ 0.02 b	1.29 $\pm$ 0.04 a	1.13 $\pm$ 0.02 b
BD (10-15 cm) ( $\text{g}\cdot\text{cm}^{-3}$ )	1.56 $\pm$ 0.03 a	1.35 $\pm$ 0.05 b	1.44 $\pm$ 0.04 a	1.34 $\pm$ 0.02 a



### 3.2 Exo-enzyme activities

The activity of  $\beta$ -glucosidase was decreased by grazing (Table 2.1). Rates ranged from 2851 to 3300  $\text{nmol}\cdot\text{g OM}^{-1}\cdot\text{h}^{-1}$  in grazed sub-plots and 3665-3820  $\text{nmol}\cdot\text{g OM}^{-1}\cdot\text{h}^{-1}$  in ungrazed sub-plots.  $\beta$ -glucosidase activity was negatively related to bulk density (Table 2.3). There was also a significant and positive relationship between  $\beta$ -glucosidase activity and belowground biomass ( $r = 0.70$ ;  $p \leq 0.001$ ). Grazing strongly increased leucine-aminopeptidase activity in both the low and high marsh (Figure 2.2b). Rates ranged from 4768 to 4943  $\text{nmol}\cdot\text{g OM}^{-1}\cdot\text{h}^{-1}$  in grazed sub-plots and 3707-3719  $\text{nmol}\cdot\text{g OM}^{-1}\cdot\text{h}^{-1}$  in ungrazed sub-plots. In contrast to  $\beta$ -glucosidase activity, leucine-aminopeptidase activity was positively related to bulk density and not related to belowground biomass (Table 2.3). Chitinase activity was eight-times lower than leucine-aminopeptidase activity. In addition, chitinase activity was unaffected by grazing, marsh zone, and their interaction (Figure 2.2c). The ratio of C- vs. N-acquiring enzymes (activity of  $\beta$ -glucosidase / activity of leucine-aminopeptidase plus chitinase) was strongly decreased by grazing (Table 2.1). Grazing strongly reduced the ratio of C- vs. N-acquiring enzymes by 38% in the low marsh and by 21% in the high marsh (Figure 2.2d). The ratio of C- vs. N-acquiring enzymes was negatively related to soil bulk density (5-10 cm and 10-15 cm) (Table 2.3) and positively related to belowground biomass ( $r = 0.66$ ;  $p \leq 0.001$ ).

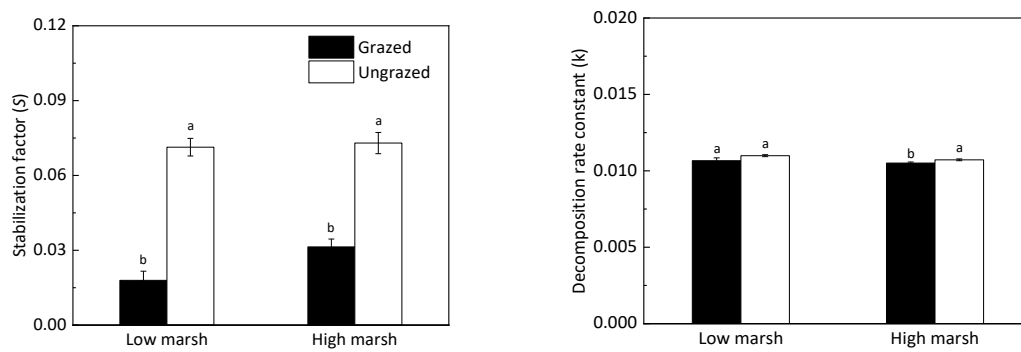


**Figure 2.2** The enzyme activity of  $\beta$ -glucosidase (GLU), leucine-aminopeptidase (LAP), chitinase (CHI), and the ratio of C- vs. N-acquiring enzymes (GLU/(CHI+LAP)) in grazed and ungrazed sub-plots of the low- and high-marsh zone. Values are means and SE ( $n=6$ ), bars not connected by the same letter are significantly different at  $p \leq 0.05$  based on Tukey's HSD.

### 3.3 Standardized litter decay based on TBI and its relation to EEAs

Both  $S$  and  $k$  were significantly decreased by livestock grazing (Table 2.1).  $S$  ranged from 0.018-0.031 in grazed sub-plots and 0.071-0.073 in ungrazed sub-plots.  $k$  ranged from 0.0105-0.0106 in grazed sub-plots and 0.0107-0.0110 in ungrazed sub-plots. Grazing significantly decreased  $S$  by 75% in the low marsh and by 57% in the high marsh (Figure 2.3). Grazing decreased  $k$  slightly, but significantly, by 3% in the low marsh and by 2% in the high marsh.  $S$  was negatively related to leucine-aminopeptidase activity, but was positively related to  $\beta$ -glucosidase activity and the ratio of C- vs. N-acquiring enzymes,

whereas  $k$  was negatively related to leucine-aminopeptidase activity and positively to chitinase activity (Table 2.3).



**Figure 2.3** Stabilization factor ( $S$ ) and decomposition rate constant ( $k$ ) in grazed and ungrazed sub-plots of the low- and high-marsh zone. Values are means and SE ( $n = 6$ ), bars not connected by the same letter are significantly different at  $p \leq 0.05$  based on Tukey's HSD.

**Table 2.3** Linear regression results for relationships between (A) bulk density at different soil depths and enzyme activities, and (B) enzyme activities in the topsoil and the TBI parameters  $S$  and  $k$ . R<sup>2</sup>-values are bold-typed at  $p \leq 0.05$ .

	GLU			LAP			CHI			GLU/(LAP+CHI)		
	R <sup>2</sup>	Slope	Intercept	R <sup>2</sup>	Slope	Intercept	R <sup>2</sup>	Slope	Intercept	R <sup>2</sup>	Slope	Intercept
<i>(A) bulk density vs. enzyme activities</i>												
BD (0-5 cm)	0.08	-2093	5994	0.10	3006	573	0.08	-1096	1912	0.12	-0.68	1.56
BD (5-10 cm)	<b>0.23</b>	-3170	7379	<b>0.41</b>	5530	-2642	<b>0.17</b>	-1480	2413	<b>0.44</b>	-1.20	2.23
BD (10-15 cm)	<b>0.35</b>	-3624	8559	<b>0.27</b>	4094	-1534	0.09	-996	1974	<b>0.50</b>	-1.17	2.39
<i>(B) enzyme activities vs. S and k</i>												
$S$	<b>0.19</b>	$2 \times 10^{-5}$	-0.01	<b>0.29</b>	$-1 \times 10^{-5}$	0.11	0.00	$3 \times 10^{-6}$	0.05	<b>0.47</b>	0.09	-0.02
$k$	0.02	$6 \times 10^{-8}$	0.01	<b>0.44</b>	$-2 \times 10^{-7}$	0.01	<b>0.26</b>	$4 \times 10^{-7}$	0.01	0.20	$1 \times 10^{-3}$	0.01

Notes:  $\beta$ -glucosidase (GLU), leucine-aminopeptidase (LAP), chitinase (CHI), the ratio of C- vs. N-acquiring enzymes (GLU/(CHI+LAP)), bulk density (BD).

## 4 Discussion

### 4.1 Grazing effects on microbial exo-enzyme activity

In line with our first hypothesis, the activity of  $\beta$ -glucosidase was lower in grazed than ungrazed sub-plots. Generally,  $\beta$ -glucosidase activity is considered the key enzyme of microbial C acquisition and therefore controls the soil C turnover of ecosystems (Sinsabaugh et al., 2008). Previous studies suggested that grazing in salt marshes affects the activity of EEAs involved in C cycling via two mechanisms, i.e. reducing soil oxygen availability as the terminal electron acceptor for microbial respiration and changing the amount or quality of OM as the microbial substrate (Mueller et al., 2017). Grazing-driven soil compaction and the resulting lower oxygen availability has previously been hypothesized as a main pathway by which grazing can reduce microbial activity in wetland soils (Mueller et al., 2017). Our data can support this mechanism, because grazed sub-plots showed significantly higher bulk density, providing evidence of trampling-driven soil compaction (Table 2.3), and more importantly, bulk density was negatively related to  $\beta$ -glucosidase activity (Table 2.3). It needs to be noted here, however, that grazing also led to lower belowground biomass. This unexpected effect is not commonly observed in salt marshes (Davidson et al., 2017), but it would lead to higher bulk density independent of trampling-driven compaction.

Our data also provides evidence for a second, alternative pathway by which grazing can reduce soil  $\beta$ -glucosidase activity. Livestock grazing can lead to large changes in plant productivity and diversity, affecting both the quantity and quality of microbial substrates. For instance, grazing effects on belowground biomass have been argued to affect the input of labile organic C compounds in the form of root exudates, thereby controlling the microbial C turnover (Olsen et al., 2011). This effect of grazing on decomposition via changes in belowground biomass production is supported by our results, which show a reduction of belowground biomass with

grazing and a positive relation between  $\beta$ -glucosidase activity and belowground biomass. Consequently, grazing-induced reduction in belowground biomass could be an alternative explanation for the observed reduction in microbial C acquisition.

Leucine-aminopeptidase and chitinase mediate the microbial N acquisition from OM and therefore reflect the microbial N demand (Moorhead et al., 2012; Sinsabaugh et al., 2008). In contrast to  $\beta$ -glucosidase activity, the activity of leucine-aminopeptidase was increased by livestock grazing. This result thus contradicts our hypothesis that lower oxygen availability in grazed soils decreases EEA. The activity of chitinase was negligibly low and therefore considered unimportant for understanding microbial N demand. Both microbial N acquisition based on leucine-aminopeptidase activity and microbial C vs. N demand based on the ratio of C- vs. N-acquiring enzymes clearly point to higher microbial N demand in grazed soils.

Microbial N demand based on EEAs is often explained by the elemental stoichiometry of C and N in soils (Sinsabaugh et al., 2009, 2008). However, in our study, soil C:N-stoichiometry cannot explain a higher microbial N demand under grazing, because soil C:N ratios are in fact markedly lower in grazed vs. ungrazed sub-plots of our field site (Yang et al., 2017).

Increased microbial N demand under grazing could also be induced by the input of labile C compounds to the soil that would not necessarily be reflected in soil C:N ratios because of their fast turnover. Plants can increase the input of C substrates to the soil via root exudation by increasing microbial nutrient acquisition and plant nutrient uptake; a mechanism previously discussed in the context of rhizosphere priming effects (Jones et al., 2004; Kuzyakov et al., 2000). Indeed, higher rates of root exudation in grazed vs. ungrazed salt marsh soils have been identified as an important control of microbial activity (Olsen et al., 2011). However, our data cannot support this mechanism because belowground biomass was decreased by grazing.

Finally, the mixing of autochthonous vs. allochthonous OM is another major control of microbial C- vs. N-acquisition activities in salt marshes, irrespective of the soil C:N stoichiometry (Mueller et al., 2020). More importantly, it is also strongly affected by grazing (Mueller et al., 2017; Mueller et al., 2019). Allochthonous OM inputs represent a major fraction of the soil OM pool in minerogenic, sediment-rich salt marshes (Mueller et al., 2020; Van de Broek et al., 2018). Considering the extreme rates of sediment-driven accretion of several centimeters per year in our study site (Yang et al., 2008), allochthonous OM input likely controls microbial C vs. nutrient acquisition to a great extent. Furthermore, grazing has been shown to reduce annual sediment deposition and accretion drastically by >30 kg dry weight m<sup>-2</sup> and 2 cm, respectively (Yang et al., 2017). It is therefore possible that lower inputs of allochthonous OM input under grazing – a mechanism previously demonstrated by Mueller et al., (2017) for European salt marshes – is also driving the stimulated microbial N demand in the grazed plots of the present study. However, additional research is needed to assess the quality and mixing of allochthonous OM in the marshes of Yangtze estuary before any conclusions can be drawn on its implications on soil microbial ecology and biogeochemistry.

#### 4.2 Litter decomposition

In line with our second hypothesis, we found a grazing-induced reduction of  $k$ , the decomposition rate constant of the deployed plant litter. Lower  $k$  in grazed sub-plots could be ascribed to lower soil oxygen availability, as we also demonstrate a negative relationship between  $k$  and soil bulk density (Table 2.3), a proxy for trampling-driven soil compaction and oxygen availability in grazed wetland soils (Elschot et al., 2015; Schrama et al., 2013). Contrary to our hypothesis, however,  $S$ , describing the stabilization of plant litter in soil, was lower in grazed sub-plots and was not inversely related to  $k$ . Even though the TBI parameters  $k$  and  $S$  do not necessarily show a strong inverse correlation (Keuskamp et al., 2013; Mueller et al., 2018), the unidirectional decrease of both parameters in response to our grazing

treatment was unexpected. Yet, this finding clearly highlights the importance of distinguishing litter decomposition rate from litter stabilization in the litter decomposition process. The factors controlling litter decomposition rate and stabilization, as well as the ecological implications of the two parameters, can be quite different (Althuizen et al., 2018). While there is a wealth of studies providing insight into the controls of litter decomposition rate, far less is known about the controls of litter stabilization (review provided by Prescott, 2010). From a C-sequestration perspective, litter stabilization is the more relevant parameter, as it describes the fraction of litter that gets ultimately transformed to stable soil OM (Keuskamp et al., 2013; Prescott, 2010).

In the present study, a lower degree of plant-litter stabilization cannot be explained by lower soil oxygen availability under grazing. We therefore argue that grazing effects on other factors controlling microbial activity are responsible for the observed effect. Specifically, *S* was negatively related to leucine-aminopeptidase activity and positively related to microbial C vs. N acquisition (based on EEA stoichiometry), suggesting a negative effect of microbial N demand on litter stabilization. In support of this notion, low stabilization potential for organic material in soils with high microbial N demand is in line with several observations on soil OM cycling in terrestrial ecosystems (Carreiro et al., 2000; Craine et al., 2007; Knorr et al., 2005). Similarly, N additions have previously been hypothesized to increase plant litter stabilization and soil OM (Prescott, 2010).

It is possible that the TBI stabilization factor, *S*, is particularly sensitive to the N demand of the soil microbial community (Keuskamp et al., 2013), given that the green-tea substrate used to assess *S* is relatively N-rich (C:N = 12). Yet, *S* has proven useful to explain variability in the soil C-sequestration capacity across ecosystems, suggesting that it can be used as a proxy for plant litter stabilization in soils (Keuskamp et al., 2013; Mueller et al., 2018). Our study



provides further support for this, because grazing-induced reductions of  $S$  are in accordance with lower soil OC contents and densities in grazed vs. ungrazed sub-plots (Yang et al., 2017).

### 4.3 Summary and implications

The present work demonstrates marked effects of livestock grazing on decomposition processes in salt marsh soils with potentially important implications for C sequestration. Negative effects of livestock grazing on soil C stocks and sequestration rates in salt marshes have previously been ascribed to reductions in plant biomass (Davidson et al., 2017). Here we suggest that livestock grazing can stimulate the early OM decomposition processes in salt marsh soils by reducing litter stabilization via increased microbial N demand, and therefore provide an alternative explanation for negative grazing effects on C stocks and sequestration. Our data show that grazing effects on EEAs and litter decomposition can only partly be explained by grazing-driven soil compaction and resulting lower oxygen availability, which has previously been hypothesized as a main pathway by which grazing can reduce microbial activity in wetland soils (Elschot et al., 2015; Mueller et al., 2017). Instead, grazing effects on microbial nutrient demand appears to be an equally important control on soil decomposition processes. In addition, the present study is the first to provide insight into the relations between soil enzymic processes and TBI, an increasingly recognized, standardized belowground litter assay used to understand soil OC formation and compare decomposition dynamics across ecosystems at a global scale (Djukic et al., 2018; Keuskamp et al., 2013). The identified interactions between microbial EEAs and TBI parameters warrant further investigation to improve the mechanistic understanding that can be derived from TBI with respect to soil OC formation. Particularly, if the observed negative effect of microbial N demand on  $S$  applies more generally, it yields important implications for linking and modeling nutrient, litter, and soil OC dynamics based on TBI.

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# 3 Plant genotype controls wetland soil microbial functioning in response to sea-level rise

## Abstract

Climate change-induced shifts in plant community composition affect the decomposition of soil organic matter via plant-microbe interactions, often with important consequences for ecosystem carbon and nutrient cycling. Given the high degree of intraspecific trait variability in plants, it has been hypothesized that genetic shifts within species yield a similar potential to affect soil microbial functioning. Here, we examined if the simulated sea-level rise and plant genotype interact to affect soil microbial communities in an experimental coastal wetland system. Two genotypes of the dominant coastal wetland grass *Elymus athericus*, characterized by differences in their sensitivity to flooding stress, were exposed to a large range of flooding frequencies. Plant genotype strongly mediated the flooding effect on soil microbial community structure and determined the presence of flooding effects on microbial exo-enzyme activities and belowground litter breakdown. We thereby present a novel mechanism by which plant processes control the effects of climate change on microbial functioning and carbon cycling in coastal wetland soils. Larger variability in microbial community structure, enzyme activities, and litter breakdown in soils planted with the unadapted plant genotype supported our general hypothesis that effects of climate change on soil microbial activity and community structure depend on plant intraspecific adaptations. We conclude that adaptive genetic variation in plants can suppress or facilitate the effects of sea-level rise on soil microbial communities. If this finding applies more generally to coastal wetlands, it yields important implications for experimental climate change research, carbon cycling, and modelling of soil organic matter accumulation.

**Keywords:** Plant-soil interaction, genotype-environment interaction, climate change, blue carbon, exo-enzyme stoichiometry, microbial community, litter breakdown, carbon sequestration

## 1 Introduction

Climate change strongly affects soil microbial decomposition, with important consequences for global carbon (C) and nutrient cycles (Davidson and Janssens, 2006; Dijkstra et al., 2010; Schaeffer et al., 2013). Plant-microbe interactions in the rhizosphere are particularly susceptible to various climate change factors (Philippot et al., 2013; Pugnaire et al., 2019; Wieder, 2014). It is therefore crucial to not only study the direct effects of climate change on soil microbial communities and resulting changes in ecosystem functioning, but also the plant-mediated, indirect effects (Bardgett et al., 2008). Indeed, several case studies from a wide range of ecosystems demonstrated how changes in plant productivity and community composition control soil microbial functioning in response to climate change, often with marked effects on ecosystem C, greenhouse-gas, and nutrient dynamics (Fuchslueger et al., 2014; Mueller et al., 2016; Teste et al., 2017; Ward et al., 2013; Wolf et al., 2007).

Climate change does not only cause shifts in plant community composition, but also affects the genetic structure within plant populations (Bustos-Korts et al., 2018; Carvalho et al., 2019; Crutsinger et al., 2006; Jump and Peñuelas, 2005). Given the high degree of intraspecific trait variability in plants, it has been hypothesized that genetic shifts within plant populations can induce important changes in soil microbial functioning (Fischer et al., 2014; Hughes et al., 2008; terHorst and Zee, 2016; Van Nuland et al., 2016; Ware et al., 2019). This hypothesis is based on studies demonstrating differences in soil microbial community structure or activity in soils of different plant genotypes (Madritch et al., 2007; Madritch and Lindroth, 2011; Schweitzer et al., 2008b, 2008a; Zogg et al., 2018). Furthermore, genotype effects on soil C

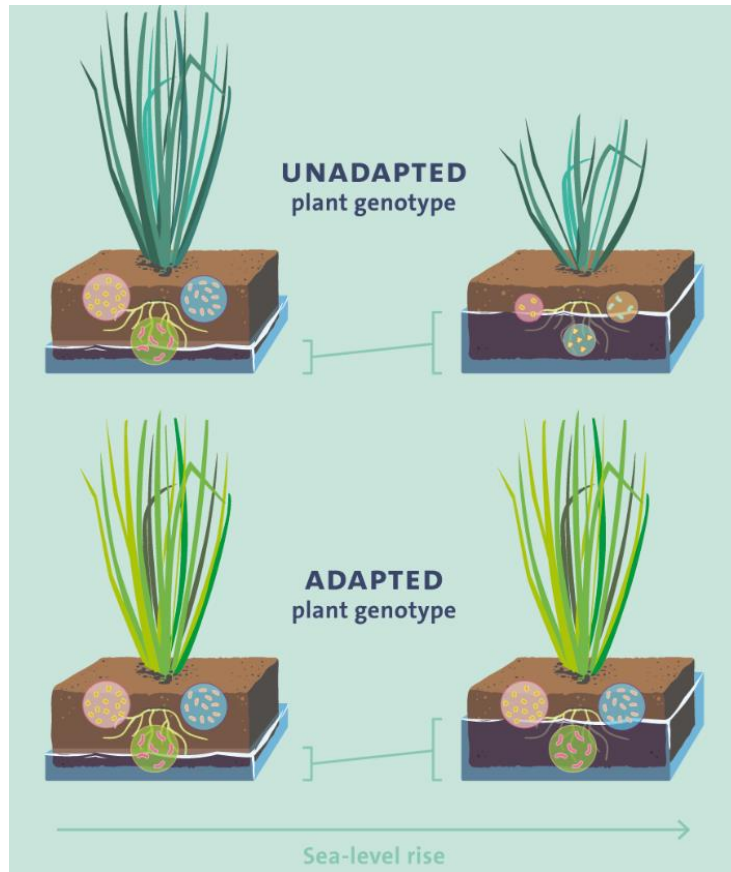
and nitrogen stocks as well as nitrogen transformations have been observed to be variable across multiple common garden sites (Pregitzer et al., 2013). However, experimental evidence for interaction effects of plant intraspecific variability and climate change factors on soil microbial processes and C cycling is virtually absent.

Plant-mediated climate change effects on soil microbial functioning are expected to be particularly pronounced in wetlands, where plants do not only control the microbial substrate (i.e. electron donor) supply, they also regulate the availability of electron acceptors by providing oxygen to an otherwise reduced rhizosphere (Kirwan and Megonigal, 2013; Mueller et al., 2016). At the same time, wetland soil microbial functioning plays a disproportionately large role in the global climate system (Freeman et al., 2001; Megonigal et al., 2003). In recent years, climate change research in tidal wetlands and other so-called blue carbon ecosystems has gained increasing attention by the scientific community (Davidson and Janssens, 2006; Kirwan and Megonigal, 2013; Kirwan and Mudd, 2012; Spivak et al., 2019). These ecosystems belong to the most effective long-term C sinks of the biosphere (Chmura et al., 2003; McLeod et al., 2011), but the impacts of accelerated rates of sea-level rise (SLR) destabilize tidal wetlands worldwide (Kirwan and Megonigal, 2013).

SLR affects the flooding frequency of tidal wetlands and represents the overriding climate change factor impacting tidal wetlands (Kirwan and Megonigal, 2013; Langley et al., 2018; Nicholls et al., 1999). Its effects on ecosystem functioning are largely plant-mediated and extremely variable, ranging from strong positive effects on soil C sequestration to ecosystem destabilization and ultimately loss (Kirwan and Megonigal, 2013; Rogers et al., 2019). SLR and the resulting flooding frequency alter plant primary production and microbial decomposition, the two primary factors controlling carbon sequestration in coastal marine ecosystems (Kirwan and Megonigal, 2013). Primary production often follows a unimodal (i.e. optimum) response to SLR, although interspecific variability is high (Kirwan et al., 2013;

Kirwan and Guntenspergen, 2012; Morris et al., 2013). The microbial decomposition response to SLR is less understood. A dominant paradigm in wetland ecosystem ecology is that decomposition rates are inversely related to flooding. However, recent studies demonstrated that the responses of decomposition and primary production to SLR are coupled (Janousek et al., 2017; Mueller et al., 2016; Stagg et al., 2017). For instance, Mueller et al. (2016) demonstrated soil microbial activity is not directly affected by SLR and its control on soil oxygen availability, but indirectly by the aboveground-biomass response to flooding frequency, which determines the input of both oxygen and labile substrates to soil microbial communities.

Considering the low plant diversity on the species level in many tidal wetland types, such as salt marshes (Semchenko et al., 2017), and the strong control of plant processes on microbial C cycling in wetland soils, it is possible that intraspecific variation and adaptive capacity functions as an important, but so far overlooked mediator of wetland-climate feedbacks. Here, we study the interaction effect of flooding frequency and plant genotype on soil microbial community structure and functioning, using the dominant tidal-wetland grass *Elymus athericus* as a model species. Two genotypes of *Elymus*, which differ in their adaptation to flooding frequency, have been identified: a flooding-sensitive genotype from the high marsh (hereafter referred to as an unadapted genotype) and a less sensitive genotype from the low marsh (hereafter adapted genotype) (Bockelmann et al., 2003; Reents et al., 2020). Given the overriding control of plant processes on microbial functioning in wetland soils, we hypothesize that flooding effects on microbial decomposition and microbial community structure are strong in soils with the unadapted plant genotype, but absent or buffered in soils of the adapted plant genotype (Figure 3.1).



**Figure 3.1** Conceptual diagram illustrating the hypothesis that effects of a changing abiotic environment on soil microbial processes are mediated by the intraspecific adaptive variation of plants. We tested this general hypothesis in a tidal-wetland system and studied the interaction effect of plant genotype and flooding frequency (a master variable in tidal-wetland ecology that will increase with accelerated sea-level rise) on soil microbial functioning. Two genotypes of the dominant tidal-wetland grass *Elymus athericus* have been identified, an unadapted plant genotype, found in high-marsh environments and an adapted plant genotype found in low-marsh environments. The adapted genotype shows no reduction of aboveground biomass even in response to extreme increases in flooding frequency (Reents et al., 2020). Given the overriding control of plant aboveground-processes on microbial functioning in tidal wetland soils, we hypothesize that the adapted genotype buffers the response of the soil microbial community to increasing flooding frequency.

## 2 Methods

### 2.1 Experimental design

The experiment was conducted from July to October 2017 (12 weeks) at the Institute of Plant Science and Microbiology (IPM), Universität Hamburg, Germany. We used platforms

positioned at three elevations in a 12 m<sup>3</sup> tidal tank to induce three flooding frequency treatments capturing the full range of flooding frequencies of a typical Northwest European salt marsh: daily (two floods every day, simulating pioneer-zone conditions), weekly (2 floods on one day per week, simulating low-marsh conditions), and monthly (2 floods on one day every two weeks, simulating high-marsh conditions). Similar experimental designs have previously been described as marsh organs (Mueller et al., 2016). Each platform contained 16 mesocosms ( $\varnothing = 15$  cm; h = 17 cm). The mesocosms were filled with soils collected at a salt marsh near Sönke-Nissen-Koog, Germany (DE, 54°36'N, 8°49'E). Soils were sieved using a 1 cm mesh to remove roots, rhizomes, and other coarse materials, and homogenized before being transferred to the mesocosms. The mesocosms were planted with either adapted or unadapted genotypes of the grass *Elymus*. The design included 48 mesocosms, 24 per genotype, and 16 per flooding treatment (n = 8). We additionally added four unplanted control mesocosms to each flooding treatment.

Plants were collected in April 2015 from *Elymus* stands on the island Schiermonnikoog, The Netherlands, which has previously been dominated by genetically distinct populations of *Elymus*, i.e. unadapted and adapted genotypes (Bockelmann et al., 2003). The plants were transferred to pots and kept in a common garden at Universität Hamburg for 24 months before the experiment commenced. Clonal plant growth led to the emergence of new individuals during this period, which were used for the experiment. New individuals of unadapted and adapted genotypes were still phenotypically distinct after 24 months under identical environmental conditions. Each mesocosm received one plant of similar size (compare Reents et al., 2020).

## 2.2 Soil sampling and processing

Soil sampling took place in October 2017 after 12 weeks of exposure to different flooding treatments and plant genotypes. Plant biomass and litter were removed before sampling. From



each mesocosm, one soil sample was taken as a core from the top 5 cm using a volumetric steel ring. Sub-samples of 20 g were homogenized and stored at -20 °C until used for EEA assays and DNA extraction. The residual sample was passed through a 2.5-mm sieve, air-dried at 65 °C until constant weight, and used to determine the dry mass and other soil properties.

### 2.3 Microbial exo-enzyme activity and belowground litter decomposition

Potential exo-enzyme activity (EEA) of  $\beta$ -glucosidase, cellobiosidase, leucine-aminopeptidase, and chitinase was determined in fluorometric assays following Mueller et al. (2017). Briefly, 1:20 soil slurries were produced using 50 mmol/L bicarbonate buffer (pH = 8) (Sinsabaugh et al., 2003). 96-well-plate assays were conducted to measure potential enzyme activity. Plates were incubated in the dark at 20 °C for 16 h and read on a Multi-Detection Microplate Reader (Bio-Tek Synergy HT, Winooski, USA). The emission and excitation wavelengths were set at 460 nm and 365 nm, respectively.

We assessed the decomposition of standardized plant litter in the rhizosphere to evaluate if genotype effects on soil microbial exo-enzyme activity translated into altered organic matter turnover and thus into ecosystem functioning (Ochoa-Hueso et al., 2020). The decomposition rate constant ( $k$ ) and stabilization factor ( $S$ ) were assessed following the Tea Bag Index (TBI) protocol (Keuskamp et al., 2013). The TBI is a standardized litter-decay assay which uses commercially available tea materials as standardized plant litter. The TBI has widely been applied to characterize and compare decomposition dynamics within and across ecosystems (Keuskamp et al., 2013; Mueller et al., 2018; Ochoa-Hueso et al., 2020). The advantages and limitations of the TBI and other standardized decomposition assays, such as cotton- and cellulose-strip assays, have been extensively discussed elsewhere (Clark, 1970; Mueller et al., 2018; Ochoa-Hueso et al., 2020; Risch et al., 2007). Each pot received, two polypropylene tea bags (55 mm x 50 mm), one containing green tea (EAN: 8 714100 770542; Lipton, Unilever), and one containing rooibos (EAN: 8 722700 188438; Lipton, Unilever). Bags were deployed

in 5 cm soil depth. The initial weight of the contents was determined by subtracting the mean content weight of 5 empty bags (Green tea:  $1.69 \pm 0.005$  g; Rooibos tea:  $1.79 \pm 0.009$  g). Bags were retrieved after an incubation period of 90 days, carefully separated from roots and soil, dried for 48 h at 70 °C, and weighed. The TBI parameters  $k$  and  $S$  were calculated following the tidal-wetland-adapted TBI protocol (Mueller et al., 2018).

#### 2.4 Microbial community structure - Illumina sequencing

Soil DNA was extracted from three randomly chosen mesocosms per treatment combination using the PowerSoil DNA extraction kit (Quiagen). From each mesocosm, two samples (technical replicates) were taken to assess within-mesocosm variability. DNA quality and yield were assessed using a fluorometer (Qubit 2.0, Thermo Fisher Scientific). PCR amplification of the prokaryotic 16S rRNA gene region was conducted using the barcoded primers 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') (Caporaso et al., 2010). The PCR protocol (PCR mix and cycling conditions) followed Meier et al. (2019). PCR products were purified using the Agencourt AMPure XP– PCR purification kit (Beckman Coulter, Inc.), and were pooled into a single sequencing library at equimolar concentrations (20 ng DNA per sample). Sequencing was conducted by Eurofins Scientific (Konstanz, Germany) using an Illumina HiSeq platform and Miseq v3 kits (2 x 300 bp). Sequence analysis and bioinformatics followed Holm et al. (2020). Briefly, the library was demultiplexed using Cutadapt (Martin, 2011), and samples were error-corrected using the DADA2 pipeline (Callahan et al., 2016). Paired-end reads were merged, and low-quality sequences and chimeras were removed. Amplicon sequence variants (ASV) were assigned to the SILVA database (version 132) (Quast et al., 2013) applying vsearch (Rognes et al., 2016) as implemented in the QIIME2 framework (Bolyen et al., 2019). Taxonomic assignment of sequences was based on a 99% similarity threshold. Raw sequencing data is available at the

European Nucleotide Archive (ENA) under BioProject accession number PRJEB38150 and sample accession numbers ERS4541081-ERS4541134.

## 2.5 Statistical analyses

One-way ANOVA was used to test for effects of plant presence on exo-enzyme activities. Subsequently, a two-way ANOVA was conducted to test for effects of flooding frequency, plant genotype, and their interaction on exo-enzyme activities. A paired t-test was used to test for a genotype effect on the average effect size of flooding frequency across all enzyme activities. Two-way ANOVA was used to test for effects of flooding frequency, genotype, and their interaction on *k* and *S*. One-way PERMANOVA was used to test for effects of plant presence on soil microbial community structure. Two-way PERMANOVA was used to test for effects of flooding frequency and genotype on microbial community composition. Data were visualized using NMDS. PERMANOVAs and NMDS were based on Bray-Curtis dissimilarities. Canonical Correspondence Analysis (CCA) was used to relate the microbial community composition to plant biomass, microbial enzyme activity, and litter decomposition parameters.

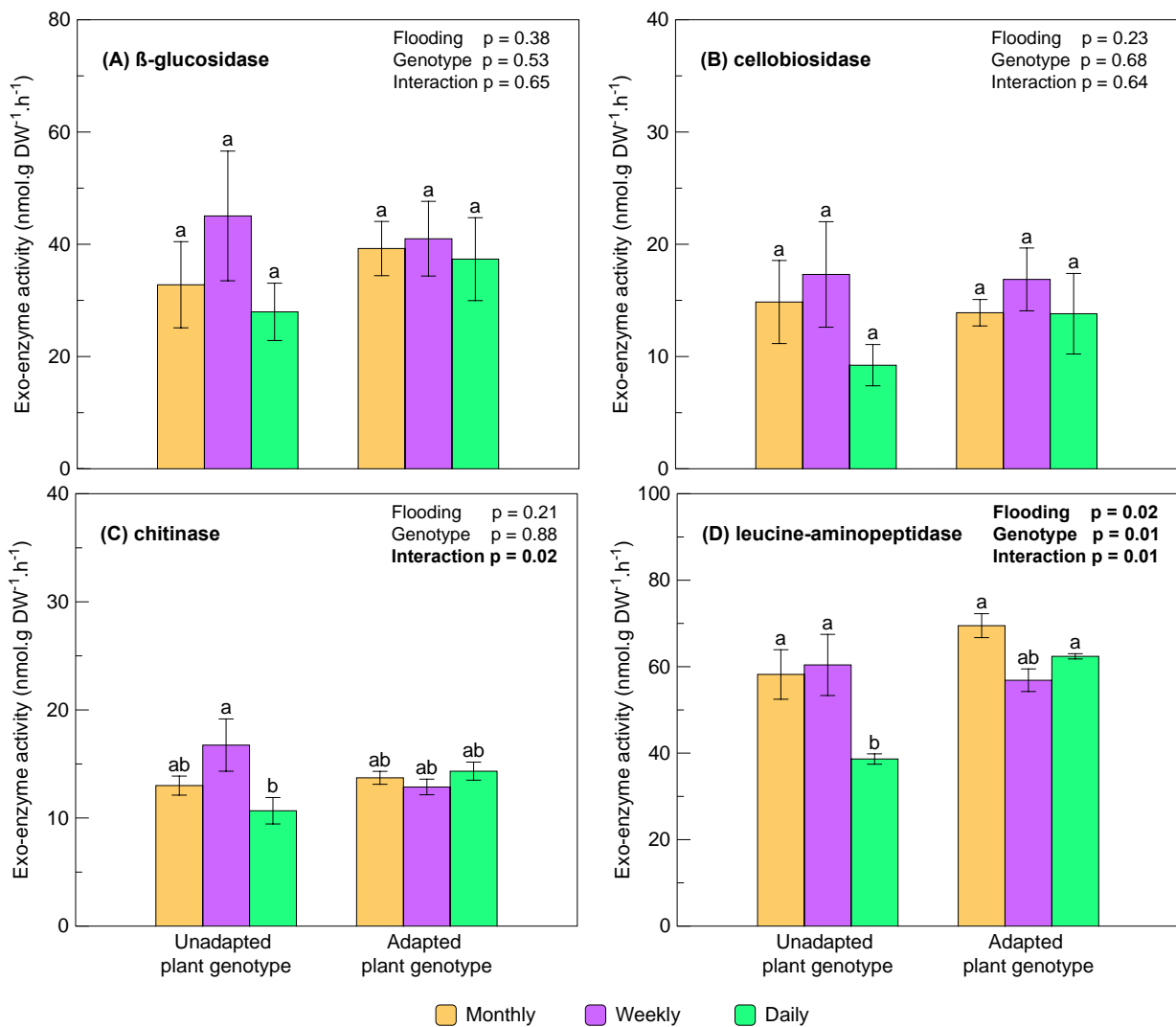
# 3 Results

## 3.1 Soil microbial enzyme activity and litter decomposition

In the absence of plants, flooding frequency did not affect any of the four exo-enzymes assayed (all  $F$ -values  $\leq 0.36$ , all  $p$ -values  $\geq 0.71$ , Figure S3.1). In the presence of plants, enzyme activities were only affected by flooding frequency in soils planted with the unadapted genotype, whereas none of the four enzyme activities were affected in soils planted with the adapted genotype (Figure 3.2). In soils of the unadapted genotype, enzyme activities were always highest at the intermediate (i.e. weekly) flooding frequency and always lowest at the

highest (i.e. daily) flooding frequency, whereas no consistent pattern was found in soils of the adapted genotype (Figure 3.2). The effect size of flooding frequency (i.e. the difference between highest and lowest mean activity of the three flooding treatments) was 1.7 - 4.7 times greater in the unadapted vs. adapted genotype (paired t-test, n = 4 enzymes, p = 0.02).

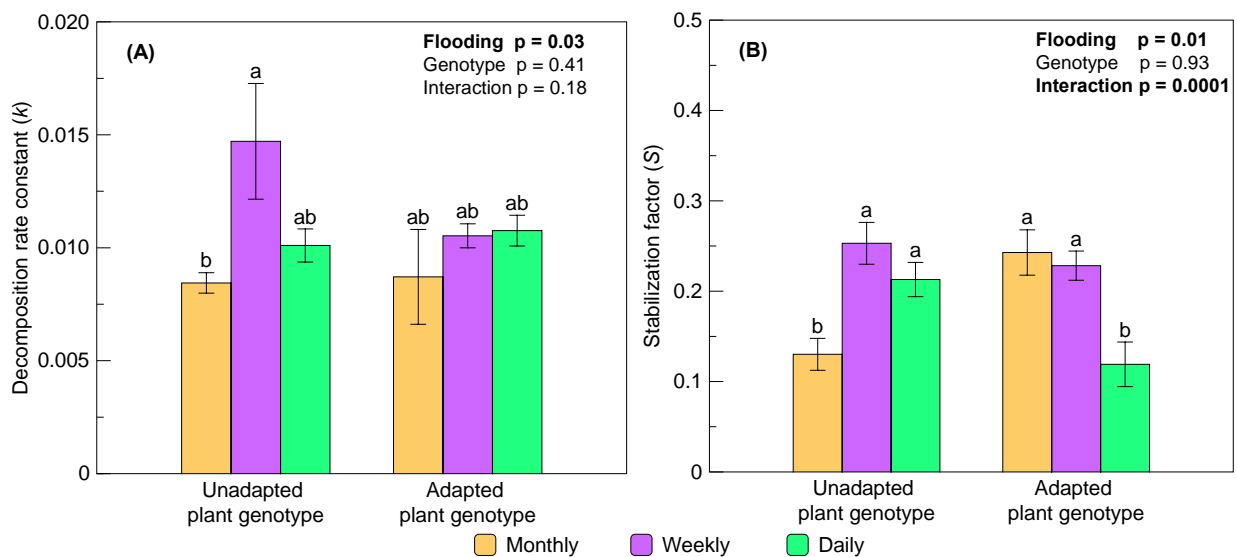
C-acquisition enzymes ( $\beta$ -glucosidase and cellobiosidase) showed different responses than N-acquisition enzymes (leucine-aminopeptidase and chitinase). The activity of C-acquisition enzymes was not affected by flooding frequency, genotype, and their interaction (Figure 3.2 A-B), whereas N-acquisition enzymes were significantly reduced by the highest flooding frequency (Figure 3.2 C-D). The reduction of N-acquisition activities by increasing flooding frequency was only observed in the unadapted genotype, whereas activities remained unchanged throughout flooding treatments in the adapted genotype (Figure 3.2 C-D). Significant differences between genotypes were only found at the highest flooding frequency (Figure 3.2).



**Figure 3.2** Activities of the exo-enzymes  $\beta$ -glucosidase (A) and cellobiosidase (B) (microbial C acquisition) as well as chitinase (C) and leucine-aminopeptidase (D) (microbial N acquisition) in soils planted with unadapted and adapted plant genotypes of *Elymus athericus* exposed to three different flooding frequencies (monthly, weekly, and daily). Values are means and SE ( $n = 8$ ). Two-way ANOVA results are included, bars not labeled by the same letter are significantly different at  $p \leq 0.05$  based on Tukey's HSD.

The initial belowground litter decomposition rate,  $k$  (*sensu* Keuskamp et al. (2013)), was significantly affected by flooding frequency. However, based on pairwise comparisons, this effect was only significant in the flooding-sensitive, unadapted plant genotype (Figure 3.3 A), reflecting the results on microbial enzyme activities. Indeed,  $k$  was significantly positively

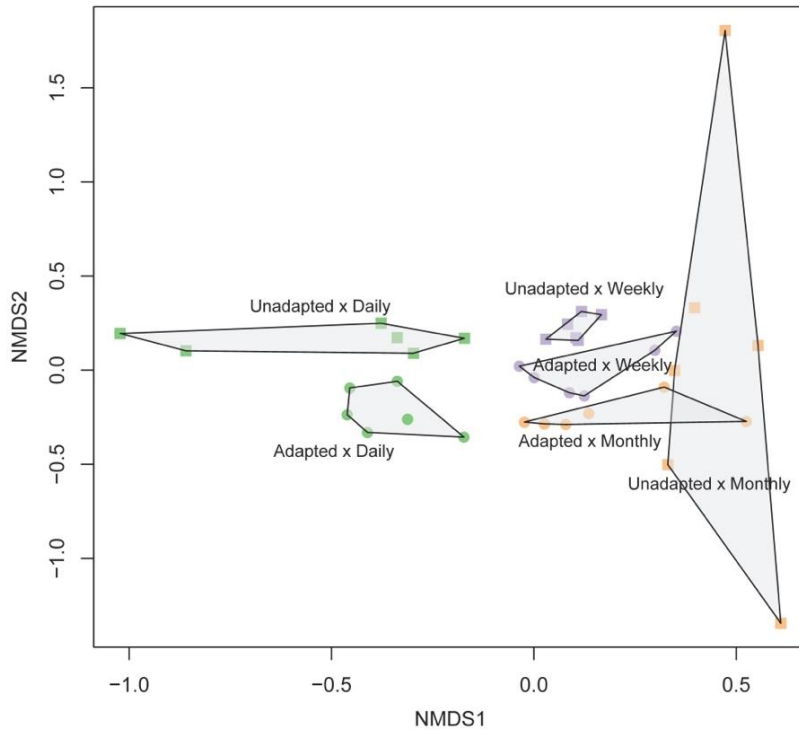
correlated with the activities of  $\beta$ -glucosidase ( $r^2 = 0.25$ ;  $p \leq 0.001$ ), cellobiosidase ( $r^2 = 0.19$ ;  $p \leq 0.01$ ), and chitinase ( $r^2 = 0.10$ ;  $p \leq 0.05$ ). Furthermore, a strong interaction effect of flooding frequency and genotype was detected on the litter stabilization factor,  $S$  (Keuskamp et al., 2013) (Figure 3.3 B). At the highest flooding frequency,  $S$  was markedly lower in the rhizosphere of the adapted versus unadapted genotype, whereas the reversed pattern was found at our lowest (i.e. monthly) flooding frequency (Figure 3.3 B). Across flooding treatments, no correlations between  $k$  or  $S$  and any enzyme activities were found (all  $r^2 \leq 0.02$ , all  $p$ -values  $\geq 0.34$ ), and no correlation was found between  $k$  or  $S$  and plant biomass parameters (from Reents et al., 2020), demonstrating that soil litter decomposition dynamics are driven by genotype effects which are unrelated to biomass parameters (Table S3.1).



**Figure 3.3** Initial decomposition rate constant ( $k$ ) and stabilization factor ( $S$ ) (*sensu* Keuskamp et al. (2013)) in soils planted with unadapted and adapted plant genotypes of *Elymus athericus* exposed to three different flooding frequencies (monthly, weekly, and daily). Values are means and SE ( $n = 8$ ). Two-way ANOVA results are included, bars not labeled by the same letter are significantly different at  $p \leq 0.05$  based on Tukey's HSD.

### 3.2 Soil microbial community structure

There was no effect of flooding frequency on the soil microbial community structure in the absence of plants (1-way PERMANOVA,  $F = 1.18$ ,  $p \geq 0.1$ ; Figure S3.2), but there were significant effects of flooding frequency in the presence of both plant genotypes (1-way PERMANOVAs, adapted genotype:  $F = 1.49$ ,  $p \leq 0.05$ ; unadapted genotype:  $F = 1.89$ ,  $p \leq 0.01$ ; Figure 3.4). Plant genotype significantly affected the microbial community structure (2-way PERMANOVA,  $F = 2.09$ ,  $p \leq 0.001$ ; Figure 3.4). In accordance with the findings on microbial enzyme activities, genotype effects were most pronounced at the highest, i.e. daily flooding frequency treatment (Figure 3.4). By contrast, differences between genotypes were absent at the lowest, i.e. monthly flooding frequency, suggesting an interaction of genotype and flooding frequency on soil microbial community structure (Figure 3.4), which was, however, not statistically significant based on the two-way PERMANOVA ( $F = 1.08$ ;  $p \geq 0.1$ ). Overall, variability in microbial community structure across flooding treatments was higher in the unadapted vs. adapted plant genotype (Figures 3.4 and Figure S3.3), reflecting the findings on enzyme activities and  $k$ . The CCA (Figure S3.3) indicates that soil microbial community is significantly related to plant biomass parameters as well as to microbial C and N demands (based on enzyme activities, *sensu* (Sinsabaugh et al., 2009, 2008)). Aboveground biomass exerted the strongest effect on the community structure (Figure S3.3). Figure S3.4 provides an overview of the most abundant prokaryotic taxa found.



**Figure 3.4** NMDS plot showing prokaryotic (bacterial + archaeal) community composition in soils planted with unadapted and adapted plant genotypes of *Elymus athericus* exposed to three different flooding frequencies (monthly, weekly, and daily).

## 4 Discussion

The present study provides experimental evidence of genotype-environment interaction effects on soil microbial enzyme activity (Figure 3.2) and belowground litter breakdown (Figure 3.3), two key processes controlling C and nutrient cycling in ecosystems. Plant genotype determined the presence or absence of climate change effects (here increasing flooding frequency) on microbial enzyme activities and litter breakdown. This result yields important implications for experimental climate change research and modeling, because it shows that plant-genotype controls can completely mask or enhance the effects of changing abiotic conditions on soil microbial processes. Our data furthermore suggest genotype-environment interaction effects on the soil microbial community structure (Figure 3.4). This finding is in agreement with a



recent observational study suggesting that climate-driven reduction of genetic variation in *Populus angustifolia* phenology affects soil fungi-to-bacteria ratios (Ware et al., 2019), and a laboratory experiment demonstrating interaction effects of drought and rapid evolution in *Brassica rapa* on soil microbial community structure (terHorst et al., 2014). Overall, larger variability in microbial community structure (Figure 3.4), enzyme activities (Figure 3.2), and litter decomposition (Figure 3.3 A) in soils planted with the unadapted plant genotype support our general hypothesis that effects of climate change on soil microbial activity and community structure depend on plant intraspecific adaptations.

The majority of studies on genotype-environment interactions are concerned with plant responses to temperature or latitudinal climate gradients in terrestrial ecosystems (Bauerle et al., 2007; Curasi et al., 2019; Huang et al., 2015; Taylor et al., 2019; Walker et al., 2019; Ware et al., 2019). Here, we manipulated flooding frequency to simulate SLR, the most commonly studied climate change factor in coastal ecosystems such as tidal wetlands. The effects of SLR on soil microbial activity can be tightly controlled by the plant response to changes in flooding frequency, as demonstrated by recent studies showing strong positive correlations between aboveground biomass and soil litter decomposition (Janousek et al., 2017), cellulose decomposition (i.e. tensile strength loss; (Jones et al., 2018)), or recalcitrant SOM decomposition (Mueller et al., 2016). The importance of plant processes in controlling soil microbial functioning in response to changing flooding frequency is reflected in the findings of the present study: In the absence of plants, flooding frequency neither affected soil microbial enzyme activities nor the soil microbial community structure (Figure S3.1, Figure S3.4). In the presence of plants, however, flooding frequency, genotype, and genotype-induced variability in plant biomass exerted significant effects on soil microbial activity and community structure. Most notably, microbial enzyme activities only responded to changes in flooding frequency when aboveground biomass responded. Aboveground and belowground biomass across flooding treatments was unchanged in the adapted genotype, whereas the unadapted genotype

showed a strong reduction of aboveground biomass at our highest flooding treatment (Reents et al., 2020). Consequently, only the flooding-sensitive unadapted genotype showed changes in soil microbial activity and community structure, whereas the adapted genotype was able to maintain microbial enzyme activities at a constant level over the entire flooding gradient (Figure 3.2).

In support of our notion that the soil microbial activity response to increasing flooding frequency follows the response of plant aboveground processes, we found a significant relationship between aboveground biomass and microbial N-acquisition activity (aminopeptidase + chitinase activity) (Sinsabaugh et al., 2009, 2008) across all flooding treatments ( $r^2 = 0.17$ ;  $p \leq 0.01$ , Table S3.1) and to an even larger degree within the daily flooding treatment ( $r^2 = 0.40$ ;  $p = 0.01$ ), where effects on aboveground biomass and N acquisition activity existed (Figure 3.2 D; Reents et al., 2020). Soil enzyme activity is tightly controlled by the balance of nutrient supply and demand (Mueller et al., 2020; Sinsabaugh et al., 2012, 2008). It is therefore possible that the maintenance of N-rich aboveground plant biomass increased the soil microbial N demand and thus stimulated the mineralization of N from soil organic matter, a mechanism that has been discussed in the context of rhizosphere priming effects (Kuzyakov, 2002).

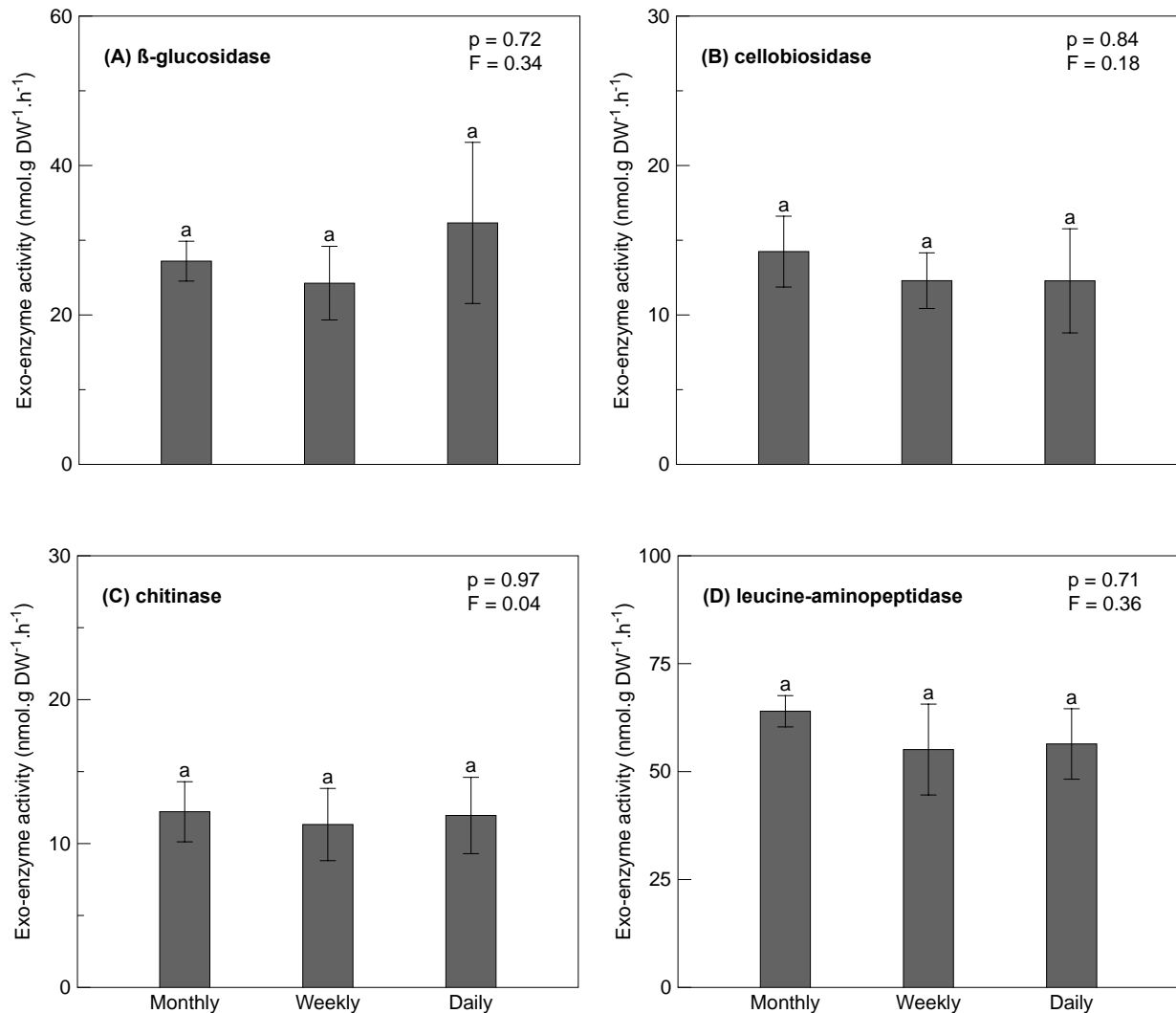
To evaluate if genotype effects on soil microbial communities translate into altered organic matter turnover and thus ecosystem functioning, we assessed the decomposition of standardized plant litter in the rhizosphere. The parameters  $S$  and  $k$  describe the initial transformation process of biomass to soil organic matter, which is a key component of many tidal-wetland resiliency models that have highlighted the critical role of the organic contribution to wetland elevation gain (Schile et al., 2014; Swanson et al., 2014). Although actual rates of  $S$  and  $k$  cannot be inferred from TBI assays using standardized litter, the approach has proven a powerful tool to characterize the potential of the soil environment to

transform organic matter inputs (Mueller et al., 2018). Effect sizes of the flooding treatment on  $S$  and  $k$  observed here are similar in range to those reported from field sites (Mueller et al., 2018; Tang et al., 2020), and genotype effect sizes were surprisingly large. Specifically, differences in  $S$  between genotypes within flooding treatments corresponded to c. 20% of the total range reported for tidal wetlands worldwide (Mueller et al., 2018). This result illustrates that the effects of plant genotype and genotype-climate change interactions on the C balance of tidal wetlands are not restricted to shifts in plant performance and primary production (Reents et al., 2020), but also concern parts of the soil C turnover.

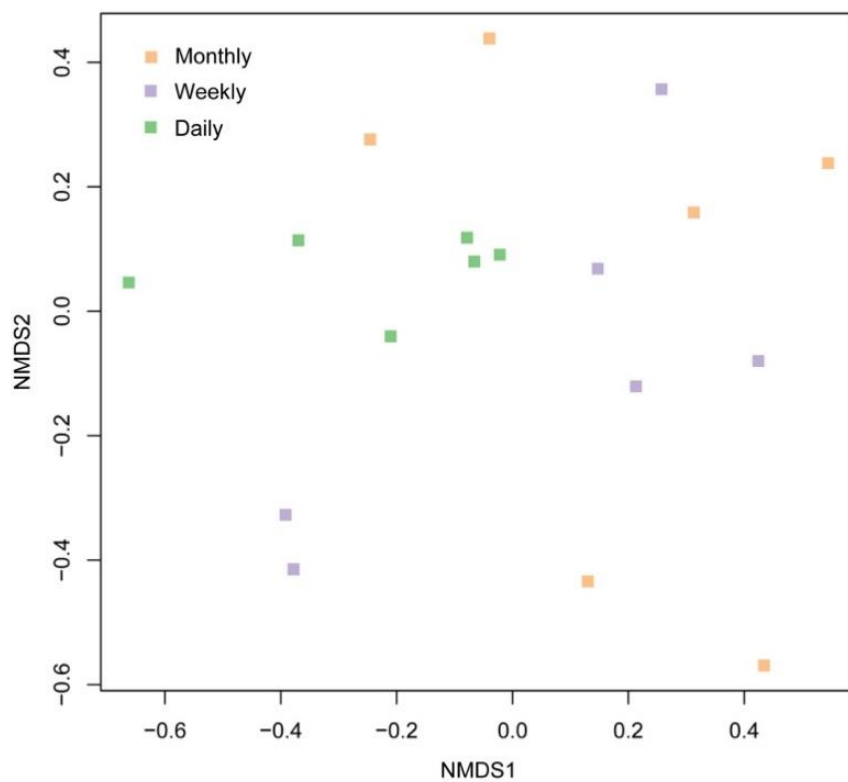
No correlations between the decomposition parameters  $S$  or  $k$  and plant biomass parameters were found, demonstrating that litter decomposition in response to flooding was controlled by genotype effects that are unrelated to plant biomass parameters. The two primary processes by which wetland plants control soil decomposition processes are root oxygen loss and root exudation (Wolf et al., 2007). Root oxygen loss is only relevant in oxygen-deficient soils, but strong genotype effects on decomposition were also present in our well-aerated monthly-flooding treatment (Figure 3.3). Therefore, root oxygen loss is unlikely to represent the primary driver of the observed genotype effects. Instead, we hypothesize that differences in root exudation patterns between genotypes affected soil litter decomposition. Root exudates are a key component of the plant control on soil decomposition processes in terrestrial soils, and their quantity and quality are not necessarily related to plant biomass parameters (Jones et al., 2004; Koelbener et al., 2010). Furthermore, differences in root-exudation patterns between genotypes are known to alter microbial community structures in other ecosystems (Micallef et al., 2009). However, our current mechanistic understanding of root-exudate effects on wetland soil microbial functioning is insufficient to explore this hypothesis more thoroughly without additional research.

In conclusion, this study adds to a growing body of research illustrating the overriding importance of plant processes in controlling the effects of climate change on microbial functioning and C cycling in tidal wetland soils. We argue that intraspecific adaptive variation in plants is critical for the regulation of blue C cycling under SLR. Future research will need to focus on intraspecific trait variability as well as climate-change driven intraspecific shifts in tidal wetland plant communities, neither of which were part of the present investigation.

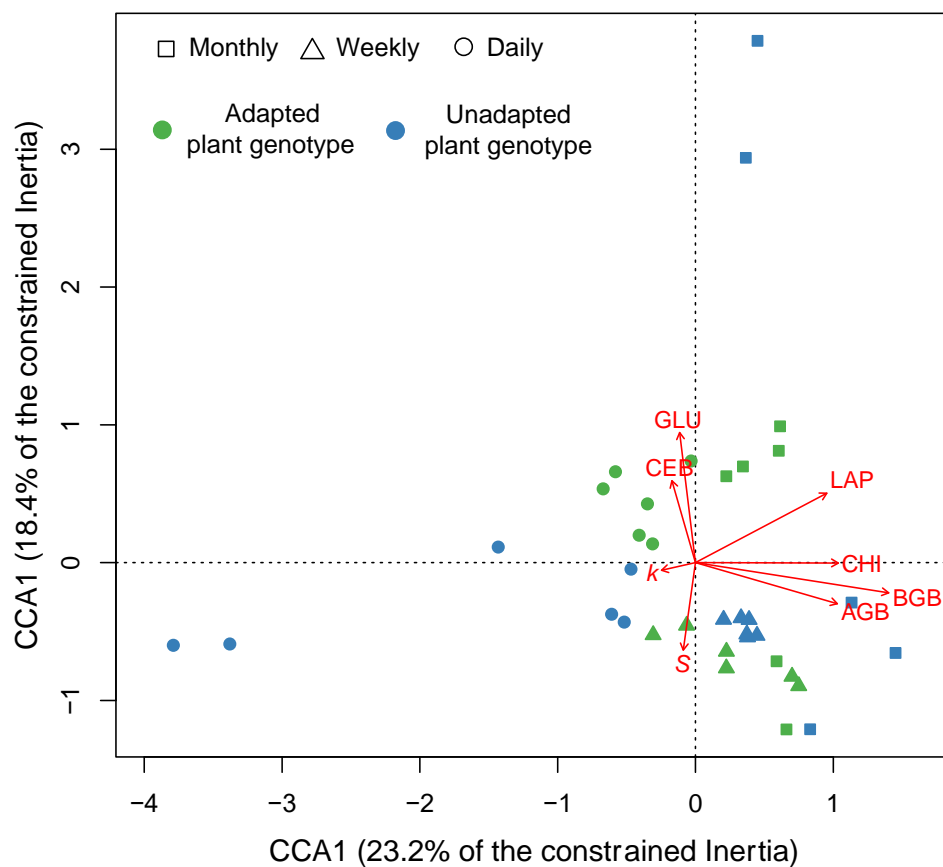
## 5 Supplementary materials



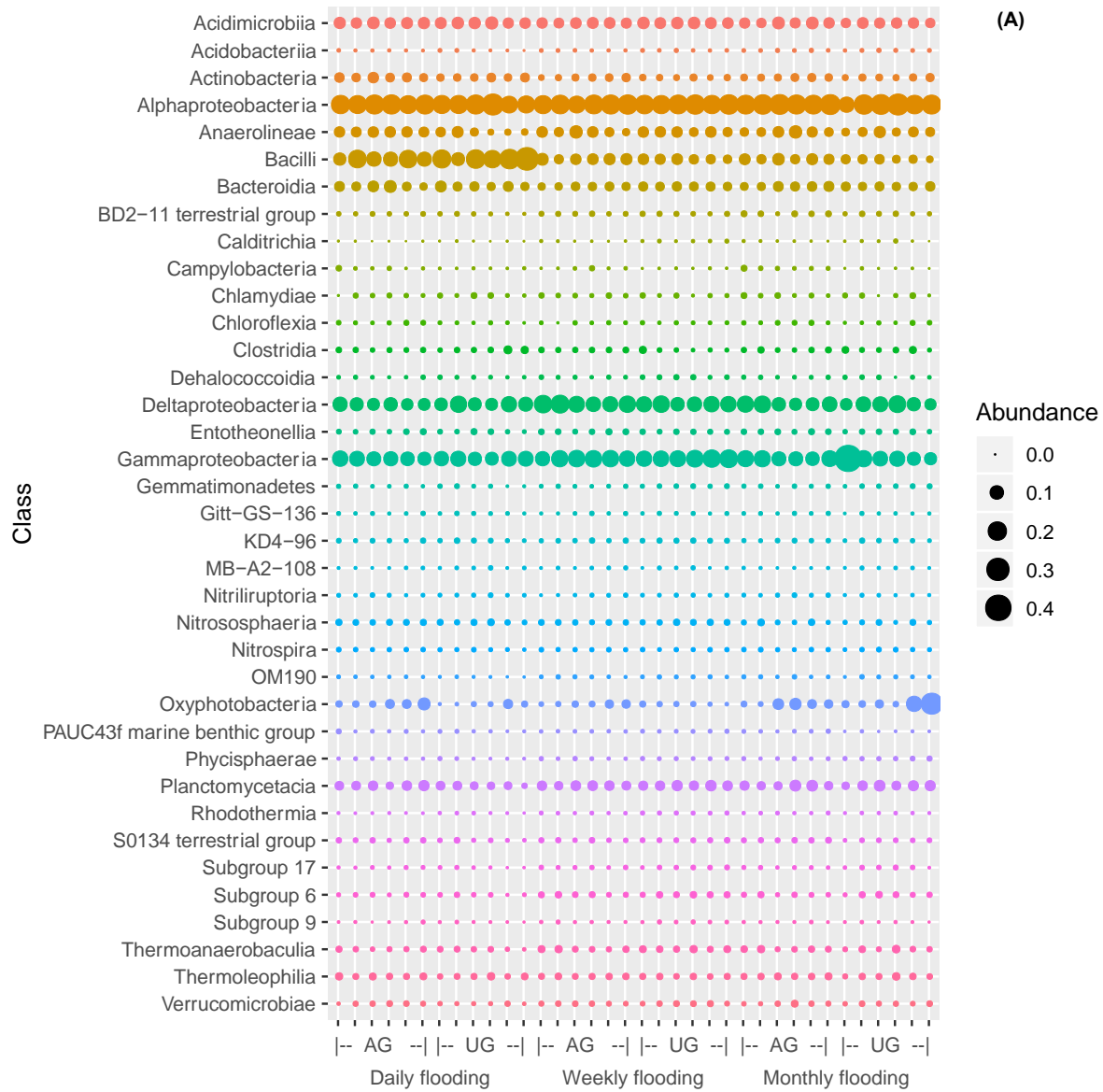
**Figure S3.1** Activities of the exo-enzymes  $\beta$ -glucosidase (A) and cellobiosidase (B) (microbial C acquisition) as well as chitinase (C) and leucine-aminopeptidase (D) (microbial N acquisition) in mesocosms containing soils without plants exposed to three different flooding frequencies (monthly, weekly and daily). Values are means and SE (n=4). No significant differences at  $p \leq 0.05$  based on Tukey's HSD were detected.



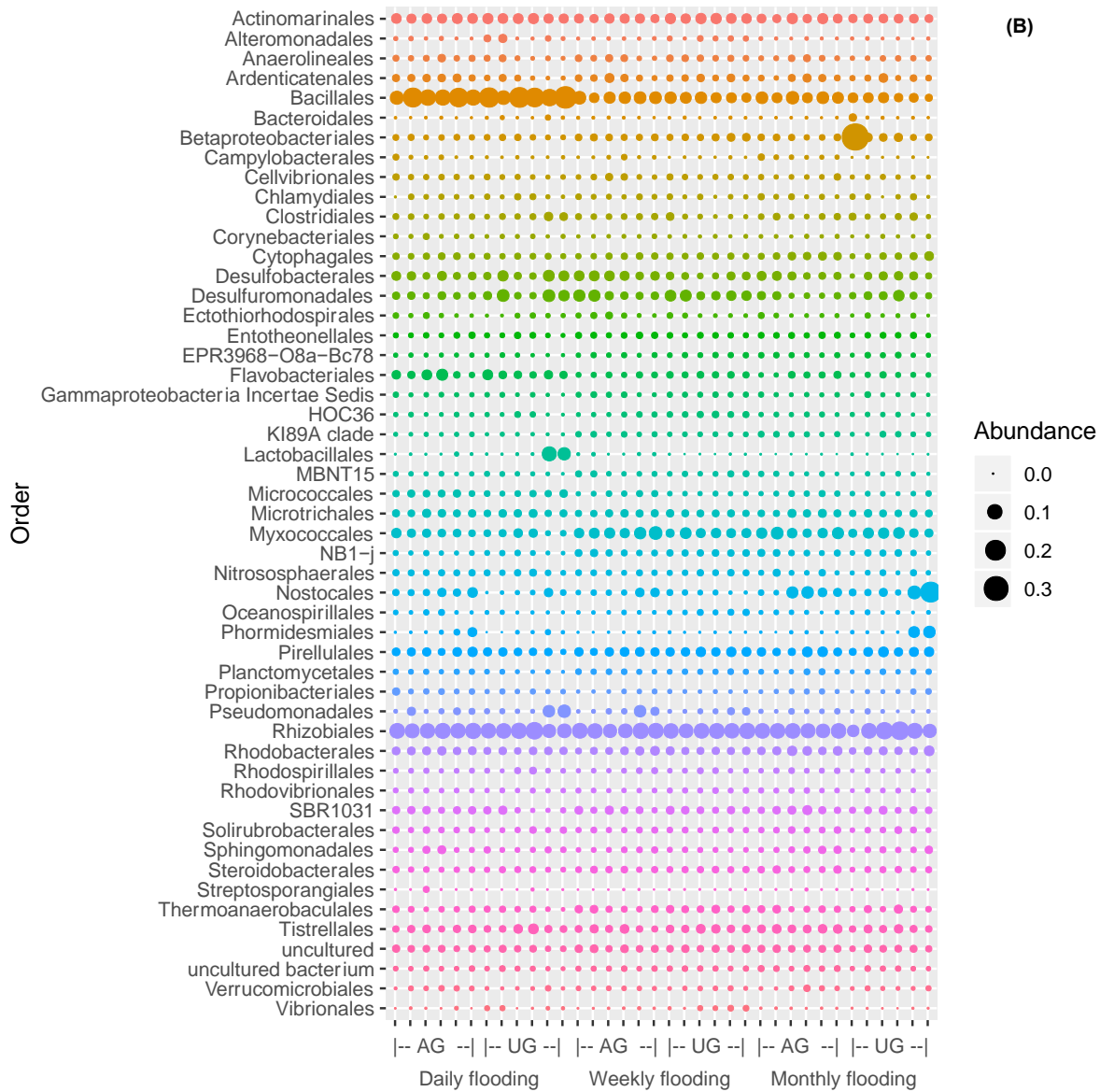
**Figure S3.2** NMDS plot showing prokaryotic (bacterial + archeal) community composition in mesocosms containing soils without plants exposed to three different flooding frequencies (monthly, weekly, and daily).

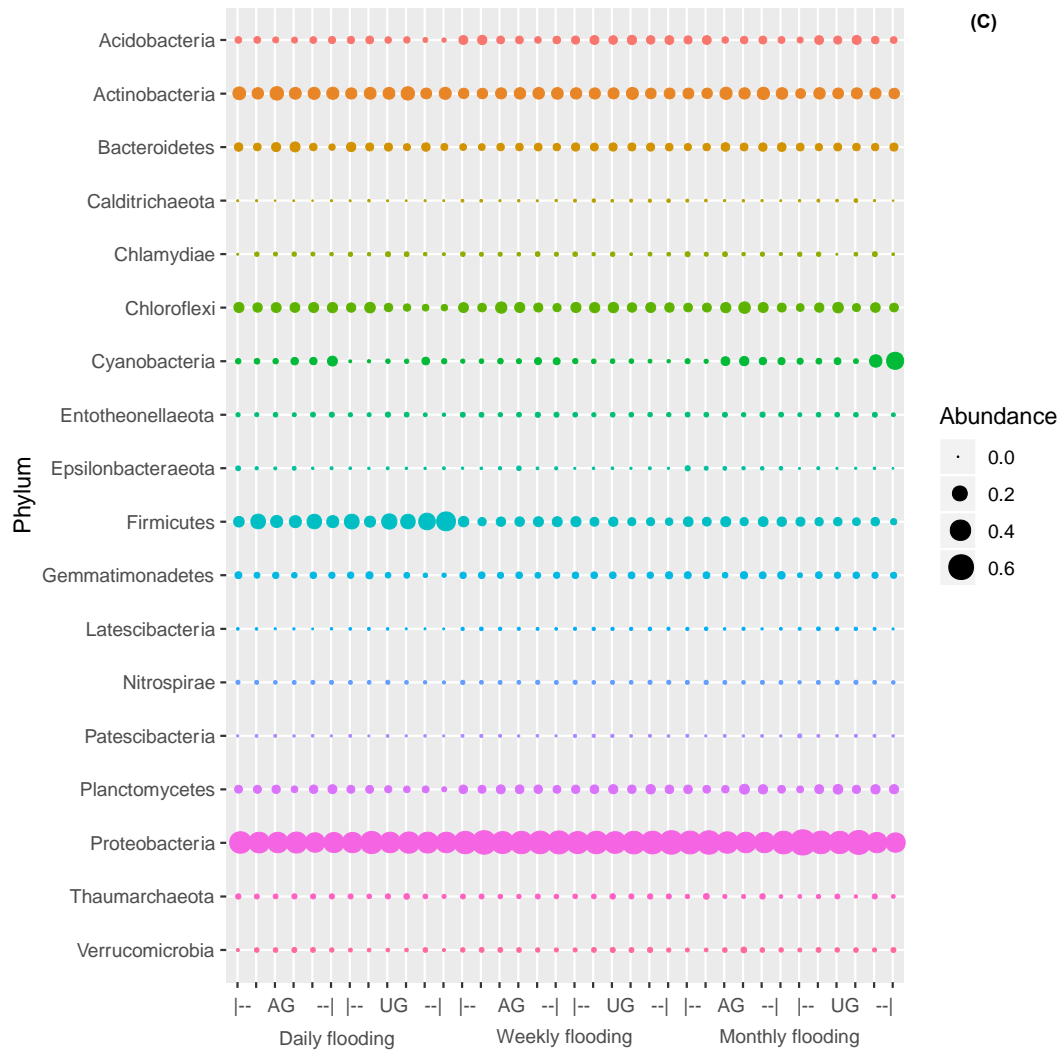


**Figure S3.3** CCA plot showing the relation of microbial community composition to plant biomass, microbial enzyme activity, and litter decomposition parameters. Notes: AGB = aboveground biomass, BGB = belowground biomass, GLU =  $\beta$ -glucosidase, CEB = cellobiosidase, CHI = chitinase, LAP = leucine-aminopeptidase,  $k$  = initial decomposition rate constant,  $S$  = stabilization factor. ANOVA-like permutation tests for Constrained Correspondence Analysis: AGB:  $F = 1.72$ ,  $p = 0.001$ ; BGB:  $F = 1.70$ ,  $p = 0.001$ ; GLU:  $F = 1.41$ ,  $p = 0.003$ ; CEB:  $F = 1.18$ ,  $p = 0.073$ ; CHI:  $F = 1.44$ ,  $p = 0.001$ ; LAP:  $F = 1.35$ ,  $p = 0.005$ ;  $k$ :  $F = 1.31$ ,  $p = 0.015$ ;  $S$ :  $F = 1.42$ ,  $p = 0.003$









**Figure S3.4** Relative abundance of different microbial lineages. Along the horizontal axis samples are arranged according to adapted (AG) and unadapted (UG) plant genotypes of the *Elymus athericus* exposed to three different flooding frequencies. The rank order along the vertical axis is shown for the class (A), order (B), and phylum (C).

**Table S3.1** Results of Pearson correlation analyses between plant biomass and exo-enzyme activities (GLU =  $\beta$ -glucosidase, CEB = cellobiosidase, CHI = chitinase and LAP = leucine-aminopeptidase), and between plant biomass and litter breakdown parameters ( $S$  = stabilization and  $k$  = decomposition rate constant). Shown are R-values bold-typed at  $p \leq 0.05$ .

	Exo-enzyme activity				Litter breakdown	
	GLU	CEB	CHI	LAP	$S$	$k$
Aboveground biomass	0.021	0.040	<b>0.293</b>	<b>0.418</b>	-0.053	-0.111
Belowground biomass	-0.026	0.005	0.023	0.272	-0.149	-0.063
Aboveground/Belowground ratio	-0.019	-0.047	-0.010	<b>-0.296</b>	0.164	0.110



# 4 Warming stimulates microbial activity in salt-marsh soils

## Abstract

Salt marshes play an important role in the global carbon (C) cycle due to the large amount of C stored in sediments, but global warming is expected to affect their C-sink capacity. Most studies concerned with global warming effects on soil C cycling in salt marshes have used laboratory incubations or field experiments that only manipulated soil surface temperature. No study has yet examined the response of soil microbial activity and litter breakdown to warming at different soil depths in salt marsh ecosystems. Thus, I investigated soil microbial functioning, including soil exo-enzyme activity, the concentration of microbial biomass carbon and nitrogen (N), and litter decomposition in both topsoil and subsoil across three marsh zones (pioneer zone, low marsh, and high marsh) under different warming treatments (ambient, + 1.5 °C and + 3 °C) in a Northwest European salt marsh. Warming strongly increased soil exo-enzyme activity, microbial biomass C and N, and the decomposition rate of standardized belowground litter. Both top- and subsoil microbial biomass C and N, and exo-enzyme activity were strongly affected by warming. Warming effects on soil microbial functioning was greater in the drier high marsh than in the frequently inundated pioneer zone. My results thereby suggest that warming effects are not just restricted to the topsoil. Higher soil microbial functioning in deeper soils, and the indirect effects of warming via soil moisture could increase microbial decomposition in salt marsh soils and thus decrease their C-sink capacity with global warming.

**Keywords:** climate change, soil exo-enzyme activity, Tea Bag Index, organic matter decomposition, carbon cycle, whole-soil profile, blue carbon, tidal wetland

# 1 Introduction

Salt marshes supply several valuable ecosystem services, such as wildlife conservation, flood protection, and improvements of water quality (Barbier et al., 2011; Kirwan and Megonigal, 2013). Recently salt marshes have additionally been recognized for their ability to store the amount of carbon (C) in soils. Soil C sequestration rate of salt marshes, mangroves, and seagrass beds is higher than in many other ecosystems, which is acknowledged by using the term “blue carbon” ecosystems (Chmura, 2013; McLeod et al., 2011). However, ecosystem services in general and especially C sequestration might be strongly affected by climate change. Global mean temperature is predicted to increase by 1.8-4.0°C until the end of the century (IPCC, 2007). Higher temperature yields the potential to change C sequestration in salt marshes by affecting the balance between organic matter input, through primary production, and output, via microbial decomposition (Kirwan and Mudd, 2012). Most of the current debate regarding the temperature-sensitivity of C sequestration is dealing with plant primary production (Hamann et al., 2018; Kirwan and Blum, 2011; Liu et al., 2018; Noyce et al., 2019), but the response of microbial decomposition to warming in salt marshes is still largely unknown.

The microbial decomposition in soils is strongly influenced by temperature through its effects on soil microbial functioning, including soil exo-enzyme activity and the concentration of microbial biomass C and nitrogen (N) (Allison et al., 2010; Razavi et al., 2017; Stone et al., 2014). Generally, soil exo-enzyme activity is regarded as the rate-limiting step of the decomposition process and gives insight into microbial C and nutrient demands (Sinsabaugh et al., 2008). Soil microbial biomass plays a key role in the terrestrial C and N cycles, and it regulates the decomposition of organic matter and nutrient transformations (Sorensen et al., 2018). Furthermore, the relationship between the soil exo-enzyme activity and temperature follows an optimum curve. In the relatively cold temperate zone, rising temperature is generally expected to increase the decomposition of organic matter via direct kinetic effects

on the metabolic activity of microorganisms (Davidson and Janssens, 2006). However, in warmer climates, a rising temperature could slow down the soil microbial activity indirectly via reductions in soil water availability (Di Nardo et al., 2004; Krivtsov et al., 2006). It is still unclear if a reduction of soil microbial activity due to suboptimum soil moisture levels is also relevant in the soils of frequently flooded salt marshes. In fact, it may also be possible that warming-driven reductions in soil moisture increase soil oxygen availability and thus microbial activity (Kirwan et al., 2014).

In addition to these general effects of warming on soil microbial functioning, there is still little known about the response of soil microbial activity to temperature in different soil depths. Most of the studies on organic matter decomposition have focused on the topsoil, because it has higher content of organic matter and higher soil microbial activity (Rumpel and Kögel-Knabner, 2011). More importantly, it is technically extremely challenging to induce whole soil warming. However, factors controlling the rate of organic matter decomposition in topsoil and subsoil may be different. For instance, soil moisture and the supply of fresh organic compounds are important in the topsoil (Fierer et al., 2003; Salomé et al., 2010), whereas temperature and the physical accessibility of organic substrates are considered as the main regulatory mechanisms of organic matter decomposition in the subsoil (Wild et al., 2019). These different factors may have resulted in limiting our understanding of the microbial decomposition response to climate change. It is thereby essential to investigate the response of these soil microbial parameters to temperatures both in topsoil and subsoil, and supply accurate predictions for soil microbial decomposition under global warming.

Here, I utilized a unique field warming experiment that uses active soil temperature manipulation up to 1 m soil depth in a Northwest European salt marsh. I assessed the effects of higher temperatures on the concentration of microbial biomass C and N, exo-enzyme activity, and litter breakdown at different soil depths in three different marsh zones (pioneer

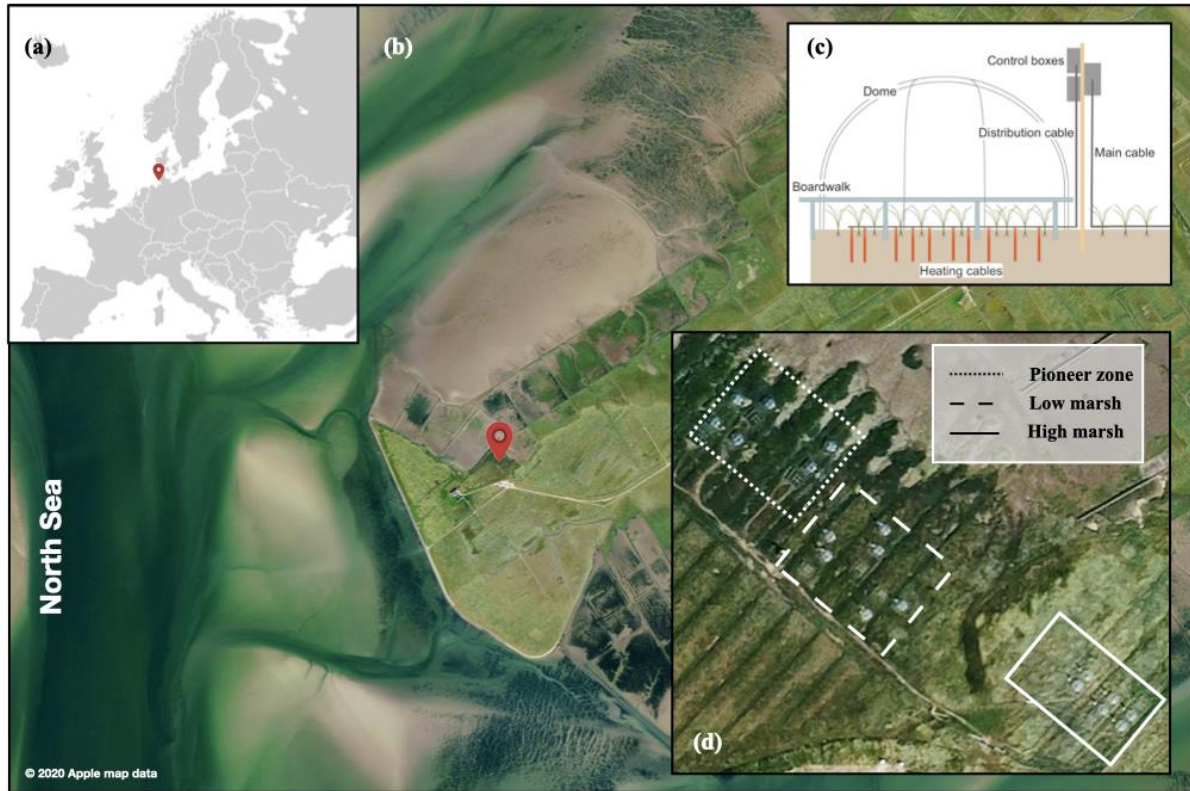
zone, low marsh, high marsh). I hypothesize (1) that warming will increase microbial biomass C and N, soil exo-enzyme activity, and the rate of litter breakdown. Furthermore, in salt marsh ecosystems, the effect of temperature on soil microbial functioning might be depending on marsh zones, and therefore flooding frequency and soil moisture conditions. Here, I hypothesize (2) that marsh zones determine the effect of temperature on soil microbial functioning and litter breakdown, and that warming effects on soil microbial functioning and litter breakdown are greater in the pioneer zone than in the high marsh, because of the limiting soil moisture conditions in the high marsh. I further hypothesize (3) the moisture-depending differences in the warming effects on microbial decomposition across marsh zones will be limited/restricted to the topsoil, due to more constant moisture regimes in the subsoil across zones.

## 2 Methods

### 2.1 Site description and experimental design

The field warming experiment operates in a Northwest European salt marsh at Hamburger Hallig, Germany (54°36'06.2"N, 8°49'00.1"E). The site is part of the Schleswig-Holstein Wadden Sea (Figure 4.1 a and b) and has been protected as a National Park since 1985. The study area is exposed to a temperate maritime climate, the annual mean temperature and mean precipitation are 10 °C and 850 mm, respectively. The salt marsh has a meso-tidal regime with a mean tidal amplitude of about 3 m (Stock, 2011). The mean elevation of the pioneer zone, low marsh, and high marsh are 136 cm, 174 cm and 212 cm, respectively. *Spartina anglica* is the dominant plant species of the pioneer zone. The low marsh is characterized by a mix of species, including *Halimione portulacoides*, *Puccinellia maritima*, and *Limonium vulgare*. The dominant plant species of the high marsh is *Elymus athericus*.





**Figure 4.1** Location of experimental site (a and b), diagram of belowground and aboveground heating (c) and plot distribution in the pioneer zone, low marsh, and high marsh (d).

In this field warming experiment (Marsh Ecosystem Response to Increased Temperatures; MERIT), belowground active temperature manipulation is conducted using heating cables inserted into the ground vertically up to 1.0 m soil depth, and aboveground temperature manipulation is achieved by passive open-top chambers (Figure 4.1c). The experiment includes the three marsh zones (pioneer zone, low marsh, and high marsh) and three temperature treatments (ambient, +1.5°C, and +3 °C) as factors. In 2018, three replicate plots per temperature treatment were established in each of the marsh zones (Figure 4.1d), hence the total number of plots is 27 (3 replicates x 3 marsh zones x 3 temperature treatments).

## 2.2 Soil sampling

Soil samples were collected on the 18<sup>th</sup> of July 2019. In each plot, one 50-cm deep soil core was taken using a soil corer and divided into 5-cm increments. Samples were stored at -20 °C before analysis.

## 2.3 Exo-enzyme assays

The quantification of exo-enzyme activity followed Tang et al. (2020). For C acquisition enzyme activity, I measured the soil  $\beta$ -glucosidase and cellobiosidase activity. Leucine-aminopeptidase and chitinase activity were measured to quantify microbial N acquisition. In brief, a 20 g of homogenized soil sample from each depth was proportionally mixed with 20 mL deionized water and stored at -20 °C. A 2 g soil slurry was mixed with 20 mL buffer. Then, 200  $\mu$ L buffered soil slurry was separately added to 50  $\mu$ L buffer, fluorophore standard, and substrate in a 96 well microplate. Plates were incubated in the dark at 20 °C for 16 h and read on a Multi-Detection Microplate Reader (Bio-Tek Synergy<sup>TM</sup> HT, Winooski, USA) with an excitation/emission setting around 360/460 nm. I refer to exo-enzyme activity normalized by the organic matter content of the sample (Morrissey et al., 2014; Mueller et al., 2017), which was determined via loss on ignition (5 hours at 550°C, following Wang et al. 2011),

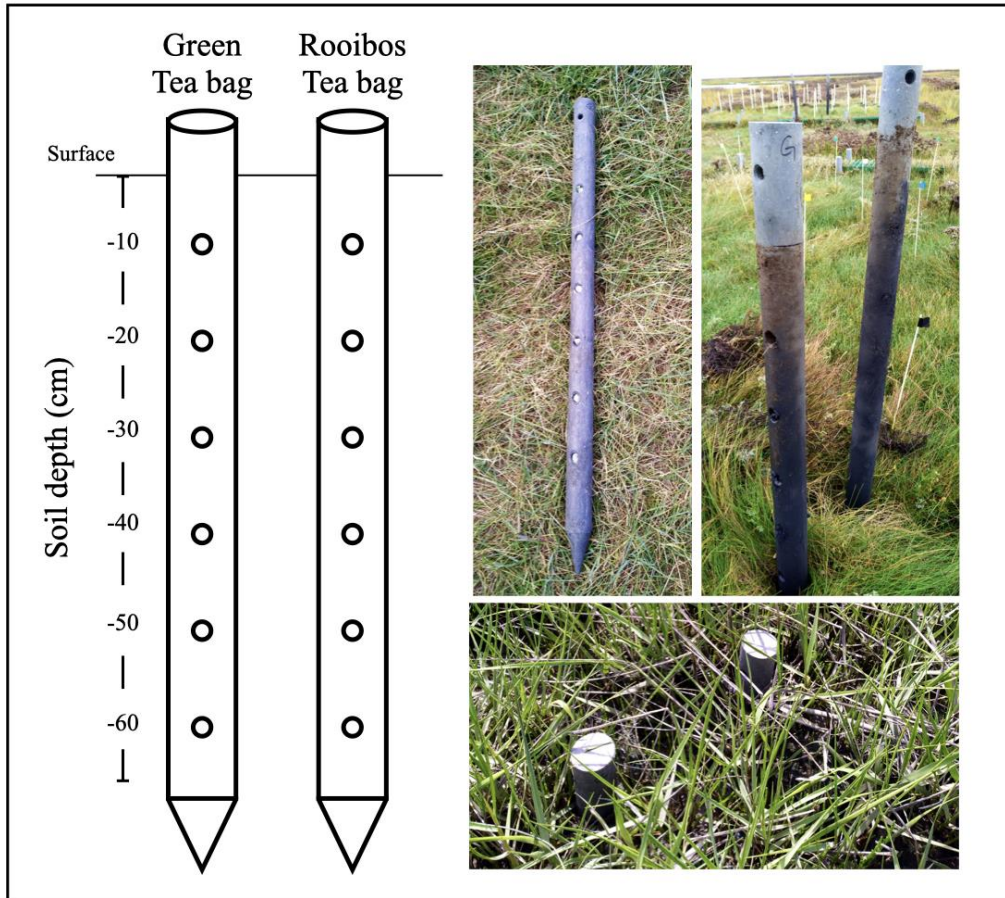
## 2.4 Soil microbial biomass C (MBC) and N (MBN)

The determination of soil MBC and MBN was using a chloroform fumigation-extraction method (Vance et al. 1987, Brooks et al. 1985). I took two 10-g sub-samples from each soil depth. One sub-sample was fumigated with chloroform, the other sub-sample was used as a control. Both sub-samples were incubated 24 h and extracted with 0.5 M K<sub>2</sub>SO<sub>4</sub> in an end to end shaker for 1 h, and subsequently filtered through a Whatman no. 42 filter. The amount of total soluble C and N in the fumigated and non-fumigated soil extracts were determined using

an Elemental Analyzer (EURO-EA 3000, Euro Vector, Italy). Soil MBC and MBN ( $\mu\text{g}\cdot\text{g}^{-1}$  FW) were calculated from the differences in the quantity of soluble organic C and N between the fumigated and non-fumigated samples.

## 2.5 Decomposition of standardized plant litter

The decomposition rate constant ( $k$ ) and stabilization factor ( $S$ ) were assessed following the Tea Bag Index (TBI) protocol (Keuskamp et al. 2013), which uses commercially available tea bags to compare the decomposition dynamics across ecosystems.  $k$  describes the labile fraction of the deployed material and  $S$  presents the part of labile fraction that did not decompose and stabilize organic matter in soils. Twelve polypropylene tea bags (six green tea and six rooibos tea bags, size: 55 mm x 50 mm) were put into the salt marsh soil using two solid posts per plot with six holes at a distance of -10 cm up to -60 cm. One post per plot contained six green tea bags (EAN: 8 714100 770603; Lipton, Unilever) and another contained six rooibos tea bags (EAN: 8 711200 875665; Lipton, Unilever) (Figure 4.2). The initial weight of the tea bag content was determined by subtracting the mean content weight of 5 empty bags from the total tea bag weight (Green tea: 1.59 g; Rooibos tea: 1.80 g). Tea bags were incubated from 8<sup>th</sup> April to 10<sup>th</sup> June, for 93 days. Afterward, the tea bags were taken out of the soil, carefully separated from roots and soil, dried for 48 h at 70 °C, and weighed. The calculation of  $k$  and  $S$  was done following the tidal-wetland-adapted TBI protocol (Mueller et al. 2018).



**Figure 4.2** Procedure for measuring standardized litter breakdown under in-situ warming. In each plot, two posts with six holes at a distance of -10 cm up to -60 cm were put into the soil. One post contained six green tea bags, the other one six rooibos tea bags.

## 2.6 Statistical analyses

Two-way repeated-measures ANOVA was used to test for effects of warming treatment (ambient, +1.5 and +3 °C), marsh zone (pioneer, low marsh, and high marsh), and soil depth (serving as the repeated measure/within-subject factor) on soil exo-enzyme activity ( $\beta$ -glucosidase, cellobiosidase, chitinase, and leucine-aminopeptidase), soil microbial biomass (MBC, MBN) and TBI parameters ( $k$  and  $S$ ). Pairwise comparisons were performed using Tukey's HSD tests. All analyses were conducted using the statistical software STATISTICA, version 12 (StatSoft Inc, Tulsa, Oklahoma, USA).

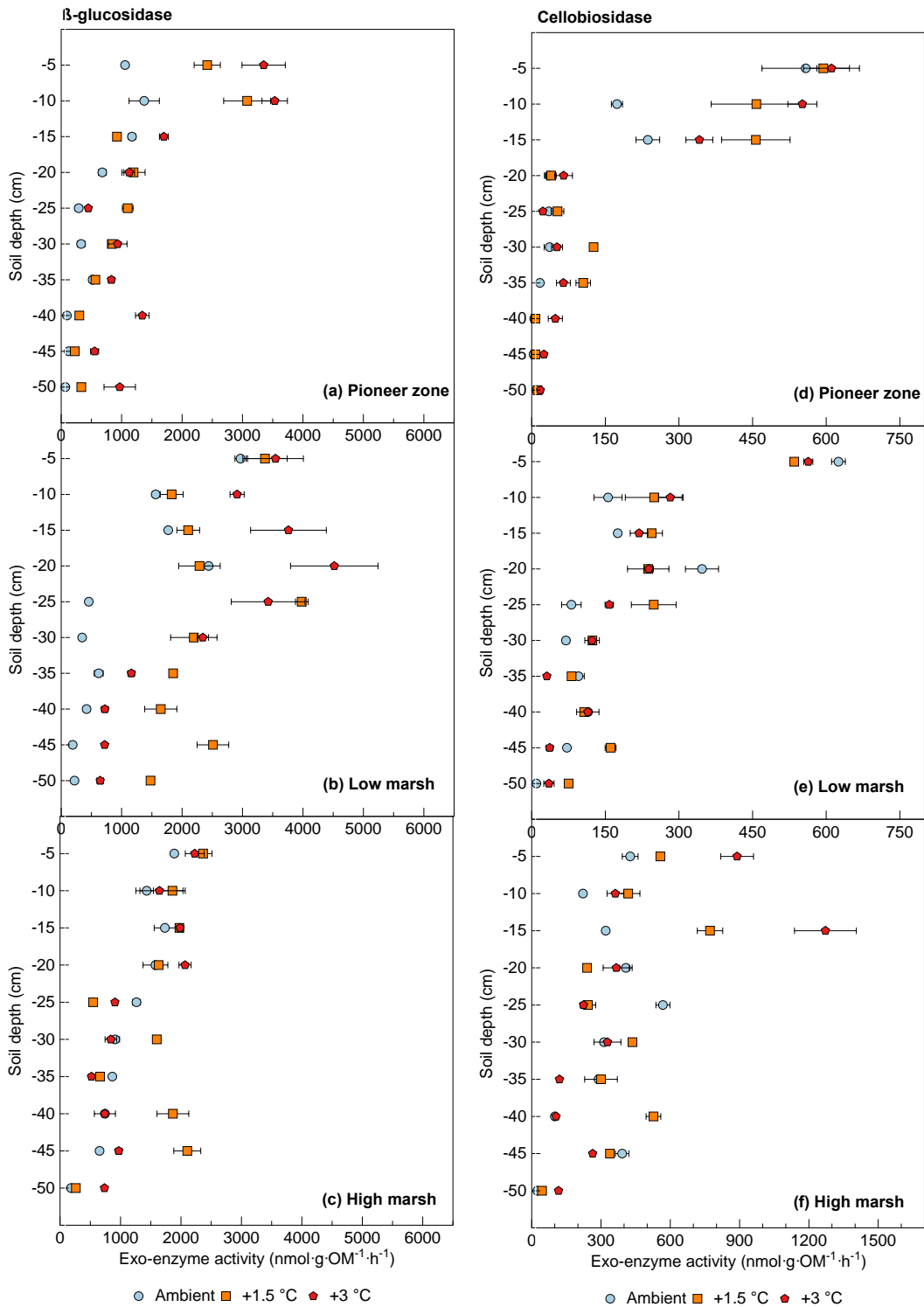
## 3 Results

### 3.1 Exo-enzyme activities

Warming strongly increased the C acquisition enzyme activity (Figure 4.3). This effect is mediated by zone, depth, and their interaction (Table 4.1). Warming effects on  $\beta$ -glucosidase activity were higher in the pioneer zone and low marsh than in the high marsh. In the pioneer zone,  $\beta$ -glucosidase activity was strongly increased by warming at -5 cm, -10 cm, and even in deeper soil depths (Figure 4.3 a). In the low and high marsh,  $\beta$ -glucosidase activity was strongly increased by warming at below -25 cm soil depths (Figure 4.3 b, Table S4.1, Table S4.2), but it was decreased by warming at below -25 cm in the high marsh (Figure 4.3 c, Table S4.2). Cellobiosidase activity was strongly increased by warming in all three marsh zones. In the pioneer zone and low marsh, cellobiosidase activity was strongly increased by warming only at -25 cm to -50 cm (Figure 4.3 d and e). In the high marsh, cellobiosidase activity was increased consistently throughout the soil profile by warming, except at -20 cm by +1.5 °C and -25 cm by +3 °C treatment (Figure 4.3 f and Table S4.2).

**Table 4.1** Results of two-way repeated measures ANOVA testing for effects of warming, marsh zone, soil depth, and their interactions on soil exo-enzyme activity (GLU =  $\beta$ -glucosidase, CEB = cellobiosidase, CHI = chitinase, and LAP = leucine-aminopeptidase), microbial biomass (MBC = microbial biomass carbon and MBN = microbial biomass nitrogen) and TBI parameters ( $k$  = decomposition rate constant and  $S$  = stabilization factor).

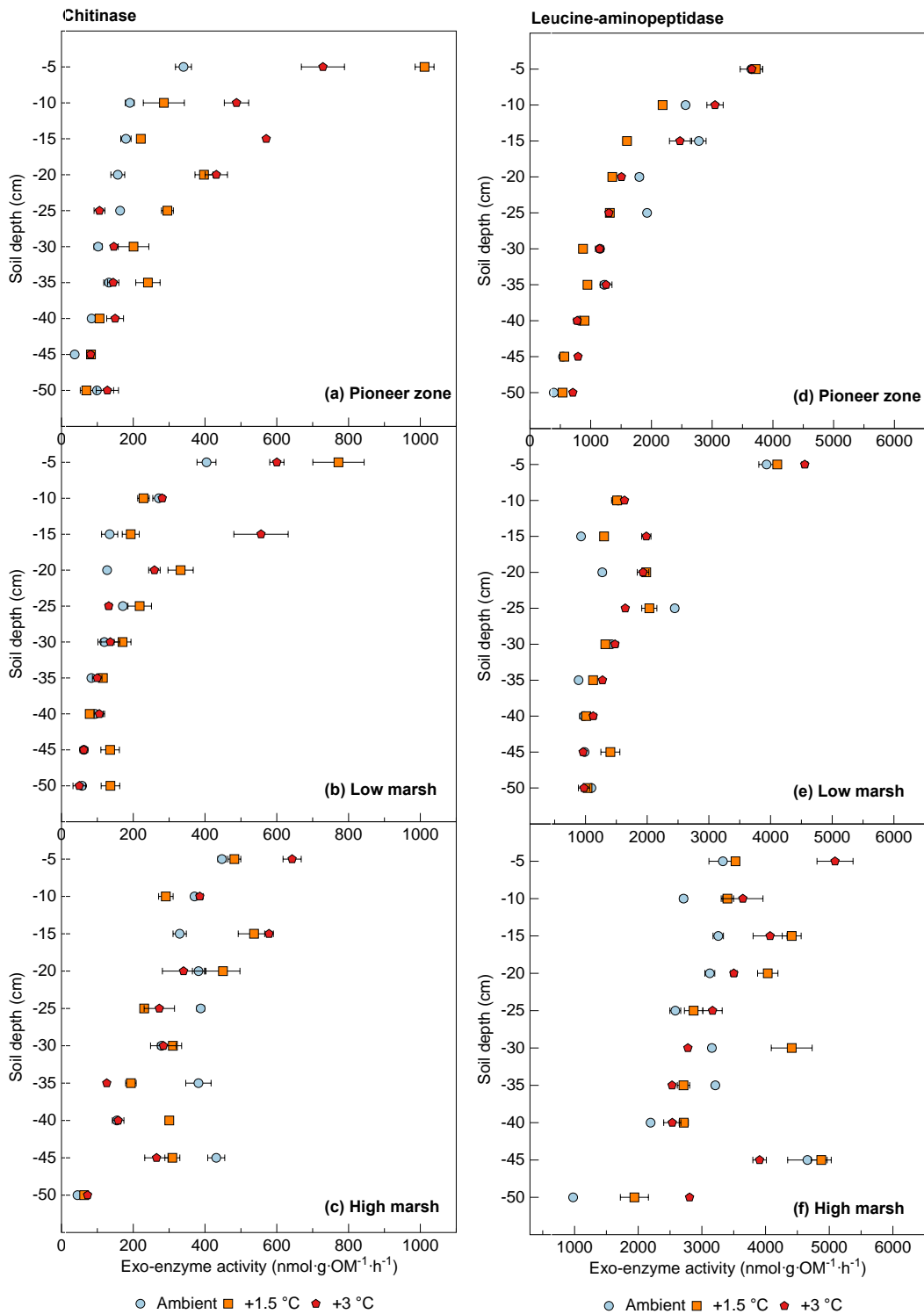
	Between subject				Within-subject									
	Warming		Zone		Warming x zone		Depth		Depth x Zone		Depth x Warming		Depth x zone x Warming	
	F value	p value	F value	p value	F value	p value	F value	p value	F value	p value	F value	p value	F value	p value
GLU	153.60	<b>0.00</b>	110.60	<b>0.00</b>	26.60	<b>0.00</b>	319.90	<b>0.00</b>	28.30	<b>0.00</b>	24.90	<b>0.00</b>	20.40	<b>0.00</b>
CEB	84.91	<b>0.00</b>	818.90	<b>0.00</b>	20.82	<b>0.00</b>	281.27	<b>0.00</b>	49.11	<b>0.00</b>	7.95	<b>0.00</b>	10.88	<b>0.00</b>
CHI	42.00	<b>0.00</b>	120.50	<b>0.00</b>	15.40	<b>0.00</b>	218.60	<b>0.00</b>	18.40	<b>0.00</b>	11.10	<b>0.00</b>	5.50	<b>0.00</b>
LAP	29.00	<b>0.00</b>	1640.00	<b>0.00</b>	21.00	<b>0.00</b>	798.00	<b>0.00</b>	185.00	<b>0.00</b>	20.00	<b>0.00</b>	21.00	<b>0.00</b>
MBC	2156.00	<b>0.00</b>	12342.00	<b>0.00</b>	83.00	<b>0.00</b>	3007.00	<b>0.00</b>	180.00	<b>0.00</b>	30.00	<b>0.00</b>	26.00	<b>0.00</b>
MBN	3703.00	<b>0.00</b>	13468.00	<b>0.00</b>	45.00	<b>0.00</b>	1626.00	<b>0.00</b>	100.00	<b>0.00</b>	25.00	<b>0.00</b>	28.00	<b>0.00</b>
$k$	36.32	<b>0.00</b>	0.17	0.85	0.58	0.68	10.92	<b>0.00</b>	1.77	0.10	1.76	0.10	2.23	<b>0.01</b>
$S$	2.64	0.10	35.67	<b>0.00</b>	0.45	0.77	68.87	<b>0.00</b>	5.33	<b>0.00</b>	0.45	0.89	2.06	<b>0.02</b>



**Figure 4.3** The C-acquisition enzyme activity of  $\beta$ -glucosidase (a, b, and c) and cellobiosidase (d, e, and f) at different soil depths of the pioneer zone, low marsh, and high marsh under three temperature treatments (ambient, +1.5 °C and +3 °C). Value are means  $\pm$  SE (n =3).

Warming strongly increased N acquisition enzyme activity (Figure 4.4). This effect was mediated by zone, depth, and their interaction (Table 4.1). Irrespective of soil depth, the activity of chitinase was always strongly increased by warming in the pioneer zone and low marsh. A less consistent warming effect was found in the high marsh. The activity of chitinase in the pioneer zone and low marsh was strongly increased by warming at -5 cm to -20 cm (Figure 4.4 a and b, Table S4.2). In the high marsh, warming strongly increased the chitinase activity at -15 cm and below -35 cm (Figure 4.4 c, Table S4.2). By contrast, the warming-induced stimulation of leucine-aminopeptidase activity was greater in the high marsh than in the pioneer zone and low marsh. The activity of leucine-aminopeptidase was generally increased by warming at -5 cm to -20 cm, -35 cm, -45 cm, and -50 cm in the high and low marsh (Figure 4.4 e and f, Table S4.2). A less consistent pattern of the leucine-aminopeptidase activity was found in the pioneer zone. Here, leucine-aminopeptidase was strongly increased by warming only at -45 cm and -50 cm (Figure 4.4 d, Table S4.2).

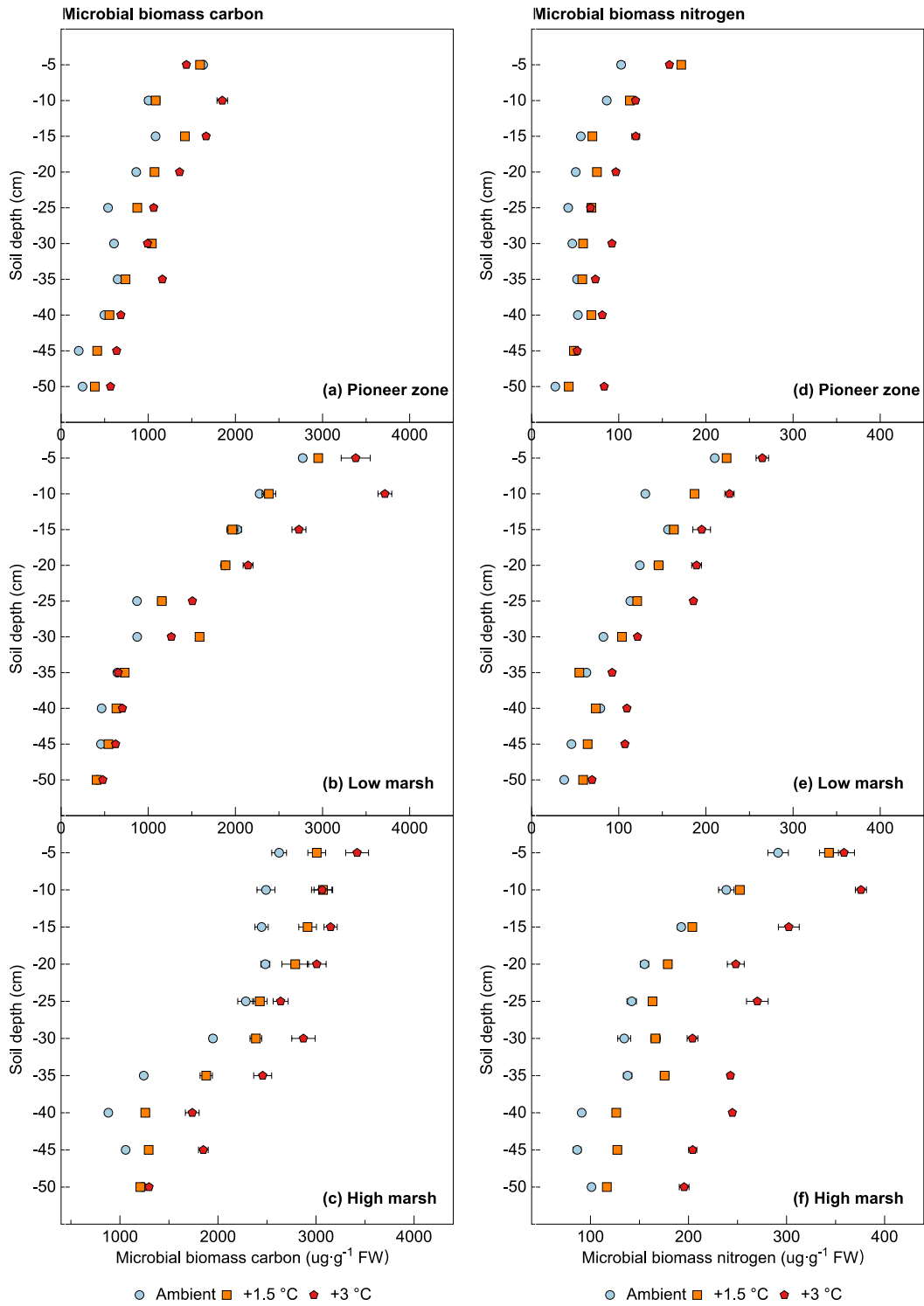




**Figure 4.4** The N-acquisition enzyme activity of chitinase (a, b, c) and leucine-aminopeptidase (e, d, f) at different soil depths of the pioneer zone, low marsh, and high marsh under three temperature treatments (ambient, +1.5 °C and +3 °C). Value are means  $\pm$  SE (n =3).

### 3.2 Microbial biomass carbon (MBC) and nitrogen (MBN)

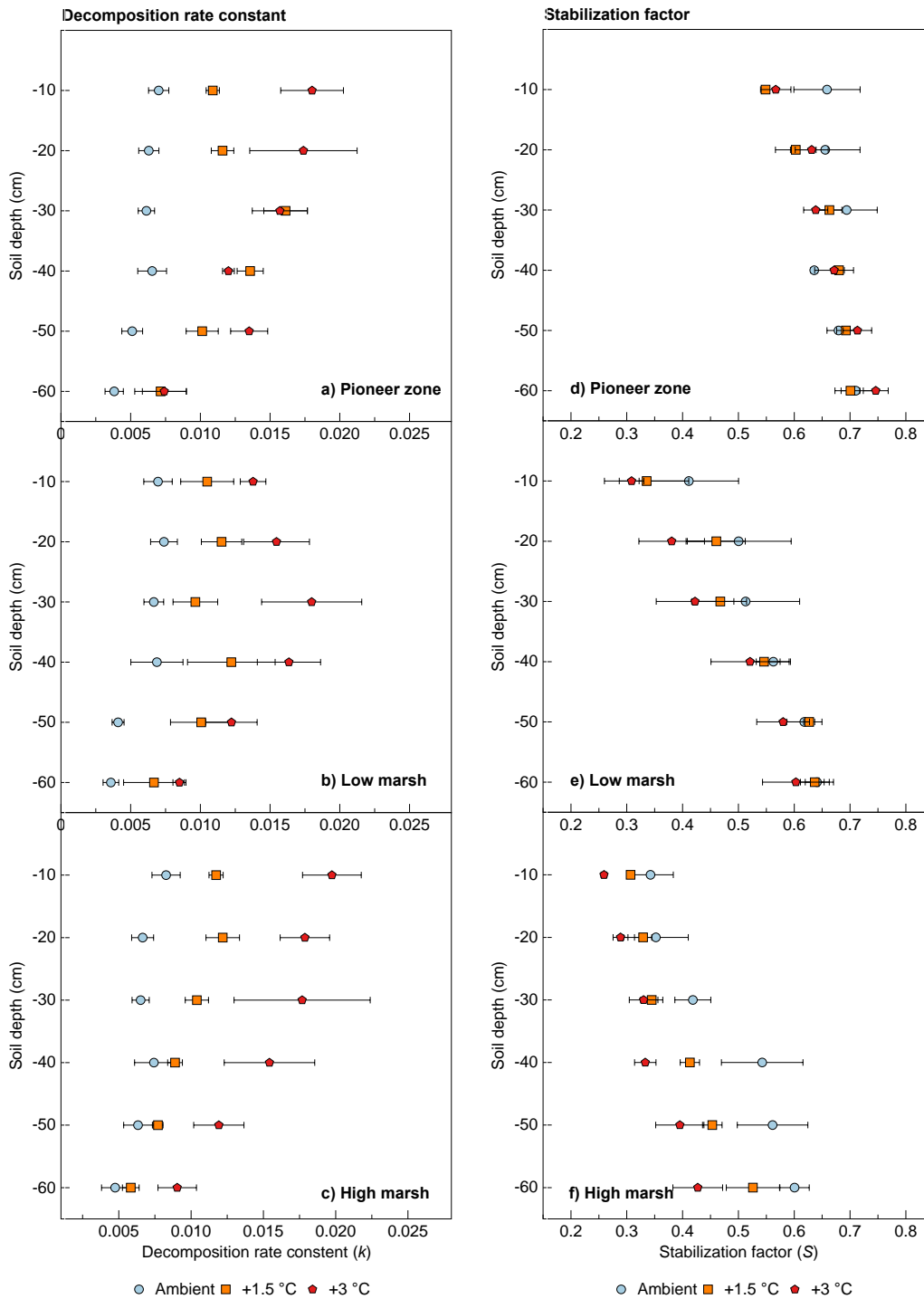
MBC and MBN were strongly increased by warming, and these changes were mediated by zone, depth, and their interaction (Figure 4.5, Table 4.1). Furthermore, MBC and MBN increased with rising elevation from pioneer zone to high marsh. Yet, I found varying patterns in different marsh zones. In the pioneer zone, warming strongly increased the soil MBC at different soil layers, except for the -5 cm layer (Figure 4.5 a, Table S4.2). In the low marsh and high marsh, soil MBC was strongly increased by warming, and its content at each soil depth was on average 1.5 times higher than under ambient conditions (Figure 4.5 b and c, Table S4.2). Likewise, soil MBN was strongly increased by warming in the pioneer zone, except for the -45 cm soil depth (Figure 4.5 d). In the low marsh and high marsh, warming also largely increased the MBN, these warming effects on MBN were 1.6 times higher than under the ambient treatment (Figure 4.5 e and f).



**Figure 4.5** The soil microbial biomass carbon (a, b, c) and microbial biomass nitrogen (d, e, f) at different soil depths of the pioneer zone, low marsh, and high marsh zones under three temperature treatments (ambient, + 1.5 °C and + 3 °C). Value are means  $\pm$  SE (n =3).

### 3.3 TBI parameters

Warming strongly increased the decomposition rate constant ( $k$ ).  $k$  was further affected by the depth and the depth x zone x treatment interaction (Figure 4.6, Table 4.1). In particular, in the pioneer zone and high marsh, warming strongly increased  $k$  by 110% and 125% under +1.5 °C and +3 °C, respectively (Figure 4.6 a and c). However, the higher temperature only significantly increased  $k$  at -30 cm in the low marsh (Figure 4.6 b, Table S4.3). In contrast, the stabilization factor ( $S$ ) was not affected by warming separately, but strongly affected by the warming x zone x depth interaction (Figure 4.6, Table 4.1).  $S$  decreased with rising elevation from pioneer zone to high marsh.  $S$  was not affected by warming with soil depths in the pioneer zone (Figure 4.6 d), but it was decreased by warming with soil depths in the low and high marsh (Figure 4.6 e and f, Table S4.3).



**Figure 4.6** The decomposition rate constant (a, b, c) and stabilization factor (d, e, f) at different soil depths of the pioneer zone, low marsh, and high marsh zones under three temperature treatments (ambient, + 1.5 °C and + 3 °C). Value are means  $\pm$  SE (n =3). The decomposition rate constant describes the labile fraction of the deployed material and the stabilization factor presents the part of labile fraction that did not decompose and stabilize organic matter in soil.

## 4 Discussion

### 4.1 Warming

In line with my first hypothesis, exo-enzyme activity, microbial biomass, and decomposition rate constant ( $k$ ) in marsh soils were strongly increased by warming. As soil exo-enzyme activity is associated with microbial C and nutrient demands (Sinsabaugh et al., 2009), this finding agrees with studies suggesting that a temperature-induced increase in soil exo-enzyme activity has the potential to increase microbial nutrient demand (Steinweg et al., 2012). Furthermore, warming effects on soil exo-enzyme activity were found to scale positively with the rate of litter decomposition (Kirschbaum, 1995; Wilson et al., 2016), which is supported by my results with higher decomposition rate constant ( $k$ ). Furthermore, three major mechanisms need to be considered for explaining the temperature effects on soil microbial functioning. First, in accordance with kinetic effects in wetland soils, higher temperatures should directly stimulate soil exo-enzyme activity (Davidson and Janssens, 2006), which could be linked to changes in soil microbial physiology (Blagodatskaya et al., 2016). Second, warming may also lead to limiting soil moisture conditions, and thus indirectly controls the soil microbial activity in terrestrial soils via low water availability (Blagodatskaya et al., 2016; Conant et al., 2008; Min et al., 2019). Third, it has been suggested that a temperature-induced increase in soil exo-enzyme activity may be caused by an increase in microbial biomass (Conant et al., 2011). My finding agrees with the first and third point, because warming not just strongly increased the soil exo-enzyme activity, but also dramatically increased soil MBC and MBN by over 50% (Figure 4.5). However, regarding the soil moisture effects on soil microbial activity (mechanism 2), my results do not support this point. In addition, there was no change in the stabilization factor ( $S$ ) under warming. This finding is surprising, because  $S$  describes the fraction of litter that gets ultimately transformed to stable soil organic matter, which is expected to inversely relate to  $k$  (Keuskamp et al., 2013; but see Tang et al., 2020).

Thus, this finding highlights that temperature effects on litter decomposition rate and stabilization might be quite different. One potential reason is, in accordance with microbial efficiency-matrix stabilization, it suggests the higher soil microbial biomass could stabilize soil organic matter (Cotrufo et al., 2013) and this agrees with my result about the temperature-induced increase in MBC.

#### **4.2 Warming and marsh zone**

My results also partly support the second hypothesis, because marsh zone determined the effects of warming on soil exo-enzyme activity and microbial biomass. However, there was no such interaction effect on the parameters of litter breakdown. Interestingly, this effect of the marsh zone varied between the enzymes studied here. The activity of the C acquisition enzymes ( $\beta$ -glucosidase and cellobiosidase) and chitinase showed greater warming stimulation in the pioneer zone than in the high marsh (Figure 4.2). This pattern supported previous work showing that soil moisture may determine the effects of temperature on soil enzyme activity (de Nijs et al., 2019; Wang et al., 2018). In the pioneer zone of salt marshes, a high flooding frequency leads to preferable moisture conditions, which probably facilitates warming effects on exo-enzyme activity. By comparison, in the high marsh, high temperatures could have induced suboptimum moisture levels and thereby reduced soil exo-enzyme activity. However, one of the enzymes assayed (leucine-aminopeptidase) as well as MBC and MBN responded completely differently. That is, warming strongly increased the activity of leucine-aminopeptidase, MBC, and MBN in the high marsh compared to the pioneer zone (Figure 4.4 and Figure 4.5). This is especially relevant, as the activity of leucine-aminopeptidase was six-time higher than the chitinase activity, the second N acquisition enzyme studied. This low chitinase activity can thus be considered as unimportant for understanding microbial N demand (Tang et al., 2020). Therefore, I argue that marsh zone mediates the response of C and N acquisition enzyme activities to higher temperatures. The C acquisition enzymes, represented

here by  $\beta$ -glucosidase and cellobiosidase, had higher activity in the frequently flooded pioneer zone. In contrast, the N acquisition enzyme activity and microbial biomass were found to be higher in the high marsh under limited water availability.

### **4.3 Interactive effects with soil depth**

In contrast to my third hypothesis, soil exo-enzyme activity and microbial biomass in both topsoil and subsoil were strongly affected by warming. Generally, it has been assumed that subsoil organic matter is relatively stable and unresponsive to warming due to its long turnover time (Harrison, Footen, & Strahm, 2011). Recent research shows that subsoils are projected to warm at roughly the same rate as topsoils (Hicks Pries et al., 2017). Thus, two potential mechanisms could explain my results. First, while warming increases the soil exo-enzyme activity and microbial biomass in the topsoil, it also supplies improved conditions for plant growth, especially for root biomass and total root length (Jia et al., 2019a; Lin et al., 2018; Mueller et al., 2018). Thus, a temperature-induced increase in root biomass and length could lead to higher oxygenation through root oxygen loss and also to a higher root exudation and thus nutrient availability in the subsoil (Rumpel et al., 2012). This in turn may potentially enhance these microbial biomass and exo-enzyme activity in the subsoil. Secondly, Conant et al. (2008) concluded that in the subsoil the temperature-sensitivity of recalcitrant organic compounds is greater than that of the more labile substrates. Unfortunately, I do not have any data to support these points. Thus, based on my results, I suggest that the response of soil exo-enzyme activity and microbial biomass in the topsoil and subsoil to warming are not just depending on soil moisture conditions, but that further factors like the quality of organic substrates and plant growth may also play a major role.

In conclusion, my study assesses soil microbial activity and biomass in whole-soil profiles under the in-situ warming of a temperate salt marsh. Warming strongly increased these parameters of soil microbial functioning and also of standardized litter decomposition rate in



both the topsoil and the subsoil. These findings 1) support kinetic effects of warming on soil exo-enzyme activity and microbial biomass; 2) suggest that marsh zones differently mediate the effects of warming on exo-enzyme activity: Higher C acquisition enzyme activity was found in the regularly flooded pioneer zone, higher N acquisition enzyme activity was detected in the high marsh under low water availability; 3) indicate the indirect effects of temperature via soil moisture could potentially influence soil microbial activity and litter breakdown. Taken together, this study improves the understanding of how global warming affects soil microbial decomposition in the whole-soil profile. This knowledge could be used to improve predictions of soil C turnover in tidal marshes under global warming.

## 5 Supplementary materials

**Table S4.1** Results of two-way repeated measures ANOVA testing for effects of warming, marsh zone, soil depth, and their interactions on soil exo-enzyme activity (GLU =  $\beta$ -glucosidase, CEB = cellobiosidase, CHI = chitinase, and LAP = leucine-aminopeptidase), microbial biomass (MBC = microbial biomass carbon and MBN = microbial biomass nitrogen) and TBI parameters ( $k$  = decomposition rate constant and  $S$  = stabilization factor) in the pioneer zone, low marsh, and high marsh.

	GLU		CEB		CHI		LAP		MBC		MBN		$k$		$S$	
	F value	P value	F value	P value	F value	P value	F value	P value	F value	P value	F value	P value	F value	P value	F value	P-value
<b>Pioneer zone</b>																
Warming	90.17	<b>0.000</b>	46.80	<b>0.000</b>	29.44	<b>0.000</b>	25.20	<b>0.001</b>	2875.00	<b>0.000</b>	1681.00	<b>0.000</b>	24.38	<b>0.003</b>	0.28	0.770
Depth	112.08	<b>0.000</b>	118.89	<b>0.000</b>	68.37	<b>0.000</b>	772.90	<b>0.000</b>	1038.00	<b>0.000</b>	414.00	<b>0.000</b>	4.93	<b>0.006</b>	0.03	<b>0.000</b>
Warming x depth	14.31	<b>0.000</b>	5.23	<b>0.000</b>	5.80	<b>0.000</b>	20.10	<b>0.000</b>	48.00	<b>0.000</b>	35.00	<b>0.000</b>	4.67	<b>0.003</b>	2.31	<b>0.050</b>
<b>Low marsh</b>																
Warming	71.45	<b>0.000</b>	27.50	<b>0.000</b>	19.33	<b>0.002</b>	16.10	<b>0.004</b>	346.00	<b>0.000</b>	1612.00	<b>0.000</b>	7.86	<b>0.020</b>	0.54	0.610
Depth	139.68	<b>0.000</b>	126.10	<b>0.000</b>	69.24	<b>0.000</b>	297.30	<b>0.000</b>	2187.00	<b>0.000</b>	1111.00	<b>0.000</b>	2.66	0.060	27.81	<b>0.000</b>
Warming x depth	36.82	<b>0.000</b>	13.46	<b>0.000</b>	6.62	<b>0.000</b>	17.20	<b>0.000</b>	25.00	<b>0.000</b>	25.00	<b>0.000</b>	0.98	0.470	0.46	0.870
<b>High marsh</b>																
Warming	10.31	<b>0.010</b>	78.50	<b>0.000</b>	4.80	<b>0.060</b>	26.60	<b>0.001</b>	399.00	<b>0.000</b>	947.80	<b>0.000</b>	17.28	<b>0.006</b>	3.44	0.100
Depth	143.10	<b>0.000</b>	177.70	<b>0.000</b>	196.40	<b>0.000</b>	136.50	<b>0.000</b>	406.00	<b>0.000</b>	388.30	<b>0.000</b>	9.63	<b>0.000</b>	55.07	<b>0.000</b>
Warming x depth	24.36	<b>0.000</b>	33.90	<b>0.000</b>	15.50	<b>0.000</b>	26.00	<b>0.000</b>	14.00	<b>0.000</b>	20.70	<b>0.000</b>	2.09	0.090	4.04	<b>0.004</b>

**Table S4.2** Tukey HSD test for the pairwise comparisons through the two-way repeated-measures ANOVA. (\* and \*\* represent that + 1.5 or + 3 °C was significantly affected by warming treatment compare to ambient temperature. \* p < 0.05 and \*\* p < 0.001).

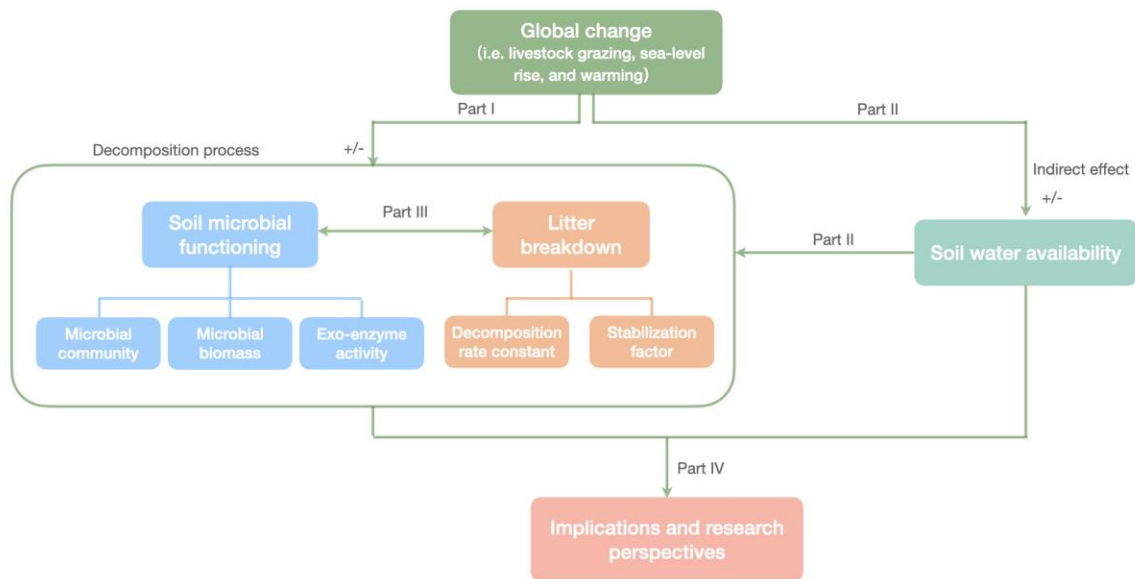
Marsh zone	Soil depth (cm)	β-glucosidase		Cellobiosidase		Chitinase		Leucine-aminopeptidase		Microbial biomass carbon		Microbial biomass nitrogen	
		+ 1.5 °C	+3 °C	+ 1.5 °C	+3 °C	+ 1.5 °C	+3 °C	+ 1.5 °C	+3 °C	+ 1.5 °C	+3 °C	+ 1.5 °C	+3 °C
Pioneer zone	-5	*	**			*						**	**
	-10	*	*				*				**	**	**
	-15						**	**		**	**	**	**
	-20					*	*	**		**	**	**	**
	-25	**						**	**	**	**	**	**
	-30	*	**	*				*		**	**	**	**
	-35			**				*			**		**
	-40	**	**		**						**	**	**
	-45		**		**				**	**	**	**	
	-50	**	**					**	**	**	**	**	**
Low marsh	-5										**	**	**
	-10										**	**	**
	-15		*				**	**	**	**	**	**	**
	-20					*		**	**	**	*	**	**
	-25	**	**	**	*				**	**	**	**	**
	-30	**	**							**	**	**	**
	-35	**			**				**		*	**	**
	-40	**								**	**		**
	-45	**	**	*		*		**		**	**	**	**
	-50	**	**	**	**	*					**	**	**
High marsh	-5				**				**	**	*	**	**
	-10			*				*		**	**	**	**
	-15			**	**	*	**	*		**	**	**	**
	-20			*				*				**	**
	-25	**		**	**	*				**	**	**	**
	-30	*						*		**	**	**	**
	-35		*		**	**	**				**	**	**
	-40	**		**		**				**	**	**	**
	-45	**					*		**	**	**	**	**
	-50		**	*	**	*	*	**	**	**	**	**	**

**Table S4.3** Tukey HSD test for the pairwise comparisons through the two-way repeated measures ANOVA. (\* and \*\* represent that + 1.5 or + 3 °C was significantly affected by warming treatment compare to ambient temperature. \*  $p < 0.05$  and \*\*  $p < 0.001$ ).

Marsh zone	Soil depth (cm)	Decomposition rate constant		Stabilization factor	
		(k)		(S)	
		+ 1.5 °C	+3 °C	+ 1.5 °C	+3 °C
Pioneer zone	-10		*		
	-20		*		
	-30	*	**		
	-40	*			
	-50		*		
	-60		*		
Low marsh	-10				
	-20				
	-30		*		
	-40				
	-50				
	-60				
High marsh	-10		*		
	-20		*		
	-30		*		
	-40		*		
	-50				
	-60		*		

# 5 General discussion

In the first part of this synthesis, I will summarize and generally discuss effects of global change drivers on soil microbial functioning (i.e. soil microbial community composition, microbial C and N biomass, and exo-enzyme activity) and standardized litter breakdown (i.e. decomposition rate constant  $k$  and stabilization factor  $S$ ) in salt marshes. In the second part, I will further discuss possible indirect effects of global change on soil microbial activity via alterations in soil moisture. Then, in the third part, I will link soil exo-enzyme activity to the parameters of standardized litter breakdown and explore their relationship under global change. In the end, I will conclude with implications and possible applications, and give directions for future research.



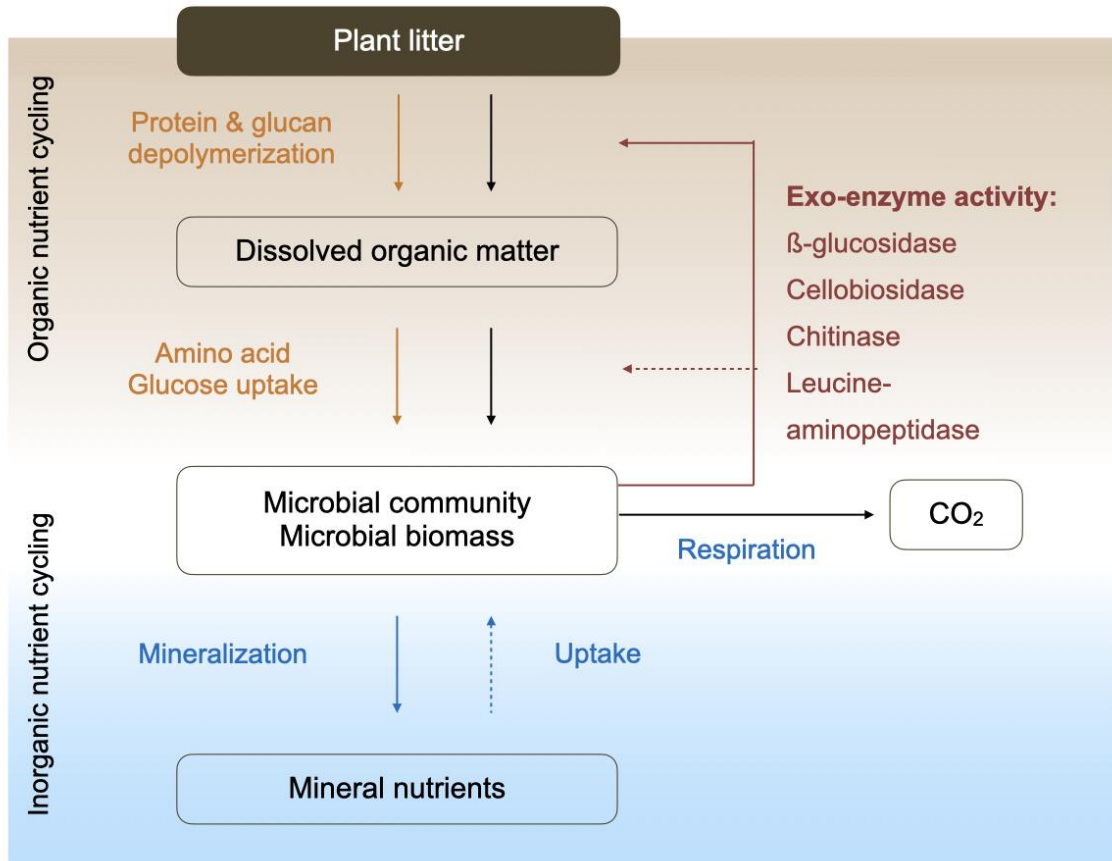
**Figure 5.1** Conceptual diagram of the general discussion outline. Part I summarizes and generally discusses how global change affects soil microbial functioning and litter breakdown. Part II develops a concept about the indirect effects of global change via soil moisture on soil microbial activity. Part III links the soil exo-enzyme activity to litter breakdown parameters. Part IV presents implications and recommendations for future research.

## **5.1 Effects of global change on microbial functioning and litter breakdown in salt marsh soils**

Previous studies found that global change factors, such as sea-level rise, global warming, and livestock grazing, can have positive influences on organic matter decomposition in salt marshes (Kirwan et al., 2014; Kirwan and Mudd, 2012; Mueller et al., 2017; Mueller et al., 2018; Spivak et al., 2019). Yet, few studies have paid attention to microbial decomposition processes in salt marshes (Mueller et al., 2017; Tang et al., 2020), which are mainly depended on soil microbial activity and litter quality. Thus, in this part, I discuss the effects of global change factors on both soil microbial functioning and parameters of standardized-litter breakdown.

### **Global change affects microbial functioning in salt marshes**

The soil microbial community plays an important role in soil C turnover and nutrient transformation (Figure 5.2) (Doetterl et al., 2018; Sinsabaugh et al., 2009; Stone et al., 2014; Thakur et al., 2019). Their soil exo-enzyme activities are regarded as the rate-limiting step of the decomposition process and give insight into microbial C and nutrient demands (Mueller et al., 2017; Sinsabaugh et al., 2008). In addition, soil microbial biomass acts as a pivotal part in controlling the decomposition of soil organic matter and nutrient transformation (Kuzyakov et al., 2000; Sorensen et al., 2018; Walker et al., 2018).



**Figure 5.2** Schematic representation of the soil microbial processes studied (modified from Mooshammer et al., 2017). Soil microbial community and biomass are the main drivers of the microbial decomposition process. In the organic nutrient cycling, their related soil exo-enzyme activity process plant litter into dissolved organic matter in the soil. In the inorganic nutrient cycling, soil microbial biomass, as the source and sink of soil nutrients, and its stoichiometry play a key role in soil organic carbon and nitrogen mineralization.

Chapter 2 of this thesis presents results of a study on effects of livestock grazing on soil exo-enzyme activities in a salt marsh of Chongming Island, China (Figure 5.3A). This study describes mechanisms by which livestock grazing can strongly affect soil exo-enzyme activities through soil redox condition and plant primary production. Furthermore, these findings demonstrate that grazing-induced soil compaction leads to an increase in bulk density (Chapter 2, Table 2.2), which could indirectly decrease  $\beta$ -glucosidase activity. I also present the negative effect of grazing on  $\beta$ -glucosidase activity via a decrease in plant belowground

biomass (Chapter 2, Table 2.3). In contrast, livestock grazing unexpectedly strongly increased leucine-aminopeptidase activity. I speculate this effect may be driven by lower inputs of allochthonous organic matter input under grazing (Mueller et al., 2017; Yang et al., 2017).



**Figure 5.3** Display of field and mesocosm experiments used to study several global change factors. (A) Livestock grazing affects soil exo-enzyme activity and litter decomposition in a salt marsh of Chongming Island, China; (B) The interactive effect of plant genotype and flooding frequency on soil microbial functioning and litter decomposition in a mesocosm experiment at the Institute of Plant Science and Microbiology, Hamburg University; (C and D) Warming effects on microbial activity, microbial biomass and litter breakdown in a Northwest European salt marsh at Hamburger Hallig, Germany.

In the case of different flooding frequencies, adapted plant genotypes (Figure 5.3B) can maintain soil microbial communities and exo-enzyme activities at a constant level under high flooding frequency (Chapter 3, Figure 3.2 D and Figure 3.4). I thereby highlight the importance of different plant genotypes and their related plant growth in controlling the effects of sea-level



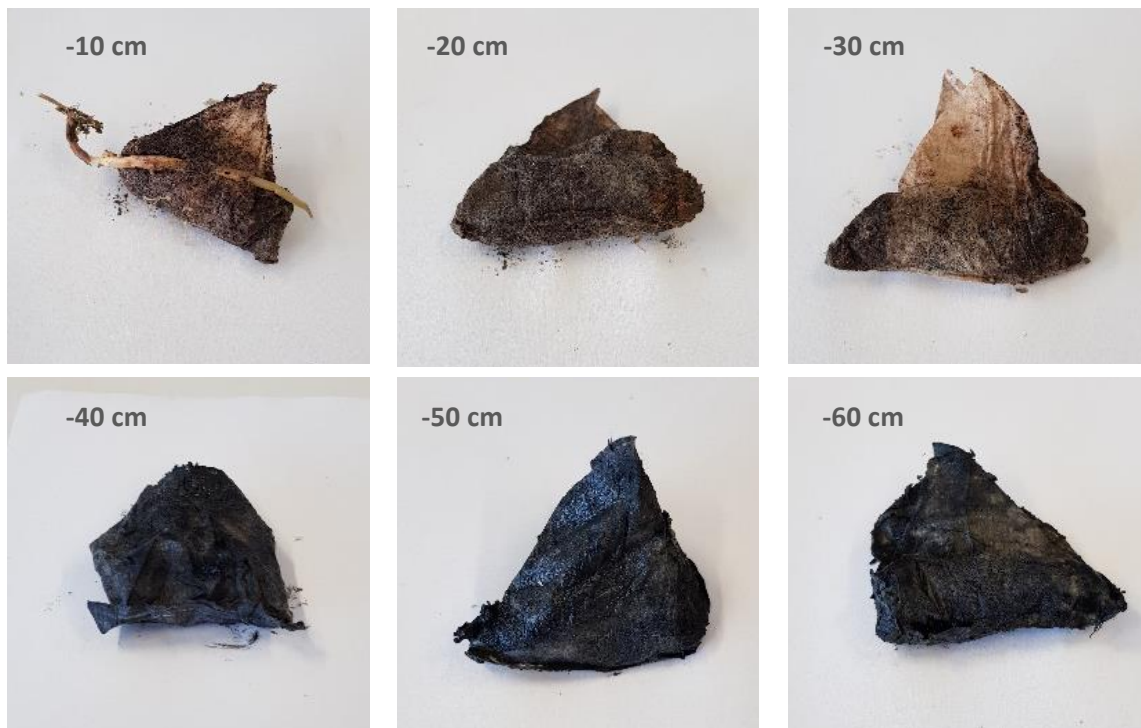
rise on microbial functioning and C turnover in salt marsh soils. In chapter 4 (Figure 5.3C), I was able to gain important insights into the warming effects on soil exo-enzyme activities and microbial biomass in salt marshes. This study supports the hypothesis that warming stimulates soil exo-enzyme activities and the concentration of microbial biomass C and N via kinetic effects (Davidson and Janssens, 2006). Furthermore, I also found that warming increased the soil microbial activity in both topsoil and subsoil, and this effect might be ascribed to soil moisture, organic substrates, and plant growth (Jia et al., 2019b, 2017; Lin et al., 2018).

### **Global change affects litter breakdown in salt marshes**

Litter-bag method is typically used to assess the rates of organic matter decomposition *in situ* (Crossley and Hoglund, 1962), and it is determined by the quality and quantity of plant litter. Yet, complex interactions between plant litter quality and global change factors hamper a straightforward assessment of decomposition rate (Duan et al., 2018; Orwin et al., 2010; Petraglia et al., 2019). Thus, it is necessary to use standardized litter to assess the litter decomposition in inter-site comparisons (Keuskamp et al., 2013). In the Tea-Bag Index approach, two different types of commercial tea bags are utilized to calculate the parameters of litter breakdown, namely the stabilization factor ( $S$ ) and the decomposition rate constant ( $k$ ) (Keuskamp et al., 2013). These two parameters are generally coupled, and we usually find an increase in the stabilization factors ( $S$ ) together with a decrease in the decomposition rate constant ( $k$ ) (Keuskamp et al., 2013). Therefore, these two parameters can be used to assess effects of global change on the initial transformation of plant litter to soil organic matter in salt marshes (Mueller et al., 2018).

In chapter 2, grazing reduced the decomposition rate constant ( $k$ ), which could be ascribed to higher soil bulk density. Yet, the stabilization factor ( $S$ ) was also decreased under grazing. This finding highlights the importance to distinguish between the litter decomposition rate and the litter stabilization in the plant litter decomposition process (Althuizen et al., 2018). In chapter

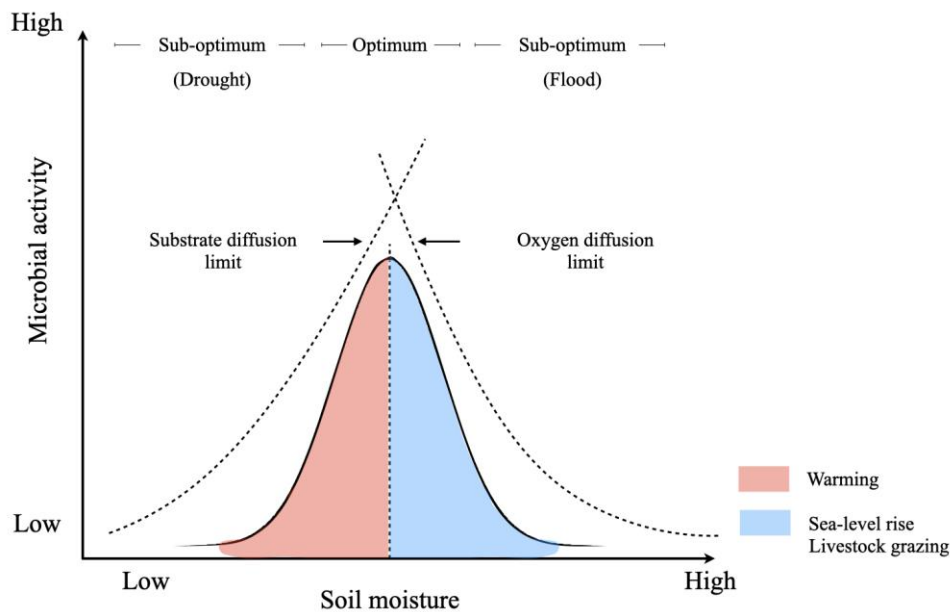
3, only flooding frequency affected the decomposition rate constant ( $k$ ), whereas the stabilization factor ( $S$ ) was strongly affected by the interactive effects of flooding frequency and plant-genotype. That is, the effects of sea-level rise on litter decomposition are not restricted to shifts in plant growth, but are also affected by the plant intraspecific genetic variation, because plant genetic identity can affect soil microbial activity by enhancing plant productivity and litter decomposition. Moreover, high temperatures increased the decomposition rate constant ( $k$ ) (Figure 5.4), while it just slightly decreased the stabilization factor ( $S$ ) (Chapter 4, Figure 4.6). The results of these three studies indicate an uncoupled response of litter decomposition rate ( $k$ ) and stabilization factor ( $S$ ) to global change.



**Figure 5.4** Tea bags were deployed at different soil depths for three months. Twelve polypropylene tea bags (55 mm x 50 mm) were put into two solid posts with six holes at a distance of 10 cm. Posts were inserted into the soil reaching a total soil depth of 60 cm. One post per plot contained six green tea bags and another contained six rooibos tea bags.

## 5.2 Indirect effects of global change via soil moisture on soil microbial activity in salt marshes

Soil moisture is one of the main abiotic factors determining the microbial decomposition in terrestrial soils (Brockett et al., 2012; de Nijs et al., 2019; Skopp et al., 1990). It controls the availability of oxygen for microorganisms, regulates the plant litter breakdown, and ultimately influences the organic matter decomposition (Henry, 2012; Prescott, 2010; Sierra et al., 2017). Previous studies suggest the relationship between soil moisture and microbial activity follows an optimum curve (Skopp et al., 1990). Low moisture levels can inhibit microbial activity by lowering intracellular water potential and restricting substrate supply, thus reducing microbial activity. In contrast, high moisture level leads to anaerobic conditions and thus decrease soil microbial activity (Figure 5.5).



**Figure 5.5** A conceptual model of soil microbial activity influenced by soil moisture (modified from Skopp et al., 1990). In the ambient condition, soil microbial activity follows an optimum curve depending on soil substrates and oxygen availability. In the left panel (red), warming decreases soil moisture and more water

input is needed to reach the optimal level of soil microbial activity. In the right panel (blue), livestock grazing and sea-level rise increase soil moisture, and slow down the soil microbial activity.

Global change factors, such as livestock grazing, sea-level rise, and warming, have been recognized for affecting soil moisture and thus indirectly affecting the microbial decomposition process (Brockett et al., 2012; Gavazov et al., 2014; Green et al., 2019; Steinweg et al., 2012). For instance, warming may lead to low soil moisture or drought conditions, which further affects soil microbial activity through changes in soil organic substrates and oxygen availability (Dijkstra et al., 2010; Henry, 2012; Kirschbaum, 1995). Likewise, grazing-induced soil compaction and sea-level rise can decrease soil oxygen availability due to the high soil moisture, and subsequently slow down soil microbial activity (Figure 5.5) (Brockett et al., 2012; Gavazov et al., 2014; Green et al., 2019; Steinweg et al., 2012). Thus, the sensitivity of soil moisture to global change is a topic of prominent relevance for soil microbial activity and their related microbial decomposition in salt marshes.

In my research, I also found that effects of global change on soil microbial activity could be potentially related to changes in soil moisture. For instance, I was able to demonstrate that livestock grazing could lead to a decrease in plant belowground biomass and high soil bulk density, which had previously been hypothesized as main pathways to reduce microbial activity in wetland soils (Elschot et al., 2015; Mueller et al., 2017). Furthermore, changes in soil bulk density are also positively related to soil moisture conditions in salt marsh soils (Risch et al., 2007; Wang et al., 2014). I thereby suggest that livestock grazing could increase soil moisture due to the high soil bulk density and indirectly decrease soil microbial activity.

Sea-level rise may also increase soil moisture and thus lead to decreases in soil exo-enzyme activity (Chapter 3). Addressing the warming effects, rising temperatures have been recognized to limiting soil moisture, and thus indirectly control the soil microbial activity (Dijkstra et al., 2010; Henry, 2012; Kirschbaum, 1995). However, my results suggest that soil microbial

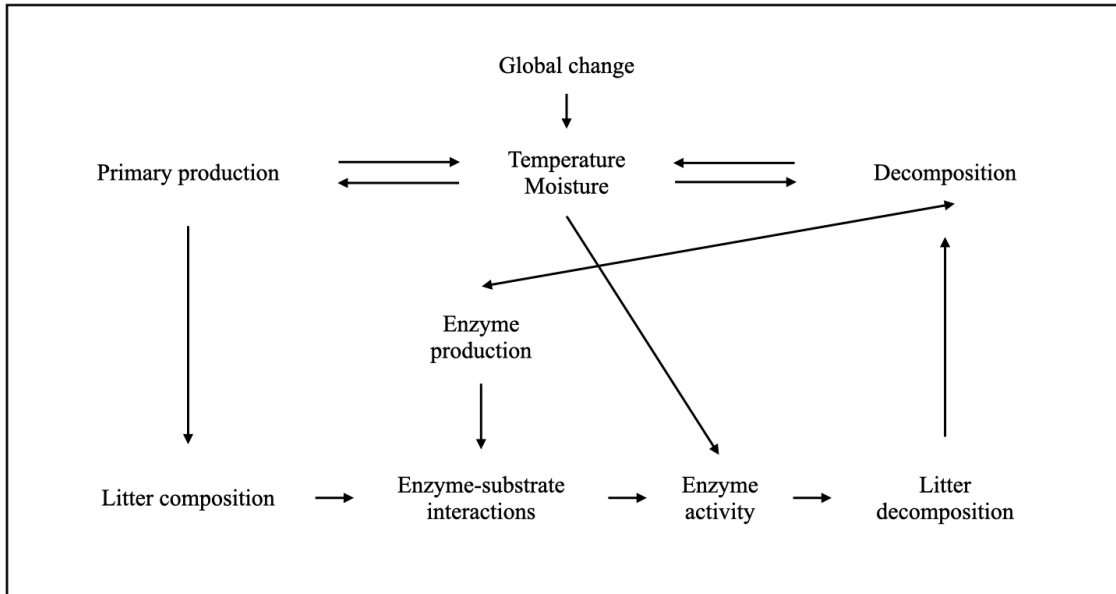
activity is strongly increased by rising temperatures (Chapter 4). This effect could be attributed to the regular flooding in salt marshes, which leads to preferable moisture conditions and may stimulate soil microbial activity.

Global change-induced indirect effects of soil moisture can also be mediated by plant species and soil depth. My results in chapter 3 show that the response of soil exo-enzyme activity to higher flooding frequency is distinctly mediated by plant-genotypes. I found a reduction of soil exo-enzyme activities under higher flooding frequency only in the unadapted genotype (Chapter 3, Figure 3.2D). In addition, factors controlling the soil microbial activity in the whole-soil profile were also different. In the topsoil, soil moisture and the supply of fresh organic compounds are the main factors to regulate soil microbial activity (Fierer et al., 2003; Salomé et al., 2010), whereas temperature and organic substrates are considered as the main regulatory mechanisms of microbial activity in the subsoil (Wild et al., 2019). Yet, my study demonstrates that soil microbial activity in both topsoil and subsoil are strongly affected by warming (Chapter 4). Overall, these findings suggest that indirect effects of global change via soil moisture changes can have both a positive or a negative influence on microbial activity.

### **5.3 Linking soil exo-enzyme activity and standardized litter breakdown parameters**

Litter decomposition is affected by plant litter quality, microbial community composition, microbial activity, and soil properties (Figure 5.6). Soil exo-enzymes have been widely recognized for their importance in litter decomposition (Moorhead et al., 2012; Sinsabaugh et al., 2002; Zhou et al., 2018). Furthermore, differences in litter composition lead to differences in enzyme activity through enzyme-substrate interactions (Burns et al., 2013). In turn, differential enzyme activity leads to temporal differences in decomposition among litter types

(Sinsabaugh et al., 1991). However, no study has yet examined the relationship between soil exo-enzyme activity and standardized-litter breakdown under several global change factors.



**Figure 5.6** A conceptual model of plant litter decomposition from an enzymic perspective. Global change leads to changes in temperature and soil moisture, which affect plant primary production and organic matter decomposition. Differences in litter composition (e.g. lignin and cellulose) lead to differences in enzyme activity through enzyme-substrate interactions. In turn, varied enzyme activity induces temporal differences in plant litter decomposition. Thus, differences in rates of litter decomposition are caused by variations in enzyme production and by direct effects of global change on soil enzyme activity (modified from Sinsabaugh et al., 1991).

To gain insight into the response of litter breakdown to global change, I combined my results from all three studies and re-analyzed the relationship between exo-enzyme activity and the litter breakdown parameters ( $k$  and  $S$ ). In the case of livestock grazing, the stabilization factor ( $S$ ) is positively related to  $\beta$ -glucosidase activity and is negatively related to the leucine-aminopeptidase activity (Table 5.1). Likewise, the decomposition rate constant ( $k$ ) is positively associated with chitinase activity and is negatively related to the leucine-aminopeptidase activity. In chapter 2, I discuss the activity of chitinase is negligibly low and consider it unimportant for understanding microbial N demand (see Chapter 2, Figure 2.2 C, and D).

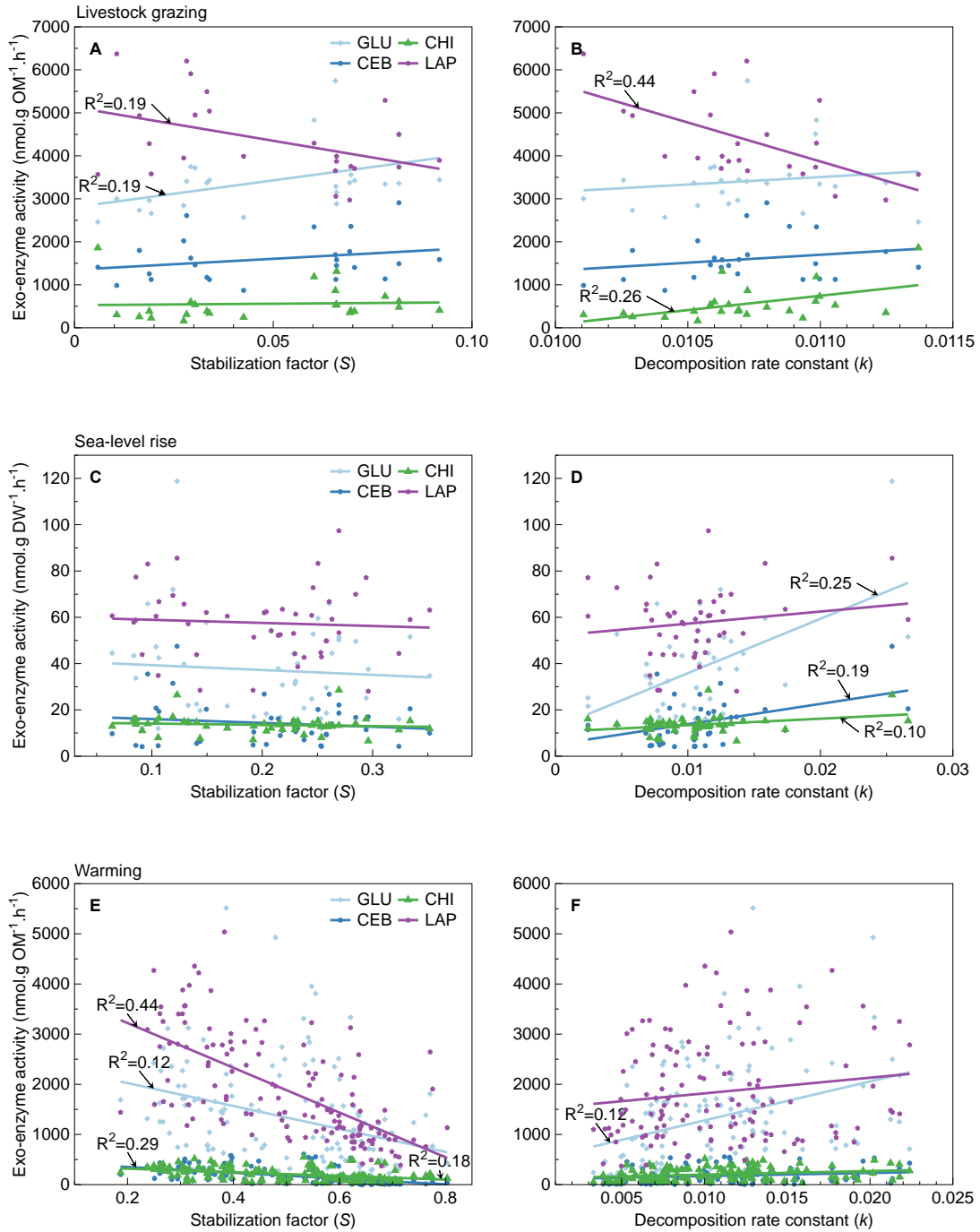
Microbial N acquisition based on leucine-aminopeptidase activity and microbial C vs. N demand based on the ratio of C- vs. N-acquiring enzymes points to a higher microbial N demand in grazed soils (see Chapter 2, Figure 2.2). Thus, this finding suggests grazing-induced negative effects of microbial C and N demand on litter decomposition and stabilization (Figure 5.7 A and B). With the interactive effects of plant genotype and sea-level rise (Chapter 3), there is no relationship between stabilization factors ( $S$ ) and soil exo-enzyme activities. This result could be ascribed to the response of plant genotypes to different flooding frequencies, because the stabilization factor ( $S$ ) was markedly lower in the adapted versus unadapted genotype, but the reversed pattern was found at the lowest flooding frequency (see Chapter 3, Figure 3.3B). In addition, the decomposition rate constant ( $k$ ) is positively related to the activity of  $\beta$ -glucosidase, cellobiosidase, and chitinase (Table 5.1, Figure 5.7 D). This finding suggests that sea-level rise decreases litter decomposition with the reduction of soil exo-enzyme activities. Addressing the warming effects, the stabilization factor ( $S$ ) is negatively related to all four exo-enzyme activities (Table 5.1, Figure 5.7 E). This finding suggests that higher temperatures could increase the C and N acquisition enzyme activity and ultimately decrease the stabilization of organic matter. Similarly, the decomposition rate constant ( $k$ ) is positively related to exo-enzyme activities, except for leucine-aminopeptidase activity (Table 5.1, Figure 5.7 F). This indicates that higher temperature stimulates the soil microbial activity and subsequently increases the rate of litter decomposition.

**Table 5.1** Combine results from all three studies and used the linear regression to re-analyze the relationship between exo-enzyme activities and parameters of standardized litter breakdown ( $S$  and  $k$ ) under global change. R values are in bold type at  $p \leq 0.05$ . (GLU =  $\beta$ -glucosidase, CEB = cellobiosidase, CHI = chitinase, LAP = leucine-aminopeptidase,  $S$  = stabilization factor, and  $k$  = decomposition rate constant).

	Livestock grazing				Sea-level rise				Warming			
	$S$		$k$		$S$		$k$		$S$		$k$	
	R-value	p-value	R-value	p-value	R-value	p-value	R-value	p-value	R-value	p-value	R-value	p-value
GLU	<b>0.44</b>	0.03	0.14	0.51	-0.08	0.61	<b>0.50</b>	0.00	<b>-0.34</b>	0.00	<b>0.35</b>	0.00
CEB	0.25	0.23	0.21	0.32	-0.14	0.34	<b>0.44</b>	0.00	<b>-0.54</b>	0.00	<b>0.19</b>	0.04
CHI	0.04	0.84	<b>0.51</b>	0.01	-0.11	0.47	<b>0.32</b>	0.02	<b>-0.43</b>	0.00	<b>0.26</b>	0.00
LAP	<b>-0.43</b>	0.04	<b>-0.58</b>	0.00	-0.07	0.65	0.15	0.30	<b>-0.67</b>	0.00	0.14	0.12

Furthermore, the relationship between parameters of litter breakdown and soil exo-enzyme activity is different under each global change factor. For example, while the decomposition rate constant ( $k$ ) is negatively related to the activity of leucine-aminopeptidase under livestock grazing, it is positively related to four exo-enzyme activities with increasing flooding frequency and warming. These findings highlight that soil exo-enzyme activities determine the rate of litter decomposition under global change. However, the effects of the studied global change factors on the link between soil exo-enzyme activity and stabilization factor ( $S$ ) are quite different. On the one hand, the activity of leucine-aminopeptidase is negatively related to the stabilization factor ( $S$ ) under livestock grazing and warming. On the other hand,  $\beta$ -glucosidase activity is positively related to the stabilization factor ( $S$ ) under livestock grazing, but it is negatively related to stabilization factor ( $S$ ) under warming. That is, these results agree with my finding in chapter 2, which suggests that the factors controlling litter decomposition rate and stabilization, as well as the ecological implications of the two parameters, can be quite different, and it is still hard to interpret which exo-enzymes regulate the stabilization factor in marsh soils.





**Figure 5.7** Relationships between parameters of standardized litter breakdown and exo-enzyme activities under livestock grazing (A and B), sea-level rise (C and D), and warming (E and F). (GLU =  $\beta$ -glucosidase, CEB = cellobiosidase, CHI = chitinase, LAP = leucine-aminopeptidase,  $S$  = stabilization factor, and  $k$  = decomposition rate constant).

### **5.3 Implications and research perspectives**

My research is one of the first to provide insight into the effects of global change on soil exo-enzyme activities and parameters of standardized litter breakdown in salt marshes. However, soil microbial activity and litter breakdown show different responses to each single global change factor. For instance, effects of livestock grazing on exo-enzyme activity and litter decomposition can just partly be explained by grazing-induced soil compaction (Chapter 2), and the lower stabilization factor could be ascribed to higher microbial N demand (Craine et al., 2007; Knorr et al., 2005). Concerning the effect of sea-level rise in chapter 3, the adaptive genetic variation in plants can suppress or facilitate the effects of sea-level rise on soil microbial functioning and litter breakdown. Similarly, warming also stimulates soil microbial activity and litter decomposition rate in the whole-soil profile (Chapter 4). Although I discuss how the response of the microbial decomposition process to global change could be influenced by and related to soil moisture (see part 5.2), there is no consensus that soil moisture is a key factor to determine the response of decomposition processes to global change in salt marsh soils (see Chapter 2, but compare Chapter 4). In fact, my research focuses on the response of soil enzyme activity and litter breakdown to several global change factors, but the mechanisms by which these and other global change factors affect soil microbial activity and litter decomposition still need further research. Future studies should consider the effects of plant characteristics (i.e. plant growth and rhizosphere processes), soil moisture, and their interactions under global change.

Furthermore, it should be noted that my study utilizes commercially available tea materials as standardized plant litter to assess the parameters of litter breakdown. Although this method has been widely applied to characterize and compare organic matter decomposition within and across ecosystems, it mainly describes the fraction of labile and rapidly decomposable organic matter that becomes stabilized during deployment. However, litter decomposition includes

these easily decomposed components as well as material resistant to decomposition (i.e. recalcitrant compounds). In the early-stage decomposition processes, there are plenty of labile compounds, which are fast and easily degraded. Yet, decomposition of recalcitrant compounds is an important process in the C and N cycling during the later-stage decomposition processes, and also controls the decomposition rate in salt marsh ecosystems (Mueller et al., 2017; Wang et al., 2019). For instance, most of the mineralized soil organic matter is derived from the recalcitrant compounds, which are difficult and take a long time to break down (i.e. lignin) (Benner et al., 1984; Wang et al., 2012). However, the “Tea Bag Index” calculates the stabilization and decomposition of organic matter by using materials with a high hydrolyzable fraction. It thus mainly describes the decomposition of labile compounds over a relatively short time. Thus, additional studies should address the decomposition of recalcitrant compounds and extend the incubation time.

In conclusion, the majority of studies on C sequestration in salt marshes are focused on the process of organic matter input (i.e. plant primary production), which considers the feedback between plants and soil C turnover. Yet, little is known about the organic matter decomposition. My research suggests differential effects of global change on microbial decomposition processes in salt marshes. This knowledge improves our understanding of the response of organic matter decomposition to global change in blue carbon ecosystems.



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## Summary

Salt marshes are known to sequester “blue carbon” at high rates in soils. Global change yields the potential to change the carbon sequestration potential of salt marshes by affecting the balance between organic matter input, through plant primary production, and output, through decomposition processes. Yet, most of the current debate regarding the global change-induced change in carbon sequestration has been dealing with plant production, while the dynamics of organic matter decomposition are still largely unknown. Thus, this thesis focuses on the effects of several global change factors (livestock grazing, sea-level rise, and warming) on soil microbial activity and litter breakdown in salt marshes, aiming to understand their role in the decomposition process, with critical implications for carbon sequestration. Five chapters are included in this thesis: **Chapter 1** presents a general introduction to the topic, **Chapter 2** to **Chapter 4** are three manuscripts as the main part, **Chapter 5** is a general discussion, which explores how the findings of the main chapters (2 to 4) relate to each other on a spatial broader scale and give directions for future research.

**Chapter 2** presents a grazing-exclusion experiment in a Chinese salt marsh ecosystem of the Yangtze estuary. Here, I assess soil exo-enzyme activity to gain insight into the microbial carbon and nitrogen demand, and use standardized litter to evaluate soil decomposition dynamics. The findings suggest that the effects of livestock grazing on soil exo-enzyme activity and litter decomposition can just partly be explained by grazing-driven soil compaction resulting in lower oxygen availability. Instead, grazing effects on microbial nutrient demand seems to be an at least equally important control on decomposition processes in salt marshes.

**Chapter 3** assesses the interactive effects of plant genotype and flooding frequency on soil microbial community structure, soil exo-enzyme activity, and litter breakdown in a controlled mesocosm experiment. This study concludes that adaptive genetic variation in plants can

suppress or facilitate the effects of sea-level rise on soil microbial communities and microbial activity.

**Chapter 4** investigates soil exo-enzyme activity, microbial biomass, and litter decomposition at both topsoil and subsoil under field warming in a Northwest European salt marsh. Results show that the stimulating effect of temperature on soil microbial functioning is greater in the dry high marsh than in the frequently inundated pioneer zone. Besides, both topsoil and subsoil of soil exo-enzyme activity and microbial biomass are also strongly affected by warming. This finding suggests warming effects are not just restricted to the topsoil. Higher soil microbial functioning in deeper soils and the indirect effects of warming via soil moisture could increase microbial decomposition in salt marsh soils and thus decrease their C-sink capacity with global warming.

My works show that global change strongly affects soil microbial functioning (i.e. soil microbial community composition, soil microbial biomass, and exo-enzyme activity), with important implications for carbon and nitrogen microbial demand in salt marsh soils. Besides, even though the litter breakdown parameters (decomposition rate constant and stabilization factor) respond differently to each global change factor, they also reveal new insights into decomposition dynamics. In conclusion, this thesis improves the understanding of global change effects on decomposition processes via soil microbial activity and litter breakdown in salt marshes.

# Zusammenfassung

Salzwiesen sind dafür bekannt, Kohlenstoff in hoher Rate im Boden festzulegen. Verschiedene Faktoren des Globalen Wandels haben das Potenzial, diese wichtige Ökosystemleistung von Salzwiesen zu verändern, indem sie das Gleichgewicht zwischen dem Eintrag an organischer Substanz durch die Primärproduktion der Pflanzen und dem Austrag durch Zersetzungsprozesse beeinflussen. Der größte Teil der aktuellen Debatte, über die durch den Globalen Wandel verursachte Veränderung der Kohlenstoff-Festlegung hat sich jedoch mit der Pflanzenproduktion befasst, während die Dynamik des Abbaus organischer Substanz noch weitgehend unbekannt ist. Daher konzentriert sich diese Arbeit auf die Auswirkungen mehrerer Faktoren des Globalen Wandels (Nutztierhaltung, Meeresspiegelanstieg und Erwärmung) auf die mikrobielle Aktivität des Bodens und den Streuabbau in Salzwiesen, mit dem Ziel, ihren Einfluss auf den Zersetzungsprozess zu verstehen, was entscheidende Auswirkungen auf die Kohlenstoff-Festlegung hat. Fünf Kapitel sind in dieser Arbeit enthalten: **Kapitel 1** enthält eine allgemeine Einführung in das Thema. **Kapitel 2** bis **Kapitel 4** sind drei Manuskripte als Hauptteil. **Kapitel 5** ist eine allgemeine Diskussion, in der untersucht wird, wie die Ergebnisse der Hauptkapitel (2-4) auf einer räumlich breiteren Skala miteinander in Beziehung stehen und in den Richtungen für die zukünftige Forschung angegeben werden.

**Kapitel 2** stellt die Ergebnisse eines Beweidungsexperiments in einer Salzwiese des chinesischen Jangtse-Ästuars dar. Hier betrachte ich die Exo-Enzymaktivität des Bodens, um einen Einblick in den mikrobiellen Kohlenstoff- und Stickstoff- Bedarf zu gewinnen und um die Zersetzungsdynamik standardisierter im Boden zu verstehen. Die Ergebnisse zeigen, dass die Auswirkungen der Nutztieraktivität auf die Exo-Enzymaktivität des Bodens und den Abbau von Streu nur teilweise durch die trittbedingte Bodenverdichtung und dem resultierenden Sauerstoffmangel erklärt werden kann. Die Auswirkungen der Beweidung auf die mikrobielle

Nährstoffbedarf scheinen eine mindestens ebenso wichtige in Rolle im Zersetzungsprozess zu spielen.

**Kapitel 3** untersucht wie die Interaktion von Pflanzengenotyp und Meeresspiegelanstieg sich auf die Struktur der mikrobiellen Bodengemeinschaft, die Exo-Enzymaktivität und den Streuabbau in einem kontrollierten Mesokosmos-Experiment auswirkt. Diese Studie kommt zu dem Schluss, dass die adaptive genetische Variation in Pflanzen die Auswirkungen des Meeresspiegelanstiegs auf die mikrobiellen Bodengemeinschaften und die mikrobielle Aktivität sowohl unterdrücken als auch verstärken kann.

**Kapitel 4** untersucht den Einfluss von Erwärmung auf die Exo-Enzymaktivität die mikrobielle Biomasse und die Zersetzung von Streu sowohl im Ober- als auch im Unterboden in einer europäischen Salzwiese. Die Ergebnisse zeigen, dass die stimulierende Wirkung der Erwärmung auf die mikrobielle Bodenfunktion in der trockeneren oberen Salzwiese größer ist als in der häufig überfluteten Pionierzone. Außerdem sind Erwärmungseffekte auf die Exo-Enzymaktivität und mikrobielle Biomasse sowohl im Ober- als auch im Unterbodendeutlich. Eine höhere mikrobielle Aktivität in tieferen Bodenschichten und die indirekten Auswirkungen der Erwärmung über die Bodenfeuchte könnten die mikrobielle Zersetzung in Salzwiesenböden erhöhen und damit deren C-Senken-Kapazität mit im Zuge der Globalen Erwärmung verringern.

Meine Arbeiten zeigen, dass der Globale Wandel die mikrobielle Funktionsfähigkeit des Bodens (d.h. die Zusammensetzung der mikrobiellen Bodengemeinschaft, die mikrobielle Bodenbiomasse und die Exo-Enzymaktivität) stark beeinflusst. Auch wenn die Streu-Abbauparameter (Zersetzungsratenkonstante und Stabilisierungsfaktor) auf jeden Faktor des globalen Wandels unterschiedlich reagieren, zeigen sie doch neue Erkenntnisse über die Zersetzungsdynamik. Zusammenfassend verbessert diese Arbeit das Verständnis der

Auswirkungen des Globalen Wandels auf Zersetzungsprozesse durch Betrachtungen der bodenmikrobiellen Aktivität und des Streuabbaus in Salzwiesen.



## **Author contributions**

**Chapter 1** H. Tang wrote this chapter.

**Chapter 2** H. Tang conducted the exo-enzyme activity measurements, analyzed all data (including statistics), and wrote the initial manuscript. J.H. Wu and Z.C Yang designed the experiment. S. Nolte collected all samples, conducted soil properties measurements. P. Mueller reviewed and edited the manuscript with H. Tang.

**Chapter 3** H. Tang conceived and designed the experiment, set up the controlled mesocosms experiment, collected all samples, conducted enzyme, and decomposition assays. Analyzed enzyme and decomposition assays data (including statistics), and wrote the initial manuscript. P. Mueller conducted the molecular microbial lab work and wrote the initial manuscript with H. Tang. F. Horn carried out the bioinformatics and analyzed the molecular data.

**Chapter 4** H. Tang conceived and designed the experiment, collected all samples, conducted all measurements, analyzed all data (including statistics), and wrote the initial manuscript.

**Chapter 5** H. Tang wrote this chapter. All findings presented in section 5.3 are based on research and data analysis by H. Tang.





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To whom it may concern,

As a native English speaker, I do hereby declare that the Ph.D. thesis: “Global Change Effects on Decomposition Processes in Salt Marshes” has been written in concise and correct English (US).

Sincerely,

Heather Shupe

# Eidesstattliche Versicherung

## Declaration on oath

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

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

**Hamburg, den 28th October, 2020**

**Unterschrift (Hao Tang) \_\_\_\_\_**