The structure of coastal invertebrate communities and their temporal and spatial dynamics

Dissertation

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Den Tod als Gewissheit.

Geringe Aussicht auf Erfolg.

Worauf warten wir noch?"

Summary

The Wadden Sea is one of the largest tidal flats in the world, stretching across the coasts of the Netherlands, Germany and western Denmark. Along sheltered coastlines lie salt marshes which are essential habitats for a variety of organisms. Salt marshes are considered Blue Carbon ecosystems due to their high carbon storage capacity. This is mainly due to the influx of carbon from the ocean and burial of local plant materials in anoxic sediments. Salt marshes show a gradient in shore height, producing distinct zones, each with separate abiotic conditions. In the lowest reaches of the marsh, called the pioneer zone (PZ), inundation occurs daily for several hours. Above the PZ lies the lower salt marsh (LSM), which is inundated during spring tides. Above lies the upper salt marsh (USM) which is only inundated during storm surges. Shore height not only alters inundation frequencies, but it also creates a gradient of allochthonous marine input from the Wadden Sea. Furthermore, due to seasonal changes in tidal range, inundation frequencies across these zones may change.

Despite these spatial and temporal changes in abiotic conditions and allochthonous influx, little is known about how this influences soil microorganisms and soil meso- and macrofauna. Soil microorganisms as well as soil fauna are essential in the cycling of nutrients and thus impact soil health and productivity. Soil microbial communities decompose litter material, making nutrients available for plants or other microbes. In contrast, meso- and macrofauna break down litter, making it accessible to microbes or consume microbes as secondary decomposers. Within salt marshes research of microbial soil communities has largely focused on their composition in relation to abiotic conditions, litter decomposition rates and bacterial or fungal contributions to vascular plant decomposition. However, these studies have predominantly focused on microbial communities associated with the PZ cordgrass (*Spartina* sp.). Only recently the focus has shifted towards microbial decomposer communities across all salt marsh zones within the Wadden Sea. With indications of spatial changes in abiotic conditions influencing the presence of fungi as well as the exoenzyme activity of bacteria.

Within the soil decomposer system two distinct energy channels exist, the bacterial and the fungal channel. Generally, the bacterial channel is considered to have fast nutrient turnover rates and to depend on easily available resources, whereas the fungal channel has slow turnover rates, processes more recalcitrant resources and may be characterized by top-down control. However, the relative importance of these channels and how the spatiotemporal dynamics of the salt marsh impact the channelling of resources has not been investigated in depth.

The aims of this thesis were to investigate the spatiotemporal dynamics of microbial basal resources, their channelling through the food web as well as changes in trophic position of consumers. In addition, I investigated the use of allochthonous marine resources and their channelling through the soil decomposer food web of the Wadden Sea salt marsh. For this

purpose, I sampled soil microorganisms and soil meso- and macrofauna of a Spiekeroog salt marsh in April (spring), July (summer) and October (autumn) 2019. Basal resources and microbial communities were investigated using phospholipid fatty acid patterns in Chapter 2. Channelling of these basal resources to higher consumers was investigated using neutral lipid fatty acids in Chapter 3. Lastly, Chapter 4 investigated spatiotemporal changes in trophic position and allochthonous resource consumption using the stable isotopes ^{15}N and ^{13}C of the soil macrofauna.

The study presented in Chapter 2 showed that the dominant microbial group within the salt marsh are Gram-positive bacteria, with peak fungi concentrations in the LSM and peak algal concentrations in PZ. The microbial communities showed remarkable temporal stability, despite marked seasonal changes in abiotic conditions. Spatial dynamics, i.e. shore height and sediment depth, were the dominant factors shaping the soil microbial community, with bacteria increasing with sediment depth.

The results shown in Chapter 3 demonstrated that the bacterial channel and associated plant resources form the dominant basal resources of the soil fauna, regardless of shore height and season. The contribution of allochthonous marine resources to consumer nutrition was generally low, but depended on season, presumably linked to inundation frequencies and algal productivity.

Lastly, Chapter 4 showed that the salt marsh soil macrofauna food web is simpler than expected, with three trophic levels, regardless of shore height. However, trophic positions of taxa/species varied across shore height and season. Furthermore, Chapter 4 indicated a wider range of resources in the LSM, presumably due to marine influx. However, Bayesian mixing models indicate that autochthonous vascular plant resources are the dominant basal resource for macrofauna consumers of the salt marsh.

Overall, this thesis is the first to investigate spatiotemporal dynamics of basal resources and their channelling through the food web of the Wadden Sea salt marsh. Chapter 2 demonstrated that the dominant microbial decomposer are bacteria. Chapter 3 showed that the dominant basal channel is autochthonous plants and associated bacteria. Lastly, Chapter 4 showed that while spatial dynamics did not impact the number of trophic levels, taxa/species changed their trophic levels across temporal scales. In addition, resource use became more variable with reduced shore height, but overall allochthonous marine resources were a minor addition to the diet of macrofauna consumers.

Chapter 1 – General Introduction

Wadden Sea

The Wadden Sea is the largest coherent tidal flat in the world, stretching across the coastlines of the Netherlands, Germany and western Denmark (Reise et al. 2010, Kabat et al. 2012). The flats formed when melting of the Fennoscandian and Canadian ice sheet slowed, allowing soil accretion and creating a belt of barrier islands sheltering the tidal flats from the open North Sea (Flemming and Jr Davis 1994, Reise et al. 2010, Kabat et al. 2012). Thereby, a tidal flat covering 4700 km² was formed which emerges twice a day during low tide (Reise et al. 2010, Kabat et al. 2012). Changes in sediment accretion across locality and sea level have since shaped the coastline of the Wadden Sea (Reise et al. 2010, Kabat et al. 2012). At the same time constant exchange of water and sediment between the North Sea and the Wadden Sea occurs, driven by tidal rhythm and wind direction (Postma 1981, Bartholomä et al. 2009), resulting in constantly changing structure and position of the islands over time. This dependence on sediment input separates European marshes from most North American marshes, which acquire their shore height predominantly through organic matter input (organogenic), rather than sediment (minerogenic) input (Allen 2000, Kirwan and Megonigal 2013, Kearney and Turner 2016). Because these islands are accretions of sediment, they are continually eroded by ocean movements in the west with sediment build up in the east (Flemming and Jr Davis 1994). Barrier islands are subject to multiple constraints, including their shore gradient, sediment budget, wind and wave climate, tidal prism and sea level (Flemming 2002, Bartholomä et al. 2009). Because of this, concerns over sea-level rise migrating the islands toward the mainland shoreline are high (Flemming 2002, Kirwan and Megonigal 2013). Vegetation has been shown to allow soil accretion by reducing flow speed and turbulence, causing sediment to settle (Dijkema et al. 1990, Mudd et al. 2010). However, models predict that this is only applicable if the vegetation cover is closed and the sea level remains below the vegetation height (Dijkema et al. 1990, Temmerman et al. 2005). Vegetation cover - especially at the transition to tidal flats - is disjointed and thus susceptible to erosion (Dijkema et al. 1990, Rodriguez and McKee 2021). Despite the uncertainty, habitats at the interface of the tidal flats and the terrestrial islands are acknowledged as essential for the persistence of the Wadden Sea. One of the key habitats on barrier islands is the salt marsh.

Salt marshes

Salt marshes exist between the marine mudflats and the terrestrial system, along a gradient of shore height ranging from below mean high-water level (MHWL) to the upper storm tide limit (Dinter 2018). Salt marshes belong to the so called "blue carbon ecosystems" alongside other wetlands such as mangroves because, despite their small global area, their contribution to global carbon sequestration is high (McLeod et al. 2011, Mueller et al. 2019a, b). However, most carbon stored in salt marshes originates from allochthonous marine material buried in the soil, not by atmospheric carbon taken up by primary producers (McLeod et al. 2011, Schrama et al. 2013, Mueller et al. 2019b). This can be

attributed to three factors: high rates of organic input, reduced rates of decomposition and constant burial of organic carbon by the tide (McLeod et al. 2011). Furthermore, the continual accretion of soil in marshes prevents carbon saturation of the soil, unlike terrestrial systems (Mueller et al. 2019a). However, recent research suggests sequestered organic matter in deeper soils to decline, possibly related to changes in the soil during succession - such as higher aeration of the soil and reduced soil accretion with elevation - but remain far above those of tidal mudflats (Mueller et al. 2019b).

The Wadden Sea is home to the back-barrier island salt marshes (Adam 2002, Bakker et al. 2015, Winter et al. 2018). Back-barrier marshes exist on the landward facing side of Wadden Sea islands and are essential for shoreline and tidal mudflat protection (Barbier et al. 2011, Haynert et al. 2017, Winter et al. 2018). Salt marshes exist along a gradient of shore heights and resultant physical conditions (salinity redox potential, oxygen availability), leading to the zonation of salt marsh plants specific to these zones (Adam 2002, Bakker et al. 2015, Collin et al. 2010, Davy 2002, Winter et al. 2018). In the lower parts of the marsh, plants experience prolonged and regular inundation with seawater, whereas at higher elevations they may experience either hypo- or hypersaline conditions (Jefferies and Davy 1979). These gradients in conditions give rise to three vegetation zones in the Wadden Sea. The highest zone lies >35 cm above the mean high-water level (MHWL) and is called the upper salt marsh (USM). It is dominated by *Elymus athericus* (*Elytrigia atherica*) (salinity 5 to 20 ‰) and is only flooded during storm surges (Niedringhaus 2009, Dinter 2018, Winter et al. 2018). Below the USM, at a shore height of 0-35 cm above MHWL recognisable by *Atriplex portulacoides* and *Puccinellia maritima*, and with a salinity between 20 and 26 ‰, lies the lower salt marsh (LSM) (Niedringhaus 2009, Dinter 2018, Winter et al. 2018). Below the MHWL is the pioneer zone (PZ), it is inundated twice a day, dominated by *Salicornia stricta* and the only C4 plant *Spartina anglica* with salinity between 26 to 32 ‰ (Haynert et al. 2017, Dinter 2018, Winter et al. 2018). Furthermore, allochthonous marine resources, such as micro- and macroalgae, may be flushed into the lowest reaches of the marsh (Riera et al. 1999, Schrama et al. 2013, Winter et al. 2018). In coastal systems marine detrital material enhances nutrients and energy of adjacent coastal habitats (Polis and Hurd 1996, Schrama et al. 2013), with previous studies pointing to strong dependence on aquatic resources by coastal soil communities (Korobushkin et al. 2016, Mueller et al. 2019a, 2020). Specifically in salt marshes the close proximity of diverging ecosystems creates exchange between two nutritionally different sources. On the terrestrial side of the salt marsh carbon-rich nutrient-poor vascular plant resources are deposited, whereas allochthonous marine input is nutrient-rich (nitrogen and phosphorus) and low in carbon (Tobias and Neubauer 2009, Mueller et al. 2020). Despite the importance of the marshes for shoreline protection as well as long term carbon storage, little is known about them, in particular the ecology of soil decomposer communities and the influence of marine allochthonous resources.

Soil communities

Soil communities are essential in nutrient cycling and the breakdown of organic material which forms the basal resource channelled to higher trophic levels (Terborgh and Estes 2010, Sofo 2020). Soil communities range from microbes to mesofauna, such as oribatid mites and collembolans, to macrofauna, such as earthworms, diplopods and beetles (Scheu et al. 2005, Sofo 2020). The interplay of these communities can shape the productivity of soil and plant health.

Bacteria and Fungi

In the soil basal resources are comprised of dead organic matter (typically vascular plants), which is densely colonized by microbes (Wardle and Yeates 1993, Scheu et al. 2005, Zieger et al. 2017). Understanding these basal resources and their channelling through the food web shows which resources the system relies on. Microbial assemblages comprise a variety of organisms, ranging from viruses to microalgae, fungi and bacteria (Sattley and Madigan 2015, Sofo 2020). Within the detrital food web two distinct basal channels exist, the bacterial and fungal channel (Wardle and Yeates 1993, Scheu et al. 2005, Zieger et al. 2017). Bacteria-dominated channels have fast nutrient turnover and are controlled by the availability of resources (bottom up), whereas fungi-based channels have slow nutrient turnover and are controlled by predators (top down) (Scheu et al. 2005). Therefore, knowledge of these basal channels gives insight into the nutrient cycling capacity of the soil.

Bacteria break down autotroph material, may be linked in symbiotic relationships with plants or animals as well as prey on other microbes (Moore and de Ruiter 2012, Steffan et al. 2015, Steffan and Dhrampal 2018). Despite their importance, and their potential to regulate higher trophic levels (Steffan and Dhrampal 2018), molecular methods still show a large quantity of unidentified bacterial groups with unknown importance to the soil food web (Mora et al. 2011, Louca et al. 2019). Furthermore, bacteria are a major sink of essential nutrients, such as nitrogen (N) and phosphorus (P) (Sattley and Madigan 2015), or are able to fix atmospheric nitrogen needed for plant establishment and growth (Orr et al. 2011, Mus et al. 2016). Due to their ability to break down organic and inorganic material and provide essential nutrients for plants, bacteria serve an important role in nutrient cycling and soil fertility (Graham et al. 2016, Sofo 2020). However, the function of salt marsh soil bacteria differs with shore height because of spatial differences in nutrient availability (Mueller et al. 2020). Assimilation of N into biomass (assimilatory nitrate reduction) increases with shore height, whereas denitrification increases with declining shore height (Tebbe et al. 2022). In contrast, exoenzymes related to carbon acquisition increase with decreasing shore height (Mueller et al. 2020). Indicating that bacterial communities change their function in relation to shore height, nutrient availability, inundation frequency and resultant soil anoxia. In addition to nutrient cycling, bacteria help aggregate the soil thus improving aeration, root penetration and water holding potential of the soil due to excretion of extracellular polymeric substances (Costa et al. 2018). Furthermore, once organic matter is incorporated into salt marsh soils bacteria become the

dominant decomposers (Benner et al. 1984, Cortes-Tolalpa et al. 2018, Calado et al. 2019, Leadbeater et al. 2021).

Fungi fulfil very similar roles, including nitrogen transformations, breakdown of organic material and nutrient cycling (Sofo 2020). However, unlike bacteria fungi are exclusively heterotrophs and therefore restricted to the use of carbon resources, heavily relying on plant residues (Sofo 2020). Compared to bacteria, fungi are important for the breakdown of complex carbon structures in litter, including lignocellulose (Benner et al. 1984, Newell et al. 1996, Calado and Barata 2012, Calado et al. 2019). Most organic carbon in salt marsh systems originates from local lignocellulose-rich grasses (Morriss and Jensen 1998, Bouchard and Lefeuvre 2000, Leadbeater et al. 2021), including *Spartina* sp. and *E. athericus* (Hemminga et al. 1988, Newell et al. 1989, Morriss and Jensen 1998, Calado et al. 2019, Carrasco-Barea et al. 2022). Filamentous fungi are well suited to decompose lignocellulose-rich detritus due to their morphology as well as production of lignocellulose-degrading enzymes (Newell et al. 1996, Calado and Barata 2012). Investigations of the decomposition processes of the salt marsh grass *Spartina alterniflora* identified two main stages. During senescence saprotrophic fungi break down the standing grass blade accessing its nutrients; once depleted, the remaining lignocellulose-rich grass blade is incorporated into the soil (Benner et al. 1984, Newell et al. 1989, 1996, Buchan et al. 2003, Calado and Barata 2012). Similar findings have been made of *Spartina* sp. in European salt marshes (Castro and Freitas 2000, Calado et al. 2019). However, recent evidence suggests that during decomposition of *E. athericus* and *A. portulacoides* bacteria dominate with fungi becoming active once lignin content is high (Carrasco-Barea et al. 2022). However, few European studies exist which investigated the dynamics of fungi with seasonal and spatial changes in abiotic conditions. Alzarhani et al. (2019) investigated spatial and temporal patterns and found that abiotic variables were the best predictor of fungal diversity and structure.

Overall, both fungi and bacteria are important for litter decomposition and subsequent nutrient cycling; in salt marshes their dynamics are dependent on the stage of decomposition as well as plant species. However, we are aware of only one study investigating fungal and bacterial contributions to salt marsh plant litter decomposition other than *Spartina* sp. of the pioneer zone (Carrasco-Barea et al. 2022). In addition, very few studies have investigated changes in microbial community composition with season across salt marsh zones.

Algae

In addition to bacteria, micro- and macroalgae inhabit, or are deposited in the salt marsh (Nienhuis· and Simons 1971). Microalgae may inhabit the sediment below and may form scattered mats above the MHWL (Carter 1933, Nienhuis· and Simons 1971, Kaas 1987). Macroalgae on the other hand predominantly grow below the MHWL, but are also displaced to higher zones with the tide (Kolbe et al. 1995, Boyer and Fong 2005). In addition, epibenthic diatoms persists across the Wadden Sea mudflats (Scholz and Liebezeit 2012, Taylor et al. 2013, Costa et al. 2018). On the mudflats microalgal assemblages form

microphytobenthic biofilms (MPB) on the surface of the sediment thereby stabilising it (Redzuan and Milow 2019, Redzuan and Underwood 2020). While MPB are common on unvegetated sediments, their tidal displacement to salt marshes provides allochthonous organic material (Redzuan and Underwood 2020). Displacement of algae or other marine deposits to higher salt marsh zones is mediated by the tidal range (Nienhuis 1978, 1987, Boyer and Fong 2005), with algal deposits displaced up to the USM during storm surges or spring tides (Nienhuis 1978, Redzuan and Underwood 2020).

Marine algae are rich in nutrients, making them a favourable resource for mudflat invertebrates (Sullivan and Moncreiff 1990, Davies and Falconer 2001). Previously, consumption of algae has been suggested for the terrestrial amphipod *Talitrus saltator,* the staphylinid beetle *Bledius spectabilis*, as well as soil mesofauna (Wyatt 1986, Adin and Riera 2003, Winter et al. 2018). However, these reports for certain species did not elucidate the importance of algae for the saltmarsh food web and potential variations across temporal and spatial scales.

Persistence of the algae in the marshes is subject to abiotic conditions, mainly nutrients, light and desiccation. Unlike salt marshes in North America, European marshes lie well above the MHWL at neap tides, restricting moisture conditions (Beeftink 1977, Bouchard and Lefeuvre 2000). However, seasonal increases in tidal range have been shown to enrich organic matter with microalgae above the MHWL (Bouchard et al. 1998). Kaas (1987) noted that blue-green algal mats resist desiccation and recover once inundation resumes. Furthermore, grazing or removal of local vascular plants was found to not only change the composition of soil microalgae, but also their density due to light availability (Sullivan and Daiber 1975, Zedler 1980, Kaas 1987). However, to the best of our knowledge algal presence in dense *E. athericus* stands of the USM has not been investigated. Despite the variable abiotic conditions, specific algae are well suited to survive in salt marshes.

Overall, algae are present throughout the salt marsh, thanks to temporal changes in abiotic conditions. However, the influence of temporal variations in algal productivity on soil invertebrate communities of salt marshes has not been investigated.

Soil meso- and macrofauna
The soil mesofauna are strongly linked to microbial communities, both by direct consumption altering its structure, mixing of the organic material to stimulate microbes and passively dispersing microbes (Scheu et al. 2005). In addition, they may exploit the detrital material produced by the microbial communities (Scheu et al. 2005). Mites span a wide range of trophic positions, from fungivores (e.g., Oribatida) to predators (Schneider et al. 2005, Sofo 2020). Their activity has been suggested to stimulate fungal growth, enhancing decomposition processes (Scheu et al. 2005, Sofo 2020). Predatory mites (Mesostigmata) consume both microfauna, such as nematodes, as well as mesofauna, such as Oribatida and Collembola (springtails) (Scheu et al. 2005, Heidemann et al. 2014, Sofo 2020). Collembola also feed on decaying matter, microalgae, fungal hyphae and may rarely be predatory (Chahartaghi et al. 2005, Sofo 2020). In addition, they improve soil structure,

break down litter as well as provide nutrition through their faecal pellets (Sofo 2020). Both Mesostigmata and Collembola are predated by macrofauna species, providing an important link between meso- and macrofauna communities (Brose and Scheu 2014, Sofo 2020). In salt marshes species diversity declines as one moves towards the mudflats, with an increased proportion of halophilous specialists living in the PZ (Luxton 1967, Haynert et al. 2017, Winter et al. 2018). In addition, Haynert et al. (2017) demonstrated that the soil mesofauna community comprises four trophic levels with most species acting as secondary decomposers, but some also as first- and second-order predators. In addition, for some of these species, marine algae have been shown to contribute to their diet (Winter et al. 2018). However, whether marine algae form a significant basal resource for these communities has not been investigated in detail.

Unlike the mesofauna, macrofauna are the predominant long-term habitat engineers in the soil (Scheu et al. 2005). Owing to burrowing and litter breakdown they influence soil structure, shape meso- and microfauna communities, and impact decomposition rates (Scheu et al. 2005, Sofo 2020). Most salt marsh studies have focused on the effects of megafauna grazing by e.g., sheep and cattle, on invertebrate abundance (Pétillon et al. 2007, Thakur et al. 2014). Past research suggested a decline in allochthonous resource use by the soil fauna with increasing shore height (Schrama et al. 2012, 2013). With amphipods burrowing and breaking down litter material making it available for being used by other soil fauna (Schrama et al. 2012, 2013, Garcia-Hernandez 2022). However, it has also been shown that the staphylinid beetle *Bledius spectabilis* and the amphipod *T. saltator* consume algae (Wyatt 1986, Wyatt and Foster 1989, Adin and Riera 2003). However, the exploitation of resources by the soil macrofauna, both autochthonous and allochthonous, and its variation with season and shore height is little studied. This is largely due to the small size of organism with the soil matrix and flooding limiting research approaches to indirect methods instead of direct observations.

Analysing food webs

Understanding the structure of soil food webs may allow insight into resilience of trophic functions (Leibold et al. 1997, Rooney et al. 2008), as well as the rate of energy fixation and transfer to higher trophic levels (Hairston 1993). Soil food webs are essential due to the ecosystem services soil communities provide (de Vries et al. 2013, Sofo 2020, see Soil communities), with changes in soil community structure linked to differences in ecosystem services (de Vries et al. 2013, Sofo 2020). Conversion of land by humans disturbs soil structure and decreases soil organic matter (SOM) concentrations, thereby reducing habitats essential for many soil organisms (Sofo 2020). This in turn reduces the complexity of soil communities, thus reducing nutrient cycling efficiency (Sofo 2020). Therefore, understanding how external influence affects soil food webs is essential to maintain ecosystem function.

Soil food webs are difficult to study since the organisms are small and the soil matrix makes direct observations impossible. Confronted with these difficulties, a variety of indirect methods are employed including stable isotopes to investigate food web structure and

lipids (phospholipids and neutral lipids) to trace resource channelling through the food web (Juen and Traugott 2005, Traugott et al. 2013).

Food web structure

Stable isotopes are atoms of the same element with differing numbers of neutrons, altering their atomic weight, with heavier isotopes being rarer (Ehleringer and Rundel 1989). Of these isotopes the ratios of $^{15}N/^{14}N$ and $^{13}C/^{12}C$ can give insight both into the trophic position and resource consumption of organisms, foregoing direct observation (Ehleringer and Rundel 1989, David M. Post 2002, Traugott et al. 2013). This is because ^{15}N in consumers is enriched by 3.4 ‰ relative to their resource allowing distinction of trophic levels (Martínez Del Rio et al. 2009, Traugott et al. 2013, Potapov et al. 2019). By contrast, ^{13}C is conserved across trophic levels allowing e.g., to trace the channelling of resources of different ^{13}C signatures (David M. Post 2002, Traugott et al. 2013). This is especially useful in aquatic systems to differentiate between littoral algae and detritus or open water phytoplankton (France 1995, David M. Post 2002). Furthermore, it has been used to indicate transfer of allochthonous aquatic resources to terrestrial systems (Korobushkin et al. 2016, 2022). This can be attributed to the differing ^{13}C signatures of algal material, C3 photosynthesising and C4 photosynthesising plants. In Wadden Sea salt marshes, with the exception of the C4 plant *S. anglica*, all other vascular plants are C3, and are therefore significantly depleted in ^{13}C compared to *S. anglica* (Winter et al. 2018). Marine algae on the other hand, are positioned between C3 and C4 plants due to their use of bicarbonate (Maberly et al. 1992) also allowing to differentiate them from vascular plants. Stable isotopes have been used to demonstrate feeding relations of soil macro-invertebrates of salt marshes, separating vascular plants and benthic diatoms (Créach et al. 1997). Furthermore, Haynert et al. (2017) and Winter et al. (2018) used stable isotopes to determine the trophic position and the use of allochthonous marine resources by the salt marsh soil mesofauna. However, while the stable isotope method can give insight into the trophic position, precise resource use estimates are difficult. This is because isotopic values of resources need to be known which, in particular for detritus-associated bacteria and fungi but also algae, is difficult.

Channelling of resources

Phospholipids are part of the bilayer of the cell membrane and degrade quickly after death, therefore indicating live microbial mass (White et al. 1996, Kandeler 2007). Furthermore, group-specific phospholipid fatty acids (PLFA), such as methyl-branched, cyclopropyl fatty acids and 18:2ω6,9 are only or predominantly found in Gram-positive, Gram-negative bacteria and fungi, respectively, allowing to quantify these groups in soil (Bossio and Scow 1998, Zelles 1999, Kandeler 2007, Pollierer et al. 2015). This provides important insight into the dominant basal channel within the system.

In addition to feeding relations, ratios of certain PLFA markers provide insight into the composition of the microbial community, deficiencies of resources, stress and activity (Vestal and White 1989, Korner and Laczkó 1992, Bossio and Scow 1998, Zelles 1999). There are two main stress indicator ratios in PLFAs, the cyclopropyl to precursor ratio (cy/pre)

and the monounsaturated to saturated ratio (mono/sat) (Vestal and White 1989, Korner and Laczkó 1992, Bossio and Scow 1998, Zelles 1999). During periods of stress, Gram-negative bacteria enter the stationary growth phase (Wixon and Balser 2013), during which they increasingly convert monounsaturated fatty acids into cyclopropyl fatty acids (Law et al. 1963, Guckert et al. 1986, Kaur et al. 2005, Wixon and Balser 2013). Abiotic conditions which may trigger the stationary growth phase includes acidic conditions, high temperature and low oxygen availability (Knivett and Cullen 1965). While the benefit of producing these cyclopropyl lipids is not fully understood, altering their production led to susceptibility to environmental stressors and reduced fitness (Rowlett et al. 2017). Therefore, higher cyclopropyl to precursor ratios indicate the stationary growth phase in Gram-negative bacteria as a result of environmental stressors. The mono/sat ratio reflects that monounsaturated fatty acids decline in microorganisms when resources or oxygen is reduced (Bossio and Scow 1998, Gómez-Brandón et al. 2011, Pollierer et al. 2015). Therefore, the mono/sat ratio is considered an indicator of oxygen availability as well as substrate availability for microbes. Within the North Sea, the PLFA method has been used to investigate trophic position and diet of zooplankton (Kürten et al. 2013), as well as benthic microbial composition, diet and stress (Stoeck et al. 2002, Haynert et al. 2020). Overall, due to microorganisms producing group-specific phospholipids, the study of PLFAs allows quantitative insights into the dynamics of soil microbial communities and basal channels.

Tracing the channelling of basal resources through the food web is accomplished by studying the neutral lipid fatty acids (NLFA) composition of consumer (Traugott et al. 2013). NLFAs act as energy storage compounds in organisms (Ruess and Chamberlain 2010). Marine studies first showed that specific phospholipids are incorporated into consumers with little to no changes in structure (Lee et al. 1971, Graeve et al. 1994). This incorporation of basal resource lipids without structural changes became known as 'dietary routing' (Stott et al. 1997). Dietary routing is possible due to differences in biosynthetic pathways between microbes and animals, with the latter only being able to synthesize a limited spectrum of lipids by their own (Ruess and Chamberlain 2010). Meaning that higher trophic levels must source these essential lipids from their diet (Ruess and Chamberlain 2010). Thereby, tracing basal resources through the food web using lipid markers is possible (Zelles 1999, Ruess et al. 2005, Pollierer et al. 2010). Among NLFAs there are two types of markers, relative and absolute markers (Ruess and Chamberlain 2010, Pollierer et al. 2010). Relative marker lipids can be synthesised by the consumer, but accumulate with dietary supplementation such as oleic acid 18:1ω9 and linoleic acid 18:2ω6,9 for plants and fungi, respectively (Ruess and Chamberlain 2010). By contrast, absolute markers are lipids which are only synthesised by microorganisms, such as iso-, anteiso and cyclopropyl lipids by bacteria (Zelles 1999, Ruess and Chamberlain 2010). Therefore, while some lipids are absolute indicators of resources, other may suggest dietary supplementation.

Within the Wadden Sea salt marsh animal communities NLFA analysis has been used to investigate basal resource use in oribatid mites, with the results indicating that they use

both autochthonous terrestrial and allochthonous marine resources (Winter et al. 2018). Furthermore, the diet of benthic Foraminifera was investigated, proving that diet was dependent on their respective habitat (Haynert et al. 2020). Therefore, NLFA is a promising method to investigate basal resource use as well as the use of allochthonous resources for the Wadden Sea food web.

Aims and hypotheses

The aims of this thesis were to investigate the temporal and spatial dynamics of salt marsh soil communities as well as the use of allochthonous marine resources. For this purpose, in Chapter 2 I investigated the spatiotemporal dynamics of basal microbial resources using PLFA as well as their stress indicators using the following hypotheses:

1) The concentration of PLFA markers changes across salt marsh zones, with the algal marker concentration increasing toward lower zones (LSM and PZ), the stress indicator cyclic to precursor FA ratio being highest in the USM due to higher temperatures and more frequent droughts, and the monounsaturated to saturated FA ratio being highest in the USM due to greater availability of oxygen.

2) Due to changes in abiotic conditions and tidal regime in the marsh, PLFA marker concentration varies across seasons with the cyclic to precursor FA ratio increasing in summer due to increased stress by high temperature and drought, and the monounsaturated to saturated FA ratio increasing due to increased oxygen supply.

3) The concentration of PLFA markers decreases with sediment depth due to reduced resource input and redox potential; due to the association of monounsaturated FAs with aerobic growth, we hypothesized the monounsaturated to saturated FA ratio to decrease with sediment depth, whereas the cyclic to precursor FA ratio to increase.

Moving up the food chain, I investigated spatiotemporal changes in channelling of these basal resource in Chapter 3. To do so, I assessed the NLFA composition of salt marsh meso- and macrofauna using the following hypotheses:

1) The contribution of vascular plants, bacteria and fungi as basal resources of soil taxa increases with shore height, because of the increasing aerobic environment with shore height. Conversely, we hypothesized the contribution of algae as basal resource to increase with decreasing shore height due to increased input with inundation frequency.

2) The marker concentration of soil animal taxa to vary with season due changes in temperature and inundation frequency affecting resource availability, with algal markers increasing in summer, due to high temperature and light conditions, and fungi, bacteria and plant markers increasing in autumn, due to increased input of plant litter material.

Finally, in Chapter 4 I investigated spatiotemporal changes in food web structure as well as allochthonous resource use in soil macrofauna. Assessment of the food web structure and resource use was accomplished with stable isotopes (^{15}N and ^{13}C) and the following hypotheses:

1) The consumption of allochthonous marine algal resources increases with greater tidal influence i.e., the enrichment of ^{13}C in consumers of the LSM is higher than in consumers of the USM.

2) Resembling terrestrial habitats, salt marsh soil macrofauna communities consist of four trophic levels including primary decomposers, secondary decomposers, first order predators and second order predators.

3) Resource use of soil macrofauna varies across seasons (spring, summer, autumn) and zone with allochthonous marine resources being especially important for the salt marsh macrofauna food web in autumn due to heavy storms carrying large amounts of marine resources to higher positions in the salt marsh.

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Chapter 2 - Spatial and temporal variations in salt marsh microorganisms of the Wadden Sea

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Abstract

Salt marshes exist at the interface of the marine and the terrestrial system. Shore height differences and associated variations in inundation frequency result in altered abiotic conditions, plant communities, and resource input into the belowground system. These factors result in three unique zones, the upper salt marsh (USM), the lower salt marsh (LSM), and the pioneer zone (PZ). Marine detritus, such as micro-and macroalgae, is typically flushed into the PZ daily, with storm surges moving both salt marsh detritus and marine detritus into higher salt marsh zones. Microbial assemblages are essential for the decomposition of organic matter and have been shown to sensitively respond to changes in abiotic conditions such as oxygen supply and salinity. However, temporal and spatial dynamics of microbial communities of Wadden Sea salt marshes received little attention. We investigated the dynamics of soil microbial communities across horizontal (USM, LSM, and PZ), vertical (0–5 and 5–10-cm sediment depth), and temporal (spring, summer, and autumn) scales in the Wadden Sea salt marsh of the European North Atlantic coast using phospholipid fatty acid (PLFA) analysis. Our results show strong spatial dynamics both among salt marsh zones and between sediment depths, but temporal dynamics to be only minor. Despite varying in space and time, PLFA markers indicated that bacteria generally were the dominant microbial group across salt marsh zones and seasons, however, their dominance was most pronounced in the USM, whereas fungal biomass peaked in the LSM and algal biomass in the PZ. Only algal markers and the stress marker monounsaturated to saturated fatty acid ratio responded to seasonality. Overall, therefore, the results indicate remarkable temporal stability of salt marsh microbial communities despite strong variability in abiotic factors.

Introduction

Salt marshes predominantly form along the interface between the marine and terrestrial system, where erosion by seawater is reduced and sediment gradually accumulates (Adam, 1993; Allen, 2000; Pennings & Bertness, 2001). With the establishment of vascular plants, the sediment is stabilized and continued external addition increases shore height (Adam, 1993; Allen, 2000). Wadden Sea salt marshes are essential in protecting mudflats and the coastline from erosion by the North Sea (Reed et al., 2018; Wang et al., 2012). Furthermore, they sequester large amounts of carbon, thereby functioning as blue carbon systems (McLeod et al., 2011; Mueller et al., 2019). Due to the gradual build-up, inundation frequency declines with shore height (Bockelmann et al., 2002; Caçador et al., 2007; Roozen & Westhoff, 1985). Changes in inundation frequency result in separate habitats driven by abiotic stressors, such as salinity, water logging, and soil anoxia, as well as competition

(Bockelmann et al., 2002; Buth, 1987; Pennings & Bertness, 2001). Salt marshes of the Wadden Sea are split into three separate zones distinguished by their vegetation: the upper salt marsh (USM), the lower salt marsh (LSM), and the pioneer zone (PZ) (Haynert et al., 2017; Winter et al., 2018).

The functioning of salt marshes as carbon sinks results from slow decomposition of highly productive, lignocellulose-rich terrestrial plants by microbial communities (Hemminga & Buth, 1991; Hemminga et al., 1988; Leadbeater et al., 2021). In addition, inundation of the salt marsh brings allochthonous marine material, such as algal wrack and microphytobenthos, to the marsh adding to the autochthonous organic matter input to the system (Polis & Hurd, 1996; Redzuan & Underwood, 2020; Zong & Horton, 1998). Recent studies indicated the use of these marine resources by soil mesofauna of the salt marsh (Haynert et al., 2017; Winter et al., 2018). The activity of microbes breaking down plant material is influenced by abiotic factors such as soil temperature, water concentration, nutrient addition, and soil anoxia (Hanley et al., 2021; Hemminga & Buth, 1991). Therefore, changes in abiotic pressures across marsh zones may influence microbial processes via changes in microbial community composition and thereby their functioning as blue carbon system. However, most studies focusing on the breakdown of detrital material and microbial activity, investigated North American salt marshes (Gandy & Yoch, 1988; Hanley et al., 2021; Newell et al., 1989; Sherr & Payne, 1978), differing from Wadden Sea marshes in their microtidal range (<2 m) and their organogenic nature resulting in the accretion of organic material, rather than sediment (Allen, 2000; Kearney & Turner, 2016). Studies on European marshes focused on the rate of decomposition, the contribution of fungi or bacteria, and the fate of detrital material of salt marsh plants (Benner et al., 1984; Bouchard et al., 1998; Bouchard & Lefeuvre, 2000). In addition, the importance of benthic microphytobenthos for benthic macroinvertebrates and bacteria of the Wadden Sea mudflats has been extensively studied (Christianen et al., 2017; Middelburg et al., 2000; Taylor et al., 2013). Until today, however, the structure of microbial communities within European salt marshes has not been investigated in detail. Soil microbial communities exist at the base of the food web and are key in the breakdown of organic matter and nutrient cycling (Bardgett et al., 1999; Buckley & Schmidt, 2001; Gray et al., 2011; Vestal & White, 1989). Microbial communities include bacteria, archaea, protozoans, fungi, micro-metazoans, and algae (Vestal & White, 1989). The composition of these communities determines the rate of substrate utilization, and also the type of organic and inorganic material used as substrate (Gray et al., 2011). For example, Gram-positive bacteria may break down recalcitrant materials, whereas Gram-negative bacteria rely on labile root exudates (Fanin et al., 2019). Studies have demonstrated the sensitivity of microbial communities to external factors such as temperature, precipitation, pH, and atmospheric CO_2 concentrations (Alzarhani et al., 2019; Buckley & Schmidt, 2001; Gray et al., 2011). In salt marshes of Europe, research thus far has focused on the decomposition of residues of specific vascular plants, such as *Spartina alterniflora*, *S. anglica*, and *Atriplex portulacoides* (Benner et al., 1984; Bouchard et al., 1998; Buchan et al., 2003; Hemminga & Buth, 1991; Hemminga et al., 1988), the species and biomass of bacteria and fungi (Buchan et al., 2003;

Carvalho et al., 2004; Castro & Freitas, 2000; Leadbeater et al., 2021) as well as which group predominantly breaks down lignocellulose (Benner et al., 1984; Calado et al., 2019; Cortes-Tolalpa et al., 2018; Leadbeater et al., 2021). In contrast, the overall microbial community composition and their dynamics over time and zonation in the salt marsh received little attention. Therefore, knowledge of these communities is still limited, mainly due to the vast numbers of microbial species and difficulties in cultivating and quantifying them (Buckley & Schmidt, 2001; Vestal & White, 1989). Recently, DNA metabarcoding has been used to identify spatial dynamics influencing benthic diatom communities in salt marshes (Plante et al., 2021). However, lipid analysis, more specifically phospholipids, allowing the quantification of microbial biomass, community composition, substrate availability, and metabolic status (Bossio & Scow, 1998; Vestal & White, 1989; Zelles, 1999), has not been used for analyzing variations in microbial communities across salt marsh zones.

Phospholipid fatty acid (PLFA) analysis is based on the fact that some fatty acids (FAs) are only produced by certain microorganisms (Vestal & White, 1989; Zelles, 1999). In addition, specific PLFA ratios serve as indicators of physiological stress and nutrient availability (Bossio & Scow, 1998; Pollierer et al., 2015; Wixon & Balser, 2013). Physiological stress in Gram-negative bacteria is indicated by the ratio of cyclopropyl lipids to their monounsaturated precursors (Gray et al., 2011; Pollierer et al., 2015; Willers et al., 2015). With increased stress, such as temperature rise or lack of oxygen, microorganisms enter a state of reduced growth (Wixon & Balser, 2013) associated with increased conversion of monounsaturated lipids to cyclopropyl lipids (Guckert et al., 1986; Kaur et al., 2005). Furthermore, monounsaturated lipids were shown to increase with greater substrate availability and decrease with flooding intensity (Bossio & Scow, 1998; Pollierer et al., 2015). As a result, the ratio of monounsaturated to saturated precursor lipids is indicative of aerobic activity as well as substrate availability to microorganisms (Bossio & Scow, 1998; Pollierer et al., 2015). Previous research has indicated seasonal changes in microbial PLFA signatures with changing abiotic conditions (Moore-Kucera & Dick, 2008; Pollierer et al., 2015), confirming that microbial community changes can be assessed across temporal scales. Therefore, phospholipid analysis allows the quantification of functional groups such as fungi, Gram-positive, and Gram-negative bacteria as well as their variations with abiotic conditions. Measuring phospholipids across habitats and time allows insight into the dynamics of these functional groups.

The aims of this study were to investigate the structure of microbial communities, the use of allochthonous algal material and physiological stress indicators across the salt marsh soil, and their temporal and spatial changes using PLFAs. More specifically, we hypothesized that (1) the concentration of PLFA markers changes across salt marsh zones, with the algal marker concentration increasing toward lower zones (LSM and PZ), the stress indicator cyclic to precursor FA ratio being highest in the USM due to higher temperatures and more frequent droughts, and the monounsaturated to saturated FA ratio being highest in the USM due to greater availability of oxygen, organic material, and low inundation frequency. Furthermore, we hypothesized that (2) due to changes in abiotic conditions and tidal

regime in the marsh, PLFA marker concentration varies across seasons with the cyclic to precursor FA ratio increasing in summer due to increased stress by high temperature and drought, and the monounsaturated to saturated FA ratio increasing due to increased oxygen supply. Finally, we hypothesized (3) the concentration of PLFA markers to decrease with sediment depth due to reduced resource input and redox potential; due to the association of monounsaturated FAs with aerobic growth, we hypothesized the monounsaturated to saturated FA ratio to decrease with sediment depth, whereas the cyclic to precursor FA ratio to increase. Overall, this study is expected to contribute to the identification of the factors driving the temporal and spatial dynamics of microbial communities and carbon sequestration in salt marsh soil.

Methods

Sampling

Samples were taken across five transects on the island of Spiekeroog (Wadden Sea National Park, Germany; 53°45'2"- 53°47'1"N, 7°40'0"- 7°49'1"E) in April (16th, three days prior to spring tide), July (16th, during spring tide), and October (22nd, one day past neap tide) 2019. The three sampling dates were taken to represent three seasons, that is, spring, summer, and autumn, and thereby the temporal variation in microbial communities. Per transect and zone one soil core (ø 5 cm) was taken and separated into two sediment depths, 0–5 and 5–10 cm, resulting in 30 cores per sampling date. The cores were stored at −20°C until further processing. Prior to extraction of PLFAs, each core was sieved (2 mm) and material other than soil, such as organic material and buried animals, was removed. Samples were taken from the USM, LSM, and PZ. The USM is situated 35 cm above the mean high water level (MHWL) to the storm tide limit, it is inundated between 35 and 70 times a year with a soil salinity between 5 and 20 ‰ and is dominated by *Elymus athericus* (= *Elytrigia atherica*) (Niedringhaus, 2009). The LSM is located 0–35 cm above the MHWL and flooded between 150 and 250 times a year with a soil salinity of 20‰–26‰; it is characterized by *Atriplex portulacoides* and *Puccinellia maritima*. The PZ, situated below the MHWL, is typically inundated twice a day with a soil salinity of 26‰–32‰ and characterized by *Salicornia* sp. and *Spartina anglica* as well as macroalgae such as *Fucus* sp. and *Ulva* sp. (Haynert et al., 2017; Niedringhaus, 2009; Winter et al., 2018).

PLFA Analysis

The extraction, lipid separation, and transesterification of lipids followed the protocol of Buyer and Sasser (2012) with minor adjustments. Approximately 2 g of soil was weighed into 10-ml glass tubes, dried overnight in a vacuum centrifuge at room temperature, and the dry weight determined. For the extraction, a predetermined volume (1000 µl) of the organic phase was evaporated prior to lipid separation.

Lipid separation was performed on a 96-well plate (Thermo-Scientific, Silica 96 Well Plate, 50 mg). Once separated, 20 µl of internal standard was added to each sample. Transesterification was done with $CHCl_3$ and 0.075 M CH_3COOH with 0.7 ml of sample removed and evaporated. Samples were eluted with isooctane into vial inserts, placed into 1.5-ml vials, capped, and stored at −20°C until gas chromatography. Lipids were separated using a gas chromatograph (Clarus 500, PerkinElmer, Norwalk, USA) equipped with an Elite-5 capillary column (30 m × 0.32 mm i.d., film thickness 0.25 mm, PerkinElmer, Norwalk, USA). The analysis started with 60°C for 1 min, then increased by 30°C/min to 160°C; followed by 3°C/min increase to 260°C; the injection temperature was 250°C, the carrier gas was helium.

Lipids were identified by retention time based on standard mixtures composed of 37 fatty acid methyl esters (FAMEs; Sigma-Aldrich, St Louis, USA) ranging between C11 and C24 chain lengths, as well as 26 bacterial acid methyl esters (BAMEs; Sigma-Aldrich, St Louis,

USA), and algal standards for 16:2ω6,9 and 16:3ω3,6,9 (Larodan AB, Solna, Sweden) (Buse et al., 2013).

Fatty acids were allocated to marker groups. Vascular plant markers included 18:1ω9, 22:0, and 24:0 (Ruess & Chamberlain, 2010; Zelles, 1999). Bacterial markers included 15:0, 16:1ω7, 17:0, 18:1ω7, 2-OH-12:0, a15:0, cy17:0, cy19:0, i15:0, i16:0, and i17:0 (Haynert et al., 2020; Ruess & Chamberlain, 2010; Zelles, 1999); 18:2ω6,9 was taken as general fungal marker (Frostegard & Baath, 1996). Algal markers included 14:0, 16:2ω6,9, 16:3ω3,6,9, 20:5ω3,6,9,12,15, and 22:6ω3,6,9,12,15,18 (Buse et al., 2013; Kelly & Scheibling, 2012). Changes in the PLFA concentration of the complement of marker groups were taken to represent changes in microbial community composition. To get further insight into microbial community composition and functioning, we calculated three indices. The fungi/ bacteria ratio was calculated as 18:2ω6,9 / sum of (i15:0 + a15:0 + 15:0 + i16:0 + 16:1ω7 + i17:0 + cy17:0 + 17:0 + 18:1ω7 + cy19:0) (Frostegard & Baath, 1996; Pollierer et al., 2015; Wixon & Balser, 2013). Furthermore, two stress indicator ratios were included. The cyclic phospholipids to their precursor (cy/pre) ratio, calculated as cy17:0/16:1ω7 (cy19:0 was only present in one sample and therefore omitted) (Bossio & Scow, 1998; Moore-Kucera & Dick, 2008; Wixon & Balser, 2013) indicating physiological or nutritional stress in Gram-negative bacteria (Bossio & Scow, 1998; Wixon & Balser, 2013). The monounsaturated to saturated precursor (mono/sat) ratio, calculated as sum of monounsaturated FAs (16:1ω7 + 17:1ω7 + 18:1ω7 + 18:1ω9) / sum of saturated FAs (14:0 + 15:0 + 16:0 + 17:0 + 18:0 + 20:0), indicated aerobic growth and substrate availability for microbes (Bossio & Scow, 1998; Wixon & Balser, 2013).

Statistical analysis

PLFA marker lipids and ratios were analyzed by mixed-effects models with "Zone" (USM, LSM, and PZ), "Season" (spring, summer, and autumn), and "Depth" (0–5 and 5–10 cm) as fixed factors and "coreID" nested within "Transect" as random factor. The statistical analyses were done in R 4.0.5 (R Core Team, 2021) using packages: "emmeans," "lme4," "lmerTest," "effects," and "tidyverse." PLFA marker lipid concentrations (nmol g−1 dry weight) were log(x+1) transformed to improve homogeneity of variance, PLFA ratios were logit transformed. Means and standard deviation given in text are based on nontransformed data.

Redundancy analysis (RDA) was used for analyzing the composition of marker lipids (as percentages of total) together with abiotic data including soil salinity (Meier, Thölen, Lohmus, et al., 2020), inundation frequency (counts/month) and inundation duration (calculated from water level data; Meier et al., 2020b), soil water content (D. Meier, submitted to PANGAEA), and temperature (Meier et al., 2020a). Soil parameters were measured as described in Balke et al. (2017). Forward selection was used to determine the most important abiotic factors explaining lipid marker composition.

Results

Marker PLFA

Plant marker concentration significantly varied with Depth and Zone, but not with Season (Table 1). Generally, it was much higher in the 0–5-cm (11.21 ± 3.00 nmol g^{-1}) than in the 5–10-cm sediment depth (5.48 ± 2.01 nmol g^{-1}). Reduction of the marker with sediment depth was consistent across salt marsh zones and seasons without significant interactions. Among salt marsh zones, plant markers were highest in the USM and LSM, and lower in the PZ (Figure 1).

Similar to plant markers, bacterial marker concentration significantly varied with Depth and Zone, with Season being only marginally significant and none of the interactions being significant (Table 1). Generally, in the 0–5-cm sediment depth (74.91 ± 17.30 nmol g^{-1}) it was about twice as high as in the 5–10-cm sediment depth (38.95 ± 10.31 nmol g^{-1}). Furthermore, it declined in from the USM to the LSM to the PZ (Figure 1). Of the individual bacterial PLFAs in the upper sediment depth the concentration of 18:1ω7 was greatest (29.26 ± 9.66 nmol g^{-1}), followed by 16:1ω7 (17.02 ± 4.30 nmol g^{-1}), i15:0 (10.82 ± 2.73 nmol g^{-1}), and a15:0 (6.55 ± 1.21 nmol g^{-1}). In the deeper sediment, the concentration of 18:1ω7 also was greatest (12.11 ± 4.73 nmol g^{-1}), followed by i15:0 (7.36 ± 1.95 nmol g^{-1}), 16:1ω7 (7.30 ± 2.45 nmol g^{-1}), and a15:0 (4.65 ± 1.11 nmol g^{-1}).

Again as for the plant and bacterial markers, the fungal marker 18:2ω6,9 varied significantly with Depth and Zone, but not with Season as main factor, however, in contrast to the plant and bacterial markers, there was a significant interaction between Zone and Season (Table 1). Concentration of the marker was highest in the 0–5-cm sediment depth (4.96 ± 2.04 nmol g^{-1}) and dropped to less than half in the 5–10-cm sediment depth (2.30 ± 1.71 nmol g^{-1}). On average, the fungal marker concentration was similarly low in the USM and PZ, but higher in the LSM (Figure 1). However, in the USM fungal marker concentrations were highest in spring and gradually declined toward autumn, whereas in the LSM it was highest in summer and lower in spring and autumn; in the PZ changes were inverse to those in the LSM (Figure 2a).

Table 1: F- and p-values of linear mixed-effects models on variations in PLFA markers (plants, bacteria, fungi, algae) and marker ratios (fungi/bacteria, cy/pre, mono/sat; see Methods for details) with sediment depths (0-5 and 5-10 cm), salt marsh zones (upper salt marsh, lower salt marsh, pioneer zone) and seasons (April, July and October), and their interactions . Significant effects (P < 0.05) are highlighted in bold.

Marker	Depth (D)		Zone (Z)		Season (S)		D x Z		D x S		Z x S		D x Z x S	
	F	p	F	p	F	p	F	p	F	p	F	p	F	p
Plant	226.97	**<0.001**	39.59	**<0.001**	2.30	0.107	0.35	0.704	1.06	0.351	1.49	0.216	1.50	0.212
Bacteria	219.49	**<0.001**	13.43	**<0.001**	2.93	0.060	1.41	0.251	0.65	0.527	1.75	0.149	1.02	0.403
Fungi	103.02	**<0.001**	8.14	**0.006**	0.32	0.726	0.77	0.466	0.52	0.597	32.19	**0.018**	0.95	0.441
Algal	208.17	**<0.001**	8.05	**0.001**	4.22	**0.019**	5.29	**0.007**	1.18	0.314	1.55	0.197	2.24	0.073
Fungi/bacteria	7.90	**0.007**	9.08	**0.004**	3.13	0.051	0.00	0.997	0.09	0.912	6.53	**<0.001**	1.36	0.260
Cy/Pre	48.89	**<0.001**	15.06	**0.002**	2.47	0.093	1.18	0.315	9.36	**<0.001**	0.53	0.718	2.98	**0.026**
Mono/Sat	259.18	**<0.001**	330.98	**<0.001**	34.81	**<0.001**	8.33	**0.001**	1.88	0.161	5.42	**0.001**	0.56	0.693

Note: Significant effects (p= < 0.05) are highlighted in bold

Algal marker concentrations varied significantly across all factors, with a significant interaction between Depth and Zone (Table 1). Concentrations were higher in the 0–5-cm than in the 5–10-cm sediment depth, but the difference between the depths was most pronounced in the PZ with concentrations in the 5–10-cm sediment depth 60% less than the 0–5-cm.In the LSM, algal marker concentrations halved in 5–10c-m and reduced by 40% in in the USM (Figure 3a). Generally, however, the concentration of the algal marker was similar in the PZ and LSM, but lower in the USM (Figure 1). Across seasons, the algal marker
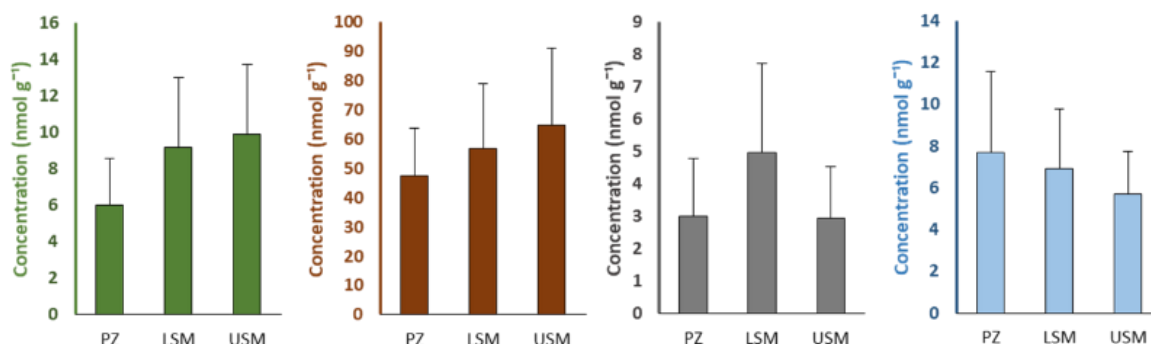

Figure 1: Concentration of marker PLFAs (nmol g^{-1}) for plants (green); bacteria (brown); fungi (grey) and algae (blue) across salt marsh zones. PZ- pioneer zone; LSM - lower salt marsh; USM - upper salt marsh. Error bars show standard deviation. Note different scales of y-axis.

concentration was highest in April (7.54 ± 3.56 nmol g^{-1}), and at a similarly low level in July (6.33 ± 2.95 nmol g^{-1}) and October (6.43 ± 2.66 nmol g^{-1}). However, individual marker concentrations declined in parallel in the 0–5- and 5–10-cm sediment depths in the order 14:0 (4.18 ± 1.00 and 2.41 ± 0.70 nmol g^{-1}, respectively) > 16:3ω3,6,9 (3.15 ± 0.89 and 1.94 ± 0.81 nmol g^{-1}) > 20:5ω3 (1.69 ± 1.76 nmol g^{-1} and 0.06 ± 0.27 nmol g^{-1}) > 22:6ω3 (0.09 ± 0.22 nmol g^{-1} and absent in 5–10 cm). The flagellate marker 22:6ω3 was only found in the 0–5-cm sediment depth of the PZ in April and July. The diatom marker (20:5ω3,6,9,12,15) concentration was highest in the 0–5-cm sediment depth of the PZ (3.48 ± 1.79 nmol g^{-1}), followed by the 0–5-cm sediment depth of the LSM (1.06 ± 0.99 nmol g^{-1}) and the 0–5-cm sediment depth of the USM (0.53 ± 0.35 nmol g^{-1}); it was very low in the 5–10-cm sediment depth (0.06 ± 0.27 nmol g^{-1}).

Redundancy analysis (RDA) of the marker fatty acids confirmed changes in the concentration of PLFA markers across the salt marsh zones, seasons, and depths (Figure 4). Forward selection indicated the strongest abiotic factors to be water content, salinity, flooding frequency, and soil temperature. The first axis explained 59.6% of the variation and separated the USM from the PZ and LSM, with the separation being mainly due to higher water content (pseudo-F= 82.8, p = .002, contribution = 70.8%), soil salinity (pseudo-F= 18.5, p = .002, contribution = 13.2%) and flooding frequency (pseudo-F= 10.1, p = .002, contribution = 6.2%) in the LSM and PZ. Bacterial and plant marker fatty acids were closely associated with the USM, whereas algal markers were closely associated with the PZ, and the fungal marker fatty acid with the LSM. Both sampling dates and sediment depths were

separated along the second axis explaining an additional 3.2% of the variation and correlating mainly with temperature (pseudo-F= 5.0, p = .004, contribution= 3.4%).
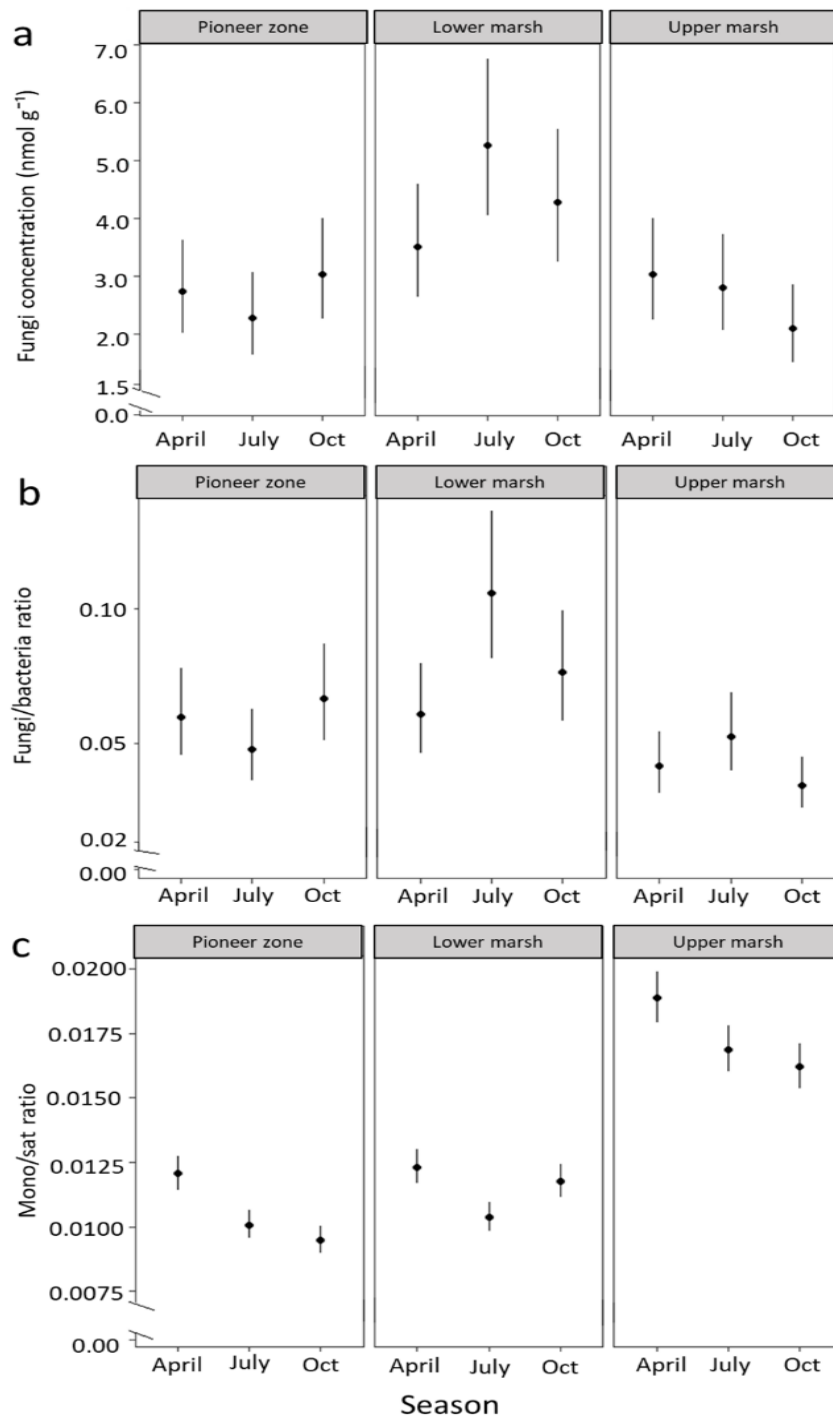
Figure 2: Changes in (a) the fungal marker concentration, (b) the fungi/bacteria PLFA ratio, and (c) monounsaturated/ saturated PLFA ratio (both logit transformed) across salt marsh zones and season (April, July, October); estimated means with 95% confidence intervals
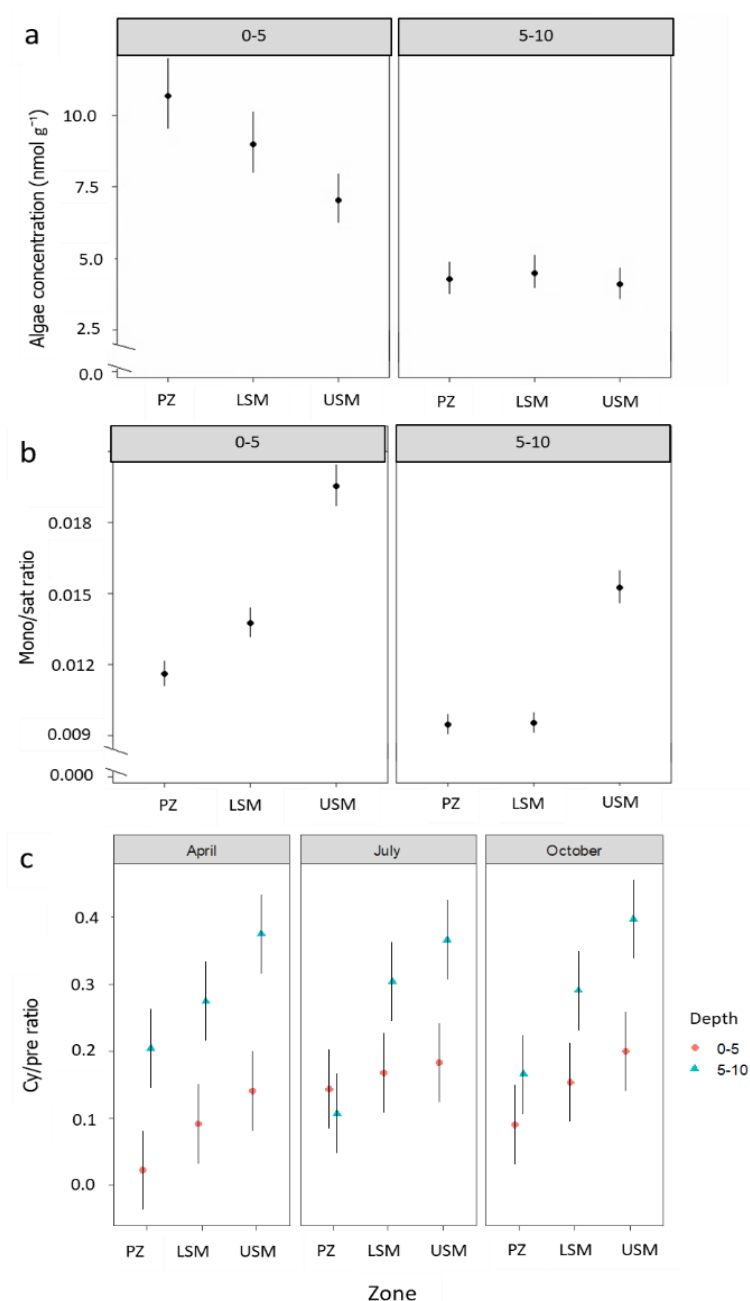
Figure 3: Changes in (a) algal marker concentration with sediment depths across salt marsh zones, (b) the monounsaturated/saturated PLFA ratio with sediment depths across salt marsh zones, and (c) the cyclic/precursor PLFA ratio with sediment depths (0-5 and 5-10 cm) across salt marsh zones (PZ, pioneer zone; LSM, lower salt marsh; USM, upper salt marsh), and season (April, July, October); estimated means with 95% confidence intervals

PLFA Ratios

The fungi/bacteria ratio varied significantly with Depth and Zone, but the effect of Zone varied with Season (Table 1). The ratio decreased significantly from 0–5-cm (0.069 ± 0.031) to 5–10-cm sediment depth (0.060 ± 0.038). Across the salt marsh zones the ratio was highest in the LSM (0.087 ± 0.042), followed by the PZ (0.062 ± 0.028) and the USM (0.044 ± 0.013), but markedly changed with season with the changes paralleling those of the fungal marker (Figure 2b).

The cy/pre ratio also varied significantly with Depth and Zone, with significant interactions for both Depth and Zone as well as Depth and Season (Table 1). Across the salt marsh zones it generally declined in the order USM (0.277 ± 0.113) > LSM (0.214 ± 0.094) > PZ (0.122 ± 0.097). This pattern was consistent in the 0–5- and 5–10-cm sediment depths, but the gradient was steeper in 5–10 cm (Figure 3c). Furthermore, the cy/pre ratio was generally higher in the 5–10-cm than in the 0–5-cm sediment depth, with the exception of the PZ in July.

The mono/sat ratio varied significantly with Depth, Zone, and Season with significant interactions for both Depth and Zone as well as Zone and Season (Table 1). Generally, it declined from 0–5 (1.506 ± 0.370) to 5–10 cm (1.150 ± 0.310), and from the USM to the LSM to the PZ. In both the USM and PZ, it declined from spring to summer to autumn, but in the LSM it was at a similar level in spring and autumn and lower in summer (Figure 2c). Furthermore, although it generally declined from the USM to the LSM to the PZ, this decline was gradual in the 0–5-cm depth, whereas in the 5–10-cm sediment depth, it dropped from the USM to a similarly low level in the LSM and PZ (Figure 3b).
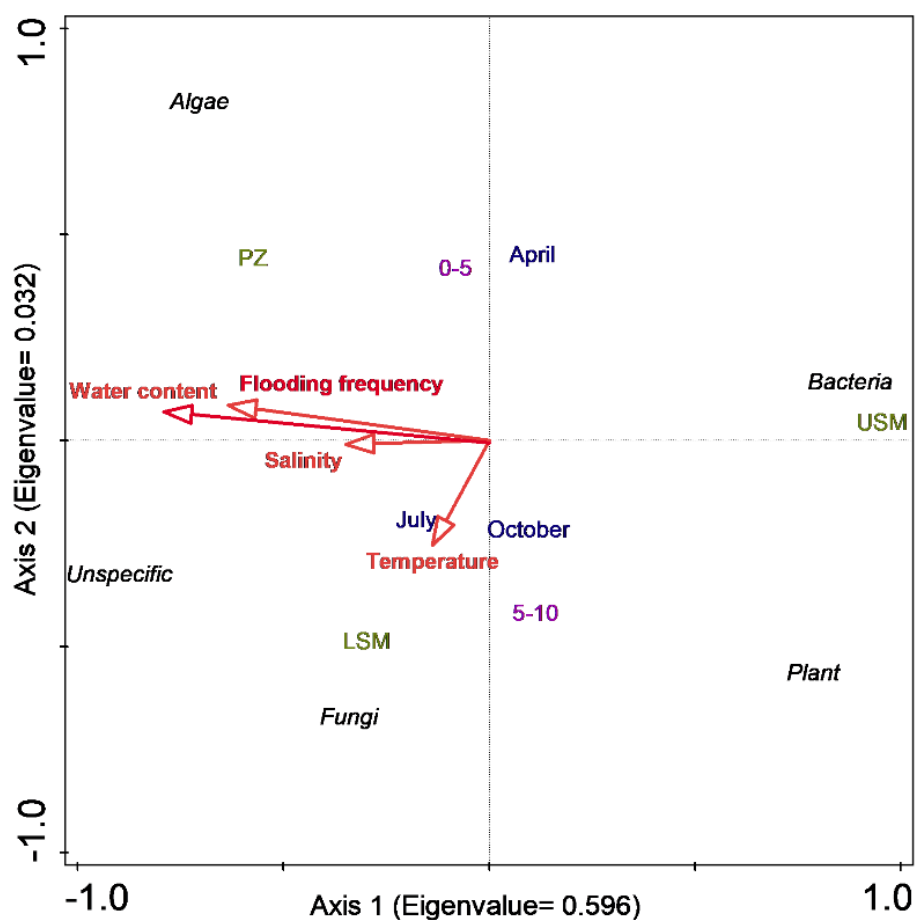

Figure 4: Redundancy analysis of PLFA marker groups with water content, inundation frequency. salinity (‰) and temperature (°C) as explanatory environmental factors. Salt marsh zones (PZ- pioneer zone; LSM - lower salt marsh; USM - upper salt marsh), soil layer (0-5 and 5-10 cm) and season (April, July, October) were included as silent variables not affecting the ordination

Discussion

In this study, we aimed to identify changes in the composition and stress indicators of microorganisms across salt marsh zones (USM, LSM, and PZ), seasons (spring, summer, and autumn), and sediment depths (0–5 and 5–10 cm) in the back-barrier salt marsh of Spiekeroog. Overall, microbial community structure and stress indicators strongly varied among salt marsh zones and between sediment depths, whereas the influence of season was minor.

Salt marsh Zones

Generally, plant and bacterial marker concentrations were highest in the USM, algal marker concentration in the PZ, and the fungal marker concentration in the LSM. Soil water content, inundation frequency, salinity, and temperature were identified as major environmental factors driving microbial community composition as reflected by PLFA composition clearly separating the three salt marsh zones.

Plant PLFA marker concentration changed significantly across zones, with high concentration in the USM and LSM, but low concentration in the PZ, confirming our first hypothesis. Consistent with previous studies these results indicate comparatively low plant productivity in the PZ with high loss of detrital material due to tidal movement (Bouchard et al., 1998; Bouchard & Lefeuvre, 2000). Similar to plant PLFA markers, the concentration of bacterial PLFA markers increased from the PZ to the LSM to the USM, again consistent with our first hypothesis. In both sediment depths Gram-positive bacteria were dominating indicating that mainly complex resources resistant to decomposition such as lignocellulose are processed (Fanin et al., 2019). The presence of litter containing complex compounds such as lignin may have contributed to the dominance of Gram-positive bacteria in the USM and LSM. Furthermore, the lack of grazing by ungulates at our study site may have contributed to the dominance of Gram-positive bacteria, as grazing typically increases root exudates favoring Gram-negative bacteria (Ford et al., 2013). However, in salt marshes Gram-negative bacteria may also produce lignocellulose-degrading enzymes (Leadbeater et al., 2021). As predicted by our first hypothesis the cy/pre ratio was highest in the USM. The high soil temperature, lack of inundation, reduced water content, and high salinity of the USM in July 2019 points to frequent evaporation of soil water, resulting in increased salinity (Meier et al., 2020a, 2020b; Meier, Thölen, Lohmus, et al., 2020; Pennings & Bertness, 2001), and this supports our assumption that high temperature and more frequent droughts function as stress for Gram-negative bacteria. High cy/pre ratio in the LSM and PZ in the upper sediment depth in July and October also point to increased stress for Gram-negative bacteria in summer and autumn due to high temperature and increased drought. Also supporting our first hypothesis, the mono/sat ratio as an indicator of substrate availability and aerobic microbial activity was highest in the USM. In addition, in the 0–5-cm sediment depth it increased gradually from the PZ to the LSM to the USM. Contrasting the USM and LSM, in the PZ only small amounts of dead organic matter such as algal wrack was present. In the PZ, both organic matter and bacteria were found to be depleted in ^{13}C relative to *Spartina* litter as C4 plant indicating the use of allochthonous

marine material (Boschker et al., 1999; Bouillon et al., 2005; Middelburg et al., 1997). In addition, anoxic conditions in the PZ likely contributed to lower bacterial biomass than in the LSM and USM (Buth, 1987; Howarth & Hobbie, 1982).

While overall low, the concentration of the fungal marker 18:2ω6.9 varied significantly across salt marsh zones being highest in the LSM. This was also reflected by the fungi/bacteria ratio, which followed the same pattern, supporting our first hypothesis. The generally low fungal marker concentration suggests that bacteria rather than fungi dominate in salt marsh soil. However, due to their lignocellulose degrading enzymes, fungi may be essential in contributing to the breakdown of salt marsh plant material (Calado & Barata, 2012; Calado et al., 2019). Presumably, fungi only dominate on plant litter material, whereas in soil, bacteria dominate (Benner et al., 1984; Calado et al., 2019; Leadbeater et al., 2021). The review by Calabon et al. (2021) suggests up to 10 fungi genera associated with *Atriplex*, but Wadden Sea vascular plant and fungi associations are understudied. In the PZ, anoxic conditions likely contributed to the low fungal biomass (Calado & Barata, 2012; Calado et al., 2019), as well as displacement of fungi-bearing litter material. However, the lower fungal marker concentration in the USM than in the LSM remains difficult to explain.

Algal marker concentrations also varied significantly across salt marsh zones. Consistent with our first hypothesis, concentrations were high in the LSM and PZ, and lower in the USM. The presence of the diatom markers 14:0 and 20:5ω3,6,9,12,15, and the green algae marker 16:3ω3 indicates that algae form an important component of the microbial community in each of the three salt marsh zones. Furthermore, the concentration of 20:5ω3,6,9,12,15 in the PZ strongly exceeded that in the LSM and PZ indicating high abundance of diatoms. Given the importance of diatoms in the stabilization of tidal flat sediments (Holland et al., 1974; Hope et al., 2020) and their addition to salt marsh soils with the tide (Redzuan & Underwood, 2020; Scholz & Liebezeit, 2012a), their high abundance in the PZ underlines their key role in frequently inundated salt marsh zones. The reduced presence of 20:5ω3,6,9,12,15 in the LSM and USM likely reflects a shift toward the dominance of heterotrophic microorganisms at higher elevation in the salt marsh. Presumably, also due to high inundation frequency, the flagellate marker 22:6ω3,6,9,12,15,18 only occurred in the PZ. Flagellates are likely to depend heavily on suspended organic marine material (Heijden et al., 2019). However, some of the markers discussed above may have alternative sources including, for example, 14:0, which can also be produced by macroalgae (Biandolino & Prato, 2006; Fleurence et al., 1994; Johnson & Calder, 1973). Furthermore, in terrestrial systems, 14:0 is used as a bacterial marker (Bossio & Scow, 1998; Pollierer et al., 2015). In addition, while diatoms are the dominant producer of 20:5ω3,6,9,12,15,18 (Goutx et al., 2005; Léveillé et al., 1997; Scholz & Liebezeit, 2013; Zhukova & Aizdaicher, 1995), Collembola such as *Folsomia candida* and *Proisotoma minuta* also biosynthesize this marker (Chamberlain et al., 2004, 2006), but this is likely to be of minor importance.

Season

As indicated above, overall, Season only moderately affected PLFA marker concentration and marker ratios. As a main effect, only the algal marker and the mono/sat ratio varied significantly with Season, but the interaction between Season and Zone was also significant for the fungal marker, the fungi/bacteria ratio, and the mono/sat ratio. Furthermore, the interaction between Season and Depth as well as the three factor interaction between Season, Depth, and Zone was significant for the cy/pre ratio.

The fact that the vascular plant marker did not vary significantly with Season disproved our second hypothesis and indicates that the input of vascular plant material to the salt marsh sediments is rather constant in time. Despite the concentration of the bacterial marker not significantly changing with season, the mono/sat ratio varied significantly with season, but the effect differed between zones. In the LSM, the ratio was highest in April, declined in July, and then increased again in October. High loss of detritus in the LSM due to tidal movements (Bouchard et al., 1998; Bouchard & Lefeuvre, 2000) and increased inundation frequency in July 2019 likely contributed to this change, with accumulation of litter contributing to the increase in October (Bossio & Scow, 1998; Meier et al., 2020b). In contrast, in the USM, the ratio was highest in April and steadily declined until October. The high ratio in April suggests an increase in aerobic activity and presence of resources, potentially related to the input of allochthonous organic matter by winter storm tides (Bossio & Scow, 1998; Bouchard et al., 1998; Bouchard & Lefeuvre, 2000). Reductions in the ratio later in the year are likely linked to high salinity and low water content in summer, and increased inundation frequency in October. Overall, changes in the mono/sat ratio appear to be linked to resource input, water content, and salinity suggesting that the response is more complicated than we hypothesized. Changes in the cy/pre ratio with season were complex and depended on both salt marsh zone and depth. In contrast to our second hypothesis, the cy/pre ratio only increased in the LSM and PZ. In July it increased strongly, coinciding with increased inundation frequency of both the LSM and PZ, likely reducing the redox potential of the soil (Bossio & Scow, 1998). In contrast, the cy/pre ratio in the USM remained relatively constant in time, suggesting that Gram-negative bacteria in the USM remain little affected, pointing to rather constant redox potential conditions across seasons. Overall, contrary to our second hypothesis, the strongest abiotic factors affecting Gram-negative bacteria appear to be anoxia due to water logging with this being restricted to the LSM and PZ.

Marker PLFAs for algae were the only signal which varied significantly with season as the main factor, confirming our second hypothesis. Concentration across seasons was highest in April and dropped by approximately 15% to a similar level in July and October. This conforms to earlier reports that the biomass of microalgae peaks in spring (Scholz & Liebezeit, 2012b) and that of macroalgae drops in autumn (Kolbe et al., 1995).

The fungal PLFA marker also varied significantly with Season, but the variations differed between salt marsh zones. Contrasting the USM and PZ, in the LSM the fungal marker peaked in July, potentially due to reduced competition with bacteria, but the pattern needs

further investigation. In the PZ it was inverse, with the fungal marker being lowest in July and increasing in autumn. Studies on *Spartina* spp. indicate that while leaves remain attached fungi dominate, but once they enter the sediment bacteria take over (Calado & Barata, 2012; Calado et al., 2019; Castro & Freitas, 2000; Newell et al., 1996). The incorporation of plant material heavily colonized by fungi may have contributed to the increased fungal marker in the PZ in autumn. However, bacterial stress indicators (cy/pre and mono/sat) in the PZ in October suggest reduced rather than increased competition with fungi. In contrast, in the USM, the fungal marker concentration was lowest in October, possibly reflecting a declining input of plant residues into the soil of the USM until autumn. Generally, the fungi/bacteria ratio followed the seasonal pattern of the fungal marker suggesting that seasonal dynamics were driven by changes in fungal rather than bacterial biomass. However, as a note of caution, we did not fully capture seasonal variations as we did not sample in winter and, therefore, may have missed variations due to low temperature and winter storms.

Sediment depth

Sediment depth was the strongest factor affecting microbial community composition of the studied salt marsh soils. Most of the PLFA markers were approximately halved in the 5–10-cm compared to the 0–5-cm sediment depth, and bacteria became more dominant deeper in soil, as indicated by the fungi/bacteria ratio. Overall, this supports our third hypothesis that due to reduced resource availability and redox potential the biomass and activity of microorganisms are declining with sediment depth.

The reduction in the bacteria marker concentration by about 50% in the 5–10-cm compared to the 0–5-cm sediment depth across salt marsh zones suggests that plant roots only play a minor role in oxygenating deeper sediment depths (Armstrong et al., 1985; Buth, 1987). The stress indicators mono/sat and cy/pre ratios also reflect increased oxygen limitation in deeper soil, supporting our third hypothesis. Across sediment depths both indicators were generally highest in the USM followed by the LSM and PZ, presumably reflecting the general decline in oxygenation at lower salt marsh zones. However, the decrease was least pronounced in the PZ, suggesting reduced impact of oxygen stress. This agrees with previous indications of anaerobic bacteria dominance and anaerobic breakdown of detritus in the PZ (Bossio & Scow, 1998; Howarth & Hobbie, 1982; Wixon & Balser, 2013). In the upper sediment depth the cy/pre ratio increased during the year in the LSM and PZ, particularly between April and July, whereas it remained constant in the USM. The changes in the PZ and LSM coincide with a stark increase in inundation frequency in these salt marsh zones in 2019 (Meier et al., 2020b), presumably resulting in waterlogging of the upper sediment depth reducing the redox potential.

The decrease in the fungal marker concentration with sediment depth presumably reflects both reduced oxygen and plant resource availability deeper in soil (Calado & Barata, 2012; Padgett et al., 1986, 1989). The decrease in fungi with sediment depth was also reflected

in the decrease in the fungi/bacteria ratio indicating that the decline in fungi is more pronounced than that of bacteria.

Algal marker concentrations also declined with sediment depth, presumably reflecting light limitation. Among the algal PLFA markers, the reduction was strongest for the diatom marker 20:5ω3,6,9,12,15, which was reduced by 95% (PZ), 100% (LSM), and 97% (USM) in the 5–10-cm compared to the 0–5-cm sediment depth. Epipelic diatoms may vertically migrate to the surface of the soil during low tide to photosynthesize (Cartaxana et al., 2016; Redzuan & Milow, 2019), thereby actively avoiding burial. In contrast, the green algae marker 16:3ω3,6,9 was only reduced by 40% (PZ), 47% (LSM) and 28% (USM) in the 5–10-cm compared to the 0–5-cm sediment depth. The fact that on average algal marker concentrations in the 5–10-cm depth still were about half of that in the 0–5-cm sediment depth presumably reflects the burial of green algal material in marine sediment due to tidal forces (Buchan et al., 2003; Currin et al., 1995). Buried organic matter probably decays slowly as has been shown for *S. anglica* roots (Hemminga et al., 1988), which is likely related to reduced decomposition of complex plant compounds such as lignocellulose at low oxygen conditions deeper in the sediment (Howarth & Hobbie, 1982). However, as discussed previously, some of these marker PLFAs may have alternative sources, which requires further investigation.

Conclusion

Overall, this study provided insight into the spatial and temporal dynamics of the structure and functioning of the soil microbial community in salt marsh soils. The results highlight strong variations among salt marsh zones and with sediment depth, both related to variations in abiotic conditions, in particular, not only inundation frequency-associated water logging and salinity but also temperature. In contrast, seasonal variations are much less pronounced and limited to algae and indicators of bacterial substrate availability (mono/sat ratio) as well as fungi and the fungi/bacteria ratio, but the latter depended on salt marsh zone. Changes in algae markers among seasons reflect changes in inundation frequency. The overall stability in PLFA patterns in time indicate little influence of temperature on the microbial communities. Instead, spatial dynamics were the strongest factor—both across vertical (sediment depth) and horizontal scales (salt marsh zones)—indicating a microbial community regulated by inundation frequency and the associated abiotic conditions including water content, salinity, and oxygen availability. These findings give insights into the dynamics of microbial communities of Wadden Sea salt marshes and associated functions, which is urgently needed in the face of global change and the potential of Wadden Sea salt marshes for blue carbon sequestration.

Acknowledgements

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Chapter 3 - Channelling of basal resources and use of allochthonous marine carbon by soil arthropods of the Wadden Sea salt marsh

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Abstract

Salt marshes are located at the border between the marine and terrestrial system. Because they are formed as sediment accumulates, they comprise a gradient of shore height with differing inundation frequencies and associated abiotic soil conditions. Along this gradient both autochthonous vascular plant resources, as well as allochthonous marine algal or detrital resources are available, with the availability of both varying with season and salt marsh zone. However, little is known on the importance of either resource for the soil animal food web. We investigated both spatial and temporal resource use of the soil macro- and mesofauna of the salt marsh using neutral lipid fatty acids (NLFAs). Generally, irrespective of season and zone the soil animal food web predominantly relied on carbon originating from autochthonous vascular plants and associated bacteria and fungi. Although being only minor, allochthonous resources of marine origin contributed to soil food web nutrition across salt marsh zones and seasons. The contribution of algae to soil food web nutrition depended on inundation frequency and season, i.e. algal productivity. Overall, the results demonstrate that the salt marsh soil fauna in large relies on autochthonous resources originating from vascular plants, with the contribution of allochthonous marine resources being only minor and restricted to few taxa.

Introduction

Studying trophic interactions allows insight into the channelling of energy through food webs (Terborgh & Estes 2010). This is important for identifying the basal resources food webs rely on and their channelling to higher trophic levels. In soil food webs basal resources typically comprise dead organic matter and associated microorganisms, with the dead organic matter originating predominantly from the local vascular plant community, i.e. autochthonous resources (Wardle & Yeates 1993, Scheu et al. 2005, Zieger et al. 2017). However, allochthonous resources may also form an important component of the nutrition of belowground food webs (Mueller et al. 2020). Allochthonous resources are defined as resources, which are not produced in the habitat consumers live in, typically originating from adjacent, more productive habitats (Adin & Riera 2003, Ingimarsdóttir et al. 2014, Neres-Lima et al. 2017). Habitats relying on allochthonous resources include glacier forelands (Ingimarsdóttir et al. 2014), streams (Neres-Lima et al. 2017) and intertidal systems (Korobushkin et al. 2016, Mueller et al. 2019). Intertidal systems are receiving allochthonous marine resources carried in by the tide and consequently benefit from this additional input.

The European Wadden Sea is one of the largest intertidal systems in the world, stretching from the Netherlands across Germany into the western coastline of Denmark (Reise et al. 2010, Kabat et al. 2012). The Wadden Sea is characterized by high exchange between riverine, marine and terrestrial resources, and is heavily influenced by currents (Lastra et al. 2008, Reise et al. 2010). The size of the Wadden Sea mudflats is owed to barrier islands, protecting them from the open ocean (Reise et al. 2010, Balke et al. 2017). Salt marshes lie at the interface between the marine mudflats and the dunes that occur at higher elevations and towards the open marine side of the islands. Because of their location, salt marshes are subject to calm currents and a gradual build-up of soil (Chapman & Steers 1958, Pennings & Bertness 2001). This gradient causes changes in flooding frequency, salinity and oxygen availability in the soil (Hedges & Oades 1997, Dinter 2018). The resulting habitat forms three distinct vegetation zones, depending on the shore height above the mean high water level (MHWL). The upper salt marsh (USM) is located >35 cm above MHWL and dominated by *Elymus athericus* (*Elytrigia atherica*) with a soil salinity of 5 to 20 ‰. The lower salt marsh (LSM) is situated between 0-35 cm above MHWL and dominated by *Atriplex portulacoides* and *Puccinellia maritima* with a soil salinity between 20 and 26 ‰. The pioneer zone (PZ) lies below the MHWL and is flooded about twice a day for six hours resulting in a soil salinity of 26 to 32 ‰; it is dominated by *Salicornia stricta* and the C4 plant *Spartina anglica* (Balke et al. 2017, Dinter 2018, Winter et al. 2018). In addition, macroalgae, such as *Rhizoclonium riparium, Fucus* sp. and *Ulva* sp., inhabit the PZ (Balke et al. 2017, Winter et al. 2018).

The tidal range of the Wadden Sea is subject to seasonal changes, related to hydrodynamic conditions and wind (Bartholomä et al. 2009). This leads to the accumulation of detrital material from the lowest salt marsh zones and marine systems in drift lines across the USM (Bouchard et al. 1998, Bouchard & Lefeuvre 2000). Due to the high production of phytoplankton in the shallow regions of the North Sea (Reid et al. 1990, Scholz & Liebezeit 2012) and the presence of macroalgae in shallow coastal waters, allochthonous resources are deposited in the salt marsh. Some studies have indicated the importance of edaphic algae for salt marsh marine invertebrates (Sullivan & Moncreiff 1990), suggesting that microalgae contribute heavily to secondary production due to their labile structure compared to *S. anglica* and other vascular plants (Buffan-Dubau & Carman 2000). In addition, macro- and microalgae as well as diatoms have been shown to be used by macroinvertebrates of French salt marshes (Riera et al. 1999, Adin & Riera 2003). However, these studies focused on marine invertebrates and not the terrestrial soil fauna.

Terrestrial soil fauna is separated into meso- (0.1 - 2 mm) and macrofauna (2- 20 mm), both are essential in the breakdown of detrital material, nutrient cycling and soil structure (Scheu et al. 2005, Sofo 2020). Mesofauna include mites – both predators and detritivores – as well as Collembola feeding on detritus, fungi, bacteria, algae and nematodes (Schneider et al. 2005, Heidemann et al. 2011, 2014, Ferlian et al. 2015, Sofo 2020). In Wadden Sea salt marshes mesofauna diversity declines towards the mudflats, favouring

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halobiont species such as the oribatid mite *Zachvatkinibates quadrivertex* (Polderman 1974, Weigmann 2009). Similarly, soil macrofauna diversity declines and thereby their role in soil structure formation and interaction with soil microorganisms and plants (Scheu et al. 2005, Sofo 2020). Despite the importance of soil fauna, little is known about their community structure and functioning in the salt marsh, and little is known on the role of allochthonous resources and the channelling of basal resources through the soil animal food web.

Neutral lipid fatty acids (NLFAs) of consumers carry information on their diet and this has successfully been used in the marsh to trace the diet of Wadden Sea salt marsh Oribatida (Winter et al. 2018). Indicating differential use of resources based on the zone where the species was sampled and resultant resource availability (Winter et al. 2018). The NLFA method is based on 'dietary routing', i.e. the incorporation of fatty acids into the deposit fat of animal consumers without major change (Ruess et al. 2005, Pollierer et al. 2010, Ruess & Chamberlain 2010, Eitzinger et al. 2013). Based on specific marker NLFAs the method allows to trace the channelling of basal resources, such as fungi, bacteria and plant detritus, through the soil animal food web (Pollierer et al. 2010, Traugott et al. 2013). Furthermore, specific markers for algae including diatoms may allow to trace the incorporation of resources of marine origin (Ruess & Müller-Navarra 2019).

Using NLFA analysis, we investigated the channelling of basal resources through the soil animal food web across salt marsh zones (USM, LSM and PZ) and seasons (spring, summer and autumn), thereby inspecting spatial as well as temporal dynamics. We hypothesised that (1) the contribution of vascular plants, bacteria and fungi as basal resources of soil taxa increases with shore height, because of the increasing aerobic environment with shore height. Conversely, we hypothesized the contribution of algae as basal resource to increase with decreasing shore height due to increased input with inundation frequency. Further, we hypothesized (2) the marker concentration of soil animal taxa to vary with season due changes in temperature and inundation frequency affecting resource availability, with algal markers increasing in summer, due to high temperature and light conditions, and fungi, bacteria and plant markers increasing in autumn, due to increased input of plant litter material.

Methods

Sampling

Samples were taken along five transects spanning across the USM, LSM and PZ on the island of Spiekeroog (Wadden Sea National Park, Germany; 53°45'2"- 53°47'1"N, 7°40'0"-7°49'1"E) in April (spring), July (summer) and October (autumn) 2019. Within each transect, one soil core of 20 cm diameter was taken per zone and separated into two layers (0-5 and 5-10 cm depth). Cores were stored in plastic containers and kept at ambient temperature until soil fauna extraction at the University of Göttingen. Soil fauna was extracted using heat (Kempson et al. 1963); animals were extracted into a 1:1 mixture of ethylene glycol and water. Once extracted, the animals were filtered through 45 μm gauze, flushed with water, placed into 70 % ethanol and stored at -20°C. Animals were identified under a stereomicroscope, macrofauna was identified to species, whereas mesofauna species were grouped to higher taxonomic units to gain sufficient material for lipid extraction. To achieve sufficient animal tissue material soil horizons and samples were in part pooled. The identification followed (Weigmann 2006) for oribatid mites and (Schaefer 2018) for the rest of the fauna. Sorting was done within 2-4 weeks after extraction to minimize the loss of lipids due to placement in ethanol; prior to storage the ethanol was evaporated and the animals placed at -20°C (Zieger & Scheu 2018).

Lipid extraction

Animals were placed into 10 ml tubes and extracted as described in Haubert et al. (2004). In brief, animals were shaken overnight in 5 ml extraction solution [chloroform/methanol/0.05 M phosphate buffer (pH 7.4), 1:2:0.8]. Then, the extract was transferred to fresh tubes with additional 2.5 ml extraction solution and shaken for 1 h. Chloroform and distilled water were added (0.8 ml each), vortexed and centrifuged at 1500 rpm at 7-10 °C for 5 min. The top phase was removed, the remaining phase was fractionated in silica columns (Chromabond® SiOH (3 ml), Machery-Nagel™, Düren, Germany) and eluted with 1.5 ml then 2 ml chloroform. Samples were then dried at 30 °C in a vacuum centrifuge before saponification with 1 ml of a sodium hydroxide – methanol solution (45 g NaOH, 150 ml CH_3OH, 150 ml distilled H_2O) at 100°C for 30 min. Followed by methylation with 2 ml HCl – methanol solution (325 ml 6.0 N HCl, 275 ml CH_3OH) at 80°C for 10 min. Finally neutral lipids were extracted into hexane – methyl tertiary butyl ether (1:1) and washed with liquid NaOH (10.8 g NaOH, 900 ml distilled H_2O). Lipids were stored in 1.5 ml GC-vials, capped and stored at -20°C until gas chromatography.

Lipids were separated using a gas chromatograph (Clarus 500, PerkinElmer Corporation, Norwalk, USA) equipped with an Elite-5 capillary column (30 m x 0.32 mm i.d., film thickness 0.25 mm, PerkinElmer, Norwalk, USA). The analysis started with 60°C for 1 min, increased by 30°C/min to 160°C; followed by 3°C/min increase to 260°C; injection temperature was 250°C, with helium as carrier gas. Lipids were identified by retention time, based on standard mixtures composed of 37 FAMEs (Fatty Acid Methyl Esters) ranging

between chain lengths of C11-C24 , as well as 26 BAMEs (Bacterial Acid Methyl Esters, Sigma-Aldrich, St Louis, USA) and algal standards for 16:2ω6,9 and 16:3ω3,6,9 (Larodan AB, Solna, Sweden) (Buse et al. 2013). Lipid concentration was calculated as percentages. Only lipids contributing >1 % of total were included in the analysis. Lipids were aggregated into six NLFA marker groups including algae (14:0; 16:2ω6,9; 16:3ω3,6,9 and 20:5ω3,6,9,12,15), animals (20:1ω9), bacteria (a15:0; i15:0; i16:0; 16:1ω7; i17:0 and 18:1ω7), fungi (18:2ω6,9), vascular plants (18:1ω9 and 24:0) and unspecific (13:0, 14:1, 15:0, 16:0, 17:0, 17:1, 18:0, 20:0, 20:2, 20:3ω6,9,12, 20:4ω6,9,12,15).

Statistical analyses

Percentage data of NLFAs were arcsine transformed and analysed by principal component analysis (PCA) in CANOCO 5 (Ter Braak & Šmilauer 2018) with Zone (USM, LSM, PZ), Season (April, July, October) and Species as passive (=supplementary) variables not affecting the ordination. Linear mixed-effects models were used to assess the influence of Zone and Season on lipid marker concentration (arcsine transformed data). First, variation of each marker with the fixed factors Zone and Season was analysed; Core ID nested within Transect as well as the Species were included as random factors to control for non-independency of species from the same soil core. This model included *Amischa* sp., *Archisotoma besselsi*, Mesostigmata, Staphylinidae larvae and *Talitrus saltator*. These taxa/species were selected because they were present across multiple zones and seasons and covered multiple trophic levels; they comprised 54 % of the total individuals. Subsequently, to assess changes within taxa/species across zone and season, linear mixed-effects models were run for each taxon with the factors Zone, Season and Marker (type of NLFA marker group) with CoreID nested within Transect to account for multiple samplings within the same transect. Linear mixed-effects models were run in R (4.1.0) (R. core Team 2021) using the packages emmeans (Version 1.6.2-1), lme4 (Version 1.1-27.1), car (Version 3.0-11), lmerTest (Version 3.1-3) and dplyr (Version 1.0.7).

Results

Generally, lipid concentrations contributing most to total NLFAs (including unspecific NLFAs) declined in the order plant marker 18:1ω9 (30.62 ± 13.92 %) > unspecific marker 16:0 (19.17 ± 9.33 %) > unspecific marker 18:0 (13.90 ± 10.53 %) > fungal marker 18:2ω6,9 (11.05 ± 10.90 %) > bacterial marker 18:1ω7 (8.99 ± 11.20 %) > bacterial marker 16:1ω7 (8.32 ± 11.22 %). None of the taxa contained marker NLFAs of Gram-negative bacteria. The PCA of the NLFA data explained 55.08% of the variation in the dataset and separated the USM from the LSM and PZ along the first two axes, whereas the seasons showed little separation (Figure 1).

Figure 1: Principle component analysis (PCA) of arcsine transformed concentrations of neutral lipid fatty acids (NLFAs) including zone (USM – upper salt marsh, LSM – lower salt marsh and PZ – pioneer zone), season (April – spring, July – summer and October - autumn) with species as passive variables.

The LSM and PZ were closely associated with Gram-positive bacterial marker NLFAs, whereas the USM clustered with the vascular plant (18:1ω9) and the fungal marker NLFA (18:2ω6,9). Unspecific NLFAs, including some algal markers (14:0, 16:2ω6,9 and 20:5ω3,6,9,12,15) clustered separately. Mesostigmata clustered with Gram-positive bacterial marker NLFAs of the PZ and LSM, together with Isotomidae and *A. besselsi*, whereas *U. repleta* clustered with the general bacteria marker NLFA 18:1ω7. Staphylinidae

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larvae, *Ochthebius* sp. and *T. saltator* clustered with the algal marker NLFAs 20:5ω3, 16:2ω2 and 14:0 as well as the animal marker 20:1ω9 and unspecific NLFAs 14:1 and 20:2. Onychiuridae, *D. gustavii* and *Amischa* sp. showed little association with any marker lipids, but clustered with the USM and the fungal marker 18:2ω6,9 and the plant marker 18:1ω9. Oppiidae, *Z. quadrivertex* and Oribatuloidea did not cluster with any marker lipids, but e.g., with the unspecific NLFA 20:0 separate from the other animal taxa.

Variations in individual NLFA markers across taxa between zones and seasons

The plant NLFA marker concentration across the five animal taxa, which occurred at each of the three seasons, varied with Zone and Season (significant Zone × Season interaction; $F_{4,63}$= 3.63, P = 0.010). In the USM it was highest in July and lower in April and October; in the LSM it was lowest in July and higher in April and October, whereas in the PZ it increased from April to October (Fig. 2a). Similarly, the bacterial NLFA marker concentration varied with Zone and Season (significant Zone × Season interaction; $F_{2,73}$= 3.42, P = 0.013). Unlike the plant marker, bacterial NLFA marker in the USM was lowest in July and higher in April and October; in the LSM it was highest in July and lower in April and October; in the PZ it declined from April to October (Fig. 2b). The fungal NLFA marker concentration did not differ with Zone but with Season ($F_{2,69}$= 3.32, P = 0.042); it was highest in October (12.12 ± 12.86 % of total), lower in April (7.55 ± 8.90 %) and lowest in July (6.33 ± 4.83). Further, unspecific NLFA markers also significantly varied with Season ($F_{2,62}$ = 6.82, P = 0.002); they were highest in July (41.74 ± 13.39 % of total), lower in April (36.88 ± 11.28 %) and lowest in October (30.60 ± 12.59 %). Neither algal nor animal NLFA marker concentration varied significantly with Zone or Season and were on average 3.65 ± 3.92 % and 0.21 ± 0.71 %, respectively.

Variations in NLFA markers of individual taxa

Of the taxa which were present at each of the three seasons the NLFA marker concentration only varied significantly among markers but not with Season in Coleoptera larvae, *T. saltator*, Isotomidae and Onychiuridae (Table 1). NLFA marker concentrations also did not vary with Zone in Isotomidae, Onychiuridae and *T. saltator*, which were present in at least two zones and all seasons (Table 1). In Coleoptera larvae NLFA marker concentration declined in the order unspecific marker (31.66 ± 17.00 %) > plant marker (28.13 ± 19.66 %) > fungal marker (25.84 ± 9.64 %) > bacterial (10.31 ± 9.26 %) > algal marker (4.06 ± 4.02 %). In *T. saltator* NLFA marker concentration declined in the order unspecific markers (35.36 ± 8.11 %) > plant markers (28.66 ± 5.30 %) > bacterial markers (17.48 ± 6.91 %) > fungal markers (11.62 ± 2.69 %) > algal markers (5.30 ± 3.25 %) > animal markers (1.57 ± 1.21 %). In Isotomidae NLFA marker concentrations declined in the order bacterial markers (37.01 ± 13.20%) > unspecific markers (33.97 ± 5.68 %) > plant markers (23.02 ± 12.53 %) > algal (3.36 ± 2.74 %) > fungal markers (2.63 ± 3.29 %). In Onychiuridae NLFA marker concentrations declined in the order unspecific markers (35.65 ± 12.07 %) > plant markers (32.23 ±10.26 %) > bacterial markers (17.24 ± 8.57 %) > fungal (9.32 ± 6.28 %) > algal markers (5.56 ± 7.59 %).
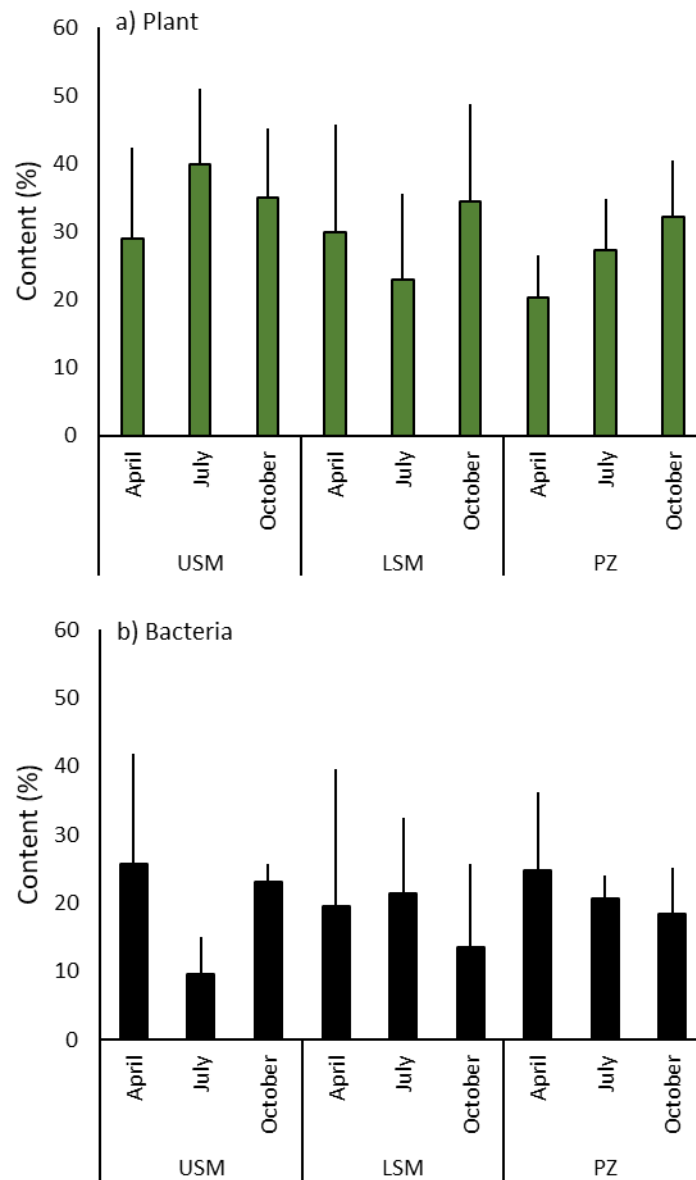
Figure 2: Variations in the neutral lipid fatty acid (NLFA) marker concentration (%) for bacteria (a) and vascular plants (b) in animal consumers averaged across consumer species with zone (USM- upper salt marsh, LSM – lower salt marsh and PZ – pioneer zone) and season (April – spring, July – summer, October – autumn); a) vascular plant NLFA marker concentration across zone and season; b) bacterial NLFA marker concentration across zone and season. Error bars denote the standard deviation.

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Table 1: Summary of Linear mixed-effects model results of NLFA marker lipids (%). including number of samples (n). F- and p-values. Not all factors and interactions could be fitted for all species/taxa beause not all species/taxa were present across all zones (USM - upper salt marsh. LSM - lower salt marsh. PZ - pioneer zone)and seasons (April - spring. July - summer. Ocotber - autumn); the seasons and zones where the species/taxa was present is given in the columns "Seasons" and "Zones". respectively.

Species	Zone	Season	n	Zone (Z) F p	Season (Se) F p	Marker (M) F p	Z x Se F p	Z x M F p	Se x M F p	Z x M x Se F p
Amischa sp.	USM / LSM	April-October / April-October	14	4.11 0.048	1.71 0.192	94.28 <0.001	1.69 0.196	7.08 <0.001	7.97 <0.001	6.84 <0.001
Archisotoma besselsi	LSM / PZ	April-October / April-October	11	7.98 0.008	0.99 0.383	205.35 <0.001	1.20 0.315	17.70 <0.001	4.75 <0.001	5.94 <0.001
Coleoptera larvae	USM	April. October	4		0.15 0.703	5.10 0.010			0.44 0.813	
Dicheirotrichus gustavii	USM	July. October	5		1.28 0.272	47.80 <0.001			8.40 <0.001	
Hypogastruridae	USM	April. October	6		0.05 0.823	24.43 <0.001			2.72 0.044	
Isotomidae	USM / LSM	April. July / July	6	2.59 0.125	0.12 0.735	30.30 <0.001	2.26 0.092		0.48 0.787	
Mesostigmata	USM / LSM / PZ	April-October	27	0.38 0.685	0.55 0.577	201.80 <0.001	0.28 0.889	2.10 0.031	1.86 0.059	1.39 0.143
Ochthebius sp.	USM / LSM	April. July / July	7	0.01 0.929	0.16 0.696	104.38 <0.001		6.79 <0.001	1.99 0.117	
Onychiuridae	USM / LSM	April. October / April. July	5	0.36 0.554	0.07 0.930	15.98 <0.001		2.61 0.060	1.38 0.266	
Oppiidae	USM	April-October	8		1.80 0.183	190.69 <0.001			2.45 0.028	
Oribatuloidae	USM	April-October	7		0.56 0.576	62.73 <0.001			0.75 0.673	
Staphylinidae larvae	USM / LSM	April. July / April-October	12	0.16 0.689	0.12 0.888	50.77 <0.001	0.03 0.866	3.29 0.01	21.08 <0.001	2.23 0.069
Talitrus saltator	USM / LSM	April-October	18	0.10 0.747	0.92 0.405	113.50 <0.001	1.25 0.293	0.71 0.621	1.90 0.059	1.69 0.099
Uropoda repleta	USM / LSM	April. October	12	0.23 0.634	0.12 0.884	144.57 <0.001	0.20 0.661	1.43 0.235	2.48 0.020	1.38 0.252
Zachvatkinibates quadrivertex	LSM / PZ	April-October / April	9	0.16 0.696	4.03 0.025	263.16 <0.001		11.10 <0.001	12.94 <0.001	

Zone

In six of the nine taxa which occurred in at least two zones and seasons NLFA marker concentrations varied with both Markers and Zone (significant Marker × Zone interaction; Table 1, Fig. 3). In *Amischa* sp., which was present in the LSM and USM, plant NLFA marker concentrations generally dominated and algae as well as animal marker NLFAs were very low or absent (Fig. 3a). By contrast, bacterial and fungal marker concentrations varied between zones with the bacterial marker increasing and the fungal marker decreasing from the LSM to the USM. In *A. besselsi*, which was present in the PZ and LSM, the unspecific markers were generally dominant, the plant NLFA marker was also high but similar in both zones, whereas animal and fungal markers were low or absent (Fig. 3b). By contrast, algal and bacterial marker concentrations were higher in the PZ than in the LSM. In Mesostigmata, which were present in each of the three zones, unspecific and plant marker were dominant. Bacterial markers were also high but similar among zones, whereas animal and algal markers were low or absent in each of the three zones (Fig. 3c). By contrast, the fungal NLFA marker increased from the PZ to the LSM to the USM. In *Ochthebius* sp., which was present in the LSM and USM, the concentrations of unspecific, plant and bacterial markers were high, with the bacterial marker differing strongly between zones, being higher in the USM than the LSM (Fig. 3d). Although concentrations of the algal and fungal marker NLFAs were generally low, the concentration of the algal marker in the USM exceeded that in the LSM, whereas the opposite was true for the fungal marker. In Staphylinidae larvae, which were present in the LSM and USM, the concentrations of unspecific and bacterial NLFA markers were dominant, but did not vary across zone. By contrast, plant and algal NLFA marker concentrations in the USM exceeded that in the LSM, whereas the opposite was true for the fungal marker (Fig. 3e). In *Z. quadrivertex*, which was present in the PZ and LSM, plant NLFA marker concentration was highest, but declined from the PZ to the LSM (Fig. 3f). Concentrations of the fungal marker was similar in the PZ and LSM, whereas the unspecific marker as well as the bacterial marker, although being generally low, increased from the PZ to the LSM. Concentrations of algal and animal markers were low or absent.
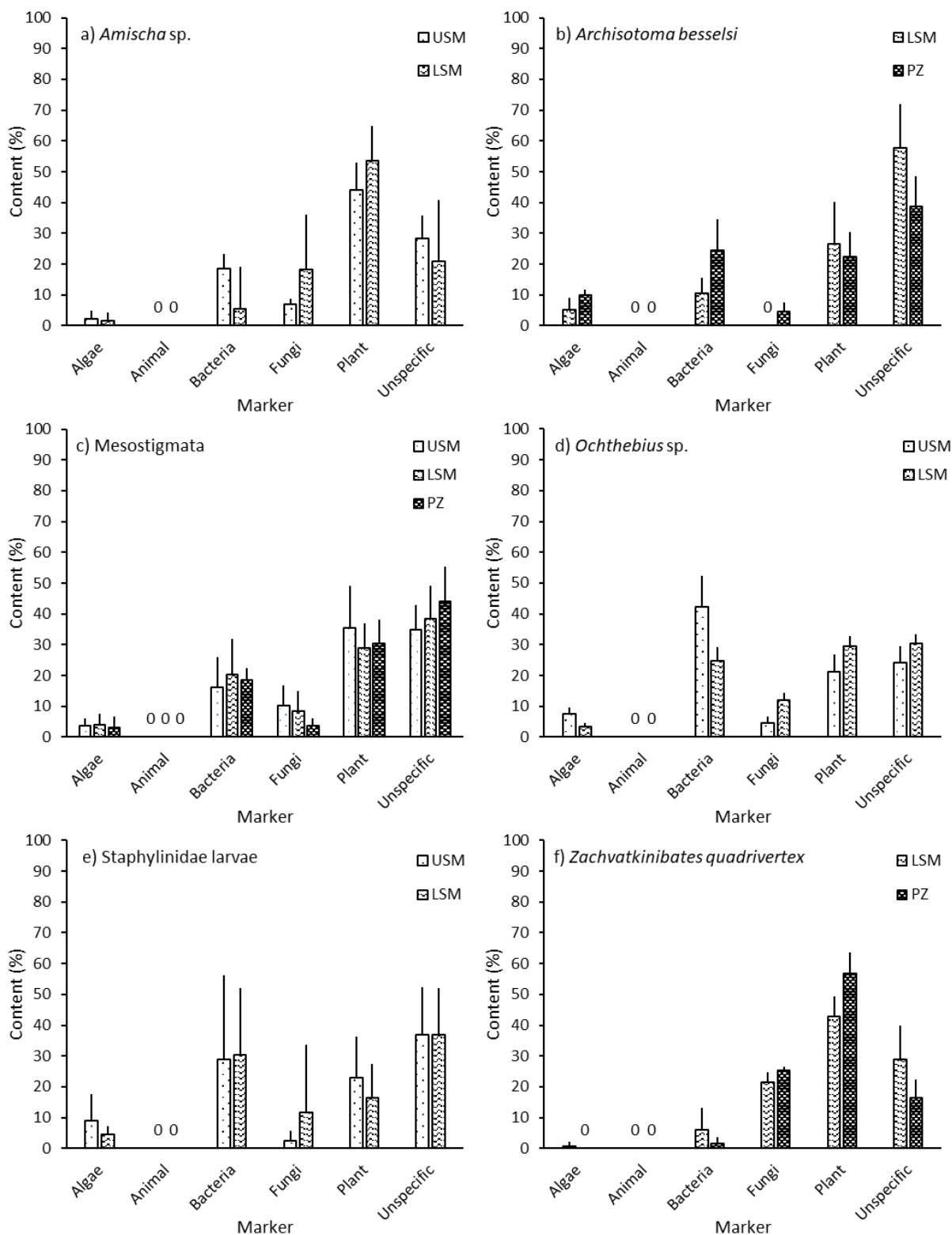
Figure 3: Variations in the neutral lipid fatty acid (NLFA) marker concentration (%) across zone (USM- upper salt marsh, LSM – lower salt marsh and PZ – pioneer zone) of a) *Amischa* sp.; b) *Archisotoma besselsi*; c) Mesostigmata; d) *Ochthebius* sp.; e) Staphylinidae larvae; f) *Zachvatkinibates quadrivertex*. Zones where taxa/species originated are indicated within the graph; error bars denote the standard deviation.

Season

In eight of the 15 taxa NLFA marker concentrations varied with Marker and Season (significant Marker × Season interaction; Table 1, Fig. 4); in two of them (*A. besselsi* and *Amischa* sp.) this depended on Zone (significant Marker × Season × Zone interaction). In *Amischa* sp. the plant marker was high throughout the seasons, whereas the bacterial marker peaked in July and the fungal marker in October (Fig. 4a); this was more pronounced in the LSM than in the USM (data not shown). The algal NLFA marker of the USM peaked in April and July, whereas in the LSM it peaked in July and October. In *A. besselsi* the plant marker peaked in April and October (Fig. 4b) and this was most pronounced in the LSM (data not shown). The algal marker was similar across seasons, whereas the bacterial marker peaked in April (Fig. 4b). In *D. gustavii* the plant marker dominated and peaked in October (Fig. 4c). Although being less abundant, the bacterial marker also peaked in October, whereas the fungal marker peaked in July. The algal marker was generally low. In Hypogastruridae plant, bacterial, fungal and unspecific marker concentrations were at a similar level, whereas algal and animal marker concentrations were low (Fig. 4d). The plant and fungal NLFA markers declined in October, whereas the bacterial NLFA marker increased. In Oppiidae plant fungal and unspecific marker concentrations dominated, whereas algal, animal and bacterial markers were low or absent (Fig. 4e). Although varying significantly with season, marker concentrations generally stayed rather constant across the three seasons. In Staphylinidae larvae plant NLFA marker increased from April to October (Fig. 4f). Bacterial and fungal marker concentrations varied strongly and in an opposite way between seasons; the bacterial marker peaked in April and was virtually absent in October, whereas the fungal marker peaked in October and was virtually absent in April and July. In *U. repleta* plant marker was generally low, whereas bacterial and unspecific markers dominated. Although varying significantly with season, the marker concentration patterns were similar across seasons. Algal, animal and fungal NLFA marker were absent. In *Z. quadrivertex* the plant marker dominated throughout the seasons, whereas concentrations of the bacterial marker were low but peaked in July (Fig. 4h). The fungal marker varied little among seasons, but generally ranked second after the plant marker. The algal and animal markers were very low or absent.
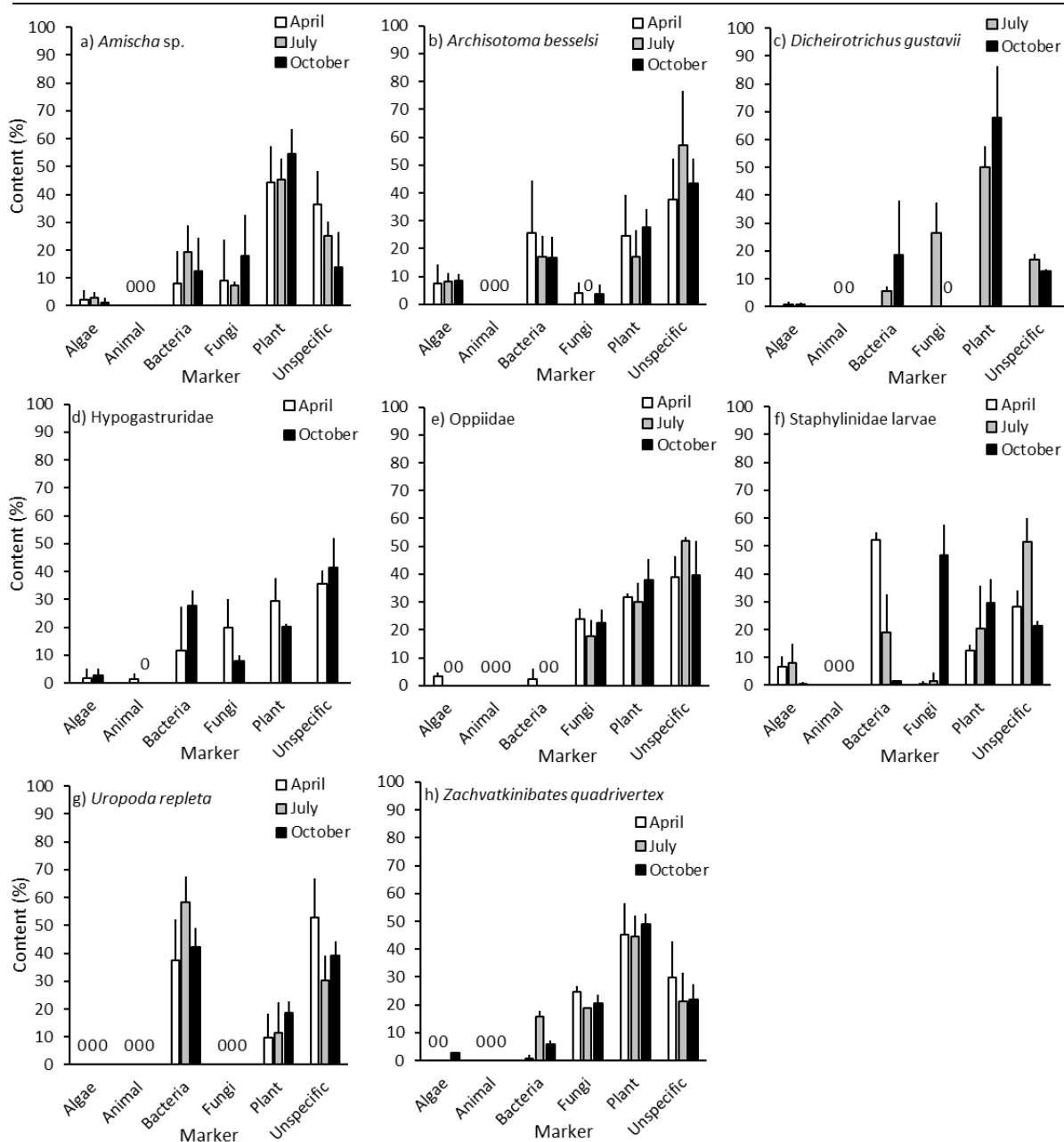
Figure 4: Variations in the neutral lipid fatty acid (NLFA) marker concentration (%) across season (April – spring, July – summer, October – autumn) of a) *Amischa* sp.; b) *Archisotoma besselsi*; c) *Dicheirotrichus gustavii*; d) Hypogastruridae; e) Oppiidae; f) Staphylinidae larvae; g) *Uropoda repleta*; h) *Zachvatkinibates quadrivertex*. Seasons where taxa/species originated are indicated within the graph; error bars denote the standard deviation.

Discussion

This study aimed at determining spatial and temporal dynamics in the channelling of basal resources through and the use of allochthonous marine carbon by the soil food web of the Wadden Sea salt marsh. Overall, NLFA markers pointed towards the dominant use of plant and bacterial resources across the studied animal taxa/species, indicating the predominant use of autochthonous resources channelled to higher trophic levels via consumption of plant detritus and bacteria. Although being less important, autochthonous plant resources in part were also channelled to higher trophic levels via fungi.

Changes in energy channelling with season and salt marsh zones across taxa

Generally, the plant NLFA marker dominated NLFA markers across salt marsh zones and seasons averaging 46.9% of total marker NLFAs (excluding unspecific NLFAs). However, it declined from the USM to the LSM to the PZ, suggesting that the use of plant-associated resources decreases with decreasing shore height supporting our first hypothesis. These findings are in line with the decline in plant marker PLFAs in soil with decreasing shore height reported by Rinke et al. (2022). However, plant biomarker NLFA concentrations in consumers also varied with season. It was generally high in autumn, but in the USM and PZ also in summer, partially supporting our second hypothesis. These patterns coincide with the seasonal biomass production of the dominant vascular plant species (Bouchard et al. 1998, Morriss & Jensen 1998, Bouchard & Lefeuvre 2000), indicating a corresponding increase in the channelling of plant-based resources through the salt marsh food web. However, seasonal patterns depended on salt marsh zone and minimum values in the LSM in July suggest that in this zone less plant detritus enters the belowground system in summer. In the LSM and PZ plant litter material is likely displaced by tides, limiting its availability for animal consumers (Bouchard et al. 1998, Bouchard & Lefeuvre 2000).

Although being generally high across salt marsh zones and seasons, averaging 30.7 % of total marker NLFAs (excluding unspecific NLFAs), bacterial NLFA marker concentration of soil animals was highest in the PZ, implying that bacteria-associated resources increase with inundation frequency. This contrasts the results of Mueller et al. (2020) suggesting that soil bacteria density declines at lower shore height, but is conform to earlier findings that generally bacteria dominate the decomposer system in salt marsh soils in particular in the PZ (Benner et al. 1984, Calado & Barata 2012, Calabon et al. 2021, Leadbeater et al. 2021, Rinke et al. 2022). Although being generally high, bacterial NLFA marker concentration in each zone varied with season indicating changes in bacterial prey with season. Notably, these changes were most pronounced in the USM, where bacterial marker NLFAs dropped in July indicating reduced use of bacteria as prey in summer. This drop coincided with high temperature, salinity and reduced water content at our study site (Meier et al. 2020c a, Pieck et al. 2021). Presumably, these factors limited the accessibility of bacteria for consumers in the USM. By contrast, in the LSM bacterial NLFA marker concentration remained at a similar high level in April and July but declined in autumn. This suggests that in the LSM the accessibility and consumption of bacteria in summer benefited

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from more frequent inundation, whereas in autumn reduced channelling of bacterial resources might be related to increased anoxia (Meier et al. 2020b, Pieck et al. 2021) as well as displacement of litter material by frequent tides (Bouchard et al. 1998). In the PZ bacterial NLFA marker concentration peaked in April and then continuously declined until October. The factors responsible for the low bacteria marker concentration in autumn might be the same as in the LSM. High channelling of bacteria in spring might reflect that the colonization of litter by bacteria increases during decomposition and this coincides with more intensive grazing by bacterivorous nematodes as shown e.g., for litter of *S. anglica* (Alkemade et al. 1994, de Mesel et al. 2003). This may generally explain the high channelling of bacterial resources in spring in each of the salt marsh zones. Displacement of bacterial grazers by tides, as shown for nematodes (Alkemade et al. 1994, de Mesel et al. 2003), may also have contributed to the low channelling of bacterial resources in autumn.

The fungal NLFA marker concentration was generally low and averaged 16.2 % of the total marker NLFAs (excluding unspecific NLFAs). Contrasting the plant and bacterial NLFA markers, it did not vary significantly between salt marsh zones but among seasons. It was highest in October and about 50% lower in April and July, suggesting maximum channelling of fungi to higher trophic levels in autumn. This contrasts low fungal marker PLFAs in soil of the USM and LSM in autumn as reported by Rinke et al. (2022), but coincides with the peak of dead organic matter of salt marsh plants in autumn (Morriss & Jensen 1998, Bouchard & Lefeuvre 2000), likely boosting saprotrophic fungi (Calado & Barata 2012, Calado et al. 2019). Therefore, increased channelling of fungi in autumn likely reflects the input of plant litter material with associated saprotrophic fungi contributing to the predominant use of autochthonous resources by the salt marsh food web.

Algal and animal NLFA marker concentrations were generally low and accounted for only 5.9 % and 0.34 % of total marker concentration (excluding unspecific NLFAs), respectively, suggesting that allochthonous and animal-based resources do not contribute substantially to the basal resources for the salt marsh soil fauna.

Overall, the results indicate that autochthonous vascular plant material forms the dominant basis of the salt marsh soil food web. Furthermore, bacteria are the dominant decomposers in the salt marsh soil, whereas fungi become more important with the input of plant litter. This suggests that plant resources are either channelled directly to animal consumers via primary decomposers feeding on plant litter or via bacteria colonizing plant organic matter and being consumed by secondary decomposers, as well as to a lesser degree by fungi associated with plant litter at early stages of decay again being channelled to higher trophic levels by secondary decomposers.

Spatial changes in energy channelling through salt marsh taxa
The channelling of energy from basal resources into animal consumers varied strongly among the taxa/species studied. Conform to the general pattern discussed above, in most

taxa/species the plant NLFA marker dominated, but in a number of taxa/species the bacterial marker exceeded the plant marker concentration including *Ochthebius* sp., Staphylinidae larvae and *Uropoda repleta*. Although the general pattern of energy channelling within taxa/species remained consistent across seasons and salt marsh zones, i.e. the dominant NLFA marker and the association of species to energy channels typically remained the same across seasons and salt marsh zones, in each of the 15 taxa/species studied, except Coleoptera larvae, Isotomidae, Onychiuridae, Oribatuloidae and *Talitrus saltator*, NLFA marker composition varied significantly with season, saltmarsh zone or their interaction.

Conform to the pattern across animal taxa/species, the plant NLFA marker declined with decreasing shore height in *Archisotoma besselsi*, Staphylinidae larvae and Mesostigmata, again pointing to the decline in vascular plant resources with increased inundation frequency (see above).

Contrasting the general pattern of increased channelling of bacterial resources with decreasing shore hight, the bacterial NLFA marker declined at lower elevations in *Amischa* sp. and *Ochthebius* sp., suggesting that the access of bacterial resources in these species declines with inundation frequency, which is consistent with changes in bacterial PLFAs across salt marsh zones (Rinke et al. 2022). Presumably, these comparatively large species are more sensitive to removal of litter due to frequent inundations, which detrimentally affects their access to bacterial resources.

Fungal NLFA patterns were not consistent among taxa/species, but conform to the general pattern they were uniformly low. Contrasting the overall uniform use of fungal resources across salt marsh zones, fungal NLFA marker concentrations declined with decreasing shore height in Mesostigmata, whereas in *Amischa* sp., Staphylinidae larvae and *Ochthebius* sp. fungal NLFA marker increased in the LSM, matching soil fungal PLFA patterns (Rinke et al 2022).

The algal NLFA marker did not change in a consistent way with shore height in the studied taxa/species, presumably the lack of pattern is related to the fact that its concentration was generally low across the taxa studied. The same applied to animal NLFA markers.

Overall, changes in NLFA patterns among the taxa/species studied reflect species specific trophic niches linked to changes in the availability of autochthonous litter resources and their predominant processing by bacteria in soil.

Temporal changes in energy channelling through salt marsh taxa
In eight of the 15 taxa/species studied NLFA marker concentrations varied with season. The dominant channel of six of these taxa/species was the plant and bacterial channel, whereas the fungal channel predominated in Oppiidae and *Z. quadrivertex.* Conform to the general pattern, plant NLFA marker concentrations peaked in October in *Amischa* sp. *A. besselsi*, *D. gustavii*, Staphylinidae larvae and *U. repleta* suggesting that these taxa/species benefit from the increased input of vascular plant litter in autumn. In fact, the increase coincides

with peak senescent biomass of dominant plant species (Morriss & Jensen 1998, Bouchard & Lefeuvre 2000).

Bacterial resource channeling in the 15 taxa/species studied also differed strongly among taxa/species. The differential channeling of bacterial-based resources also contributed to the fact that the bacterial channel across taxa varied with both salt marsh zone and season (see above). Corresponding with the general pattern of the USM, high bacterial NLFA marker concentrations in *D. gustavii* and Hypogastruridae in October may be associated with peak senescent biomass of dominant plant species in October (Bouchard et al. 1998, Bouchard & Lefeuvre 2000). Contrasting the general pattern, high bacterial NLFA marker concentrations in July for *Amischa* sp., *Z. quadrivertex* and *U. repleta* may be related to low soil water content and high salinity in the USM and LSM at that time (Meier et al. 2020c a, Pieck et al. 2021), which may have contributed to increased plant litter input and thereby boosted channeling of bacterial resources. Similarly, high bacterial NLFA marker concentrations in April for Staphylinidae larvae and *A. besselsi* may be related to the high proportion of senescent compared to living matter of dominant plants in April (Bouchard et al. 1998, Morriss & Jensen 1998, Bouchard & Lefeuvre 2000).

Fungal NLFA marker concentration also varied across seasons, but not consistently among taxa/species. Reflecting the pattern across taxa it peaked in October in *Amischa* sp. and Staphylinidae larvae , whereas in *A. besselsi* it peaked in April and October and in *D. gustavii* in July. Overall, this suggests that senescent plant materials colonized predominantly by fungi (Calado & Barata 2012, Calado et al. 2019, Calabon et al. 2021) are available throughout the year but their input peaks in October.

Although being generally low, algal NLFA marker concentrations in Oppiidae, *Amischa* sp. and Staphylinidae larvae peaked in April and July and this is consistent with the peak in algal PLFAs early in the year (Rinke et al 2022). By contrast, in *Z. quadrivertex* the algal NLFA marker peaked in October, presumably this is due to frequent inundations enriching the LSM soil with microalgae as shown by Bouchard et al. (1998). The presence of algal NLFA markers across seasons in *A. besselsi, D. gustavii* and Hypogastruridae, however, also documents that algae contributed to the diet of salt marsh consumers throughout the year, but their contribution remained generally low.

Conclusion

We investigated spatial and temporal changes in the channelling of basal resources through the soil food web of the Wadden Sea salt marsh. Overall, the results indicate that autochthonous plant litter resources and associated bacteria form the dominant basal resources of the Wadden Sea soil food web. Fungi were generally less important and the channelling of fungal resources was restricted to certain taxa/species. In six out of the 15 taxa/species NLFA marker concentrations varied significantly among salt marsh zones, indicating pronounced spatial variations in the channelling of basal resources through the salt marsh food web. Generally, plant and bacterial NLFA marker concentrations declined

towards lower salt marsh zones. Algal NLFA marker concentrations were generally low and did not vary consistently among taxa between zones and the same was true for animal NLFA markers. Similar to the spatial variation, in eight taxa/species NLFA marker concentrations varied with season, indicating that the channelling of basal resources also varies markedly among seasons. Plant NLFA marker concentrations peaked in October, coinciding with increased plant litter input, whereas bacterial and fungal NLFA marker concentrations did not vary consistently among taxa across seasons. Similar to the spatial variations, the low NLFA marker concentrations for algae and animals did not vary consistently among seasons. Overall, the results highlight that the channelling of basal resources through the salt marsh food web varies both in space and time, with the changes being mainly driven by seasonal dynamics in the input of autochthonous resources, i.e. litter material of vascular plants.

Data availability
The datasets generated and/or analysed during the current study are available in the DRYAD repository (doi:10.5061/dryad.mcvdnck3m),

https://doi.org/10.5061/dryad.mcvdnck3m

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Chapter 4 – Trophic structure and origin of resources of soil macrofauna in the salt marsh of the Wadden Sea: a stable isotope (^{15}N,^{13}C) study

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Abstract

Salt marshes exist along the gradient of the marine mudflat to the terrestrial dunes, with a gradient of shore height and associated plant zonation. The lower salt marsh (LSM) extends from the mean high tidal level to 35 cm above that level and is followed by the upper salt marsh (USM). Despite changes in the amount of allochthonous marine input and in abiotic conditions, little is known about changes in the trophic structure and used of basal resources by the soil macrofauna along marine – terrestrial boundaries. Natural variations in carbon stable isotope ratios (δ^{13}C signatures) allow insight into basal resources of consumers such as marine algae, terrestrial C3 and C4 photosynthesising plants. Furthermore, variations in nitrogen stable isotope ratios (δ^{15}N signatures) allow insight into the trophic position of consumers. We investigated spatial and temporal changes in stable isotope signatures in salt marsh soil macrofauna of the island of Spiekeroog, German Wadden Sea. The range of δ^{15}N signatures indicated no changes in food chain length across salt marsh zones with consumers in both zones comprising primary decomposer, secondary decomposer and first order predators. However, the trophic position of individual species changed between zones, but in particular with season. Contrasting δ^{15}N signatures, the range in δ^{13}C signatures in the LSM was twice that in the USM indicating a wider range of resources consumed. Bayesian mixing models indicated predominant autochthonous resource use in both the LSM and USM, with the use of marine allochthonous resources never exceeding 29.6%. However, the models also indicate an increase in the use of marine resources in certain species in the LSM with no use in the USM. Overall, the results indicate that the resource use of salt marsh macrofauna varies more in space than in time, with the food web being generally based on autochthonous rather than allochthonous resources. However, there also is trophic plasticity in certain species across both temporal and spatial scales including variations in the use of allochthonous resources. Generally, however, marine input contributes little to the nutrition of salt marsh soil macroinvertebrates.

Introduction

Ecosystems are defined by primary production and the use of those autochthonous resources by consumers such as phytophagous animals or decomposers [1, 2]. Such systems include e.g., forests and eutrophic lakes [2, 3]. By contrast, habitats such as glacier forelands, beaches and most freshwater systems are characterized by the input of external,

allochthonous resources [4–6]. This input of allochthonous resources may come from far away by wind, e.g. in glacier forelands, or from adjacent habitats with greater primary productivity, e.g. marine input on beaches [4, 5, 7]. Additionally, some systems receive both allochthonous and autochthonous resources, such as salt marshes.

Salt marshes are located at the interface between marine and terrestrial systems. As a result, they are regularly flooded resulting in a vegetation zonation associated with soil accumulation [8–10]. Salt marshes fulfil several important ecosystem services, such as shoreline protection from wave action and absorption of floodwaters during storms [11]. In addition, they are among the most productive systems of the world, which is related to anoxic conditions in the soil acting as a carbon sink [11]. Furthermore, they serve as refuge for juvenile fish and migratory birds [11]. Wadden Sea salt marshes are inhabited by a variety of vascular plants, such as *Elymus athericus* (*Elytrigia atherica*), *Atriplex portulacoides, Puccinellia maritima, Salicornia stricta* and *Spartina anglica*, contributing to high primary productivity [12, 13]. Additionally, tidal movements regularly flood parts of the marsh, depositing living and dead marine algae in the salt marsh [10]. In the North Sea, the tide transports large amounts of microalgae to the seashore including diatoms and dinoflagellates [14]. Both macroalgae and microalgae may flush into the marsh [9, 15], where they serve as resources for marine grazers occupying the lowest reaches of the marshes [16–19]. Despite this influx of marine resources to salt marshes, the relative importance of these allochthonous resources for consumers has hardly been studied.

The trophic structure of a food web determines the rate of energy fixation and transfer to higher levels in an ecosystem [20]. The trophic structure of food webs indicates its resilience to disturbances through coexisting species maintaining their trophic function and energy channelling [21–24]. Energy channels centre around basal resources such as primary producers or detritus [1, 23]. Additionally, resources from adjacent systems may affect higher trophic levels through improved primary production and associated secondary production [1, 4, 23]. These energy channels may vary in time and space, similar to the use of marine resources by terrestrial detritivores in salt marsh systems, which may vary across successional stages [13, 23]. Changes in energy channels, either through loss of a resource or addition of a new resource, influence the functional groups associated with those channels [1]. Therefore, a thorough understanding of the basal resources and the pathways they are channelled through food webs is vital to understand the functioning of the system.

Soil macrofauna includes species of a body size > 2 mm ranging from beetles to earthworms [25]. In the soil, macrofauna species are often considered ecosystem engineers because of their influence on the microbial, chemical and physical composition of the soil matrix [25–27]. Furthermore, by breaking down litter, they increase the rate of decomposition and nutrient cycling [25, 28]. The terrestrial macrofauna living in salt marshes may benefit from both autochthonous vascular plants and allochthonous algal or marine litter. This benefit may induce greater consumer production, thus influencing trophic structure [4]. Past

studies of salt marsh succession by [8] as well as [23] suggest a decline in the use of marine resources with declining inundation frequency as a result of shore height. Trophic interactions, such as consumption of larval instars of rove beetles by the carabid beetle *Dicheirotrichus gustavii* [29] as well as consumption of algal wrack by the talitrid amphipod *Talitrus saltator* on the beaches of barrier islands [4, 30], have been identified. While studying trophic interactions by direct observations is difficult due to the small size of the soil fauna and their prey, and the inaccessibility of their habitat, past studies of forest soils have suggested that food specialists are rare [26, 31]. Given the increased availability of marine resources in the lower reaches of the salt marsh and the generalist feeding nature of soil decomposers, it is likely that these allochthonous resources are exploited influencing the trophic structure and nutrient availability in the marsh. However, the trophic structure of the soil macrofauna, their resource-use and changes across Wadden Sea salt marsh zones and seasons have not been investigated.

Given the regular flooding of the marshes and the small size of consumers and prey, delineating the trophic structure of salt marsh food webs using direct observations of trophic interactions is virtually impossible. However, for studying the trophic structure of animal communities natural variations in stable isotope ratios (^{15}N/^{14}N, ^{13}C/^{12}C) are increasingly used and this also applies to salt marshes [10]. Previous studies in the salt marsh of the North Sea using stable isotopes in fact indicated increased use of marine resources by oribatid mites at the lowest reaches of the salt marsh [9]. In addition, studies based on stable isotopes allowed insight into the diet and trophic position of benthic macrofauna of salt marshes [32, 33]. Stable isotope analysis allows determining trophic levels through ^{15}N/^{14}N ratios as well as identification of the resources used through ^{13}C/^{12}C ratios [34]. ^{15}N concentrations increase with transfer to higher trophic levels, thus allowing to estimate the trophic level of consumers [34, 35]. ^{13}C concentration, on the other hand, varies between resources, specifically plants with differing photosynthetic pathways (C3 and C4) [34, 35] allowing to trace the channelling of these resources through food webs. Both of these isotopes have been used in intertidal salt marshes to understand changes in the quality of organic matter with time [36]. In addition, they have been used to delineate marine resource use by insect larvae consumed by spiders on shorelines of the Baltic Sea [37] as well as by Polychaeta, Pulmonata and Amphipoda of coastal waters [33, 38]. δ^{13}C signatures become especially useful when investigating the use of marine and terrestrial resources due to the distinct δ^{13}C signatures of algae [9, 23, 33]. Differences in δ^{13}C signatures between algae and other plants are due to algae using bicarbonate as inorganic carbon source resulting in enriched δ^{13}C signatures, which may also vary with light and nutrient availability [39–41]. Therefore, using δ^{13}C and δ^{15}N signatures may allow to discern the use of allochthonous marine and autochthonous terrestrial plant resources as well as the trophic structure of salt marsh soil macrofauna.

Here, we investigated the importance of allochthonous marine input (mainly algae) compared to autochthonous terrestrial resources for the soil macrofauna of the upper

(USM) and lower salt marsh (LSM). Furthermore, we compared the trophic structure of the salt marsh soil macrofauna across salt marsh zones as well as seasons. More specifically, we investigated the following hypotheses: (1) The consumption of allochthonous marine algal resources increases with greater tidal influence i.e., the enrichment of ^{13}C in consumers of the LSM is higher than in consumers of the USM. (2) Resembling terrestrial habitats, salt marsh soil macrofauna communities consist of four trophic levels including primary decomposers, secondary decomposers, first order predators and second order predators [26]. (3) Resource use of soil macrofauna varies across seasons (spring, summer, autumn) and zone with allochthonous marine resources being especially important for the salt marsh macrofauna food web in autumn due to heavy storms carrying large amounts of marine resources to higher positions in the salt marsh.

Materials and Methods

Study site
Sampling was performed in the Wadden Sea salt marsh of Spiekeroog, Lower Saxony, Germany, as part of the DynaCom project (https://uol.de/en/icbm/collaborative-projects/dynacom). The Wadden Sea stretches across the Netherlands to Denmark and holds a vast area of salt marshes and mudflats [42, 43]. Back-barrier marshes form on the leeward side of barrier islands, thus are sheltered from strong wave action [8]. Due to the lack of strong wave action, sediment is deposited and over time a gradient of shore height is formed [8, 10]. This gradient alters the inundation period and frequency, resulting in different vegetation zones. The USM is located 35 cm or more above the mean high-water level, with inundations occurring up to 70 times a year, whereas the LSM lies between 0-35 cm above the mean high-water level and is flooded up to 250 times a year [43,44].

Sampling
Sampling was performed on the 16th of April (spring), 16th of July (summer) and 22nd of October 2019 (autumn) during low tide along five transects (53°45'2" - 53° 47'1"N, 7° 40'0" - 7° 49'1"E). Per transect and zone one soil core (ø 20 cm, depth 10 cm) was taken. Animals were extracted by heat [44] and stored in 70 % ethanol at -20°C. Animals were determined to group or species level [45]. Additionally, litter, soil, vascular plant species and macroalgae were collected by hand and stored at -20°C (Supplementary table 2).

Sample preparation and stable isotope analysis
Taxa/species were not present in all soil cores, we attempted to analyse at least three specimens per taxon/species and sampling date originating from separate soil cores. Samples were dried at 60°C for 24 h and weighed into tin capsules using a fine scale (Cubis MSE 3.6P, Sartorius). Variations in stable isotope ratios (^{13}C/^{12}C and ^{15}N/^{14}N) were measured by an elemental analyser (Euro EA 3000, EuroVector S.p.A; Milano Italy) modified for small samples coupled with an isotope mass spectrometer (Delta V Plus, Thermo Electron, Bremen Germany) [46]. Ratios of stable isotopes were expressed as δX (‰) = [(Rsample—

Rstandard)/Rstandard] x 1000, with 'X' representing the target isotope and 'R' the heavy-to-light isotope ratios (^{13}C/^{12}C and ^{15}N/^{14}N) of the sample and standard, respectively. Vienna PD Belemnite (PDB) was used as standard for δ^{13}C and atmospheric nitrogen for δ^{15}N. Acetanilide was used for internal calibration.

Statistical analysis

Signatures of δ^{15}N and δ^{13}C of macrofauna taxa were analysed using linear mixed effects models in R (4.1.0) (R. core Team 2021) using the packages emmeans (Version 1.6.2-1), lme4 (Version 1.1-27.1), car (Version 3.0-11), lmerTest (Version 3.1-3) and dplyr (Version 1.0.7). "Zone" and "Season" were included as fixed factors and "Core ID" nested within "Transect" as random factor. Due to inconsistent occurrence across zone and season, macrofauna taxa were analysed separately. The full factorial model with Zone and Season could only be fitted for *T. saltator* (Amphipoda), *Ochthebius dilatatus* (Coleoptera, Hydraenidae) and *D. gustavii* (Coleoptera, Carabidae) present in both zones and all three seasons, except for *D. gustavii* which was not found in the USM in April. Staphylinidae larvae (April, July and October) and chalcidoid wasps (Chalcidoidea) (July and October) were only found in the LSM, and therefore only season was fitted as fixed factor. Similarly, in *Amischa* spp. (Coleoptera, Staphylinidae) only season was fitted as fixed factor as it was only found in the USM (April, July and October). To inspect variations in the length of the food chain as well as the span of resources with Season and Zone we calculated ^{15}N and ^{13}C ranges in cores where two or more species occurred as the difference between the most ^{13}C or ^{15}N enriched and the least ^{13}C or ^{15}N enriched taxa/species, and analysed the data using MANOVA in Statistica 13 (TIBCO Software Inc. 2018; http://tibco.com). To determine the dependence of species on autochthonous and allochthonous resources, Bayesian mixing models were calculated per season using FRUITS (Beta Version 2.1.1) using fractionation factors for ^{15}N and ^{13}C of 3.4 ‰ and 4.0 ‰, respectively. The fractionation factors are well established; for ^{13}C the fractionation factor is based on the 'detrital shift' as described in [47] and reflects the relative enrichment of soil animals compared to litter. For ^{15}N it reflects the average trophic level fractionation [47, 48]. For the first trophic level mean δ^{15}N of litter ± 1.7 ‰ was used. Because of lack of replicates across season for *Dyschirius* sp. (Coleoptera, Carabidae), *Argenna* sp. (Araneae, Dictynidae) and Linyphiidae (Araneae), mixing models analysing variations in stable isotope signatures in these taxa / species were only fitted with Zone as fixed factor. The vegetation at both zones was dominated by C3 plants and the mean signatures of algae and vascular plant species were used in the mixing models (Supplementary table 1). The C4 plant *Spartina anglica,* present at the transition to the mudflats, was not included in the pool of potential resources as animal signatures indicated that it did not form part of the basal resources.

Results

In total, six macrofauna species, 20 plant species (Supplementary table 2) as well as soil and litter were collected and analysed for δ^{13}C and δ^{15}N signatures. In April, animal δ^{13}C signatures ranged from -21.63 to -25.93 ‰ and δ^{15}N signatures from 5.02 to 13.97 ‰, respective ranges in July were -22.50 to -25.65 for δ^{13}C and 5.05 to 18.93 ‰ for δ^{15}N, and in October -22.83 to -26.19 ‰ for δ^{13}C and 4.10 to 14.79 ‰ for δ^{15}N (Figure 1). Respective ranges of terrestrial vascular plants for δ^{13}C and δ^{15}N were -27.15 to -26.44 ‰ and 4.01 to 10.44 ‰ in April, -27.61 to -24.44 ‰ and 5.32 to 9.08 ‰ in July, and -28.62 to -25.72 ‰ and 4.43 to 8.42 ‰ in October (Figure 1). Typically, the communities spanned three trophic levels, including primary decomposers, secondary decomposers and first order predators (Figure 1). Generally, the range in ^{15}N signatures neither varied significantly between zones nor among seasons and averaged 5.71 ± 2.43 ‰. By contrast, the range in ^{13}C signatures varied significantly with Zone, with 0.91 ± 0.40 ‰ in the USM and 2.67 ± 0.43 ‰ in the LSM ($F_{1,18}$= 15.26, p= 0.001), but not with Season.

Salt marsh zones

Among individual taxa δ^{15}N signatures between the LSM and USM were significant in *O. dilatatus* (7.81 ± 0.69 ‰ and 4.68 ± 0.70 ‰, respectively) and *T. saltator* (9.54 ± 0.95 ‰ and 6.70 ± 0.82, respectively) (Table 1). Further, δ^{13}C signatures of *O. dilatatus* were significantly higher in the LSM than the USM (-22.69 ± 0.45 ‰ and -23.65 ± 0.41 ‰, respectively), whereas in *D. gustavii* δ^{13}C signatures in the LSM were significantly lower than in the USM (-25.92 ± 0.35 ‰ and -24.73 ± 0.40 ‰, respectively).

Table 1: F- and p-values of linear mixed-effects models on variations in δ^{15}N and δ^{13}C signatures of macrofauna taxa/species with season (April. July. October) and salt marsh zone (upper salt marsh. lower salt marsh) on the island of Spiekeroog in 2019.

Species	n	Zone (Z)				Season (S)				Z x S			
		δ^{15}N		δ^{13}C		δ^{15}N		δ^{13}C		δ^{15}N		δ^{13}C	
		F	p	F	p	F	p	F	p	F	p	F	p
Talitrus saltator	15	**26.19**	**0.004**	2.40	0.156	**10.76**	**0.019**	1.23	0.336	0.53	0.620	1.71	0.235
Ochthebius dilatata	16	**61.01**	**0.001**	**7.73**	**0.003**	**7.47**	**0.039**	1.55	0.269	1.05	0.426	**7.84**	**0.013**
Dicheirotrichus gustavii	10	9.22	0.075	**37.44**	**0.001**	**9.85**	**0.021**	**8.97**	**0.024**	4.95	0.069	0.06	0.819
Chalcid wasps	6	Only LSM				11.19	0.075	0.95	0.384	N/A			
Staphylinidae larvae	7					10.99	**0.015**	2.64	0.275				
Amischa spp.	9	Only USM				0.41	0.705	**27.33**	**0.030**				

'n' number of replicates; N/A. not analysed.
Bold values indicate p = < 0.05

Season

δ^{15}N signatures among individual species, only varied significantly with season in *D. gustavii*, *O. dilatatus*, Staphylinidae larvae and *T. saltator* (Table 1). Seasonal changes are presented separately for individual taxa/species because no common patterns were evident. In *D. gustavii* δ^{15}N signatures were significantly higher in October (12.89 ± 0.64 ‰) than in July (11.89 ± 1.02 ‰), whereas in *O. dilatatus* they increased significantly from April

(5.95 ± 1.32 ‰) to July (7.06 ± 1.96 ‰) and decreased again in October (5.82 ± 1.94 ‰). In Staphylinidae larvae δ^{15}N signatures significantly increased from April (8.62 ± 0.62 ‰) to July (13.66 ± 2.15 ‰) and remained at a similar level in October (12.36 ± 1.18 ‰). In *T. saltator* δ^{15}N signatures declined from April (9.09 ± 1.61 ‰) to July (7.20 ± 1.28 ‰) and remained at a similar level in October (7.79 ± 1.38 ‰). δ^{13}C signatures varied significantly with season in *Amischa* sp. and *D. gustavii.* Furthermore, in *O. dilatatus* the effect of Season depended on Zone (Table 1). In *Amischa* sp. δ^{13}C signatures increased from April (-24.05 ± 1.04 ‰) to July (-23.97 ± 1.32 ‰) and declined again in October (-24.52 ± 1.01 ‰). Also, in *D. gustavii* δ^{13}C signatures decreased from July (-25.16 ± 0.65 ‰) to October (-25.73 ± 0.75 ‰). By contrast, in *O. dilatatus* δ^{13}C signatures changed little with Season in the USM (-23.95 ± 0.46, -23.51 ± 0.01 ‰ and -23.46 ± 0.42 ‰ for April, July and October, respectively), whereas in the LSM δ^{13}C signatures gradually declined from April (-22.19 ± 0.12 ‰) to July (-22.57 ± 0.14 ‰) to October (-23.14 ± 0.32 ‰).
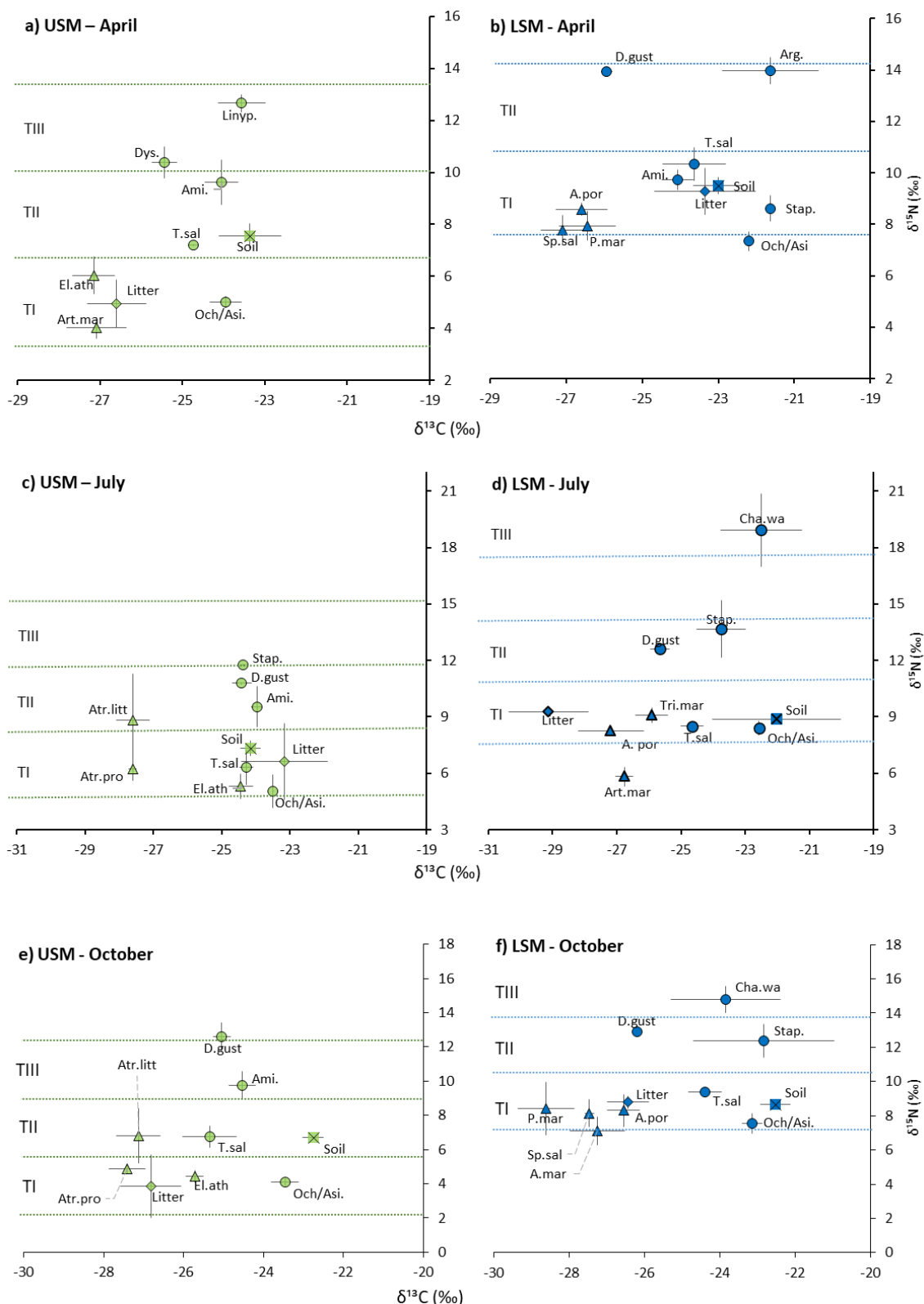
Figure 1: Scatterplot of δ^{15}N und δ^{13}C signatures of salt marsh fauna taxa / species (circles), plants (triangles), litter (diamonds) and soil (crossed squares) in the upper salt marsh (USM)= green and lower salt marsh (LSM)= blue; (a) USM in April, (b) LSM in April, (c) USM in July, (d) LSM in July, (e) USM in October and (f) LSM in October; means ± SD. Dotted lines indicate trophic levels. Animals: Ami. – *Amischa* sp., Arg. – *Argenna* sp., D.gust - *Dicheirotrichus gustavii*, Dys. - *Dyschirius* sp., Ochth. – *Ochthebius dilatatus*, Linyp. - Linyphiidae, Stap. - Staphylinidae larvae, T.sal - *Talitrus saltator*. Plants: Atr.litt – *Atriplex littoralis*, Art.mar – *Artemisia maritima*, A.por – *Atriplex portulacoides*, Atr.pro – *Atriplex prostrata*, El.ath – *Elymus athericus*, P.mar- *Puccinellia maritima*, Sp.sal – *Spergularia salina*.

Allochthonous resource use

Bayesian mixing models indicated an almost exclusive use of terrestrial resources across taxa in both the LSM and USM. In April they used 85-98 % terrestrial resources with the exception of *Argenna* sp. in the LSM (70.4 ± 17.8 %) (Figure 2a); in July they used 90-97 % terrestrial resources with the exception of the chalcidoid wasps of the LSM (74.4 ± 17.3 %) (Figure 2b). In October they used 83-97 % terrestrial resources with the exception of Staphylinidae larvae in the LSM (75.6 ± 16.2 %) (Figure 2c).
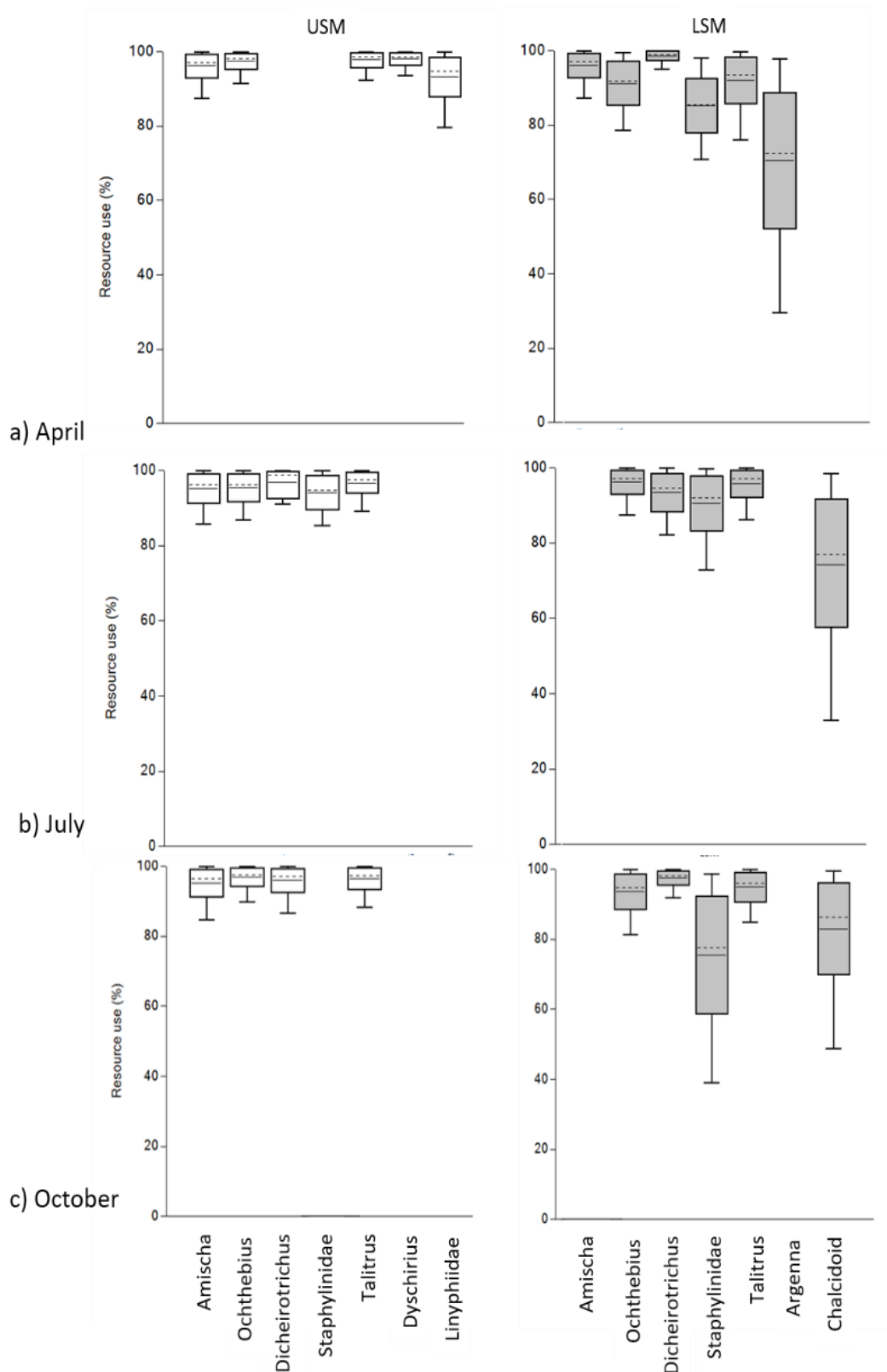
Figure2: Boxplot of results of Bayesian mixing models on the use of terrestrial vs marine resources (%) by macrofauna species in the salt marsh of Spiekeroog in (a) April, (b) July and (c) October. Left panel (white boxes) = upper salt marsh (USM), right panel (grey boxes) = lower salt marsh (LSM). Boxes represent the 68% confidence intervals, error bars 95% confidence intervals; the solid horizontal line represents the mean, the dashed horizontal line the median.

Discussion

We investigated the trophic structure and allochthonous resource use of the soil macrofauna of the Wadden Sea salt marsh. Consisting of only three trophic levels the macrofauna food web is simpler than the previously investigated mesofauna food web [10]. Further, the range of δ^{13}C signatures in the LSM significantly exceeded that in the USM indicating a wider range of basal resources used in the LSM than in the USM. However, as indicated by Bayesian mixing models, allochthonous marine resources generally are of little importance for the nutrition of soil macrofauna in both the LSM and USM. Thereby, despite the proximity of marine and terrestrial habitats, we found little evidence for the use of marine resources by the soil macrofauna.

Differences between salt marsh zones

Stable isotope signatures of soil macrofauna taxa / species differed significantly between the USM and LSM. δ^{15}N signatures were generally higher in the LSM compared to the USM and this applied in particular to *O. dilatatus* (Coleoptera, Hydraenidae) and T. *saltator* (Amphipoda) as well as in trend to *D. gustavii* (Coleoptera, Carabidae). Only δ^{13}C signatures varied significantly between salt marsh zones in *D. gustavii*, with higher enrichment in the USM than the LSM, contradicting our first hypothesis. Higher δ^{15}N signatures in macrofauna taxa / species in the LSM than in the USM contrast patterns in the salt marsh mesofauna, in which δ^{15}N signatures were higher in the USM than in the LSM [10]. Considering the higher δ^{15}N signatures of soil, plant and litter material in the LSM than the USM, the higher signatures of *O. dilatatus* and *T. saltator* in the LSM than in the USM are likely related to their basal resources, pointing to the predominant use of autochthonous resources. In *D. gustavii* δ^{15}N signatures did not differ significantly between the USM and LSM. However, in the USM *D. gustavii* occupied a higher trophic position than in the LSM, as discussed in [47], high δ^{15}N and low δ^{13}C signatures indicates consumption of resources based on freshly fixed carbon or consumers thereof, again pointing to the dominant use of autochthonous resources.

Trophic level

Regardless of season or zone, macrofauna communities consisted of three trophic levels rejecting our second hypothesis. The results suggest that the salt marsh macrofauna food web uniformly comprises primary decomposers, secondary decomposers and first order predators. The latter included chalcidoid wasps (Chalcidoidea) parasitizing a wide range of host species [49]. These findings are in contrast to [10] reporting four trophic levels for the salt marsh soil mesofauna. The lower number of trophic levels in macrofauna across both zones is likely due to high disturbance by inundation reducing soil macrofauna diversity. As proposed by the intermediate disturbance hypothesis, we expected a reduction in diversity with disturbance frequency [50]. However, while inundation frequency indeed is lower in the USM than in the LSM, soil salinity is high in both the USM and LSM [51, 52]. As discussed by [50] increased abiotic stressors may reduce diversity and favour the dominance of

specialists. Contrasting these assumptions, the wider range in δ^{13}C signatures of macrofauna species in the LSM compared to the USM points to more flexible resource use in species of the former. Overall, the results suggest that the salt marsh soil macrofauna food web is simpler than expected, presumably due to frequent flooding and associated disturbances.

Season

Although seasonal variations in stable isotope signatures generally were less pronounced than variations between salt marsh zones, δ^{15}N signatures varied significantly with season in *D. gustavii, O. dilatatus*, Staphylinidae larvae and *T. saltator*. Further, δ^{13}C signatures varied significantly in *D. gustavii, Amischa* sp. (Coleoptera, Staphylinidae) and *O. dilatatus*, but in the latter this depended on salt marsh zone. In *D. gustavii* and *Amischa* sp. δ^{13}C signatures declined from July to October, whereas in *O. dilatatus* they declined from April to October but only in the LSM, contradicting our third hypothesis. δ^{15}N signatures in *D. gustavii* of the USM changed considerably with season indicating a switch from living as secondary decomposer in April to living as predator in October, whereas in the LSM *D. gustavii* constantly lived as secondary decomposer. Changes in resource use in *D. gustavii* in the USM is supported by the decline in δ^{13}C signatures from July to October indicating a switch from decomposer prey in July to herbivore prey in October [47]. In *O. dilatatus,* δ^{15}N signatures in the LSM, but not the USM, were higher in July than in April and October. Further, δ^{13}C signatures in the LSM declined later in the year, indicating increased use of autochthonous terrestrial resources. Previous studies showed *Ochthebius* species to scrape mats of microalgae on rocky shores as well as to feed on detritus [53, 54]. In the salt marsh *Ochthebius* species occur in or near saline channels, with larval stages living submerged [55–57] suggesting that they consume resources of marine origin. Contrasting this assumption, our results based on Bayesian mixing models indicate that the resources used by *O. dilatatus* in salt marshes are primarily based on autochthonous vascular plant litter material. δ^{15}N of Staphylinidae larvae in the LSM point to a trophic change from living as primary decomposer in April, to living as secondary decomposer in July and October. These changes could point to increased consumption of partially decomposed litter colonized by microorganisms, or to later larval stages living as predators by feeding on primary decomposers. Variations in δ^{15}N signatures with season in *T. saltator* indicate trophic plasticity in this species in particular in the USM, where its trophic position changed from secondary decomposer in April to primary decomposer in July and back to secondary decomposer in October. Direct feeding on plant litter might be related to increased plant growth and litter production during summer. Overall, variations in δ^{15}N signatures in *T. saltator* suggests that in salt marshes this species lives as opportunistic omnivore with its diet including microorganisms but also plant litter material depending on resource availability. This contrasts previous studies at beaches suggesting that *T. saltator* predominantly feeds on algae [7]. In *Amischa* sp. from the USM, δ^{13}C signatures declined significantly in October, which may point to increased predation on herbivores, whereas in

April and July they may feed more on decomposer prey [47]. Overall, resource use of a number of salt marsh macrofauna species changed with season indicating trophic plasticity. However, this was restricted to few species suggesting that overall, the resources used vary little between seasons.

Allochthonous resource use

As indicated by Bayesian mixing models the salt marsh macrofauna almost exclusively exploits autochthonous C3 plant resources with the use of allochthonous marine resources being restricted to few species and not exceeding 29.6 %, contradicting our third hypothesis. Conversely suggesting that despite salt marshes being sinks for marine carbon [58] carbon of marine origin is not a predominant resource for the salt marsh soil macrofauna. Species feeding predominantly on terrestrial resources regardless of season included *D. gustavii, Amischa* sp. and *T. saltator*. In the carabid beetle *D. gustavii* this suggests predation on phytophagous species. By contrast, *Amischa* sp. likely fed on both animal prey as well as fungi as Aleocharinae rove beetles are known to function as both detritivores and predators [59]. As indicated by their δ^{15}N signatures, in the salt marsh they predominantly function as detritivores of terrestrial C3 plant-based resources, only occasionally feeding on animal prey. Unexpectedly, *T. saltator* also exclusively relied on terrestrial resources (see above), with its variable trophic position indicating that in salt marshes it lives as omnivore. On the other hand, the spider *Argenna* sp. (Araneae, Dictynidae), Staphylinidae larvae and chalcidoid wasps indicated some marine resource use at least at certain sampling dates. In April, the use of marine resources was highest in *Argenna* sp. in the LSM, presumably due to feeding on dipterans with marine larval stages [37]. In Staphylinidae larvae the use of marine resources in the LSM was highest in October; in chalcidoid wasps in April and July. These resource changes may be related to higher flooding frequency at those time periods and the associated increase in marine detritus. Staphylinidae larvae may either use fungi in the deposited detritus or prey on detritivores such as dipteran larvae. Similarly, increased use of marine resources in chalcidoid wasps, might be due to parasitizing dipteran larvae developing in deposited marine detritus as has been shown for seaweed flies on beaches [60]. Overall, the results suggest that allochthonous input of marine resources is of limited importance for the salt marsh macrofauna community regardless of season and zone.

Conclusions

We investigated the trophic structure, trophic plasticity and resource use of the salt marsh macrofauna along spatial and temporal scales. Compared to the soil mesofauna and typical terrestrial habitats, the trophic structure of the salt marsh macrofauna is more simple with only three trophic levels irrespective of zone and season, presumably reflecting disturbance frequency and harsh abiotic conditions resulting in less diverse and trophically less structured communities. δ^{13}C signatures and Bayesian mixing models indicated that the macrofauna community predominantly relies on autochthonous C3 plant-based resources, regardless of season and zone. Changes in resource use were species-specific with the use

of allochthonous marine-based resources being restricted to chalcidoid wasps, the spider *Argenna* sp. and Staphylinidae larvae. Generally, variations in stable isotope ratios suggest that spatial variation in trophic niches exceeds seasonal variation. Overall, the trophic structure of the salt marsh macrofauna is rather simple, similar to arable systems, with terrestrial C3 plants as the main resource and allochthonous marine material being of little importance. Therefore, despite their importance as blue carbon systems, the soil macrofauna of salt marshes has little association with marine carbon.

Availability of data and materials

The datasets generated and/or analysed during the current study are available in the DRYAD repository (doi:10.5061/dryad.tdz08kq1t),

https://datadryad.org/stash/share/sCK5KbHOxJzZgmCQsrYkdtJiZhH10igspyu2800HBzk

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Chapter 5 – General discussion

Soil communities are an essential link in the cycling of nutrients of all habitats. Microbial communities begin the decomposition process of litter material making nutrients available for plants; whereas meso- and macrofauna may consume microbes or break down litter material, making it accessible for microbial decomposers. This thesis aimed to uncover the spatiotemporal dynamics of the basal soil resources, their channelling through the food web and trophic dynamics of salt marsh soil meso- and macrofauna. For this purpose, salt marsh soil fauna of the island of Spiekeroog was sampled in spring, summer and autumn of 2019.

Chapter 2 showed that microbial communities are stable across temporal scales, despite changes in abiotic conditions. Instead, spatial patterns strongly affected microbial communities, including both sediment depth and zone. Chapter 3 showed that the predominant basal resources of the salt marsh soil fauna is autochthonous plant material and associated bacteria and fungi, whereas allochthonous contributions were minor and varied with seasonal algal productivity. Chapter 4 showed that $\delta^{15}N$ ranges did not change across zones, with the soil macrofauna food web generally being composed of three trophic levels. However, the trophic position of specific taxa/species varied across zones, but most notably across seasons. In addition, $\delta^{13}C$ ranges doubled in the LSM, indicating a wider range of resources. However, despite these signatures, allochthonous marine resources did not constitute a significant basal resource for soil macrofauna.

Spatial dynamics

Chapter 2 showed that across salt marsh zones Gram-positive bacteria were the dominant microbial decomposers with concentrations increasing with soil depth and shore height. Fungal marker concentrations were highest in the LSM whereas algal marker concentrations were highest in the PZ. Unlike Gram-negative bacteria, Gram-positive bacteria predominantly break down resources resistant to decomposition, such as lignocellulose (Fanin et al. 2019). Considering that the major input of carbon in salt marsh systems is lignocellulose-rich plant detritus, such as litter from *Spartina* sp. and *E. athericus* (Bouchard and Lefeuvre 2000, Leadbeater et al. 2021), these findings are as expected. Despite frequent inundations and resulting reduced redox potential and anoxia in lower zones, bacteria remained the dominant decomposers. These findings agree with Keith-Roach et al. (2002), Mueller et al. (2020) and Tebbe et al. (2022) who showed dominance of bacteria across the marsh.

Persistence of bacteria despite regular inundation is likely due to exchange of marine bacteria with the tide, as suggested by Dini-Andreote et al. (2016) as well as lack of nutrient limitation owing to marine influx (Mueller et al. 2020). Alternatively, once soils become anoxic, a sequence of anaerobic processes follows; denitrification, Mn reduction, ferric reduction, sulphate reduction and finally methanogenesis (Tobias and Neubauer 2009), providing alternative metabolic pathways. Anaerobic bacteria have been suggested to be

the dominant microbial decomposer of north American salt marshes (Howarth and Hobbie 1982). This is further corroborated by the decline of the mono/sat ratio from the USM to the PZ, indicating declining aerobic activity and substrate availability towards the mudflats (see Chapter 1 – Channelling of resources). This confirms findings by Tebbe et al. (2022) who showed increased denitrification and dissimilatory sulphate reduction in frequently inundated areas of the Spiekeroog salt marsh. Therefore, bacteria in frequently inundated zones are predominantly anaerobic. In addition, salt marshes are subject to regular N-influx in lower zones, changing the exoenzyme use of microbes accordingly (Tebbe et al. 2022). In lower zone soils phosphorous exoenzyme use increases, whereas in upper zones nitrogen exoenzyme use is high (Mueller et al. 2020, Tebbe et al. 2022). This is due to the nutrient discrepancy across the salt marsh gradient (see Chapter 1 – Salt marshes). Therefore, bacteria show adaptation to anaerobic conditions as well as specific nutrient conditions. Overall, allochthonous influx, anaerobic respiration, specific exoenzyme use and exchange with marine bacteria likely explain bacterial persistence in frequently inundated soils, albeit at lower concentrations.

While overall low, fungal marker concentrations were highest in the LSM, these findings contrast Mueller et al. (2020) who showed highest fungal presence in the USM. Fungi are the dominant microbial decomposers of complex litter materials prior to incorporation into the soil and withstand a variety of abiotic conditions (Benner et al. 1984, Newell et al. 1996, Calado and Barata 2012, Calado et al. 2019). In the Spiekeroog salt marsh the USM was the only salt marsh zone with a consistent litter layer made up of lignocellulose rich *E. athericus*. Therefore, highest fungal presence was expected there. Fungi rely on external carbon, thus are unlikely to persist in LSM and PZ soils, due to the displacement of litter material by the tide (Bouchard and Lefeuvre 2000). However, while the LSM and PZ are inundated more frequently, the USM is subject to drought conditions leading to high soil salinity, as was documented by data loggers in 2019 (Meier et al. 2020a, Pieck et al. 2021). Alzarhani et al. (2019) suggests abiotic conditions are limiting fungi in salt marsh systems, but this may vary from site to site. In addition, as discussed previously, fungi are considered the dominant decomposers of aboveground litter material prior to incorporation into the soil. However, Chapter 2 only analysed soil cores, thus abiotic conditions in the USM and removal of litter material in the LSM and PZ likely explains these findings.

Algal markers were detected throughout the salt marsh, but gradually declined from the PZ to the USM. Likely reflecting reduced persistence of autotroph algae in the upper zones, as discussed in Chapter 2. Furthermore, the diatom marker lipid 20:5ω3,6,9 was detected across zones, but concentrations were highest in the PZ, confirming prior indications of diatom presence and displacement into the marshes (Scholz and Liebezeit 2012a, 2013, Redzuan and Underwood 2020). In addition, the flagellate marker 22:6w3,6,9,12,15,18 was exclusively detected in PZ soils. While presence of diatoms in the USM is surprising, similar findings had been made in UK salt marshes (Zong and Horton 1998). However, algal mats are considered scattered in the upper regions and as of this thesis, presence of algal mats under *E. athericus* vegetation has not been investigated. Overall, across zone a gradient of

the basal resources is apparent, with fungal resources peaking in the LSM, algae in the PZ and bacteria in the USM.

Across depth, the content of all PLFA markers approximately halved, with bacteria becoming dominant in the deeper sediment depth. These findings illustrate that there is little oxygenation of the soil matrix via vascular plant roots (Armstrong et al. 1985, Buth 1987) as discussed in Chapter 2. In addition, the mono/sat ratio and cy/pre ratio declined with depth illustrating significant reduction in aerobic activity and increasing stationary growth phases in soil bacteria but this varied with zone (see Chapter 1 – Channelling of resources). The decline of the mono/sat ratio in 5-10 cm depth was lowest in the LSM and PZ, confirming the increased presence of anaerobic pathways, as discussed previously. However, the mono/sat ratio was highest in the USM, indicating better oxygenation of the sediment. In contrast, while as expected, the cy/pre ratios increased with sediment depth, across zone the highest ratios were in the USM. In the USM, cy/pre ratios remained stable throughout the sampling period, whereas in the LSM and PZ increasing ratios coincided with periods of high inundation frequency (Meier et al. 2020b), presumably reducing the availability of oxygen for Gram-negative bacteria (Chapter 2).

Alongside bacteria, fungal PLFA marker concentrations halved in the deeper sediment depths. This presumably reflects the lack of oxygenation of the deeper sediments as discussed previously. This is further supported by the fungi/bacteria ratio which reflected greater loss of fungi compared to bacteria (Chapter 2). Finally, algal PLFA markers significantly declined with sediment depth, presumably due to light restrictions, as discussed in Chapter 2. The most significant loss was observed in the diatom marker 20:5ω3,6,9,12,15 which declined by over 90 % across sediment depth. This, as discussed in Chapter 2, is likely due to the vertical migration capacity of epipelic diatoms preventing burial (Cartaxana et al. 2016, Redzuan and Milow 2019). This discrepancy suggests that the majority of this diatom marker originates from epipelic diatoms, rather than biosynthesising soil fauna. In contrast, the algal marker 16:1ω3,6,9 generally declined by approximately half with sediment depth. Presumably, these findings suggest burial of green algae due to tidal forcing which may persist due to their mixotrophic nature (Selosse et al. 2017) (see Chapter 2).

The study presented in Chapter 3 investigated the spatiotemporal dynamics of basal resource channelling in the salt marsh soil fauna. NLFA marker concentrations showed that the dominant channel was the plant-bacteria channel. Channelling of plant resources declined towards the mudflats, whereas generally bacteria channelling increased. However, channelling of both resources also depended on season. Furthermore, no Gram-negative bacterial markers were detected, as previously shown in Chapter 2. Fungal, algal and animal basal resources were low and did not vary consistently with zone across taxa/species.

The decline of vascular plant markers agrees with results of Chapter 2, where PLFA plant marker concentrations declined towards the PZ. In addition, they confirm results of Bouchard et al. (2000) who showed that saltmarsh vascular plant productivity declines with reduction in shore height. Presumably, tidal displacement of litter material as well as

reduced biomass production explains these findings. In contrast, the increased channelling of bacteria towards the PZ contrasts results of Chapter 2 as well as Mueller et al. (2020), who showed that bacterial biomass declines towards the mudflats. The increase in bacteria-associated resource consumption could be linked to consumption of secondary decomposers, such as bactivorous nematodes on *Spartina* sp. (Alkemade et al. 1994, de Mesel et al. 2003). However, this general pattern was not evident in all taxa/species. Instead, in *Amischa* sp. and *Ochthebius* sp. bacterial NLFA markers declined with reducing shore height. Both species were found in the USM and LSM, considering their larger body size and aboveground lifestyle, tidal litter displacement in the LSM likely limited the availability of these resources. Overall, these findings indicate a bacteria dominated soil decomposer system, pointing to bottom-up dynamics and fast nutrient turnover (Scheu et al. 2005).

Conform to the general patterns fungal NLFA marker concentrations were low, supporting the low PLFA concentrations found in the study presented in Chapter 2. However, in contrast to the results presented in Chapter 2, concentrations declined towards the PZ in predatory mites (Mesostigmata), whereas in *Amischa* sp., Staphylinidae larvae and *Ochthebius* sp. they increased. The latter pattern coincides with results of Chapter 2 where fungal PLFA marker concentrations peaked in the LSM. Considering that Staphylinidae may consume a wide variety of resources and that *Ochthebius* sp. is a detrital feeder (Perkins, D. 1980, Thayer 2005, Ruta et al. 2006, Jäch et al. 2014), this discrepancy is likely due to diet of the consumer or their prey. Overall, fungi comprised a minor part of the diet of soil fauna, with spatial dynamics of the marker presumably related to the diet of consumer or their prey.

Finally, algal marker concentrations were very low, but present across soil taxa/species regardless of zone. However, concentrations did not vary consistently among taxa/species. Low algal marker concentrations across all zones confirm the findings presented in Chapter 2 that algae are present throughout the marsh. This partially agrees with Schrama et al (2012, 2013) who indicated a gradient from detritivores depending on allochthonous resources to predominantly terrestrial resources with shore height. However, these findings instead suggest a low but omnipresent transfer of algae-associated resources to higher trophic levels. Presumably, as discussed previously, microalgae persist across the salt marsh but are likely to be scattered. However, the dominant algal markers detected were the microalgal and diatom markers 14:0 and 20:5ω3,6,9. Past research suggested that omega-3 polyunsaturated fatty acids (PUFA) including 20:5ω3,6,9 are produced by eukaryotic microalgae only (Kabeya et al. 2018, Ruess and Müller-Navarra 2019). Making aquatic systems the primary provider of these essential lipids. However, several soil invertebrates have recently been shown to produce omega-3 polyunsaturated fatty acids (Chamberlain et al. 2005, Menzel et al. 2018, Kabeya et al. 2018). Though, as suggested by Menzel et al (2018), de novo synthesis is costly and dietary supplementation is preferred. Furthermore, PUFA content in Collembola may vary with species and diet (Chamberlain et al. 2005). Therefore, it is plausible that some quantity of the diatom marker 20:5ω3,6,9 in

the USM originated from soil mesofauna. In addition, NLFA lipid marker 14:0 is considered a microalgal marker in aquatic systems (Winter et al. 2018, Heijden et al. 2019), but an unspecific marker in the terrestrial system (Maraun et al. 2020). Lack of contribution of allochthonous input to the USM is likely explaining the low $\delta^{13}C$ range of USM soil fauna, as shown in Chapter 4. Suggesting autochthonous origin of basal resources. Overall, Chapter 3 illustrated the low but omnipresent contribution of algae to the soil fauna diet across the marsh. Similarly, animal NLFA marker concentrations were very low, but present in fauna across the marsh. Overall, results presented in Chapter 3 showed that autochthonous vascular plants and their microbial decomposer are the dominant basal resource exploited within the salt marsh soil fauna.

Chapter 4 investigated spatiotemporal dynamics in trophic structure and allochthonous marine resource use in salt marsh soil macrofauna. No spatial variation in the range of $\delta^{15}N$ was evident. Suggesting no changes in the number of trophic levels, despite changes in inundation. In total, three trophic levels were evident across the salt marsh including primary decomposers, secondary decomposers and first-order predators. These findings contrast Haynert et al. (2017) who found four trophic levels in the salt marsh soil mesofauna. The intermediate disturbance hypothesis suggests that strong disturbances reduce diversity (Connell 1978). However, while inundation between the zones differed, soil salinity was similar across seasons (Meier et al. 2020b, Pieck et al. 2021), as discussed in Chapter 4. Therefore, inundation is unlikely to be the only factor shaping the community. Presumably, soil salinity acts as an additional abiotic disturbance factor, favouring habitat specialists (Connell 1978). Considering the behavioural and physical adaptations of salt marsh spiders and beetles to inundation (Wyatt 1986, Pétillon et al. 2009) this appears likely. Therefore, abiotic factors such as water content, anoxia and soil salinity limit the trophic structure of salt marsh soil macrofauna but favours habitat specialists.

However, generally $\delta^{15}N$ signatures of taxa/species in the LSM were greater than in the USM. This again, contrasts findings by Haynert et al. (2017) who noted higher $\delta^{15}N$ signatures in USM soil mesofauna. The higher $\delta^{15}N$ signatures of LSM soil fauna may be due to external nitrogen influx from the ocean, as mentioned in Chapter 1 – Salt marshes (Tobias and Neubauer 2009, Mueller et al. 2020). Such enrichment is evident when considering the $\delta^{15}N$ signatures of the basal resources of the LSM soil fauna, which are generally markedly higher in $\delta^{15}N$ (Chapter 4). Overall, there were no changes in the number of trophic levels across zones, but taxa/species-specific differences were evident due to enrichment of basal resources.

In contrast to $\delta^{15}N$, the range in $\delta^{13}C$ of consumers doubled in the LSM, suggesting greater marine input into the diet of consumers. However, as indicated by the Bayesian mixing model, consumption of allochthonous marine resources was limited, never exceeding 29.6 %. In addition, soil was generally enriched in $\delta^{13}C$ compared to litter material. Confirming Mueller et al. (2017), who showed that salt marsh belowground biomass and bulk soil was enriched in $\delta^{13}C$ compared to aboveground plant biomass. Therefore, the reduced USM $\delta^{13}C$ range may indicate predominant consumption of aboveground

resources, rather than belowground organic matter. However, Mueller et al. (2019) suggested that distance from the edge of the marsh reduced allochthonous carbon contribution, but due to inundation this was dependent on elevation. This was further shown by Bouchard et a (2000) who noted microalgal enrichment of soil organic matter during frequent inundations. Therefore, the intermittent elevation and inundation frequency of the LSM may explain the $\delta^{13}C$ enrichment of LSM litter. However, despite these findings Bayesian mixing models showed predominant consumption of autochthonous resources, with increases in marine contribution of individual species in the LSM. This finding partially agrees with results presented in Chapter 3, where the contribution of allochthonous marine resources was minor. However, the reduced $\delta^{13}C$ range and Bayesian mixing model for USM taxa/species contrasts the presence of algae across the marsh, shown in Chapter 3, suggesting that algal materials consumed are either more similar to C3 plant isotopic signatures or originate from soil fauna. Overall, Chapter 4 showed that the trophic structure of the salt marsh soil macrofauna is simpler than anticipated. In addition, the predominant resources consumed are of autochthonous origin, regardless of zone.

Temporal dynamics

The results presented in Chapter 2 illustrated that bacterial and plant PLFA concentrations did not vary significantly across temporal scales, suggesting that neither vascular plant input nor bacterial presence in the soil is influenced by seasonal changes. However, the bacterial stress ratio mono/sat significantly varied across seasons, but this response differed among the salt marsh zones. In the USM and PZ the ratio was lowest in October, whereas in the LSM it was lowest in July. Temporal variations of the mono/sat ratio reflected seasonal changes in inundation frequency (Meier et al. 2020b) and may point to tidal displacement or deposit of litter material in the LSM. In the PZ, the decline in the ratio likely links to increasing inundation frequency later in the year (Meier et al. 2020b), increasing soil anoxia. In addition, drought conditions and later frequent inundations of the USM likely limited bacteria thus decreasing aerobic activity.

Temporal changes in the cy/pre ratio on the other hand were more complicated and depended on zone as well as soil depth. USM ratios remained relatively stable, whereas in the LSM and PZ the ratio peaked in July. Presumably, reflecting seasonal increases in inundation frequency in summer 2019 (Meier et al. 2020b). This was corroborated by the mono/sat ratio which in both zones was lowest in July 2019 (Chapter 2). However, the ratio in 5-10 cm sediment depth in the PZ declined in July 2019, coinciding with a stark increase in inundation frequency (Meier et al. 2020b). This could be linked to the aforementioned presence of anaerobic bacteria, or the oxygenation of the soil pores by inundation (Garcia-Hernandez 2022). Overall, temporal changes in the bacterial stress indicators were strongly linked to changes in abiotic conditions mainly inundation frequency, soil water content and associated salinity.

Fungal PLFA marker concentrations as well as the fungi/bacteria ratio varied with season but were additionally affected by zone (Chapter 2). USM and LSM fungal marker concentrations and fungi/bacteria ratios peaked in July, likely due to reduced competition with bacteria, as indicated by higher cy/pre and reduced mono/sat ratios for LSM bacteria in July 2019. In contrast, fungal marker concentrations and fungi/bacteria ratios in the PZ were lowest in July. The decomposition of *Spartina* sp. begins with the standing leaves which are decomposed by fungi until incorporated into the soil (Castro and Freitas 2000, Calado and Barata 2012, Calado et al. 2019). However, tidal displacement of detrital mass is highest in the PZ (Bouchard and Lefeuvre 2000). Therefore, considering the stark inundation frequency increase in July 2019, tidal displacement likely explains this reduction. In contrast, Morriss and Jensen (1998) showed high proportion of standing *S. anglica* leaves in October, thus explaining the subsequent increase in fungi due to greater substrate availability. Overall, the fungal marker concentrations suggest that fungi rely on aboveground standing plant material in the frequently inundated PZ, which is strongly affected by tidal displacement.

Lastly algal PLFA marker concentrations peaked in April and declined in July and October. Confirming observations by Scholz and Liebezeit (2012a,b) who showed that microalgae peak in spring. This likely relates to macronutrient availability, hydrodynamic conditions and sediment structure, as discussed by Scholz and Liebezeit (2012a,b). Thus, unlike bacteria and fungi temporal algal dynamics are predominantly controlled by the productivity of algae.

The results presented in Chapter 3 indicated temporal dynamics in the channelling of plant, bacterial and fungal resources to higher trophic levels; but these dynamics in part depended on salt marsh zone. In general, plant NLFA marker concentrations were highest in October, but in the USM concentrations were also high in July. Elevated plant channelling in October coincides with senescence of dominant vascular plants (Bouchard et al. 1998, Morriss and Jensen 1998, Bouchard and Lefeuvre 2000). In addition, the reduction in the channelling of plant resources in the LSM and PZ during spring and summer are presumably the result of tidal displacement (Bouchard et al. 1998, Bouchard and Lefeuvre 2000).

However, the channelling of plant resources varied among consumer species. Contrasting the general pattern, in Hypogastruridae (Collembola) plant marker concentrations peaked in April. According to Haynert et al. (2017), the trophic position of Hypogastruridae ranks between primary and secondary decomposers. In addition, their NLFA patterns indicated a strong increase in bacterial NLFA marker concentrations in October. Therefore, they likely predominantly consumed organic material and associated bacteria in the autumn. Overall, these findings indicate plant-associated resource channelling during periods of high primary productivity or plant senescence, suggesting a system depending on plant resources recently entering the belowground system rather than soil organic matter.

Similar to the plant marker, bacterial NLFA marker concentrations varied with season, but distinct patterns were evident within each zone. Generally, concentrations in the USM were highest in April and October, whereas in the LSM they peaked in April and July. In

contrast, in species of the PZ they consistently decline from April to October. Peak concentrations of bacterial NLFA markers in the USM agree with the dynamics of PLFA markers presented in Chapter 2, pointing to resource control of consumer populations. In contrast, bacterial NLFA concentrations in consumers of the LSM were high in April and July suggesting that consumption of bacteria-associated resources benefitted from inundation. Bouchard et al. (1998) showed that the majority of LSM *A. portulacoides* litter was deposited within the marsh soil, presumably supporting bacterial growth. However, in July the LSM was subject to a stark increase in inundation frequency (Meier et al. 2020b), which, as discussed previously, likely displaced litter. However, Bouchard et al. (1998) also noted that during periods of frequent inundation the soil becomes enriched with microalgae. Therefore, bacteria likely benefitted from the regular inundation and subsequent enrichment in particular during summer, but this requires further investigation.

Finally, declining concentrations bacterial NLFA concentrations in the PZ across seasons coincides with the steady increase in inundation frequency across the sampling period (Meier et al. 2020b). Bouchard and Lefeuvre (2000) showed that primary productivity is lowest and tidal displacement of litter material is highest in the PZ. Therefore, considering the steady increase in inundation frequency, removal of litter and anoxia presumably limited bacterial growth. Few taxa/species indicated alternative patterns to the ones described previously, and this is likely linked to abiotic conditions and senescence of dominant plant species (see Chapter 3). Overall, bacterial channelling within salt marsh soil fauna is suggested to be linked to plant senescence, inundation frequency and the resulting substrate availability.

Fungal NLFA marker concentrations generally peaked in October and were lowest in July (Chapter 3). The increase in October, coincides with the senescence of dominant plants, providing substrate for saprotrophic fungi. However, no such temporal variation was evident in soil taxa/species which rely on the basal plant-fungi channel. Both *Zachvatkinibates quadrivertex* and Oppiidae (Oribatida) consumed fungi at similar concentrations throughout the sampling period. In both, plants were the dominant basal resource, thus consumption of fungi-colonised detritus prior to incorporation into the soil is likely (Calado and Barata 2012, Calado et al. 2019). This could indicate resource selection, rather than generalism in these species, but this requires further investigation. Overall, consumption of fungi showed a general temporal pattern linked to inundation and plant senescence.

Finally, algal NLFA marker concentrations were very low and did not vary consistently across taxa/species. In certain taxa/species from the USM and LSM algal NLFA marker concentrations peaked in April, confirming findings in Chapter 2. However, in some taxa/species in the LSM and PZ algal NLFA marker concentrations peaked in October (*Z. quadrivertex*) or were constant (*Archisotoma besselsi*). The presence of algal NLFA markers confirms previous observations of diatom remains in salt marsh collembolans (Mertens et al. 2007), suggesting selective consumption of algal resources by the collembola. But again, the plant-bacterial channel dominated in this species. Generally, these dynamics suggests

that allochthonous basal resources appear to be of little importance for the diet of salt marsh soil fauna, regardless of seasonality.

Results presented in Chapter 4 showed no temporal dynamics in the number of trophic levels, but both $\delta^{15}N$ and $\delta^{13}C$ signatures of taxa/species varied with season. *T. saltator* switched trophic position between secondary decomposer to primary decomposer across the sampling period. Presumably, due to a dietary switch from decomposing organic matter to fresh plant material in summer. However, no temporal variation in $\delta^{13}C$ was apparent, and Bayesian mixing model results show exclusive use of autochthonous resources (Chapter 4). Contrasting previous findings showing algal consumption by *T. saltator* on beaches (Adin and Riera 2003), as discussed in Chapter 4. However, the results confirm findings of the study presented in Chapter 3 showing the reliance of *T. saltator* on the plant-bacteria channel.

In *Ochthebius* sp. $\delta^{15}N$ and $\delta^{13}C$ varied across season, with $\delta^{13}C$ signatures additionally affected by zone (Chapter 4). In the USM, both isotopes remained stable across the sampling period. However, in LSM $\delta^{15}N$ signatures of *Ochthebius* sp. peaked in July, coinciding with the decline in $\delta^{13}C$ across the sampling period. Bayesian mixing models showed exclusive consumption of autochthonous resources in both zones. While these findings contrast prior indications of algal and detrital consumption by *Ochthebius* sp. on rocky shores (Beier and Pomeisl 1959, Perkins, D. 1980), they confirm the low algal NLFA marker concentrations shown in Chapter 3. Presumably, increasing inundation of the LSM limited the availability of detritus, thus promoting herbivory in this hydraenid beetle. Therefore, considering that *Ochthebius* sp. is a detrital feeder its isotopic composition depends on the constitution of detrital resources.

In Staphylinidae larvae, a temporal shift from primary decomposer (April) to secondary decomposer (July, October) was evident (Chapter 4). These changes coincide with greater production of litter of dominant plant species (Bouchard et al. 1998, Bouchard and Lefeuvre 2000) pointing to the consumption of little decomposed litter. Similarly, *D. gustavii* as well as *Amischa* sp. likely increased the consumption of litter in autumn. Both were only found in the USM, where a constant litter layer is present. *D. gustavii* lives as predator, targeting beetles such as *Bledius spectabilis* (Wyatt and Foster 1989). Whereas the trophic position of Aleocharinae such as *Amischa* sp. may vary from herbivore to predator (Thayer 2005). Finally, Bayesian mixing models indicated exclusive use of autochthonous resources in both *D. gustavii* and *Amischa* sp. (Chapter 4).

By contrast, consumption of some allochthonous material was evident in *Argenna* sp. (Araneae, Dictynidae) and Chalcidoid wasps in the LSM. *Argenna* sp. were only found in April and incorporated a significant amount of marine resources. These findings are in line with prior indications suggesting consumption of dipterans with marine larval stages (Hambäck et al. 2016). Chalcidoid wasps on the other hand were found in July and October and are known to parasitize dipterans, such as seaweed flies developing in marine detritus

(Notton 1996). Therefore, allochthonous signatures in both taxa/species occurred indirectly through their prey/host.

Overall, the findings presented in Chapter 4 are in line with those presented in Chapter 3 in showing the dominant dependence of salt marsh soil invertebrates on autochthonous resources. With changes in trophic position depending on the production/biomass of detritus and inundation frequency determining the input of litter material. These findings support earlier suggestions (Schrama et al. 2017) that the diversity of herbivore and detrital taxa/species in salt marshes are decoupled in time but with both depending on the productivity of local vascular plants.

The studies presented in this thesis reflect a soil decomposer system reliant on autochthonous resources and their microbial decomposers, mainly bacteria (bottom-up). Dominance of bacteria, as mentioned previously, suggests high nutrient input and fast turnover (Wardle et al. 2004, Scheu et al. 2005).

Microbial communities and their channelling were strongly affected by abiotic conditions within each zone. Similar to forest systems, where site conditions affect the susceptibility of microbial communities to stress (Moore-Kucera and Dick 2008, Pollierer et al. 2015). Despite the lack of a litter layer in frequently inundated zones, bacteria remained the dominant microbial decomposers, pointing to a continuous influx of easily available resources nourishing these microbes, as suggested by Mueller et al. (2020). As shown by stable isotopes and fatty acid analysis, these resources originate predominantly from autochthonous resources, contrasting results of Polis and Hurd (1996) who suggested that allochthonous input to marine islands contributes substantially to the diet of detritivores.

Ecosystems relying heavily on allochthonous resources include glacier forelands, freshwater systems and coastal areas (Polis and Hurd 1996, Doi 2009, Ingimarsdóttir et al. 2014, Neres-Lima et al. 2017, Minshall 2018). However, as evidenced across the previous chapters allochthonous resources such as marine algae contribute little to the salt marsh food web. As suggested by Huxel and McCann (1998) low allochthonous input may stabilise food web dynamics.

In particular in terrestrial systems of the temperate and boreal zone such as forests and grasslands, temporal dynamics of basal resources, driven by seasonal changes in abiotic conditions, contribute to the temporal dynamics of animal food webs (Pollierer et al. 2015, Bayranvand et al. 2017). Unlike these systems, salt marsh microbial communities as well as the channelling of basal resources to higher trophic levels varied remarkably little across temporal scales. This may also reflect that bacteria which dominated microbial communities of the salt marsh may be less sensitive to changes in abiotic conditions than fungi (Bossio and Scow 1998, Alzarhani et al. 2019). This lower sensitivity combined with constantly available autochthonous resources may contribute to the remarkable temporal constancy of salt marsh soil food webs. Alternatively, delayed response by the soil biota to changes in resources, as described by Eisenhauer et al. (2012) may explain these findings.

Concluding remarks

This thesis aimed to uncover spatiotemporal variations of salt marsh soil food webs including basal resources, energy channels and trophic structure. The results illustrated that spatial rather than temporal dynamics are the dominant factor in the salt marsh soil system. Basal resources were remarkably stable across temporal scales but varied across both sediment depth and shore height. Channelling of basal resources to higher trophic levels was restricted to autochthonous plant material colonized by microbial decomposers, in particular bacteria, with temporal dynamics restricted to few taxa/species. Furthermore, trophic dynamics showed that the number of trophic levels did not change, but in certain taxa/species the trophic position varied across seasons.

Temporal dynamics were likely driven by inundation frequency and associated variations in the deposition of detritus as well as plant or algae productivity. The dominance of spatial variations, however, reflect that salt marsh soil food webs are structured predominantly by abiotic factors driven by inundation frequency and resulting plant zonation. Furthermore, the results showed that marine carbon contributes little to the salt marsh soil food web. While algae were present across salt marsh zones and seasons, their contribution to the diet of consumers was low. Instead, autochthonous plant material colonised by bacterial decomposers was the dominant resource consumed. The dominance of the plant-bacteria channel points to a decomposer system relying on autochthonous resources with fast nutrient turnover which may contribute to the remarkable stability of the salt marsh food web.

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Appendix - Supplementary materials for Chapter 4

"Trophic structure and origin of resources of soil macrofauna in the salt marsh of the Wadden Sea: a stable isotope (^{15}N,^{13}C) study"

Supplementary table 1: Summary table of resource isotope values used for each Bayesian mixing model. Separated into Marine - meaning algae - and terrestrial - meaning vascular C3 plant species. Terrestrial vascular plants were collected across the USM - upper salt marsh and LSM - lower salt marsh. For the Bayesian mixing model only overall mean values of marine resources and terrestrial resources were used.

Season	Zone	Resource type	Species	^{15}N	^{13}C
	USM	Terrestrial	*Artemisia maritima*	4.01	-27.08
	USM	Terrestrial	*Elymus athericus*	6.04	-27.15
	LSM	Terrestrial	*Atriplex portulacoides*	8.59	-26.59
	LSM	Terrestrial	*Puccinellia maritima*	7.94	-26.44
	LSM	Terrestrial	*Spergularia salina*	7.78	-27.09
April			**Terrestrial mean =**	**6.873**	**-26.868**
	N/A	Marine	*Cystoclonium purpureum*	11.24	-18.37
	N/A	Marine	*Fucus vesiculosus*	10.04	-20.54
	N/A	Marine	*Rhizoclonium riparium*	10.70	-19.84
	N/A	Marine	*Ulva lactuca*	9.85	-18.44
			Marine mean =	**10.458**	**-19.295**
	USM	Terrestrial	*Atriplex littoralis*	8.83	-27.60
	USM	Terrestrial	*Atriplex prostrata*	6.23	-27.61
	USM	Terrestrial	*Elymus athericus*	5.32	-24.44
	LSM	Terrestrial	*Artemisia maritima*	5.82	-26.76
	LSM	Terrestrial	*Atriplex portulacoides*	8.27	-27.19
	LSM	Terrestrial	*Spergularia salina*	7.31	-27.17
July	LSM	Terrestrial	*Triglochin maritima*	9.08	-25.91
			Terrestrial mean =	**7.266**	**-26.667**
	N/A	Marine	*Cystoclonium purpureum*	13.81	-18.85
	N/A	Marine	*Fucus sp.*	11.65	-17.567
	N/A	Marine	*Rhizoclonium riparium*	13.24	-18.752
	N/A	Marine	*Ulva lactuca*	12.45	-14.658
			Marine mean=	**12.787**	**-17.457**
	USM	Terrestrial	*Atriplex littoralis*	6.80	-27.12
	USM	Terrestrial	*Atriplex prostrata*	4.89	-27.41
October	USM	Terrestrial	*Elymus athericus*	4.43	-25.72
	LSM	Terrestrial	*Artemisia maritima*	7.10	-27.26

Supplementary Table 1: continued

	LSM	Terrestrial	*Atriplex portulacoides*	8.31	-26.55
	LSM	Terrestrial	*Puccinellia maritima*	8.42	-28.62
	LSM	Terrestrial	*Spergularia salina*	8.14	-27.47
			Terrestrial mean =	**6.869**	**-27.165**
October	N/A	Marine	*Chaetomorpha linum*	11.94	-18.01
	N/A	Marine	*Cystoclonium purpureum*	10.17	-19.44
	N/A	Marine	*Enteromorpha sp.*	11.66	-16.47
	N/A	Marine	*Fucus sp.*	11.50	-19.26
	N/A	Marine	*Rhizoclonium riparium*	9.21	-20.13
	N/A	Marine	*Ulva lactuca*	9.283	-16.933
			Marine mean=	**10.627**	**-18.373**
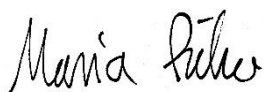
Supplementary table 2: Summary of Macrofauna, terrestrial plants and marine algae species collected on the island of Spiekeroog saltmarsh in 2019.

	Order	Family	Sample
Fauna	Amphipoda	Talitridae	*Talitrus saltator*
	Aranea	Linyphiidae	*Linyphiidae* spp.
		Dictynidae	*Argenna* spp.
	Coleoptera	Hydraenidae	*Ochthebius dilatatus*
		Carabidae	*Dicheirotrichus gustavii*
			Dyschirius spp.
		Staphylinidae	*Amischa* spp.
			Staphylinidae larvae
	Hymenoptera	Chalcididae	*Unidentified chalcid wasp*
Flora	Alismatales	Juncaginaceae	*Triglochin maritima*
	Asterales	Compositae	*Artemisia maritima*
			Tripolium pannonicum
	Caryophyllales	Amaranthaceae	*Salicornia europaea*
			Salicornia spp.
			Sueda maritima
		Caryophyllaceae	*Spergularia salina*
		Chenopodiaceae	*Atriplex littoralis*
			Atriplex portulacoides
			Atriplex prostrata
	Cladophorales	Cladophoraceae	*Chaetomorpha linum*
			Rhizoclonium riparium
	Fucales	Fucaceae	*Fucus* spp.
			Fucus vesiculosus
	Gigartinales	Cystocloniaceae	*Cystoclonium purpureum*
	Poales	Poaceae	*Elymus athericus*
			Puccinellia maritima
			Spartina anglica
	Ulvales	Ulvaceae	*Enteromorpha* spp.
			Ulva lactuca

Thesis declaration

Plagiarism declaration

I declare that I have written this doctoral dissertation independently. All persons contributing to the manuscripts have been named so. All sentences or passages quoted from other people's work have been specifically acknowledged by clear cross-referencing. I have not submitted this dissertation in any form for another degree at any university or institution. I bindingly confirm that the contents of the digital version are identical with the written version.

Maria Rinke

Göttingen, Tuesday, 27 September 2022

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