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Modification of benthic food web structure by recovering seagrass meadows, as revealed by trophic markers and mixing models

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ABSTRACT

Seagrass meadows are among the most diverse and productive coastal ecosystems in the world. Currently, the accelerating loss of these habitats is recognized worldwide. In the southern Baltic Sea, a natural recovery of *Zostera marina* meadows has occurred after a dramatic reduction within the last century. The aim of this study is to understand if and how the recovering eelgrass meadows affect the functioning of benthic ecosystems. The trophic links within the benthic food webs in the seagrass meadows and bare sandy bottoms were depicted and compared. The trophic connections were examined by combining stable isotope (SI) composition ($\delta^{13}C$, $\delta^{15}N$) and fatty acid (FA) profiles of meio- and macrofauna consumers and of potential food sources (particulate organic matter, surface sediment organic matter, epiphytes, microphytobenthos/bacteria and macrophytes) in a Bayesian mixing model framework (MixSIAR). Significantly higher amounts of the FA bacterial marker (C18:1ω7) were observed in meiofauna (approximately 40%) than in the macrofauna (1% on average), suggesting that bacteria are an important part of the meiofauna diet. The mixing model results indicated that the benthic consumers in the vegetated habitat utilized more food sources (e.g., epiphytes in the diets of meiofauna and macrofaunal grazers) and thus had a more diverse diet. Macrofaunal omnivores relied to a larger degree on animal-derived organic matter in vegetated habitat, which could be linked to higher invertebrate prey availability. The results highlight the importance of recovering seagrass meadows in driving the mechanisms responsible for food web organization. Any type of change to the state of seagrass meadows is crucial to the functioning and stability of marine ecosystems.

1. Introduction

Seagrass meadows are among the most diverse and highly productive coastal ecosystems in the world (Hemminga and Duarte, 2000). The plants play an important role as foundation species of seagrass habitats, by creating three-dimensional structures (leaves, rhizomes, roots). Hence, they increase the complexity of the seabed architecture and provide shelter and numerous niches for other organisms (Gartner et al., 2013). Moreover, seagrasses and associated macrophytes or algae can be direct food sources for faunal consumers; thus, they sustain populations of commercially important vertebrate and invertebrate species (Hemminga and Duarte, 2000).

Over the past few decades, significant decreases in seagrass abundance and aerial cover have been recorded worldwide (Waycott et al., 2009). Changes in the state of seagrasses affect coastal systems and may result in a reduction in fauna abundance and diversity, as well as in modification of food web structures and overall functioning (Boström et al., 2014). The mechanisms driving food web organization within seagrass meadows remain poorly understood as they may depend on local conditions, meadow features and consumer species pools (Boström et al., 2014). The trajectory of eelgrass meadows extent in the southern Baltic Sea off the Polish coast has shown dramatic changes over the past 60 years. Before the 1950s, most of the seafloor of the Inner Puck Bay (103 km^2) was covered by the meadows. A significant decrease (most likely caused by eutrophication and massive growth of filamentous algae) of *Zostera marina* in the area was observed in 1987, when the eelgrass area declined to only 16 km^2 (Kruk-Dowigałło, 1991).

Recently, the natural recovery of eelgrass-dominated underwater meadows has occured in several locations in the Gulf of Gdańsk

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(Jankowska pers. observation). The coverage of the seagrass beds increased rapidly, but the density and biomass of the plants remain low compared to other temperate *Z. marina* meadows (Jankowska et al., 2014).

The aim of the present study is to investigate if and how the recovering eelgrass meadows affect the overall functioning of the benthic systems in the Gulf of Gdańsk (southern Baltic Sea) in terms of food web organization and thus energy flow. The Gulf of Gdańsk serves as an example of a system characterized by both a low level of vegetation development (low density and biomass of macrophytes) and a low faunal diversity (defined by the Baltic Sea species pool). We hypothesize that recovering (low density) eelgrass meadows are capable of shaping the food web structure in similar way as that observed in well-developed meadows, i.e., by increasing the consumer pools and the number of food sources in the diets of benthic consumers.

Most of the effects of seagrass on food webs are reported from well-developed meadows and diverse benthic communities (Lepoint et al., 2000; Vizzini et al., 2002; Leduc et al., 2006, 2009; Baeta et al., 2009; Lebreton et al., 2011, 2012; Michel et al., 2015; Vafeiadou et al., 2013, 2014). The present study documents the structure of the benthic food web in two contrasting habitats: vegetated sediments of recovering meadows and the adjacent bare sands. This study includes all major possible food sources (bacteria/microphytobenthos, epiphytes, macroalgae, seagrass, particulate organic matter, and surface sediment organic matter) and most consumers are analyzed at the species level (meiofauna copepods, macrofauna and fish). Stable isotopes and fatty acids biomarkers are used in Bayesian mixing models (MixSIAR) to estimate the relative contribution of food sources in the consumers' diet. This study is the first field study in a seagrass habitat integrating all components of a benthic system and using both types of biomarkers in Bayesian mixing models to achieve an as accurate as possible depiction of the trophic links in a benthic food web. Moreover, the focus on recovering seagrass meadows is a novel approach that is now crucial because dramatic changes of seagrass cover are occurring, and intensive restoration programs are being undertaken worldwide.

2. Materials and methods

2.1. Study area

The study took place in the Gulf of Gdańsk, located in the southern Baltic Sea, off the Polish coast where salinity reaches approximately 8 (Nowacki, 1993). A recent inventory of the seabed habitats in the Polish Exclusive Economic Zone documented that areas covered by *Z. marina* meadows are rapidly growing (water.iopan.gda.pl/projects/Zostera/index-pl.html). The areal distribution of eelgrass beds, estimated in 2009, was 48 km^2 for inner Puck Bay (Węsławski et al., 2013). Currently, the actual eelgrass-covered area may be even greater, as new locations of seagrass areas in the Gulf of Gdańsk were observed during sampling campaigns in 2012–2014 (Jankowska, pers. observation). Meadow density in the summer seasons reached an average of 202 shoots m^{-2} with a biomass of 40 g dw m^{-2}, which is lower compared to other temperate eelgrass meadows in the Northern Hemisphere (Jankowska et al., 2014).

2.2. Field sampling

Two sampling stations were selected in the Gulf of Gdańsk – one within the extensive eelgrass meadows and one in the neighboring large unvegetated area; both stations were located at similar depths (1.5–2 m), approximately 2.3 km from each other (Fig. S1, Supporting Information). Sampling (by scuba divers) occured in the summer (August 2014) when seagrass vegetation development is at its maximum in the Gulf of Gdańsk (Jankowska et al., 2014). Six replicates (3 replicates for stable isotope (SI) analyses and 3 replicates for fatty acid (FA) analyses) for each source at each station were collected in the summer (Table S2, Supporting Information). The same replication protocol was used for the consumers (it was not achieved in case of rare taxa). Sampled potential food sources included: SSOM (surface sediment organic matter, collected using sediment cores), POM (particular organic matter from the water column, 6 liters of water collected at a depth of 1 m), microphytobenthos (upper 2 cm sediment layer, collected using a syringe and extracted in a laboratory), filamentous algae (*Pylaiella littoralis*), benthic macrophytes including eelgrass below- and aboveground structures and epiphytes (on the surface of the eelgrass leaves). Samples of consumers included meiofauna (taxa associated with the seagrass leaves collected with a 42 μm mesh size net and seabed taxa collected in the upper 2 cm of sediments), macrofauna and fish (collected with a sediment corer (upper 10 cm) and a small dredge, respectively). For the meiofauna, only two harpacticoid copepod species – *Paraleptastacus spinicauda* (family Leptastacidae) and *Tachidius discipes* (family Tachidiidae) – were chosen for analysis since only they were abundant enough to collect a sufficient number of individuals.

2.3. Laboratory analysis

Samples were processed in the laboratory immediately after sampling. Water was prefiltered on a 320 μm sieve to eliminate large zooplankton and then filtered through GF/F Whatman glass fiber filters (0.7 μm porosity). Macrophytes were identified, and epiphytes were detached from the leaves by shaking using a vortex mixer (10 min) and sonicating (2 × 60 s, using Sonifier Tansonic Labor 2000) the seagrass leaves and macrophytes in prefiltered seawater. Then, the water containing detached epiphytes was filtered through GF/F Whatman glass fiber filters (0.7 μm porosity). Fresh microphytobenthos was collected by transferring the upper 1 cm of the sediment to plastic boxes. Then, the sediment was covered with a 100 × 150 mm Whatman lens cleaning tissue and cover glass, and exposed to artificial white light source, to enable diatom migration. After 24 h, the microphytobenthos were scraped off the cover slides and transferred to vials with prefiltered seawater. However, the FA analyses indicated that these samples contained large quantities of bacteria; thus they were treated as a mixture of bacteria and microphytobenthos.

Adult meiofauna individuals were extracted alive from the sediment using decantation and attraction of positive phototactic copepods with artificial white light. Copepods were placed alive in a petri dish with pre-filtered seawater for a few hours to allow gut clearance. Afterwards, individuals of the two species were picked under the stereomicroscope to make several replicate samples, each replicate sample consisting of 200 adults. Macrofauna and fish were kept for 24 h in prefiltered seawater to purge their gut contents. Then, they were identified to the species level. When individuals were too small to provide enough tissue for one sample, several individuals (from 1 to 60, detailed information presented in Table S2) were pooled to obtain sufficient biomass for a sample. Samples of the fish consisted of parts of their muscles (each sample representing one individual).

All samples were placed in glass vials and stored at −80 °C. Afterwards, samples were freeze-dried and ground for further analysis and then kept at −80 °C. Subsequently, all samples were analyzed for total fatty acids content and stable isotopes ($\delta^{13}C$ and $\delta^{15}N$ [‰]). Lipid extraction, FA methylation and an analysis of fatty acid methyl esters (FAMEs) were performed in a one-step method according to Abdulkadir and Tsuchiya (2008) and De Troch et al. (2005) at the Marine Biology Research Group (Ghent University). The fatty acid nonadecanoic acid (C19:0) was added (to copepods and microphytobenthos – 20 μl, to the other samples – 40 μl, Fluka 74208) as an internal standard to allow quantification later. The FA shorthand notation A:BωX was used, where A represents the number of carbon atoms, B gives the number of double bounds and X is the position of the double bound closest to the terminal methyl group (Guckert et al., 1985). The results for each FA were expressed as the relative percentage [%] of the total FA content ± standard deviation. Total FA content refers to the FA content detected

with the applied method. Accordingly, all samples were analyzed for $\delta^{13}C$ [‰] and $\delta^{15}N$ [‰] via continuous flow – elemental analysis – isotope ratio mass spectrometry (CF-EA-IRMS) at the University of Liège using a vario MICRO cube elemental analyzer (Elementar Analysen systeme GmBH, Hanau, Germany) coupled to an IsoPrime100 mass spectrometer (Isoprime, Cheadle, United Kingdom). Prior to the analyses, to remove inorganic carbon for the measurements, the sediment, filters, gastropods and fish samples were acidified with the direct addition of HCl (Hedges and Stern, 1984) and then dried again at 60 °C for 24 h. Sucrose (IAEA-C6, $\delta^{13}C = -10.8 \pm 0.5$‰, mean ± st.dev.) and ammonium sulfate (IAEA-N_2, $\delta^{15}N = 20.3 \pm 0.2$‰, mean ± st.dev.) were used as the certified reference materials (CRMs). Both CRMs were calibrated against international isotopic references, i.e., the Vienna Pee Dee Belemnite (VPBD) for carbon and atmospheric air for nitrogen. The standard deviations of the multi-batch replicate measurements of lab standards (amphipod crustaceans) as well as glycine (Merck, Darmstadt, Germany) interspersed among the samples were 0.1‰ for $\delta^{13}C$ and 0.2‰ for $\delta^{15}N$.

2.4. Data analysis

The dataset consisted of data on relative FA and SI composition in three main groups of samples: potential food sources (11 sources), meiofauna (2 copepod species), macrofauna (19 species) and fish (3 species) (Table S2, Supporting Information).

Macrofauna species were assigned to four trophic groups based on published literature: suspension feeders (feeding on suspended organic matter particles), suspension/detritus feeders (facultatively feeding on organic matter particles suspended in water or deposited on the bottom of the sea), grazers/herbivores (feeding on many possible sources but mainly on plant-derived material) and omnivores (with diets based on both plant and animal material, Table S2, Supporting Information).

2.4.1. Fatty acids and stable isotopes composition

To explore the patterns of similarity in FA compositions among the sources and consumers and between the two habitats (vegetated vs. unvegetated), Bray-Curtis similarities were calculated for log(x + 1) transformed data of the relative total FA concentrations. PERMANOVA tests were used to test the differences in a relative FA composition among groups of samples (PsF standing for pseudo F-statistic; Anderson et al., 2008). To test for differences in $\delta^{13}C$ and $\delta^{15}N$ among the sources and the consumers from two habitats, univariate PERMANOVA tests based on Euclidean distances (non-transformed data) of $\delta^{13}C$ and $\delta^{15}N$ values in samples were applied. One-way tests were performed to test for differences among sources (in groups defined in Table S2, Supporting Information). Two-way tests were performed to identify contrasts among the consumer groups (CG, meiofauna and four macrofaunal feeding groups) and between the two habitats (H).

FA trophic markers (FATMs) were identified and assigned to specific food sources (bacteria, diatoms, flagellates, vascular plants, detritus, terrestrial vegetation) based on the literature (Table 1). A one-way PERMANOVA test on Euclidean distances (untransformed data) was used to compare the relative contribution of selected FATMs in the FA profile of different sources and consumer groups.

Table 1
Fatty acids trophic markers (FATMs) used as tracers of particular food sources within this study; based on published literature.

Food source	FATMs	Reference
Bacteria	14:0, 18:1ω7	Jaschinski et al. (2008)
Diatoms	16:1ω7	Dalsgaard et al. (2003)
	20:5ω3	Kelly and Scheibling (2012)
Macroalgae	20:5ω3	
Flagellates	22:6ω3	Nelson et al. (2002)
Detritus	18:0, 18:1ω9	Søreide et al. (2008)
Marine vascular plants	18:2ω6	Richoux and Froneman (2008)
	18:3ω3	Kelly and Scheibling (2012)
Terrestrial vegetation	22:0, 24:0	Budge and Parrish (1998)

2.4.2. Mixing models

Bayesian mixing models were applied to calculate relative contributions of potential food sources to the diets of meio- and macrofauna consumers from the two habitats (vegetated and unvegetated) based on SI and FA data. Models were run using MixSIAR (R package, Stock and Semmens, 2013) with two factors (fixed factor habitat (H) and random factor species (Sp)). To meet the methodological requirements of the model, the potential food sources were grouped. The groupings were based on the similarity of the FA composition and SI values of the sources (tested by PERMANOVA). The final set of sources used as input for the mixing models included plants (all macrophytes and *Zostera* tissues), epiphytes (filamentous algae and epiphytes), microorganisms (microphytobenthos and bacteria) and POM/SSOM (grouped as have overlapping isotopic composition), meiofauna prey (mean values of markers of all meiofauna individuals) and macrofauna prey (mean values of markers of all macrofauna individuals, except omnivores). Different potential food sources were used in the model for each consumer group based on general knowledge about their feeding preferences (Jaschinski et al., 2008; Baeta et al., 2009; Lebreton et al., 2011; Vafeiadou et al., 2013; Michel et al., 2015).

To improve the precision in discriminating sources by the model and to fulfil the model assumptions regarding the numbers of sources and markers, both SI and FA data were used as tracers (Stock and Semmens, 2013). The FAs used in the models were selected based on the assignment of certain FAs to particular food sources (FATMs, Table 1) and assuming that they belong to different biosynthetic pathways of animals and represent basic dietary FA sources (Kelly and Scheibling 2012): diatom FATMs (16:1ω7 and 20:5ω3) and detritus FATMs (18:0, 18:1ω9). The relative FA data were log-transformed following the recommendations by Budge et al. (2006). Applied fractionation factors for SI were based on the meta-analysis of McCutchan et al. (2003) i.e. 0.4 ± 1.2‰ for carbon and 2.3 ± 1.6‰ for nitrogen. For the FAs, no fractionation factors were used, as the FAs used in the models were basic dietary FA sources that were abundant both in the sources' and consumers' tissue, and it was assumed that bioconversion by consumers was negligible. Models were run on 100 000 iterations, with no resource contribution data defined a priori (uninformative prior). Several diagnostics were used to define whether the model was run correctly: Gelman-Rubin Diagnostics smaller than 1.05 and Geweke diagnostics similar among 3 chains. The model solutions (95%, 75%, 25% Bayesian credibility intervals, mode solutions [%]) are presented. Additionally, when differences between the two habitats in the source contributions to consumer diet were detected, a direct pairwise comparison of the model solutions was applied to check the probability that a certain source contribution is higher in consumers from one habitat than in those from the other (1 is the highest possible value).

3. Results

3.1. FA and SI biomarkers in food sources

In total, nineteen FA have been identified and used for the food source composition analysis. The identified FA included seven saturated FAs (SAFAs), seven monounsaturated FAs (MUFAs) and five polyunsaturated FAs (PUFAs). There were significant differences in the relative FA composition ($P < 0.001$) and in selected FATM contributions among potential food sources ($P < 0.05$, PERMANOVA, Table 2).

The FA composition of plant species was clearly distinguished from all other sources. Each of the plants contained large amounts (average of 56.3% of the total FA) of the two vascular plant FATMs (18:2ω6 and 18:3ω3, Fig. 1).

Filamentous algae and epiphytes contained the highest proportions

Table 2
Results of two-way PERMANOVA tests for differences in fatty acids (FAs) composition (multivariate tests), trophic markers (FATMs) contributions and stable isotopes composition ($\delta^{13}C$, $\delta^{15}N$) (univariate tests) in consumer groups (CG, meiofauna species or macrofauna, fish trophic groups) from the two habitats (H) collected in summer in the Puck Bay (Baltic Sea). Significant main tests (PsF) are indicated by $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.5$.

Factors	PERMANOVA main test, PsF								
	FA composition	FATM						$\delta^{13}C$	$\delta^{15}N$
		Bacteria	Diatoms	Flagellates	Detritus	Vascular plants	Terrestrial vegetation		
H	0.8	0.9	0.1	0.5	0.3	0.1	1.8	6.7	0.0
CG	8.0^{***}	2.0	8.5^{***}	20.1^{***}	6.8^{***}	0.4	0.7	12.4^{***}	48.9^{***}
H × CG	2.7^{***}	0.3	2.9	2.4	3.6^{*}	1.9	1.4	3.5^{**}	0.8

of diatom FATMs – 20:5ω3 (36.2% and 14.5%, respectively). The FA composition of microphytobenthos/bacteria was dominated by the bacterial marker – 18:1ω7 (41.0%). The SSOM had very similar FA profiles at the two sampling locations (vegetated, unvegetated) and were characterized by high proportions of three FAs (18:1ω7, 18:1ω9 and 16:1ω7 – markers of bacteria, detritus and diatoms, respectively). The POM samples had high proportions of SAFA 18:0 (10.3%) and 16:0 (7.6%) (Fig. 1, Table S3, Supporting Information).

The composition of both carbon and nitrogen stable isotopes differed significantly among sources ($P < 0.001$, PERMANOVA, Table 2). The mean $\delta^{13}C$ values of the sources ranged from −25.0‰ (POM) to −10.6‰ (plants) (Fig. 2, Table 3). The second most $\delta^{13}C$-enriched source was microphytobenthos/bacteria (−15.3‰). Epiphytes and filamentous algae had very similar carbon isotopic composition (−19.3‰ and −18.7‰, respectively). The $\delta^{13}C$ values of SSOM were comparable for the two habitats; however the $\delta^{15}N$ value was higher for the SSOM in the vegetated bottom than the bare bottom (by 2.5‰). The mean $\delta^{15}N$ values ranged from 1.0‰ (SSOM unvegetated) to 6.4‰ (filamentous algae) (Fig. 2).

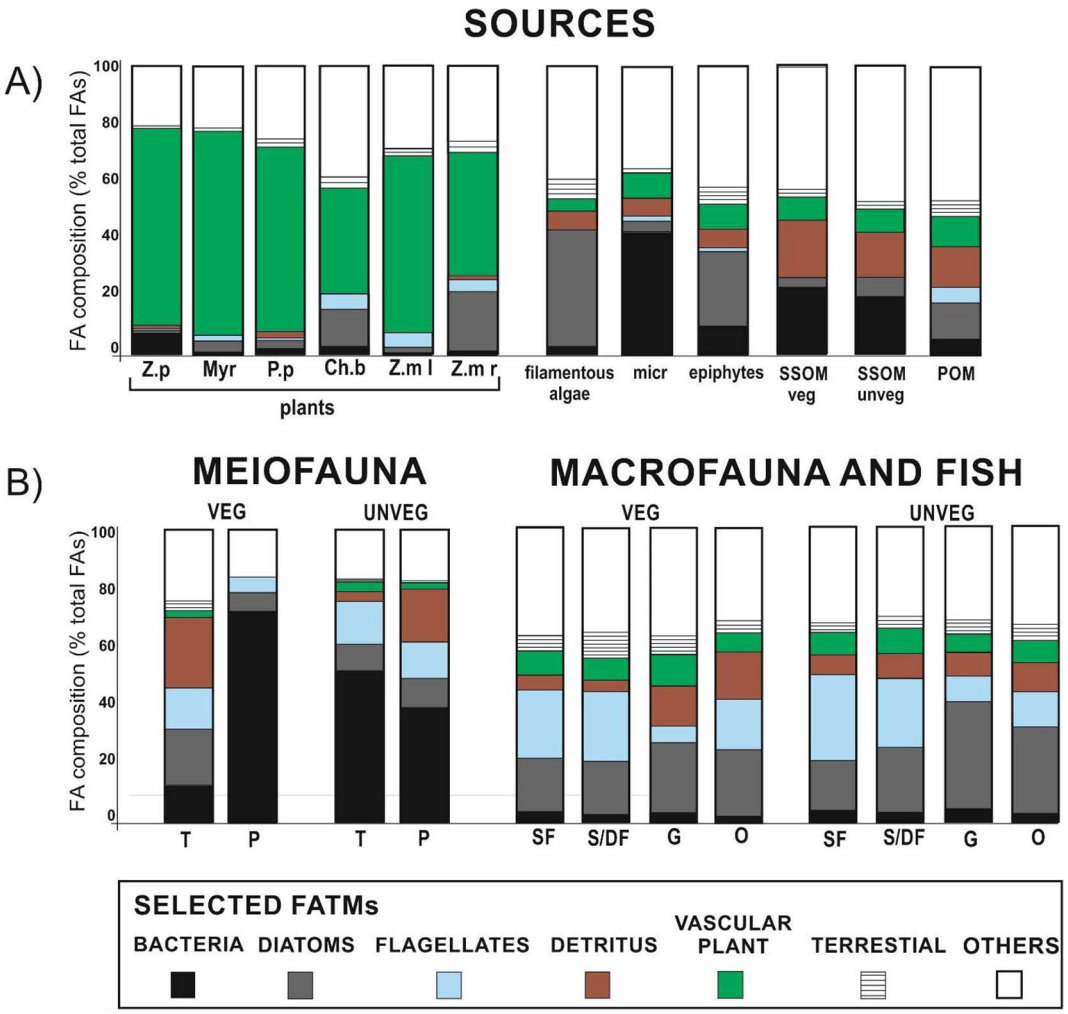

Fig. 1. Relative FA composition (expressed as FATMs, see Table 1) in samples of A) potential food sources and B) consumers collected in summer in the Puck Bay (Baltic Sea). Abbreviations: Z.p – *Zannichellia palustris*, Myr – *Myriophyllum* spp., P.p – *Potamogeton pectinatus*, Ch.b – *Chara baltica*, Z.m l – *Zostera marina* leaves, Z.m r – *Zostera marina* roots, micr – microphytobenthos/bacteria, SSOMveg/unveg – surface sediment organic matter of the vegetated/unvegetated bottom. abbreviations: T – *Tachidius discipes*, P – *Paraleptostacus spinicauda.*, SF – suspension feeders, SDF –suspension/detritus feeders, G – grazers, O – omnivores.

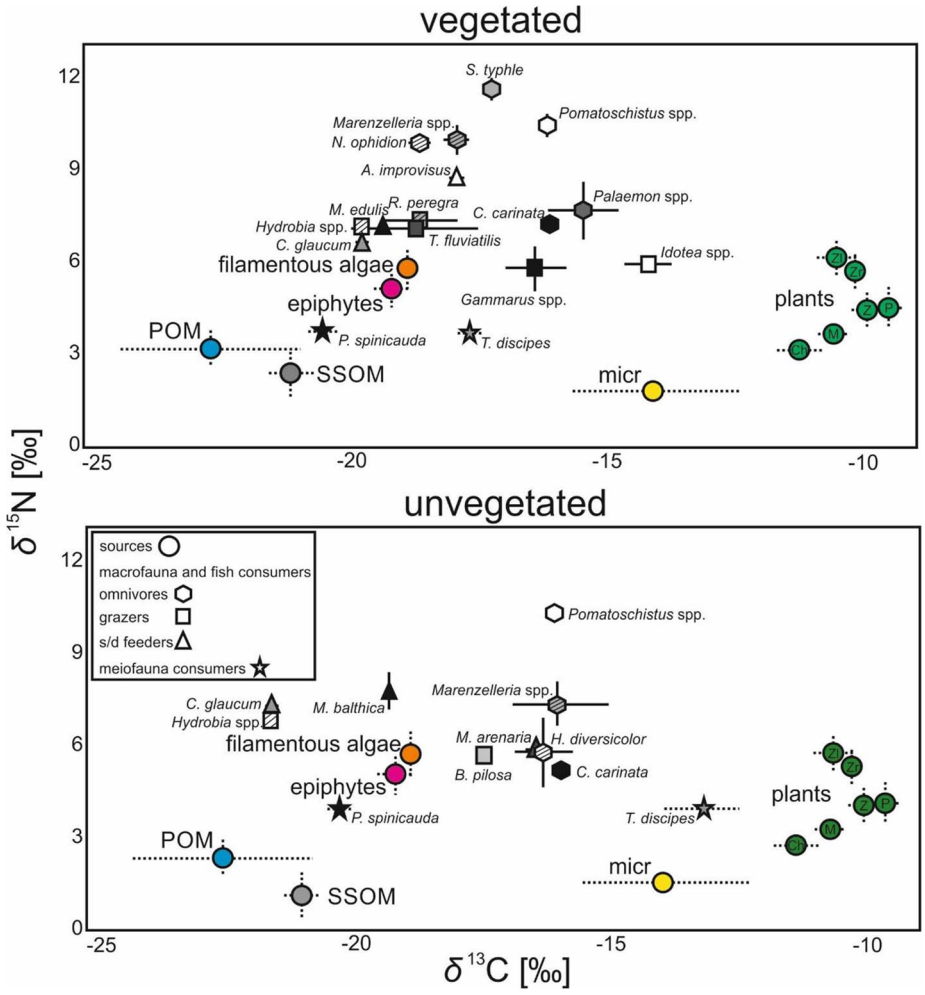

Fig. 2. Bi-plot of carbon and nitrogen isotope (mean ± SD) composition for two meiofauna, macrofauna and fish species from the vegetated and unvegetated habitats with possible food sources collected in summer in the Puck Bay (Baltic Sea). Sources abbreviations: micr – microphytobenthos and bacteria, Zl – *Zostera marina* leaves, Zr – *Zostera marina* roots, Z – *Zannichellia palustris*, P – *Potamogeton pectinatus*, M – *Myriophyllum* spp., Ch – *Chara baltica*.

3.2. FA and SI biomarkers in consumers

There were significant differences in the relative FA composition and in contributions of 4 FATMs (diatoms, flagellates, detritus, vascular plants) among the consumer groups ($P < 0.05$), but there were no significant contrasts between the habitats ($P > 0.05$, PERMANOVA, Table 2). Additionally, significant H × CG interactions were also identified in some cases (Table 2).

The FA composition of the two meiofauna (copepod) species was clearly different from those of all the macrofauna consumer groups (Fig. 1). The meiofauna species contained large amounts of the bacteria marker 18:1ω7, encompassing on average 70.7% and 37.0% in *P. spinicauda* and 11.3% and 49.6% in *T. discipes* (from the vegetated and unvegetated habitat respectively). The detritus marker, 18:1ω9, was found in high concentrations in *T. discipes* collected in the seagrass meadows (22.2% on average) and in *P. spinicauda* from the bare bottom (17.5% on average).

Suspension and suspension/detritus feeding macrofaunal species had very similar FA profiles in the two habitats, with two FAs (20:5ω3, 22:6ω3) having the highest contribution. The concentrations of the flagellate marker 22:6ω3 ranged from 22.6% in the suspension/detritus feeders in the seagrass beds to 29.0% in the suspension feeders at the bare bottom (Table S4, Supporting Information).

The FA profiles of the grazers were dominated by the diatom marker 20:5ω3 (on average 19.7% in the vegetated habitat and 15.3% in the unvegetated habitat). The grazers from seagrass meadows also had considerable proportions of 18:1ω9 (9.0%, detritus marker) and 18:2ω6 (8.9%, vascular plant marker).

The omnivores contained the highest proportion of 20:5ω3 (diatom marker, 15.3% in the vegetated and 19.8% in the unvegetated habitat) and 22:6ω3 (flagellates marker, 16.9% in the vegetated and 11.8% in the unvegetated habitat) (Fig. 1).

The carbon and nitrogen stable isotopes in the consumers differed significantly among the consumer groups but not between the habitats, with a significant interaction between the two factors ($P < 0.05$, PERMANOVA, Table 2).

The two copepod species differed in δ^{13}C values between the two habitats. There was no significant difference between the two habitats for *P. spinicauda* (average δ^{13}C values approximately −20.5‰ in both habitats), but significant contrasts for *T. discipes* were detected (−17.7‰ on average in the vegetated, −13.3‰ in the unvegetated seabed, Fig. 2, Table 3).

For the macrofaunal consumer groups, the δ^{13}C values ranged from −20.4‰ (suspension/detritus feeders) to −16.3‰ (omnivores, both from the unvegetated habitat), and the δ^{15}N values ranged from 5.9‰ (suspension/detritus feeders in the unvegetated) to 8.9‰ (omnivores in the vegetated habitat, Fig. 2). There were significant differences in δ^{13}C and δ^{15}N values among macrofaunal consumer groups ($P < 0.05$, PERMANOVA, Table 2).

For suspension/detritus feeders, the δ^{13}C values varied from −21.7‰ (*C. glaucum*) to −16.6‰ (*M. arenaria*) in the unvegetated seabed. At the vegetated bottom *C. glaucum* and *M. edulis* had similar

Table 3
Carbon and nitrogen stable isotopes ratios (mean ± SD) of consumers collected in summer in the Puck Bay (Baltic Sea), in the two habitats (vegetated, unvegetated). Trophic groups of consumers were used according to available literature (see Table S2, Supporting Information).

Consumers	Habitat	Species	δ^{13}C (‰)	δ^{15}N (‰)
Meiofauna copepods	Vegetated	*P. spinicauda*	−20.8 ± 1.0	3.3 ± 0.0
	Unvegetated		−20.5 ± 0.5	3.3 ± 0.0
	Vegetated	*T. discipes*	−17.7 ± 0.6	3.3 ± 0.0
	Unvegetated		−13.3 ± 2.1	3.3 ± 0.0
Macrofauna suspension feeders	Vegetated	*A. improvisus*	−18.0 ± 0.1	8.5 ± 0.1
		M. edulis	−19.4 ± 0.1	6.9 ± 0.0
	Unvegetated	*M. arenaria*	−16.6 ± 0.1	5.9 ± 0.1
Macrofauna suspension/ detritus feeders	Vegetated	*C. glaucum*	−19.8 ± 0.1	6.5 ± 0.0
	Unvegetated	*C. glaucum*	−21.7 ± 0.1	7.2 ± 0.0
		M. balthica	−19.4 ± 0.1	7.7 ± 0.6
Macrofauna grazers	Vegetated	*Gammarus* spp.	−16.5 ± 0.6	5.6 ± 0.7
		Hydrobia spp.	−19.8 ± 0.1	7.0 ± 0.1
		Idotea spp.	−14.2 ± 0.5	5.7 ± 0.1
		R. peregra	−18.7 ± 0.8	7.1 ± 0.1
		T. fluviatilis	−18.8 ± 1.3	6.9 ± 0.1
	Unvegetated	*B. pilosa*	−17.6 ± 0.0	5.7 ± 0.1
		Hydrobia spp.	−21.7 ± 0.1	6.9 ± 0.1
Macrofauna and fish omnivores	Vegetated	*C. carinata*	−16.2 ± 0.1	7.0 ± 0.3
		Marenzelleria spp.	−18.0 ± 0.2	9.7 ± 0.5
		N. ophidion	−18.7 ± 0.2	9.7 ± 0.1
		Palaemon spp.	−15.5 ± 0.7	7.5 ± 0.9
		Pomatoschistus spp.	−16.2 ± 0.1	10.2 ± 0.3
		S. typhle	−17.3 ± 0.1	11.3 ± 0.1
	Unvegetated	*C. carinata*	−16.1 ± 0.0	5.2 ± 0.0
		H. diversicolor	−16.4 ± 0.6	5.8 ± 1.1
		Marenzelleria spp.	−16.1 ± 1.0	7.3 ± 0.7
		Pomatoschistus spp.	−16.2 ± 0.1	10.2 ± 0.3

average carbon (−19.6‰) and nitrogen (6.7‰) isotopic composition, while *A. improvisus* had higher values for both isotope ratios (δ^{13}C = −17.9‰, δ^{15}N = 8.5‰, Fig. 2).

The grazer species had higher δ^{13}C values in the vegetated habitat than the unvegetated habitat. The highest carbon isotope ratio was noted for *Idotea* spp. collected in the seagrass beds (−14.2‰). The δ^{13}C values in the species from the bare seabed shifted toward depleted values with the lowest value noted for *Hydrobia* spp. (−21.7‰, Fig. 2).

Among the omnivores, the most ^{13}C-enriched values in the vegetated habitat were noted for *Palaemon* spp. (−15.5‰). The mean δ^{15}N value for the omnivores (in both habitats) was approximately 8–9‰ and was considerably higher than in the other consumer groups (Fig. 2). The most ^{15}N-enriched values were noted for *S. typhle* (11.3‰), whereas the most depleted values were found for *Palaemon* spp. (6.9‰), which were both from the seagrass beds.

3.3. Sources contributions to the diet of consumers (MixSIAR models)

The MixSIAR mixing models showed that the diet of *P. spinicauda* was composed of SSOM (mode of solutions = 99%, Fig. 3), regardless of the habitat. In contrast, the SSOM contribution to the diet of *T. discipes* was negligible. The other two sources contributed greatly to the diet of *T. discipes* but with different proportions in the two habitats. Individuals collected in the vegetated bottom had a higher proportion of epiphytes (73% mode and 33–72% Bayesian credibility intervals 95% (CI_{95})), than those in the unvegetated habitat (20%, 5–41% CI_{95}) in 66% of the model runs (Pr = 0.34). However, individuals collected in the unvegetated habitat had a higher microphytobenthos/bacteria proportion (68%, 41–83% CI_{95}) than individuals in the seagrass meadows (30%, 13–62% CI_{95}) in 90% of the model runs (Pr = 0.1) (Fig. 3).

Within the macrofaunal groups of suspension feeders and suspension/detritus feeders, the main food source differed among some species and between habitats. For individuals collected in the vegetated habitat, epiphytes were the sole food source for *A. improvisus* (99%, 91–100% CI_{95}) and were a considerable proportion of the food source for *C. glaucum* and *M. edulis* (proportions 54%, 41–66% CI_{95} and 61%, 50–70% CI_{95} accordingly) together with POM/SSOM (46%, 34–59% CI_{95} and 37%, 28–45% CI_{95} accordingly). POM/SSOM was the sole food source for *C. glaucum* collected in the unvegetated habitat (98%, 92–100% CI_{95}). Representatives of two other species collected in the bare seabed had other main food sources; *M. arenaria* from the unvegetated habitat had epiphytes (40%, 50–52% CI_{95}) and microphytobenthos/bacteria (42%, 30–50% CI_{95}) whereas *M. balthica* had epiphytes (61%, 19–69% CI_{95}) and POM/SSOM (38%, 31–63% CI_{95}, Fig. 3) as food sources. There was only one taxon collected both in the vegetated and unvegetated habitats, *C. glaucum,* and the proportion of food sources in its diet differed between habitats; individuals collected in the vegetated habitat had epiphytes in their diet.

Epiphytes were the main food source in the diet of four grazer species collected in the vegetated habitat – *Gammarus* spp. (mode: 55%, 41–69% CI_{95}), *Hydrobia* spp. (75%, 41–83% CI_{95}), *R. peregra* (80%, 28–100% CI_{95}) and *T. fluviatilis* (72%, 47–97% CI_{95}). Another important food source was SSOM for two grazers from the seagrass beds – *Hydrobia* spp. (30%, 11–50% CI_{95}) and *T. fluviatilis* (23%, 0–41% CI_{95}). Moreover, a considerable contribution of microphytobenthos/bacteria was noted for *Idotea* spp. (45%, 15–57% CI_{95}) and *Gammarus* spp. (38%, 25–47% CI_{95}) in the vegetated habitat. Microphytobenthos/bacteria was the main food source for *B. pilosa* from the unvegetated habitat (29%, 0–50% CI_{95}) together with SSOM (33%, 0–68% CI_{95}). SSOM was the sole food source for another grazer – *Hydrobia* spp. (80%, 43–97% CI_{95}) in the unvegetated habitat. The proportion of epiphytes and plants were negligible for all grazer taxa collected in the unvegetated habitat. There was only one taxon present in both the vegetated and unvegetated habitat, and the proportion of food sources in its diet differed between habitats; *Hydrobia* spp. from the vegetated habitat had a higher proportion of epiphytes in 82% of the model runs (Pr = 0.18) whereas specimens from the unvegetated habitat had a higher contribution of SSOM in 89% of the model runs (Pr = 0.11).

Meiofauna made considerable contributions to the diet of the four omnivore species collected in the vegetated habitat: *C. carinata* (77%, 35–100 CI_{95}), *Marenzelleria* spp. (43%, 12–77% CI_{95}), *Palaemon* spp. (99%, 55–100% CI_{95}) and *N. ophidion* (80%, 59–100% CI_{95}). Macrofauna was the main food source for *S. typhle* (98%, 61–100% CI_{95}) and *Pomatoschistus* spp. (78%, 52–97% CI_{95}) and a considerable food source for *Marenzelleria* spp. (45%, 22–78% CI_{95}), all collected in the vegetated seabed. For specimens collected in the unvegetated habitat, macrofauna prey was an important food source only for *Pomatoschistus* spp. (81%, 31–100% CI_{95}). In the same habitat, meiofauna contributed with large credibility intervals to the diet of *Marenzelleria* spp. (36%, 0–93% CI_{95}), while SSOM made up the highest proportion of the diets of *C. carinata* (40%, 0–53% CI_{95}) and *H. diversicolor* (41%, 0–70% CI_{95}) (Fig. 3, Table S5, Supporting Information). There were three species present in both the vegetated and unvegetated habitats, and the proportion of food sources in their diets differed between the habitats for two of them. *C. carinata* from the seagrass meadows had a higher proportion of meiofauna in 71% of the model runs (Pr = 0.29) whereas a species from unvegetated habitat had a higher proportion of SSOM in 63% of the model runs (Pr = 0.37). *Marenzelleria* spp. from the seagrass meadows had a higher proportion of macrofauna prey in 77% of the model runs (Pr = 23).

4. Discussion

4.1. Food source distinction

All food sources had lower or similar δ^{15}N compared to macrofauna consumers, confirming that they were at the base of the studied food

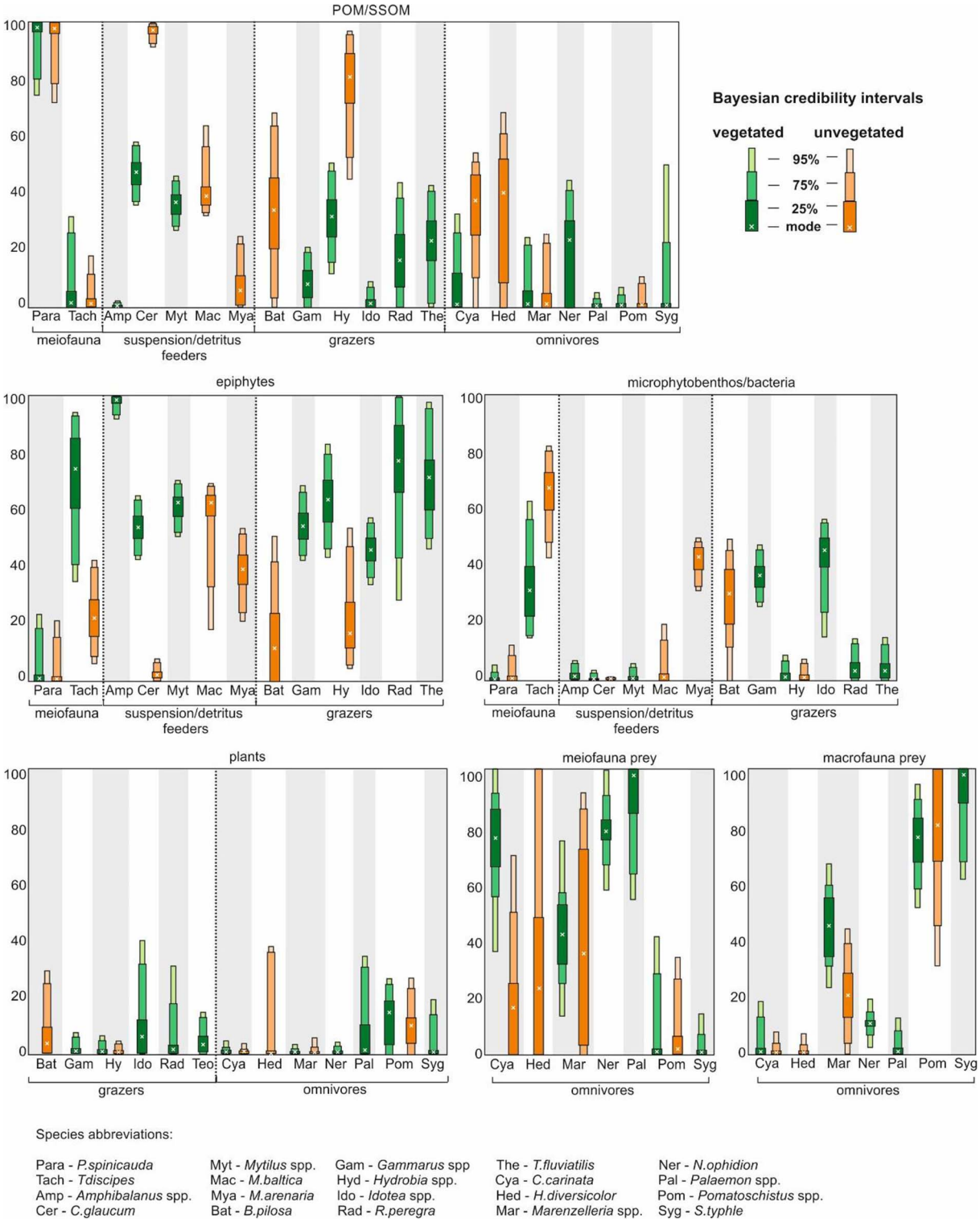

Fig. 3. Relative contributions of the food sources (plants, SSOM, meiofauna prey, macrofauna prey) to diet of meiofauna, macrofauna and fish sampled in the vegetated (green lines) and unvegetated (orange lines) habitats collected in summer in the Puck Bay (Baltic Sea). The lines indicate Bayesian credibility intervals, crosses represent modes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

web. All plant sources (including the four vascular plants *Z. marina*, *Myriophyllum* spp., *Z. palustris*, *P. pectinatus* and charophyte C*h. baltica*) had very similar SI and FA compositions, with the vascular plant FATMs being substantially dominant (18:2ω6, 18:3ω3, respectively). Filamentous algae and epiphytes reflected almost identical FA and SI compositions with the diatom and macroalgae markers being dominant (16:1ω7 and 20:5ω3, respectively). In the microphytobenthos samples collected during this study, the contribution of FA typical for diatoms was relatively low. On the other hand, the samples contained a high percentage of bacterial FATMs (18:1ω7), suggesting that they largely consisted of bacteria. In terms of the SI composition, the values obtained for the microphytobenthos samples in this study were within the wide range (−15 to −20‰) of carbon isotope ratios previously reported (Lebreton et al., 2011, 2012; Jaschinski et al., 2008).

Particulate (POM) and sediment surface (SSOM) organic matter are two composite food sources that share similar SI and FA compositions (a mixture of different FA markers). This similarity may be explained by the mixing of the water column and resuspension of organic matter particles from the seabed, which is common in the shallow (only 1.5 m) and dynamic (due to both waves and along-shore upwelling, Nowacki, 1993) waters in the study area. SSOM composition in the vegetated bottom was ^{15}N-enriched compared to the unvegetated habitat. This result indicates that more active decomposition processes may occur at the bottom of seagrass beds; indeed, significantly higher bacteria density and biomasses were documented in the vegetated sediments than in the bare sands of the study area (Jankowska et al., 2015).

4.2. Diet of benthic consumers in vegetated vs. unvegetated habitat

The present study shows that the diet composition of meiofauna copepods differs between the habitats, but these differences are species-specific. The FA profiles, carbon isotopic composition and mixing model results (indicating SSOM as the sole food source) of *P. spinicauda* were very similar in the two habitats. In contrast, according to the mixing models, epiphytes were the main food source in the diet of *T. discipes* from the vegetated habitat, whereas in the other habitat, this copepod species consumed mostly microphytobenthos/bacteria. The difference in the diets of the two studied species are most likely related to the differences in occupied microhabitats. *P. spinicauda* has an elongated, cylindrical body shape and lives in the interstitial spaces within the sediment grains, whereas *T. discipes* has a cyclopoid body shape and stays at the surface of the sediment and/or among seagrass leaves (Giere, 2009). As *P. spinicauda* remains within the sediment, it mostly has access to the SSOM regardless of the habitat. *T. discipes* probably stays on seagrass leaves and feeds on epiphytes (growing on the leaves) in the vegetated habitat, whereas in unvegetated habitat it uses mostly microphytobenthos that occurs with higher biomass at the bare sandy bottom (as indicated by higher chlorophyll *a*/POC proportion in the surface sediments; Jankowska et al., 2016). Evidently, the effects of seagrass vegetation on the harpacticoid feeding strategies depend on species lifestyle. These results also indicate that grouping meiofaunal consumers into higher taxa or into functional groups should be avoided in food web studies, as their responses to changing environments can vary considerably among species.

Copepod consumers had higher amount of bacteria FA markers (18:1ω7) in comparison to their food sources – SSOM or microphytobenthos/bacteria. This result may be derived from the selective grazing and the strong preference for bacteria among the available pool of microphytobenthos/bacteria/detritus components. Lab experiments showed that copepods can selectively graze on unicellular organisms (diatoms) of different cell size or growth phases (De Troch et al., 2006, 2012) as well as consume diatoms cells overgrown by bacteria but digest only bacteria and excrete living diatoms as fecal pellets (De Troch et al., 2005). The strong interactions between meiofauna and bacteria in the studied habitats are supported by a positive correlation between bacterial and meiofaunal abundance and biomass documented by Jankowska et al. (2015). The isotopic composition of bacteria usually reflects the composition of their substrate (Danovaro et al., 1998), therefore the selective feeding of copepod consumers in this study could be detected only by the application of the FA analyses. This result shows that the combined use of SI and FA markers in food web studies is crucial to obtain a comprehensive understanding of the consumers' diet composition, particularly when composite food sources are considered.

The effect of an increased number of food sources in a seagrass system was the strongest in the case of macrofaunal grazers. The main difference between the two habitats was the important contribution of epiphytes (together with filamentous algae) to the diet of grazers collected in the vegetated habitat, whereas in the bare sand habitat, the grazers preferred to feed on microphytobenthos/bacteria (as indicated by the model). The importance of epiphytes in the benthic consumers' diets in other studies differs among the regions and dominant seagrass species. Epiphytes were the main food source in the seagrass dominated systems from the west Baltic Sea (Jaschinski et al., 2008) and French Atlantic coast (Ouisse et al., 2012). On the other hand, microphytobenthos had the highest contributions to invertebrate diets in the intertidal temperate *Z. noltii* meadows that are characterized by significantly smaller leaves and lower biomass of epiphytes (Lebreton et al., 2011). The biomass of filamentous algae in the Puck Bay seagrass meadows increases dramatically in the summer (Jankowska et al., 2014), and several grazer species from the vegetated habitat (*Hydrobia* spp., *R. pergra*, *T. fluviatilis*) take advantage of the high availability of this food source. Microphytobenthos is rarely considered as a separate potential food source in benthic food web studies, mostly due to the difficulties in obtaining samples for tracer analyses (Ouisse et al., 2011). However, it has been regarded as a 'hidden garden' in the unvegetated habitats, strongly supporting the local zoobenthic communities (Miller et al., 1996). Apparently, when confronted with high accessibility of microphytobenthos and epiphytes, grazers preferred to feed selectively on the fresh components of the organic matter rather than the degraded organic matter of SSOM.

The differences in food source contributions between the habitats were also documented for omnivores. Organic matter of animal origin (indicator of carnivory or scavenging) was the basis of diet in the vegetated habitat, while it had smaller contributions to diets in the bare sands. This finding clearly contrasts with a common notion that there is lower predation pressure in vegetated habitats compared to unvegetated habitats (due to protective role of macrophytes, Baeta et al., 2009). Both meiofauna and macrofauna prey served as the main food sources consumed by omnivores from the vegetated bottom (all fish species, *C. carinata* and *Marenzelleria* spp.) in Puck Bay. In the unvegetated habitat, macrofauna were the main food source for only one fish species (*Pomatoschistus* spp.), whereas the diet of the other omnivores species was based on POM/SSOM. Lower abundance, biomass and diversity of macrofauna were noted for the unvegetated bottom compared to seagrass meadows in Puck Bay (Włodarska-Kowalczuk et al., 2014). Therefore, it is possible that the same omnivorous species feed as carnivores when confronted with high prey accessibility in the vegetated habitat but shift to deposit feeding in the unvegetated habitat. Moreover, the relatively weakly developed seagrass vegetation in Puck Bay might be not effective enough in protecting meio- and/or macrofauna from omnivore predation. Similarly, intensive feeding on fish by large amphipods was documented in the mixed *Zostera* spp. meadows along the Swedish west coast (Moksnes et al., 2008).

In macrofaunal suspension and suspension/detritus feeders, the dominant food sources differed among species, but no consistent trend of inter-habitat differences were noted based on the modeling results. Only one species was collected in both habitats (*C. glaucum*). In the vegetated habitat, its diet was based on epiphytes and POM/SSOM, while in the unvegetated bottom, its sole food source was POM/SSOM. According to the mixing model results, the common feature of the diet of almost all species in this group was that included a considerable proportion of POM/SSOM, supported by the high contribution of the flagellates' FA marker (marker of pelagic production; Kelly and Scheibling, 2012).

4.3. Modification of benthic food webs by recovering eelgrass meadows

The present study shows that recovering seagrass meadows modify the functioning of benthic ecosystems. Little evidence of direct consumption of seagrass tissues was documented, but the seagrass vegetation could impact benthic food web functioning in several indirect ways. Seagrass vegetation increased the number of food sources utilized by meiofauna copepods and macrofaunal grazers (i.e., epiphytes consumed by the copepod *T. discipes* and grazers only in the vegetated habitat). Moreover, seagrasses could support larger standing stocks of prey organisms and thus increase the feeding efficiency at higher

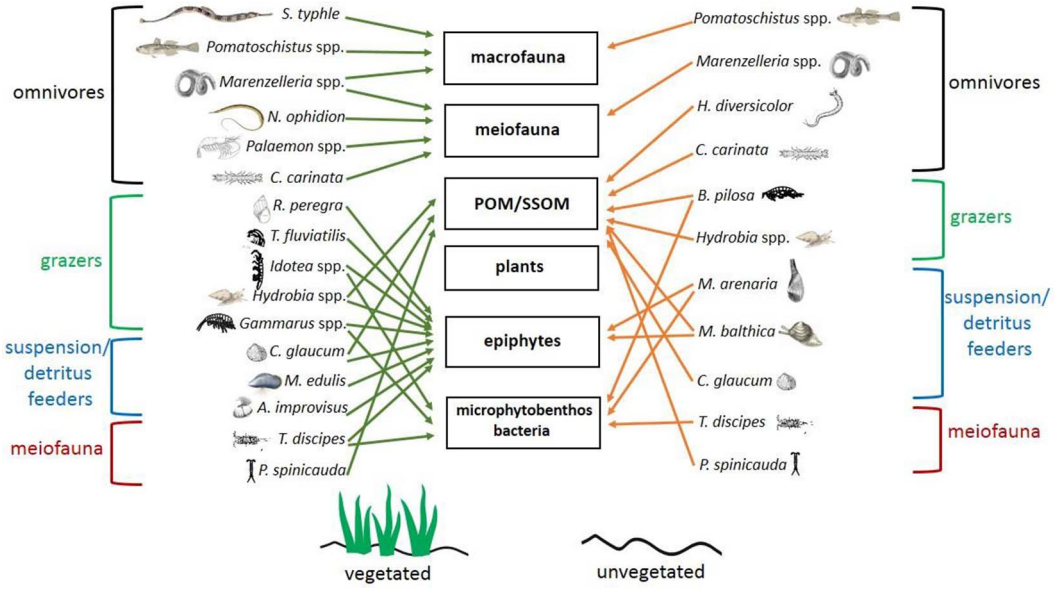

Fig. 4. Conceptual model of trophic connections in vegetated (green arrows) and unvegetated (orange arrows) habitats in summer in the Puck Bay (Baltic Sea) based on MixSIAR results. Arrows indicate food sources that had over 30% of contribution in the particular species diet as revealed by MixSIAR. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

trophic levels in omnivores. This result is indicated by the higher number of carnivorous species as well as higher meiofauna and macrofauna prey consumption in the vegetated habitat (Fig. 4). Evidently, recovering seagrass meadows, despite their low plant density, can increase trophic complexity of associated communities and thus play a key role in the energy flow through benthic ecosystems.

A study of macrofauna consumers in the Sea of Japan and New Zealand showed that their diet inside the *Zostera* meadows was based on epiphytes growing on seagrass leaves or seagrass itself, whereas the same macrofaunal species dwelling outside the meadows fed on epilithon or microphytobenthos (Hoshika et al., 2006; Leduc et al., 2006). On the other hand, several studies reported no difference in the food web structure between the vegetated and unvegetated habitats (Baeta et al., 2009; Lebreton et al., 2011, 2012; Vafeiadou et al., 2013; Vafeiadou et al., 2014). Similar trophic preferences of macrofauna and meiofauna inhabiting the bare sands and *Z. noltii* vegetated sediments have been documented along the Portugese (Baeta et al., 2009; Vafeiadou et al., 2013; Vafeiadou et al., 2014) or French (Lebreton et al., 2011, 2012) Atlantic coast. Similarly, in southeastern New Zealand, microphytobenthos were the most important food source for meiofauna communities regardless of the habitat being studies (Leduc et al., 2009). Different results on the seagrass presence effects on food web functioning given in previous reports may result from the high variability of the studied vegetated systems (climate, region, species, density) but may also result from the incomplete picture derived from only one type of biomarkers (e.g., only stable isotopes). This study proves that the usage of FA and SI together in recently released Bayesian mixing models is crucial for a detailed description of trophic connections in complex food webs.

The strong signal of food web modification by the presence of seagrasses detected within this study indicates that recovering seagrasses are capable of prompting mechanisms that drive food webs on multiple trophic levels. Any type of change in the state of seagrass meadows is therefore crucial for food web organization and for stability of coastal marine ecosystems.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ecolind.2018.02.054.

References

Abdulkadir, S., Tsuchiya, M., 2008. One-step method for quantitative and qualitative analysis of fatty acids in marine animal samples. J. Exp. Mar. Biol. Ecol. 354, 1–8.
Anderson, M.J., Gorley, R.N., Clarke, K.R., 2008. PERMANOVA for PRIMER: Guide to Software and Statistical Methods. PRIMER-E Ltd, Plymouth.
Baeta, A., Valiela, I., Rossi, F., Pinto, R., Richard, P., Niquil, N., Marques, J.C., 2009. Eutrophication and trophic structure in response to the presence of the eelgrass *Zostera noltii*. Mar. Biol. 156 (10), 2107–2120.
Boström, Ch., Baden, S., Bockelmann, A.-Ch., Dromph, K., Fredriksen, S., Gustafsson, C., Krause-Jensen, D., Möller, T., Laurentius Nielsen, S., Olesen, B., Olsen, J., Pihl, L., Rinde, E., 2014. Distribution, structure and function of Nordic eelgrass (*Zostera marina*) ecosystems: implications for coastal management and conservation. Aquat. Conserv. Mar. Freshwater Ecosyst. 3, 410–434. http://dx.doi.org/10.1002/aqc.2424.
Budge, S.M., Parrish, C.C., 1998. Lipid biogeochemistry of plankton, settling matter and sediments in Trinity Bay, Newfoundland. II. Fatty acids. Org. Geochem. 29, 1547–1559.
Budge, S.M., Iverson, S.J., Koopman, H.N., 2006. Studying trophic ecology in marine ecosystems using fatty acids: a primer on analysis and interpretation. Mar. Mammal Sci. 22, 759–801.
Dalsgaard, J., St. John, M., Kattner, G., Müller-Navarra, D., Hagen, W., 2003. Fatty acid trophic markers in the pelagic marine environment. Adv. Mar. Biol. 46, 225–340.
Danovaro, R., Croce, Della, N., Fabiano, M., 1998. Biochemical composition of particulate organic matter and bacterial dynamics at the sediment – water interface in a Mediterranean seagrass system. Hydrobiology 363, 241–251.
De Troch, M., Steinarsdóttir, M.B., Chepurnov, V., 2005. Grazing on diatoms by harpacticoid copepods: species-specific density-dependent uptake and microbial gardening. Aquat. Microb. Ecol. 39, 135–144.
De Troch, M., Chepurnov, V., Gheerardyn, H., Vanreusel, A., Ólafsson, E., 2006. Is diatom size selection by harpacticoid copepods related to grazer body size? J. Exp. Mar. Biol. Ecol. 332, 1–11.
De Troch, M., Vergaerde, I., Cnudde, C., Vanormelingen, P., Vyverman, W., Vincx, M.,

2012. The taste of diatoms: the role of diatom growth phase characteristics and associated bacteria for benthic copepod grazing. Aquat. Microb. Ecol. 67, 47–58.
Gartner, A., Tuya, F., Lavery, P.S., McMahon, K., 2013. Habitat preferences of macroinvertebrate fauna among seagrasses with varying structural forms. J. Exp. Mar. Biol. Ecol. 439, 143–151.
Giere, O., 2009. Meiobenthology. The Microscopic Motile Fauna of Aquatic Sediments, second ed. 528 pp.
Guckert, J.B., Antworth, C.P., Nichols, P.D., White, D.C., 1985. Phospholipid ester-linked fatty acid profiles as reproducible assays for changes in prokaryotic community structure of estuarine sediments. FEMS Microbiol. Ecol. 31, 147–158.
Hedges, J.I., Stern, J.H., 1984. Carbon and nitrogen determinations of carbonate-containing solids. Limnol. Oceanogr. 29, 657–663.
Hemminga, M.A., Duarte, C.M., 2000. Seagrass Ecology. Cambridge University Press, Cambridge, pp. 298.
Hoshika, A., Sarker, M.J., Ishida, S., Mishima, Y., Takai, N., 2006. Food web analysis of an eelgrass (*Zostera marina* L.) meadow and neighbouring sites in Mitsukuchi Bay (Seto Inland Sea, Japan) using carbon and nitrogen stable isotope ratios. Aquat. Bot. 85 (3), 191–197.
Jankowska, E., Włodarska-Kowalczuk, M., Kotwicki, L., Balazy, P., Kuliński, K., 2014. Seasonality in vegetation biometrics and its effects on sediment characteristics and meiofauna in Baltic seagrass meadows. Estuarine Coast. Shelf Sci. 139, 159–170.
Jankowska, E., Jankowska, K., Włodarska-Kowalczuk, M., 2015. Seagrass vegetation and meiofauna enhance the bacterial abundance in the Baltic Sea sediments (Puck Bay). Environ. Sci. Pollut. Res. 22 (18), 14372–14378.
Jankowska, E., Michel, L.N., Zaborska, A., Włodarska-Kowalczuk, M., 2016. Sediment carbon sink in low density temperate eelgrass meadows (Baltic Sea). J. Geophys. Res. Biogeosci. 121. http://dx.doi.org/10.1002/2016JG003424.
Jaschinski, S., Brepohl, D., Sommer, U., 2008. Carbon sources and trophic structure in an eelgrass *Zostera marina* bed, based on stable isotope and fatty acid analyses. Mar. Ecol. Prog. Ser. 358 (1), 103–114.
Kelly, J.R., Scheibling, R.E., 2012. Fatty acids as dietary tracers in benthic food webs. Mar. Ecol. Prog. Ser. 446, 1–22.
Kruk-Dowigałło, L., 1991. Long term changes in the structure of underwater meadows of the Puck lagoon. Acta Ichtiol. Piscatoria XXI Supplement.
Lebreton, B., Richard, P., Galois, R., Radenac, G., Pfléger, C., Guillou, G., Mornet, F., Blanchard, G.F., 2011. Trophic importance of diatoms in an intertidal *Zostera noltii* seagrass bed: Evidence from stable isotope and fatty acid analyses. Estuar. Coast. Shelf Sci. 92 (1), 140–153.
Lebreton, B., Richard, P., Galois, R., Radenac, G., Brahmia, A., Colli, G., Grouazel, M., André, C., Guillou, G., Blanchard, G.F., 2012. Food sources used by sediment meiofauna in an intertidal *Zostera noltii* seagrass bed: a seasonal stable isotope study. Mar. Biol. 159, 1537–1550.
Leduc, D., Probert, P.K., Duncan, A., 2009. A multi-method approach for identifying meiofaunal trophic connections. Mar. Ecol. Prog. Ser. 383, 95–111.
Leduc, D., Probert, P.K., Frew, R.D., Hurd, C.L., 2006. Macroinvertebrate diet in intertidal seagrass and sandflat communities: a study using C, N, and S stable isotopes. New Zealand. J. Mar. Freshwater Res. 40 (4), 615–629.
Lepoint, G., Nyssen, F., Gobert, S., Dauby, P., Bouquegneau, J.M., 2000. Relative impact of a seagrass bed and its adjacent epilithic algal community in consumer diets. Mar. Biol. 136, 513–518.
McCutchan, J.H., Lewis, W.M., Kendall, C., McGrath, C.C., 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen and sulfur. Oikos 102, 378–390.
Michel, L.N., Dauby, P., Gobert, S., Graeve, M., Nyssen, F., Thelen, N., Lepoint, G., 2015. Dominant amphipods of *Posidonia oceanica* seagrass meadows display considerable trophic diversity. Mar. Ecol. 36 (4), 969–981.
Miller, D.C., Geii, R.J., MacIntyre, H.L., Geider, R.J., MacIntyre, H.L., 1996. Microphytobenthos: the ecological role of the "secret garden" of unvegetated, shallow-water marine habitats. II. Role in sediment stability and shallow-water food webs. Estuaries 19 (2), 202.
Moksnes, P.O., Gullström, M., Tryman, K., Baden, S., 2008. Trophic cascades in a temperate seagrass community. Oikos 117 (5), 763–777.
Nelson, M.M., Leighton, D.L., Phleger, C.F., Nichols, P.D., 2002. Comparison of growth and lipid composition in the green abalone, Haliotis fulgens, provided specific macroalgal diets. Compar. Biochem. Physiol. Part B: Biochem. Mol. Biol. 131, 695–712.
Nowacki, J., 1993. Stany wód. In: Zatoka Pucka, K. (Ed.), Korzeniewski red. Fundacja Rozwoju Uniwersytetu Gdańskiego, Gdańsk, pp. 206–221.
Ouisse, V., Riera, P., Migne, A., Leroux, C., Davoult, D., 2011. Freshwater seepages and ephemeral macroalgae proliferation in an intertidal bay: I effect on benthic community structure and food web. Estuarine Coast. Shelf Sci. 91, 272–281.
Ouisse, V., Riera, P., Migné, A., Leroux, C., Davoult, D., 2012. Food web analysis in intertidal Zostera marina and Zostera noltii communities in winter and summer. Mar. Biol. 159 (1), 165–175.
Richoux, N.B., Froneman, P.W., 2008. Trophic ecology of dominant zooplankton and macrofauna in a temperate, oligotrophic South African estuary: a fatty acid approach. Mar. Ecol. Prog. Ser. 357, 121–137.
Søreide, J.E., Falk-Petersen, S., Hegseth, E.N., Hop, H., Carroll, M.L., Hobson, K.A., Blachowiak-Samolyk, K., 2008. Seasonal feeding strategies of Calanus in the high-Arctic Svalbard region. Deep Sea Res. Part II 55, 2225–2244.
Stock B.C., & Semmens B.X. (2013). MixSIAR GUI User Manual. Version 3.1. https://github.com/brianstock/MixSIAR. doi: 10.5281/zenodo.56159.
Vafeiadou, A.M., Materatski, P., Adão, H., Troch, M., Moens, T., 2013. Food sources of macrobenthos in an estuarine seagrass habitat (*Zostera noltii*) as revealed by dual stable isotope signatures. Mar. Biol. 160 (9), 2517–2523.
Vafeiadou, A.M., Materatski, P., Adão, H., De Troch, M., Moens, T., 2014. Resource utilization and trophic position of nematodes and harpacticoid copepods in and adjacent to *Zostera noltii* beds. Biogeosciences 11 (14), 4001–4014.
Vizzini, S., Sara, G., Michener, R.H., Mazzola, A., 2002. The role and contribution of the seagrass *Posidonia oceanica* (L.) Delite organic matter for secondary consumers as revealed by carbon and nitrogen stable isotopes analysis. Acta Oecol. 23, 277–285.
Waycott, M., Duarte, C., Carruthers, T., Orth, R.J., Dennison, W.C., Olyarnik, S., Calladine, A., Fourqurean, J.W., Hesk, K.L., Hughes, A.R., Kendrick, G.A., Kenworthy, W.J., Short, F.T., Williams, L., 2009. Accelerating loss of seagrasses across the globe threatens coastal ecosystems. Proce. Natl. Acad. Sci. 106, 12377–12381.
Węsławski, J.M., Kryla-Straszewska, L., Piwowarczyk, J., Urbański, J., Warzocha, J., Kotwicki, L., Wiktor, J., 2013. Habitat modelling limitations – Puck Bay, Baltic Sea – a case study. Oceanologia 55 (1), 167–183.
Włodarska-Kowalczuk, M., Jankowska, E., Kotwicki, L., Balazy, P., 2014. Evidence of season-dependency in vegetation effects on Macrofauna in temperate seagrass meadows (Baltic Sea). PLoS One 9 (7), e100788. http://dx.doi.org/10.1371/journal.pone.0100788.