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 use of basal resources by the soil macrofauna along marine – terrestrial boundaries. Natural variations in carbon stable isotope ratios ($\delta^{13}\text{C}$ 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 ($\delta^{15}\text{N}$ 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. The range of $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ signatures, the range in $\delta^{13}\text{C}$ 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 allochthonous resource use 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 time than in space, 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]. 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 competing 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. Despite this, few studies have investigated the change in basal resources across zones and the associated changes in trophic structure in Wadden Sea salt marshes.

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 Bakker et al. (2015) as well as Schrama et al. (2013) 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 *Dicheirotichus 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 have suggested that 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. For studying the trophic structure of animal communities natural variations in stable isotope ratios ($^{15}\text{N}/^{14}\text{N}$, $^{13}\text{C}/^{12}\text{C}$) 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]. However, the trophic structure of the soil macrofauna, their resource-use and changes across 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, studies based on stable isotopes allowed insight into the diet and trophic position of benthic macrofauna and mesofauna of salt marshes [9, 32, 33]. Stable isotope analysis allows determining trophic levels through $^{15}\text{N}/^{14}\text{N}$ ratios as well as identification of the resources used through $^{13}\text{C}/^{12}\text{C}$ 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]. $\delta^{13}\text{C}$ signatures become especially useful when investigating the use of marine and terrestrial resources due to the distinct $\delta^{13}\text{C}$ signatures of algae [9, 23, 33]. Differences in $\delta^{13}\text{C}$ signatures between algae and other plants are due to algae using bicarbonate as inorganic carbon source resulting in enriched $\delta^{13}\text{C}$ signatures, which may also vary with light and nutrient availability [39–41]. Therefore, using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures may allow discerning 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]. Two types of marshes exist, the "back-barrier" and "foreland" salt marshes [8]. 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 [43] and stored in 70 % thanol at -20°C. Animals were determined to group or species level [44]. Additionally, litter, soil, vascular plant species and macroalgae were collected and stored at -20°C (Supplementary table 2).

Sample preparation and stable isotope analysis

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 ($^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$) 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) [45]. Ratios of stable isotopes were expressed as $\delta X (\text{‰}) = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$, with 'X' representing the target isotope and 'R' the heavy-to-light isotope ratios ($^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$) of the sample and standard, respectively. Vienna PD Belemnite (PDB) was used as standard for $\delta^{13}\text{C}$ and atmospheric nitrogen for $\delta^{15}\text{N}$. Acetanilide was used for internal calibration.

Statistical analysis

Signatures of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ of macrofauna taxa were analysed using linear mixed effects models with "Zone" and "Season" 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*, *Ochthebius/Asiobates dilatatus* and *D. gustavii* 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 (July and October) were only found in the LSM, and therefore only season was fitted as fixed factor. Similarly, in *Amischa* spp. only season was fitted as fixed factor as it was only found in the USM (April, July and October). In cores where two or more species occurred, the ranges in ^{13}C and ^{15}N signatures of the species were calculated (most ^{13}C or ^{15}N enriched – least ^{13}C or ^{15}N enriched) and analysed 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 3.5‰ 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 Potapov et al. (2019) and reflects the relative enrichment of soil animals compared to litter. Because of lack of replicates across season for *Dyschirius* sp. *Argenna* sp. and Linyphiidae, mixing models analysing variations in stable isotope signatures in these species / taxa 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. To determine changes in the isotope range of animal species / taxa with Zone and Season, the range of ^{15}N and ^{13}C signatures of animal species / taxa per plot was used.

Results

In total, six macrofauna species, 20 plant species (Supplementary table 2) as well as soil and litter were collected and analysed for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures. In April, animal $\delta^{13}\text{C}$ signatures ranged from -21.63 to -25.93‰ and $\delta^{15}\text{N}$ signatures from 5.02 to 13.97‰, respective ranges in July were -22.50 to -25.65 for $\delta^{13}\text{C}$ and 5.05 to 18.93‰ for $\delta^{15}\text{N}$, and in October -22.83 to

-26.19‰ for δ¹³C and 4.10 to 14.79‰ for δ¹⁵N (Fig. 1). Respective ranges of terrestrial vascular plants for δ¹³C and δ¹⁵N 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 (Fig. 1).

Salt marsh zones

Among individual taxa δ¹⁵N 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, δ¹³C 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* δ¹³C signatures in the LSM were significantly lower than in the USM (-25.92 ± 0.35‰ and -24.73 ± 0.40‰, respectively) (Table 1).

Table 1

F- and p-values of linear mixed-effects model on variations in δ¹⁵N and δ¹³C signatures of macrofauna taxa with season (April, July, October) and salt marsh zone (upper salt marsh, lower salt marsh) on the island of Spiekeroog in 2019; 'n' number of replicates; ' ' not significant, * p < 0.05, ** p < 0.01, *** p < 0.001; N/A, not analysed.

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
<i>Talitrus saltator</i>	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
<i>Ochthebius dilatata</i>	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
<i>Dicheirotrichus gustavii</i>	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				
<i>Amischa</i> spp.	9	Only USM				0,41	0,705	27,33	0,030				

Trophic levels

Typically, the communities spanned three trophic levels, including primary decomposers, secondary decomposers and first order predators (Fig. 1). Generally, the range in ¹⁵N signatures neither varied significantly between zones nor among seasons and averaged 5.18 ± 0.26‰ in the USM and 6.21 ± 1.42‰ in the LSM. By contrast, the range in ¹³C signatures varied significantly with Zone, with 0.91 ± 0.40‰ in the USM and 2.67 ± 0.43‰ in the LSM (F_{1,10} = 15.26, p = 0.001), but not with Season. In addition, in the USM *T. saltator* changed its trophic level from secondary decomposer in April and October to primary decomposer in July (Fig. 1a, c, e). Furthermore, in the USM *Amischa* sp. changed its trophic level from secondary decomposer in April to first order predator in October.

Season

Among individual species, δ¹⁵N signatures only varied significantly with season in *D. gustavii*, *O. dilatatus*, Staphylinidae larvae and *T. saltator* (Table 1). In *D. gustavii* δ¹⁵N 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‰). Staphylinidae larvae δ¹⁵N significantly increased from April (8.62 ± 0.62‰) to July (13.66 ± 2.15‰) and remained stable in October (12.36 ± 1.18‰). In *T. saltator* δ¹⁵N declined from April (9.09 ± 1.61‰) to July (7.20 ± 1.28‰) and remained stable in October (7.79 ± 1.38‰). δ¹³C signatures varied significantly with season in *D. gustavii* and *Amischa* sp. Furthermore, in *O. dilatatus* the effect of Season depended on Zone (Table 1). In *D. gustavii* δ¹³C signatures significantly decreased from July (-25.16 ± 0.65‰) to October (-25.73 ± 0.75‰). For *Amischa* sp. δ¹³C signature increased from

April ($-24.05 \pm 1.04\text{‰}$) to July ($-23.97 \pm 1.32\text{‰}$) and declined again in October ($-24.52 \pm 1.01\text{‰}$). By contrast, in *O. dilatatus* $\delta^{13}\text{C}$ signatures changed little with Season in the USM (-23.95 ± 0.46 , $-23.51 \pm 0.01\text{‰}$ and $-23.46 \pm 0.42\text{‰}$ for April, July and October, respectively), whereas in the LSM $\delta^{13}\text{C}$ signatures gradually declined from April ($-22.19 \pm 0.12\text{‰}$) to July ($-22.57 \pm 0.14\text{‰}$) to October ($-23.14 \pm 0.32\text{‰}$).

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 ($70.4 \pm 17.8\%$) (Fig. 2a); in July they used 90–97% terrestrial resources with the exception of the chalcidoid wasps of the LSM ($74.4 \pm 17.3\%$) (Fig. 2b). In October they used 83–97% terrestrial resources with the exception of Staphylinidae larvae in the LSM ($75.6 \pm 16.2\%$) (Fig. 2c).

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 (Haynert et al. 2017). Stable isotope signatures varied significantly between salt marsh zones and seasons, with a significant interaction between Zone and Season for *O. dilatatus*. Further, in the LSM the range of $\delta^{13}\text{C}$ signatures significant increased later in the year. 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.

Differences between salt marsh zones

Stable isotope signatures of soil macrofauna species / taxa differed significantly between the USM and LSM confirming our first hypothesis. $\delta^{15}\text{N}$ signatures were generally higher in the LSM compared to the USM and this applied in particular to *O. dilatatus* and *T. saltator* as well as in trend to *D. gustavii*. Only $\delta^{13}\text{C}$ signatures varied significantly between salt marsh zones in *D. gustavii*, with higher enrichment in the USM than the LSM. Higher $\delta^{15}\text{N}$ signatures in macrofauna species / taxa in the LSM than in the USM contrast patterns in salt marsh mesofauna, in which $\delta^{15}\text{N}$ signatures were higher in the USM than in the LSM (Haynert et al. 2017). Haynert et al. (2017) suggested this pattern to indicate longer food chains in the USM, which we did not find in our study. Considering the higher $\delta^{15}\text{N}$ 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 higher enrichment of basal resources, pointing to the predominant use of autochthonous resources. In *D. gustavii* $\delta^{15}\text{N}$ 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. Furthermore, in the LSM *D. gustavii* was significantly less enriched in ^{13}C compared to the USM. Predation on algal-feeding rove beetle larvae of *Bledius spectabilis* may explain the trophic position of *D. gustavii* in the LSM [29, 46]. Algae rely on atmospheric N and are generally considered to be depleted in ^{15}N (Potapov et al. 2019). However, the low $\delta^{13}\text{C}$ signatures of *D. gustavii* in the LSM suggests no consumption of marine resources. Instead, as discussed in Potapov et al (2019), low $\delta^{13}\text{C}$ and high $\delta^{15}\text{N}$ signatures indicates the consumption of resources based on freshly fixed carbon or consumers thereof, again pointing to the dominant use of autochthonous resources. Overall, we found significant species-specific increases of $\delta^{15}\text{N}$ signatures in the LSM, supporting the dominant use of $\delta^{15}\text{N}$ enriched autochthonous resources in the LSM. Enrichment in ^{13}C in *D. gustavii* in the LSM also points to the use of autochthonous C3 plant 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 [47]. These findings are in contrast to Haynert et al. (2017) 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 [48]. However, while inundation frequency indeed is lower in the USM than in the LSM, soil salinity is high

in both the USM and LSM [49, 50]. As discussed by Connell (1978) increased abiotic stressors may reduce diversity and favour the dominance of specialists. Contrasting these assumptions, the wider range in $\delta^{13}\text{C}$ signatures of macrofauna species in the LSM compared to the USM points to more flexible resource use in species of the former, presumably due to increased use of surface microalgae [12]. Overall, the results suggest that the salt marsh soil macrofauna food web is simpler than expected, presumably due to frequent flooding and associated disturbances resulting in the dominance of tolerant specialists increasing their resource spectrum in particular in the LSM.

Season

Although seasonal variations in stable isotope signatures generally were less pronounced than variations between salt marsh zones, $\delta^{15}\text{N}$ signatures varied significantly with season in *D. gustavii*, *O. dilatatus*, Staphylinidae larvae and *T. saltator*. Further, $\delta^{13}\text{C}$ signatures varied significantly in *D. gustavii*, *Amischa* sp. and *O. dilatatus*, but in the latter this depended on salt marsh zone confirming our third hypothesis. $\delta^{15}\text{N}$ 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 $\delta^{13}\text{C}$ signatures from July to October indicating a switch from herbivore prey in July to decomposer prey in October (cf. Potapov et al. 2019). In *O. dilatatus*, $\delta^{15}\text{N}$ signatures in the LSM, but not the USM, were higher in July than in April and October. Further, $\delta^{13}\text{C}$ 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 (Beier and Pomeisl 1959, Perkins 1980). In the salt marsh *Ochthebius* species occur in or near saline channels, with larval stages living submerged [54–56] 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. $\delta^{15}\text{N}$ of LSM Staphylinidae larvae point to a trophic change from primary decomposer in April, to secondary decomposer in July and October. These changes could point to increased consumption of partially decomposed litter, or the direct consumption of primary decomposer by later larval stages. Variations in $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ 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, $\delta^{13}\text{C}$ 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 (cf. Potapov et al. 2019). 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 resources 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. 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 [57]. As indicated by their $\delta^{15}\text{N}$ 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., 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 (Hambäck et al. 2016). 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 [58]. 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 communities comprising predominantly habitat specialists. $\delta^{13}\text{C}$ signatures and Bayesian mixing models indicated that the macrofauna community 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.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

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>

Competing interests

The authors declare that they have no competing interests in this study.

Relevant guidelines and regulations

All research methods performed in this study complied with the permission granted to the DynaCom research project for 2019 under §21 article 1 of the NWattNPG from the Wadden Sea National Park of Lower Saxony (Germany).

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Authors' contributions

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Sample collection: Maria Rinke, Philipp M. Bendisch

Selection of taxa: Maria Rinke

Stable isotope preparation: Philipp M. Bendisch

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Data analysis: Maria Rinke

Figure preparation: Maria Rinke

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Figures

Figure 1

Scatterplot of $\delta^{15}\text{N}$ und $\delta^{13}\text{C}$ signatures of salt marsh fauna species / taxa (circles), plants (triangles), litter (diamonds) and soil (crossed squares) in the (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 $\pm$ SD; green = upper salt marsh (USM), blue= lower salt marsh (LSM). Dotted lines indicate trophic levels. Animals: Ami. – Amischa sp., Arg. – Argenna sp., D.gust - Dicheirotichus 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.

Figure 2

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.

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