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Received: 28 July 2021 | Accepted: 7 June 2022

DOI: 10.1111/1365-2435.14117

Functional Ecology BRITISH ECOLOGICAL SOCIETY

RESEARCH ARTICLE

The important role of sponges in carbon and nitrogen cycling in a deep-sea biological hotspot

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Funding information
Fundação para a Ciência e a Tecnologia, Grant/Award Number: IF/00029/2014/CP1230/CT0002 and UID/05634/2020; Horizon 2020 Framework Programme, Grant/Award Number: 679849

Handling Editor: Enrico Rezende

Abstract

1. Deep-sea sponge grounds are hotspots of biodiversity, harbouring thriving ecosystems in the otherwise barren deep sea. It remains unknown how these sponge grounds survive in this food-limited environment.
2. Here, we unravel how sponges and their associated fauna sustain themselves by identifying their food sources and food-web interactions using bulk and compound-specific stable isotope analysis of amino and fatty acids.
3. We found that sponges with a high microbial abundance had an isotopic composition resembling organisms at the base of the food web, suggesting that they are able to use dissolved resources that are generally inaccessible to animals. In contrast, low microbial abundance sponges had a bulk isotopic composition that resembles a predator at the top of a food web, which appears to be the result of very efficient recycling pathways that are so far unknown. The compound-specific-isotope analysis, however, positioned low-microbial abundance sponges with other filter-feeding fauna. Furthermore, fatty-acid analysis confirmed transfer of sponge-derived organic material to the otherwise food-limited associated fauna.
4. Through this subsidy, sponges are key to the sustenance of thriving deep-sea ecosystems and might have, due to their ubiquitous abundance, a global impact on biogeochemical cycles.

KEYWORDS

amino acids, deep-sea sponge grounds, fatty acids, food web, stable isotope analysis

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1 | INTRODUCTION

Sponges are ubiquitous in the marine environment and are among the most common megafaunal organisms in the deep sea (Tabachnick et al., 1994). They can appear solitarily or in large aggregations, forming extensive sponge grounds, which are found globally along continental shelves, slopes, seamounts, mid-ocean ridges and canyons (Maldonado et al., 2017). Deep-sea sponge communities are represented by demosponges (Class: Demospongiae) and glass sponges (Class: Hexactinellidae), which form three-dimensional structures creating habitats that can serve as substrate, refuge and feeding ground for associated fauna as well as sponges themselves (Maldonado et al., 2017). Therefore, deep-sea sponge grounds have been recognized as hotspots of biodiversity and biomass (Buhl-Mortensen et al., 2010).

Sponges can filter up to 24 m^3 seawater per kg sponge per day (Maldonado et al., 2012; Pham et al., 2019) and thereby transfer energy from the pelagic to the benthic environment. They efficiently take up particulate food (Reiswig, 1971; Yahel et al., 2007) as well as dissolved resources, as shown for many shallow water sponges (de Goeij et al., 2008, 2017; Yahel et al., 2003) and recently also for deep-sea sponges (Bart, Mueller, et al., 2021). The abundance of sponge-associated microbes is often suggested to play an important part in the ability of sponges to use this wide variety of organic and inorganic nutrients as a food source (de Goeij et al., 2017; Freeman et al., 2014; Maldonado et al., 2012). Sponges are known to contain an abundant and diverse community of microbial symbionts like bacteria, fungi, yeast and archaea (Hentschel et al., 2003; Taylor et al., 2007; Webster & Taylor, 2012). Based on differences in the amount of symbionts, sponge species are classified as high microbial abundance (HMA) sponges or low microbial abundance (LMA) sponges (Hentschel et al., 2006; Vacelet & Donadey, 1977). In HMA sponges, symbionts account for up to 60% of the biomass and have 2–4 orders of magnitude higher microbial concentrations than the surrounding seawater, containing typically 10^8–10^{10} microorganisms per gram sponge tissue (Hentschel et al., 2006). LMA sponges contain much lower microbial abundances at concentrations similar to that of the ambient seawater, with 10^5–10^6 bacteria per gram sponge tissue (Hentschel et al., 2006).

Sponges on tropical coral reefs are shown to convert the dissolved organic matter, which is generally not available for other heterotrophic metazoans, to particulate organic carbon and return this to the benthic faunal community, a pathway named the sponge loop (de Goeij et al., 2013). Shallow water sponges are furthermore established as sources of dissolved inorganic nutrients to their environment (Keesing et al., 2013), but their influence on the wider food web is poorly known. In addition, top-down controlled resource recycling through sponge predation has been hypothesized as an alternative sponge loop pathway on tropical coral reefs, where fish are known to graze on sponges (Pawlik & McMurray, 2020). Predation on sponges was also observed in deep-sea sponge reefs on the Canadian shelf, where sponges make up an important node in the food web (Archer et al., 2020).

These different functions of sponges might be essential for deep-sea benthic communities since, in contrast to shallow water communities, they are donor controlled, that is, they depend on the delivery of organic resources from the surface, sunlit layer of the ocean and cannot feed directly on photosynthetically active organisms. The flux of this suspended particulate organic matter (SPOM) from the surface ocean rapidly decreases with depth (Suess, 1980) and it remains unclear how dense sponge grounds can thrive in the (particulate) food-limited deep sea. Neither the full potential of sponges as drivers of carbon cycling in deep-sea ecosystems, nor their actual place in the food web, have been established to date. It remains challenging to study the specific function and contribution of sponges to the deep-sea food web due to their use of multiple food resources, their poorly characterized feeding strategies and the difficulty quantifying carbon flows.

There are different methods to unravel food web interactions. Stable-nitrogen- and carbon-isotope analysis has provided much insights into organic matter resources and trophic transfers within food webs (Middelburg, 2014; Peterson & Fry, 1987). Compound-specific-isotope analyses of amino acids can be used to unravel trophic positions and food web interactions based on differential fractionation of amino acid nitrogen isotopes from food source to consumer (Chikaraishi et al., 2009; McClelland & Montoya, 2002). Fatty acids can provide complementary information on deep-sea food web functioning (Colaço et al., 2007; Parzanini et al., 2019), particularly for unravelling the transfer of organic matter from sponges to the wider food web. Sponges contain specific fatty acids that can serve as a biomarker, like mid-chain-branched fatty acids containing single methyl groups between the ω5 and ω9 positions, which are unique microbial markers of the associated microbiota of sponges that are typically not found in the environment (de Kluijver, Nierop, et al., 2021; Thiel et al., 2002). Additionally, sponges can elongate carbon chains and introduce distinct double bonds (de Kluijver, Nierop, et al., 2021; Gillan et al., 1988), resulting in specific very-long-chain fatty acids (>C24) in demosponges (de Kluijver, Nierop, et al., 2021; Thiel et al., 1999) or C28 and C32 polyenoic fatty acids in glass sponges (Thiel et al., 2002).

Therefore, in this study bulk and compound specific stable isotope analyses were combined with fatty acid biomarker analysis to elucidate the benthic food web of a sponge ground situated on an Arctic seamount, the Schulz Bank. Specifically, we aimed (a) to unravel the food sources supporting a deep-sea sponge ground and (b) to characterize the role of sponges in providing food to the associated fauna.

2 | MATERIALS AND METHODS

2.1 | Study area

The sponge ground is situated on the summit of Schulz Bank (73°50′N, 7°34′E, Figure 1), which is part of the Arctic Mid-Ocean Ridge. The highest density of sponges and associated fauna was

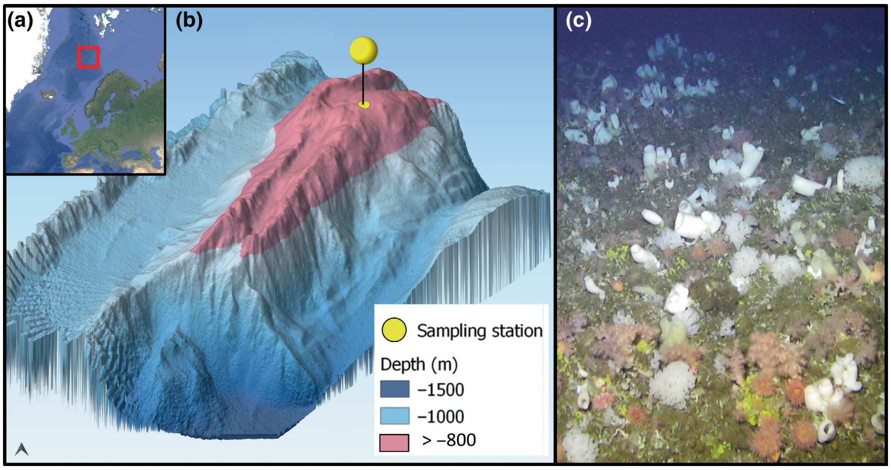

FIGURE 1 (a) Overview map with the Schulz Bank indicated, (b) the Schulz Bank summit, with the red area depicting the area with highest sponge abundances (Roberts et al., 2018), (c) the summit is covered by a dense accumulation of sponges and associated fauna.

found on the summit between 600 and 700 m water depth, forming a reef-like ecosystem (Roberts et al., 2018). The benthic fauna is growing on a thick sponge spicule layer and is dominated by glass sponges, demosponges, ascidians, cnidarians, echinoderms and demersal fish species (Meyer et al., 2019). The primary structure-forming glass sponge species are white, vase-shaped sponges like *Schaudinnia rosea* (LMA) (Figure 1c), while the demosponges are mainly represented by brown-white, round, massive sponges, like *Geodia* spp. and *Stelletta* sp. (HMA).

2.2 | Sampling

Samples were collected during three research cruises (2016–2018) with the RV *G.O. Sars*. During 2016–2017 a lander was deployed at 663 m water depth inside the sponge ground for a period of 1 year, which was equipped with a sediment trap collecting the suspended (organic) particle flux (Hanz, Roberts, et al., 2021). Fauna samples were collected from the sponge ground in 2017 and 2018 during dives with the ROV *ÆGIR* 6000 and using an Agassiz trawl. As many trophic levels as possible were targeted for collection and fauna were divided into their expected trophic positions (see Table S1). Sponges were divided into HMA or LMA species according to the available literature and different species of the same genus (e.g. *Geodia* spp.) were pooled. Smaller fauna were collected and analysed as a whole animal, whereas for larger fauna different tissue types were sampled. Muscle tissue or complete arms/legs of the organism were preferentially sampled, depending on the size of the animal. For sponges, 2×2 cm cubes of tissue were collected from different parts of the body in order to distinguish potential tissue-related effects. Seawater was drained out of the animals and potential adherent sediments were carefully removed after retrieval and before the specimens were frozen. Sediment and sponge spicule mat samples were collected with a box corer. As a primary food resource SPOM was collected with Niskin bottles attached to a Conductivity-Temperature-Depth rosette system. Water samples were collected at the surface (~40 m water depth, 5 L) and ~10 m above bottom (10 L), and filtered over pre-weighed, combusted (4 hr at 450°C) glass fibre filters (Whatman™, ~0.7 μm) and kept frozen until further analysis (−20°C).

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2.3 | Elemental and stable isotope analysis

All samples were freeze-dried before further analyses. Faunal, sediment and sediment trap ($SPOM_{trap}$) samples were homogenized, transferred into silver cups (8×5 mm Elemental Microanalysis) and were acidified in the cups (2 mol/L HCl) to remove inorganic carbonates for particulate organic carbon isotope analysis. SPOM collected on filters from water samples near the sponge ground ($SPOM_{bottom}$) and close to the surface ($SPOM_{surface}$) were exposed to a vapour of concentrated hydrochloric acid (2 mol/L HCl) to remove inorganic carbonates. Samples for total nitrogen (N) isotope analysis were not acidified, but directly transferred and then pressed into tin capsules (12×5 mm, Elemental Microanalysis). The concentration of total carbon and nitrogen and stable isotopic composition of organic carbon ($\delta^{13}C$) and total nitrogen ($\delta^{15}N$) were analysed by a Delta V Advantage isotope ratio MS coupled to a Flash 2000 Elemental Analyser (EA-IRMS) via a Conflo IV interface (Thermo Fisher Scientific Inc.). Benzoic acid and acetanilide were used as standards for $\delta^{13}C$, and acetanilide, urea, and casein for $\delta^{15}N$. Precision based on replicate measurements were ±0.15‰ for both $\delta^{13}C$ and $\delta^{15}N$. Stable isotope values are expressed in the δ notation relative to Vienna Pee Dee Belemnite ($\delta^{13}C$) and air ($\delta^{15}N$).

2.4 | Fatty acid analysis

Fatty acids were used as compound-specific source biomarkers to unravel trophic interactions. Total lipids were extracted from all samples except from sediment trap samples. Between 10 and 50 mg fauna and spicule mat sample, 60–100 mg sponge sample, 150 mg

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sediment and 2–3 filters, representing 10–40 L (0.1–0.5 mg), suspended particulate matter of bottom and surface samples were extracted according to the protocol of de Kluijver (2020). An aliquot of the total lipid extract of sponges was separated into different polarity classes, from which the phospholipid fraction was further analysed (de Kluijver, 2020).

Fatty acids were hydrolyzed from more complex intact polar lipids and esterified (de Kluijver, 2020). The fatty acid methyl esters were analysed in ethyl-acetate using gas chromatography with an apolar column (Agilent, CP-sil5 CB 25 m × 0.32 mm × 0.12 μm) coupled to a flame ionization detector for quantification and mass spectrometry for identification and isotope ratio mass spectrometry for $\delta^{13}C$ analysis. Gastropod, soft coral, sea urchin and starfish had very high sterol concentrations, hence the fatty acid methyl ester fractions were purified (de Kluijver, 2020). $SPOM_{surface}$-, anemone- and soft-coral-containing wax esters were excluded from fatty acid analysis. Since fatty acids can be metabolized and transformed by organisms only the relative abundance rather than absolute amounts were considered. The $\delta^{13}C$ values of *Geodia* spp. fatty acid methyl esters represent an average of total lipid extract and phospholipid fraction, since those isotopic results were similar (average difference 0.7–1.1‰).

2.5 | $\delta^{15}N$ analysis of amino acids

The compound-specific amino acid analysis was used to identify food web interaction that were not resolved by bulk isotope analysis. The technique relies on the different processing of amino-nitrogen groups and three groups are recognized, trophic (asparagine, glutamine, alanine, isoleucine, leucine, valine, proline), source (glycine, serine, phenylalanine, tyrosine, lysine) and metabolic (threonine) amino acids (Chikaraishi et al., 2009; O'Connell, 2017). The $\delta^{15}N$ value of source amino acids reflects the isotopic composition of nitrogen at the base of the food web since they fractionate minimally during metabolism resulting in only a small increase in $\delta^{15}N$ values with increased trophic level. Trophic amino acids, in contrast, undergo transamination and deamination processes and will increase in ^{15}N values with each trophic transfer relative to the source amino acids (Chikaraishi et al., 2009).

For each group one sample was analysed, except for *Geodia* spp. ($n = 4$), *Schaudinnia* sp. ($n = 2$) and $SPOM_{trap}$ ($n = 3$). Dried and homogenized tissue underwent acid hydrolysis followed by derivatization into n-pivaloyl isopropyl esters. These esters were subsequently analysed via gas chromatography-combustion isotope ratio mass spectrometry using a Thermo Trace 1310 GC attached to a Delta V Advantage isotope ratio mass spectrometer via an Isolink 2. Further details of sample preparation and the ramp and temperature schedule used during analysis are discussed in Riekenberg et al. (2020).

Compound-specific-stable-isotope analysis of individual amino acids provides independent information on the trophic level as well as the baseline value of $\delta^{15}N$ and shows the relative influence of the baseline $\delta^{15}N$ and trophic fractionation of consumer $\delta^{15}N$ values (McClelland & Montoya, 2002; McMahon & McCarthy, 2016). The trophic position estimation is based on the offset between the trophic amino acid glutamic acid and source amino acid phenylalanine, with a large and constant increase in ^{15}N values due to metabolism of glutamic acid relative to phenylalanine with each trophic transfer (Chikaraishi et al., 2007; McClelland & Montoya, 2002; O'Connell, 2017):

$$TP = \left(\left(\delta^{15}N_{GLU} - \delta^{15}N_{PHE} - \beta\right) / (TDF)\right) + 1,$$

where β represents the fractionation between the same amino acids in primary producers at the base of the food web ($\beta = 3.4‰$) and β has been found to be relatively consistent in marine primary producers (Chikaraishi et al., 2009; McMahon & McCarthy, 2016; Nielsen et al., 2015) and TDF is the trophic discrimination factor between consumer and their diet of 7.6‰.

The propagated standard analytical error for the trophic position is ±0.48‰ resulting from the trophic fraction uncertainty of ±0.33‰ (McMahon & McCarthy, 2016) and the measurement errors for glutamic acid (±0.41‰) and phenylalanine (±0.46‰). Therefore, robust differences in trophic position can be assumed when the trophic position difference is more than 0.48. To assess microbial re-synthesis of amino acids, the summed variance (ΣV) value was calculated (McCarthy et al., 2007). Heterotrophic reworking of proteinaceous material includes a range of processes by heterotrophic organisms. Therefore, heterotrophic processed material inherently represents a mixture of newly biosynthesized amino acid as well as remnant material. This additional processing will increase the variance in $\delta^{15}N$ of selected amino acids whereby others remain relatively unaltered. The ΣV gives the sum of variance among individual $\delta^{15}N$ values of trophic amino acids (aspartic acid, glutamic acid, alanine, leucine and proline) and can be used as a measure for total heterotrophic re-synthesis. The parameter is defined as the average deviation in the $\delta^{15}N$ values of the trophic amino acids (Calleja et al., 2013; McCarthy et al., 2007).

$$\Sigma V = \frac{1}{n} \sum |x_{AA}|,$$

where x is the deviation of each trophic amino acid from the average $\delta^{15}N$ of all trophic amino acids and n is the total number of trophic amino acids used in the calculation.

3 | RESULTS

3.1 | Stable carbon and nitrogen isotopes

Organic particles in surface water ($n = 23$) showed an average $\delta^{15}N$ of 0.6 ± 0.54‰ and $\delta^{13}C$ of −26 ± 0.35‰ ($M \pm SD$ throughout text, green squares, Figure 2). Isotopic ratios of suspended particles increased with depth to 2.5 ± 0.84‰ for $\delta^{15}N$ and −24.9 ± 0.8‰ for $\delta^{13}C$ ($SPOM_{bottom}$, green squares, Figure 2, $n = 9$). Settling particles collected with the sediment trap ($n = 12$) had $\delta^{15}N$ values between

2.2‰ and 7.5‰ with an average of 5.7 ± 1.7‰, depending on the month of collection. The average δ^{13}C value of sediment trap material (−24.9 ± 0.93‰) was similar to that of the suspended particles in the water column closest to the bottom. Bulk tissue of suspension-feeding fauna (primary consumers, dark blue dots, Figure 2), such as tunicates (*n* = 9), brittle stars (*n* = 3), soft corals (*n* = 3), small crustaceans (*n* = 7),

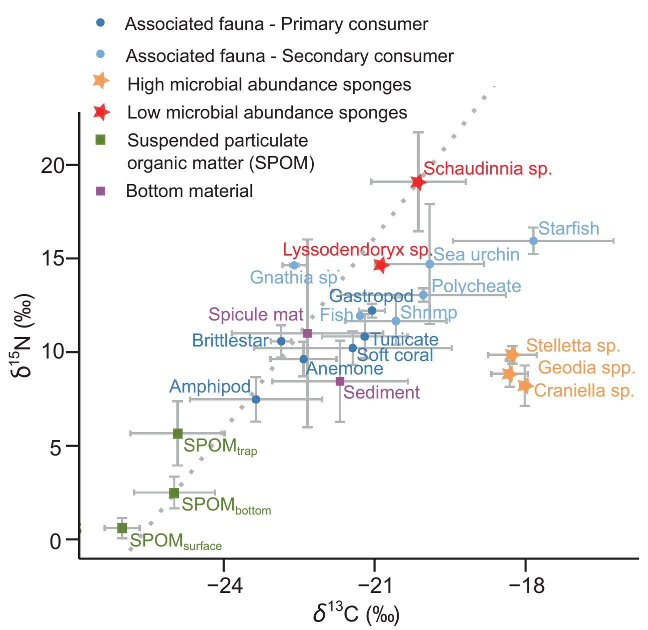

FIGURE 2 Nitrogen and carbon stable isotope ratios of bulk fauna, SPOM, sediment and spicule mat material ±*SD*. The dotted line indicates the generally anticipated enrichment in marine food webs of 3.4‰ δ^{15}N and 0.8‰ δ^{13}C (Vander Zanden & Rasmussen, 2001) with suspended particulate organic matter (SPOM) as the primary food source.

and anemones (*n* = 8) showed average δ^{15}N values of 10.1 ± 0.4‰ and δ^{13}C of −23 ± 0.4‰. This corresponded to trophic enrichment factors of about 5.1‰ for δ^{15}N and 2.6‰ for δ^{13}C compared with the presumed food source, here taken as the suspended particles at the bottom (*n* = 3). The δ^{15}N values of HMA sponges (*Geodia* spp., *n* = 8 and *Stelleta* sp., *n* = 4, orange stars, Figure 2) were similar to those of other suspension feeders (primary consumers), whereas the δ^{13}C value of HMA sponges was higher (about 4‰). Secondary consumers (light blue dots, Figure 2), such as polychaeta (*n* = 4), sea urchins (*n* = 3), fish (*n* = 1), and starfish (*n* = 2) had average δ^{15}N and δ^{13}C values of 13.6 ± 3.5‰ and −20.3 ± 2.6‰, respectively. The LMA sponge *Lyssodendorix* sp. (red star, Figure 2) showed similar isotopic values (δ^{15}N and δ^{13}C values of 14.6‰ and −20.9‰, respectively). The trophic enrichment from primary consumers (suspension feeders) to secondary consumers was about 3.6‰ for δ^{15}N and 2.6‰ for δ^{13}C. The LMA sponge, *Schaudinnia* sp., showed the highest δ^{15}N value of 19.4 ± 2.3‰, corresponding to an additional trophic enrichment of 5.8‰ with respect to the secondary consumers (*n* = 8). For the first two trophic steps (from particles in the surface layer to primary consumers and then secondary consumers at the seafloor), the average enrichments were 4.8 ± 1.2‰ for δ^{15}N and 2.6 ± 0‰ for δ^{13}C, whereby the HMA sponges are excluded from the average due to their apparent unique position in the food web.

3.2 | Compound-specific-nitrogen-isotope analysis of amino acids

The metabolic amino acid had the lowest δ^{15}N values (0.96 ± 0.28‰, Figure 3), with sponges and their spicule mat remaining above the total metabolic amino acid average (6.1 ± 2.9‰). The δ^{15}N of source amino acids was on average 8.7 ± 3‰ (Figure 3). HMA sponges

TABLE 1 Dominant fatty acids (≥10% of total fatty acids) in Schulz Bank fauna and spicule mat. Trophic position is based on compound specific isotope analysis of amino acids

Dominant fatty acids in the fauna (≥10%)		Trophic position	Mid-me-c16:0	Mid-me-C18:0	Me-24:2Δ(5,9)	26:2Δ(5,9)	30:3
Marker			Sponge associated bacteria		Sponge		
Fauna	HMA sponges	1	x	x	x	x	
	LMA sponge	2					X
	Anemone	2					
	Brittle stars	2					
	Fish	3					
	Gastropod	2					
	Polychaeta	2					
	Sea urchin	3			<10%	<10%	
	Shrimp	3					
	Soft coral	2					
	Starfish	3			<10%	<10%	
	Tunicate	2					
	Spicule mat	–				x	

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(7.2 ± 1.9‰) and sediment trap material (5.7 ± 2.9‰) were below the average of total source amino acids, while LMA sponges and spicule mats were above the total source amino acids average. Trophic amino acids had an average $\delta^{15}N$ value of 20.4 ± 7‰ (Figure 3). The HMA sponges (12.6 ± 2.1‰), as well as the sediment trap material (15.6 ± 2.9‰), showed a much lower signal than all other samples. The spicule mat material showed the highest $\delta^{15}N$ values for the trophic amino acids (30.2 ± 4.6‰).

The $\delta^{15}N$ value of source amino acids (Figure 3) of the sediment trap material and HMA sponges were similar and lower than those of suspension feeders (dark blue organisms, Figure 3), secondary consumers (light blue organisms, Figure 3), and LMA sponges. The source amino acid $\delta^{15}N$ values of the spicule mat were the highest.

The compound-specific-isotope analysis of the HMA sponges indicate that they occupy the lowest trophic position (1.3, Figure 4). Sediment trap material, LMA sponges and most associated fauna are at the second trophic level (from 2 to 2.5). Sea urchins (2.6) and tunicates (2.7) have a slightly higher trophic level. Fish (3.2) and shrimp (3.3), as well as spicule mat material (3.2) were found at the third trophic level.

The Σ*V* ranged from 0.81 to 4.12 (Figure 4), with the lowest heterotrophic enrichment for soft corals (0.81) and LMA sponges (1.22). Sediment trap material, sea urchins and shrimp (2–2.5) had intermediate values, while the spicule mat had the highest heterotrophic enrichment (4.12).

3.3 | Fatty acid analysis

The suspended particles in the sunlit surface layer were dominated by algal markers (e.g., 18:*n*ω3, 20:5ω3 and 22:6ω3; Dalsgaard et al., 2003;

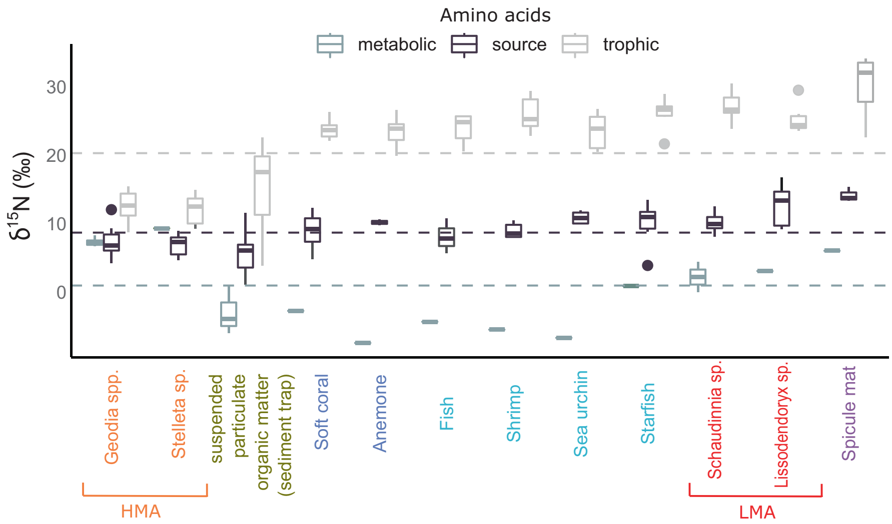

FIGURE 3 $\delta^{15}N$ values for the different amino acid classes. Horizontal dotted lines indicate the average value of all individual samples for the respective classes.

C16:0	18:1ω9	18:1ω7	C18:0	20:4ω6	20:5ω3	20:1ω	22:6ω3	22:1ω	24:5 (0r6)ω3
General	Animal	Bacteria + algae	General	Macroalgae	Algae	Zooplankton	Algae	Zooplankton	Octocorals
x						x		x	
x			x		X	x			
x			x				x		
			x		X	x			
x	x					x			
					X	x			
x	x	x			X		x		
				x		x			x
					X	x			
x		x							
x									

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Kelly & Scheibling, 2012; Table 1), accounting for 31% of all total fatty acids, whereas suspended particles at depth contained lower proportions of algal markers and relatively more mono-unsaturated and linear-saturated fatty acids. The spicule mat had a high diversity in fatty acids and contained mostly typical sponge fatty acids. Anemones included high concentrations of zooplankton markers (20:1 and 22:1; Kelly & Scheibling, 2012), which are also present in most of the associated fauna, except tunicates. Soft coral included octocoral fatty acid markers 20:4ω6 and 24:5 (Imbs et al., 2016) and brittle stars and tunicates contained substantial proportions (>1%) of bacterial markers (*i*-C15:0, *ai*-C15:0 and 18:1ω7; Dalsgaard et al., 2003; Kelly & Scheibling, 2012). The fatty-acid profiles of HMA sponges (*Geodia* spp. and *Stelletta* sp.) are dominated by mid-chain-branched fatty acids: me-C16:0 and me-C18:0. Additionally, the HMA sponges produced very-long-chain fatty acids, Me-24:2Δ(5,9) and 26:2Δ(5,9). The LMA sponge *Schaudinnia* sp. mainly contained very-long-chain fatty acids, such as 30:3, but hardly any bacterial markers, compared to the HMA sponges. Predators like sea urchins and starfish also contained substantial (>1%) bacterial markers (*i*-C15:0, *ai*-C15:0 and 18:1ω7) as well as the sponge-specific microbial markers, me-C18:0 and small amounts (<3%) of very-long-chain fatty acids. The algal-derived poly-unsaturated fatty acids (20:5ω3 and 22:6ω3) dominated the fatty acids of shrimp and fish.

To further constrain food sources and food web transfers, the δ^{13}C-isotopic values of the fatty acids in the HMA sponge were assessed. The bulk carbon-isotope value of HMA sponges was −18.1±0.1‰, whereas the value of settling suspended particles was −24.9±0.8‰ ($SPOM_{bottom}$, Figure 2). The non-specific fatty acids in *Geodia* sp. had a weighted average isotopic value of −21.7±0.8‰, the general bacterial-specific fatty acids −24.9±0.6‰ and the sponge-specific very-long-chain fatty acids −22.5±0.2‰. The symbiont-specific mid-chain-branched fatty acids had a much higher δ^{13}C of −19.2±0.6‰. The higher isotope values of these mid-chain methyl branched fatty acids were also found in both sea urchins (−19.5‰) and starfish (−19.4‰).

4 | DISCUSSION

We aimed to unravel the role of sponges in the food web of a deep-sea biological hotspot. At first glance, the bulk isotope data of the food web (Figure 2) show a traditional linear increase with a strong positive correlation between bulk δ^{15}N and δ^{13}C as is expected for a food web supported by a main primary source (Polunin et al., 2001). The base of the food web consists of near-bed SPOM, which is primarily consumed by the suspension-feeding fauna. The following trophic position is taken by secondary consumers, such as starfish, sea urchins, and fish. However, several distinct offsets from a traditional food chain are observed based on bulk isotope data: (a) The expected primary food source, settling suspended particles, is located at the second trophic position. (b) LMA sponges, presumed to be primary consumers, are observed at the top of the food web. (c) HMA sponges do not follow the linear increase of the isotopic ratios and are richer in ^{13}C compared to other suspension feeding fauna and (d) trophic enrichment factors are higher than typically expected for both carbon and nitrogen.

The position of the expected primary food source at the second trophic level can be attributed to extensive zooplankton grazing of phytoplankton, which is produced during a single annual summer bloom occurring in the Norwegian Sea (von Bodungen et al., 1995). Consequently, the remaining particles that settle and reach the bottom are already processed, and have higher δ^{15}N (2.5‰) than particles in the surface layer (0.6‰). This processing/reworking was further confirmed by the presence of zooplankton fatty-acid markers in settled particles collected near the sponge community. This is a known feature of a 'brown' food web that is based on detritus (Evans-White & Halvorson, 2017).

A striking feature of the food web observed in this study is the particularly high δ^{15}N-values for LMA sponges, which were also observed in other studies of deep-sea sponge grounds (Iken et al., 2001; Kahn et al., 2018; Polunin et al., 2001). A high inferred trophic level is inconsistent with the role of sponges as filter-feeders, but so far, no conclusive explanation has been presented. These extremely enriched ^{15}N values either indicate that sponges rely on additional or altered food sources (e.g., waste products from higher trophic levels). A sediment-trap series showed that after the summer bloom period progressively more degraded organic matter is trapped on the Schulz Bank sponge ground as shown by increasing δ^{15}N-values throughout the year (Hanz, Roberts, et al., 2021), whereas values did not reach values found in LMA sponges.

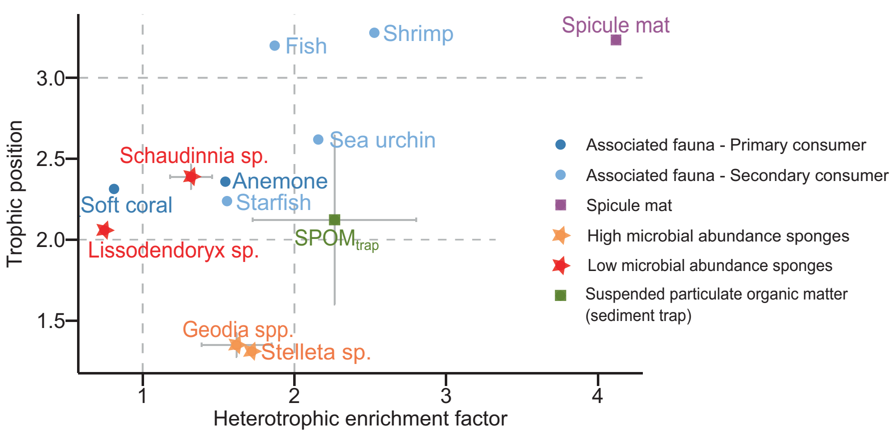

FIGURE 4 Trophic position and $\sum V$ of the benthic fauna and spicule mat samples ±*SD*.

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Alternatively, the high trophic position of LMA sponges could indicate the internal recycling of nitrogen within the sponge tissue or in the sponge microbiome. Selective uptake of microbes or uptake of microbial-derived material can affect the expected tropic enrichment (Middelburg, 2014). Kahn et al. (2018) suggested that LMA sponges take up re-suspended benthic bacteria, which are enriched in $\delta^{15}N$. Resuspension of bottom material during internal tidal-wave motions has been observed on the Schulz Bank (Hanz, Roberts, et al., 2021), which could in combination with an accumulation of degraded organic matter lead to high amounts of benthic bacteria in the water column. However, the isotopic composition of the spicule mat (i.e., including residing bacteria) measured in this study cannot explain the bulk $\delta^{15}N$-isotopic composition of LMA sponges. The spicule mat is more than one trophic level (11.0‰) lower than that of the LMA sponges (19.4‰) and the compound-specific-amino-acid data show that the spicule-mat material is already much further processed than LMA sponges themselves (Heterotrophic enrichment factor, Figure 4). Alternatively, LMA sponges may show higher $\delta^{15}N$-values due to internal recycling of nutrients, analogous to the principle of cannibalism in Arctic fishes (Hobson & Welch, 1995). Intense recycling of nitrogen within the sponge microbiome, which is consistent with molecular biology data (Kiran et al., 2018) and rate measurements (de Kluijver, Bart, et al., 2021; Rooks et al., 2020), may result in large isotopic effects with the consequence that LMA sponges obtain high $\delta^{15}N$ values.

The amino-acid values of $\delta^{15}N$ provide additional constraints on these alternative explanations. LMA sponges in this study show a much higher $\delta^{15}N$ of trophic amino acids (27.3 ± 2.2‰, Figure 2) and source amino acids (10 ± 1.6‰) than that of their assumed primary food source, the settling particles in the sediment trap (15.6 ± 2.9% and 5.7 ± 2.9‰, respectively). This is consistent with our observation that the vertical flux of settling particles does not provide the main food source for LMA sponges, since the isotopic value of source amino acids should only increase by ~0.4‰ per trophic position (Chikaraishi et al., 2009). However, the impact of intense carbon and nitrogen recycling processes within the sponge microbiome might impact the isotope values of source amino acid isotopes, e.g., via de novo synthesis of amino acids by chemoautotrophs as observed for example in cold water corals (Middelburg et al., 2015). Based on the amino-acid-isotope analysis, sponges appear to have a similar trophic position as the associated suspension-feeding fauna (Figure 4). This trophic position for LMA sponges is not only consistent with our knowledge on sponge physiology as primary consumers, but also appeared to be little affected by internal recycling processes that cause large and incompletely understood changes in bulk $\delta^{15}N$. Accordingly, LMA sponges are likely not at the top of the food chain but exhibit yet unknown processes that enrich their bulk $\delta^{15}N$ signature. We conclude that amino-acid-isotope analysis should be considered as the preferred method to analyse deep-sea ecosystem food webs that include LMA sponges.

The HMA sponges clearly deviate isotopically from the linear food chain concept because their $\delta^{13}C$ values were strongly enriched relative to the other suspension feeding fauna. This indicates that they not only rely on suspended particles but also use another carbon source that is elevated in ^{13}C value. In fact, a mismatch between particle carbon delivery and demand has been observed in multiple deep-sea sponge grounds, including the Canadian shelf and Arctic Mid-Atlantic Ridge, where sponges require between seven and up to a hundred times more carbon than is delivered by the vertical flux alone (Hanz, Beazley, et al., 2021; Hanz, Roberts, et al., 2021; Kahn et al., 2015). Uptake of dissolved organic matter likely resolves the imbalance between carbon delivery and consumption of suspended particles (Maldonado et al., 2017) because dissolved organic matter is by far the largest reservoir of organic carbon and nitrogen in the ocean (Benner et al., 1992). Recent ex situ experiments confirmed that several abundant North-Atlantic deep-sea LMA and HMA sponge species are able to take up dissolved organic carbon, which accounted to >90% of their daily organic carbon diet (Bart et al., 2020; Bart, Mueller, et al., 2021). Moreover, both HMA and LMA deep-sea sponges can subsequently release detrital particles (Bart, Hudspith, et al., 2021; Maier et al., 2020), which are in turn consumed by associated fauna (Bart, Hudspith, et al., 2021). This confirms that a sponge loop pathway can occur in deep-sea ecosystems. Future in situ experiments need to establish the uptake and recycling of dissolved organic matter in the deep sea.

Dissolved inorganic carbon could be a second additional source of carbon for deep-sea sponges. Archaea, as well as some bacteria, are able to oxidize regenerated NH_4^+ to NO_2^-, which is then used by other microbes to oxidize NO_2^- to NO_3^- (Hoffmann et al., 2009). Oxidation of NH_4^+ generates energy that is used by chemoautotrophs to fix dissolved inorganic carbon (Wuchter et al., 2006). Dissolved inorganic carbon has an elevated ^{13}C-value with typical water column values of around ~0–1‰ (Griffith et al., 2012) and chemoautotrophy thus introduces a major shift towards higher $\delta^{13}C$ values. Nevertheless, only relatively low fluxes of (dark) carbon fixation were found in other deep-sea sponges (van Duyl et al., 2008). The $\delta^{13}C$-values of the fatty acids in HMA sponges provide additional evidence for chemoautotrophic inputs (Table S2). Fatty acids produced by sponge bacterial symbionts that were linked to mixotrophs (Siegl et al., 2011) show the highest isotopic enrichment (−19.2 ± 0.6‰), compared to other bacterial fatty acids (−24.9 ± 0.6‰) and sponge-specific very-long-chain fatty acids had intermediate values, indicating transfer of carbon from symbionts to the sponge. However, the role of archaea on the isotopic composition of sponges remains unknown since they cannot be detected with fatty acid analysis.

As aforementioned, the majority of the unique carbon signal is nevertheless likely derived from the uptake of dissolved organic carbon and ^{13}C-values of dissolved organic carbon are expected to be around −23.1‰ to −22.2‰ in the North Atlantic (Hansell & Carlson, 2014; Hanz, 2021). This suggests that uptake of dissolved organic carbon together with chemoautotrophic fixation of dissolved inorganic carbon contribute to carbon supply to deep-sea sponges.

Besides carbon, also additional sources of nitrogen are used by HMA sponges. The average $\delta^{15}N$ of the trophic amino acids of HMA sponges (12.6 ± 2.1‰) is slightly lower than that of settling particles from the sediment trap (15.6 ± 5.8‰, Figure 2), yet the isotopic value of trophic amino acids is expected to increase by about 7.6‰ per

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trophic level (Chikaraishi et al., 2009) rather than decrease in $\delta^{15}N$ relative to their food source. The same holds true for the difference between source amino acids and trophic amino acids, which points towards a less processed source of amino acids for HMA sponges (+5.4‰) when compared to the settling particles ($SPOM_{trap}$, +9.8‰). Moreover, the $\delta^{15}N$-values from amino acids suggest that the HMA sponges are at the base of the food web, since only a very small trophic enrichment of the trophic amino acids compared to the source amino acids was found. *Geodia* spp. (and other HMA species) can efficiently recycle nitrogen, with active ammonia assimilation, that will result in de novo synthesis of amino acids (Hentschel et al., 2012).

This benthic-pelagic coupling via use of dissolved resources and transfer as particulate food to the associated organisms is essential, because particulate organic matter is highly limited in the deep sea due to its remineralization during the transport from the surface ocean to the seafloor (Suess, 1980). This is especially important since studies of Hanz, Roberts, et al. (2021) estimated that the Schulz Bank sponge ground only receives less than 1% of its carbon demand from the vertical flux of organic matter. This also implies that efficient recycling processes need to take place. These recycling processes are apparent when considering the larger than expected enrichment in bulk-isotopic values between each trophic level (4.7‰ for $\delta^{15}N$ and 2.6‰ for $\delta^{13}C$, Figure 2), compared to a generally expected 3.4‰ and 0.8‰ enrichments, respectively (Vander Zanden & Rasmussen, 2001). These large trophic-enrichment values are likely caused by enhanced recycling associated with the transfer of ^{13}C-enriched carbon from the HMA sponges and transfer of ^{15}N rich nitrogen mainly from LMA sponges towards the associated fauna.

The transfer of sponge-derived organic matter was further shown by the fatty acid analysis, as sponge-derived fatty acids were found in tissue of the associated fauna. This is, however, no proof for the transfer of detritus since fatty acid transfer can also be caused by top-down processes, such as predation of associated fauna on sponges, like direct grazing of sponges by fish as was observed in tropical reefs (Pawlik & McMurray, 2020). Many fish species, like cod and halibut, are known to feed on sponges (Archer et al., 2020; Mehl, 1991; Randall & Hartman, 1968). However, we found no evidence for top-down control by fish, since the surveyed fish in this study lacked sponge biomarkers. Nevertheless, we did find mid-chain-branched C18:0 fatty acids, known features of demosponges, in starfishes and sea urchins, which confirmed the trophic transfer from sponges to associated fauna. This transfer could be caused by direct feeding (predation) of starfish and sea urchins on sponges, or by feeding on the detritus released by sponges, as was recently confirmed in an ex situ experiment (Bart, Hudspith, et al., 2021). Studies in other glass sponge reefs did also observe that sponges represent a significant portion of the associated fauna's diet (Archer et al., 2020).

The relative abundance of the fatty acids in consumers confirmed that sponge-derived organic matter is directly transferred to the associated fauna and sponges thereby represent an important link in this deep-sea ecosystem. Brittle stars and tunicates do not contain sponge biomarkers, whereas they contain substantial amounts of general bacterial markers, indicating that they feed on bacteria, bacteria-derived material (from sponges), or have bacterial symbionts themselves. However, consumers may transform, catabolize or produce new fatty acids (Imbs et al., 2016). Unfortunately, trophic transfer involving archaea, which can facilitate fixation of dissolved inorganic carbon, cannot be detected with this approach since they do not use fatty acids for their membrane phospholipids (Koga & Morii, 2007).

This study shows that deep-sea sponge grounds do not follow the classical food web structure. These ecosystems are subsidized by additional food resources entering the food web via sponges (here named the sponge substitution, blue arrows Figure 5). This addition of resources appears to be mediated by both deep-sea HMA and LMA sponges, as they can directly feed on dissolved organic matter (Bart et al., 2020; Bart, Mueller, et al., 2021) and dissolved inorganic carbon (van Duyl et al., 2008) and are able to utilize inorganic nutrients (de Kluijver, Bart, et al., 2021; Hoffmann

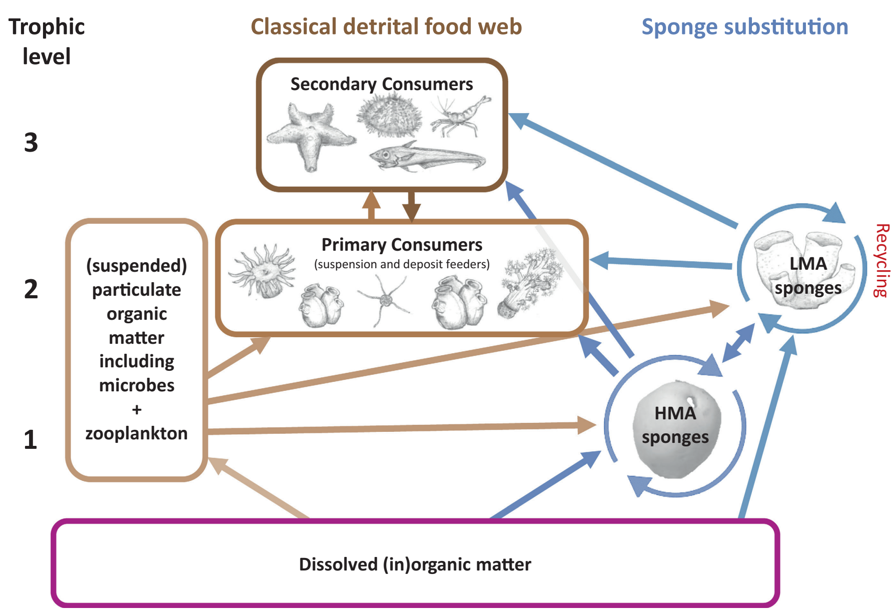

FIGURE 5 Proposed food web of the Schulz Bank sponge ground with isotopic enrichment of bulk $\delta^{15}N$ and $\delta^{13}C$. Brown arrows indicate the detrital food web, and blue arrows indicate the sponge substitution within the deep-sea biological hotspot.

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et al., 2009). Both LMA and HMA sponges are known to provide the deep-sea food web with sponge-derived organic matter as either detritus or through predation (Bart, Hudspith, et al., 2021; Maier et al., 2020) and sponge needles of both sponge groups are found in the stomach of associated fauna (Archer et al., 2020). The compound-specific-isotope analysis of amino acids of this study gave a more detailed and consistent picture of the food web structure compared with the classical bulk isotope analysis. Through the combination of bulk isotopes and amino- and fatty acid profiling, we elucidated major parts of this complex food web (Figure 5). Overall, it is likely that sponge grounds are relying on particulate as well as dissolved resources, which enables them to survive and even thrive in an environment that is otherwise considered to be food limited. We show that, intriguingly, sponges play an important role in deep-sea benthic ecosystems by positioning themselves at both to bottom and the top of the food web.

The 'sponge substitution' could also play an important role in other deep-sea ecosystems, like cold water coral reefs, where sponges are abundant and might be able to supply particulate organic carbon to the associated fauna (Rix et al., 2016). Maier et al. (2020) showed that sponges are important links in the food web for cold water coral reefs, especially during periods of low food availability. Due to the global distribution of sponges, they might play an even more important role for benthic ecosystems and accordingly for the oceanic carbon and nitrogen cycling than so far anticipated.

AUTHORS' CONTRIBUTIONS

U.H. wrote the main manuscript text, collected the samples and is responsible for the analysis and interpretation of the data; P.R. conducted the AA measurements and analysed the data; A.d.K. conducted FA measurements and analysed the data; M.v.d.M. and J.J.M. helped with the analysis and interpretation of the data; E.W. contributed to the sample collection; F.M. developed the study concept, sample collection and helped with interpretation of the data; J.M.d.G. and M.C.B. contributed to the sample collection, analysis and interpretation of the data; H.-T.R. organized the fieldwork and initiated the study. All authors reviewed the manuscript and contributed to the discussion.

ACKNOWLEDGEMENTS

This research has been performed in the scope of the SponGES project, which received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 679849. A.C. was supported by Fundação para a Ciência e a Tecnologia (FCT) through IF/00029/2014/CP1230/CT0002 to and through the strategic projects UID/05634/2020. Klaas Nierop, Desmond Eefting and Femke van Dam (Utrecht University) are acknowledged for their help with fatty acid analysis. Ronald van Bommel (NIOZ) is acknowledged for his help with the stable isotope analysis. We thank captain and crew of the G.O. Sars as well as the ROV crew for their help in obtaining the samples.

CONFLICT OF INTEREST

The author(s) declare no competing interests.

DATA AVAILABILITY STATEMENT

All data are available from the Pangaea Digital Repository https://doi.org/10.1594/PANGAEA.923765, https://doi.pangaea.de/10.1594/PANGAEA.923764 and https://doi.pangaea.de/10.1594/PANGAEA.923749 (Hanz et al., 2020a, 2020b, 2020c).

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REFERENCES

Archer, S. K., Kahn, A. S., Thiess, M., Law, L., Leys, S. P., Johannessen, S. C., Layman, C. A., Burke, L., & Dunham, A. (2020). Foundation species abundance influences food web topology on glass sponge reefs. *Frontiers in Marine Science*, *7*, 799.

Bart, M. C., de Kluijver, A., Hoetjes, S., Absalah, S., Mueller, B., Kenchington, E., Rapp, H. T., & de Goeij, J. M. (2020). Differential processing of dissolved and particulate organic matter by deep-sea sponges and their microbial symbionts. *Scientific Reports*, *10*(1), 1–13.

Bart, M. C., Hudspith, M., Rapp, H. T., Verdonschot, P. F., & de Goeij, J. M. (2021). A deep-sea sponge loop? Sponges transfer dissolved and particulate organic carbon and nitrogen to associated fauna. *Frontiers in Marine Science*, *8*, 229.

Bart, M. C., Mueller, B., Rombouts, T., van de Ven, C., Tompkins, G. J., Osinga, R., Brussaard, C., MacDonald, B., Engel, A., Rapp, H. T., & de Goeij, J. M. (2021). Dissolved organic carbon (DOC) is essential to balance the metabolic demands of North-Atlantic deep-sea sponges. *Limnology and Oceanography*, *66*(3), 925–938.

Benner, R., Pakulski, J. D., McCarthy, M., Hedges, J. I., & Hatcher, P. G. (1992). Bulk chemical characteristics of dissolved organic matter in the ocean. *Science*, *255*(5051), 1561–1564.

Buhl-Mortensen, L., Vanreusel, A., Gooday, A. J., Levin, L. A., Priede, I. G., Buhl-Mortensen, P., Gheerardyn, H., King, N. J., & Raes, M. (2010). Biological structures as a source of habitat heterogeneity and biodiversity on the deep ocean margins. *Marine Ecology*, *31*(1), 21–50.

Calleja, M. L., Batista, F., Peacock, M., Kudela, R., & McCarthy, M. (2013). Changes in compound specific $\delta^{15}N$ amino acid signatures and D/L ratios in marine dissolved organic matter induced by heterotrophic bacterial reworking. *Marine Chemistry*, *149*, 32–44.

Chikaraishi, Y., Kashiyama, Y., Ogawa, N. O., Kitazato, H., & Ohkouchi, N. (2007). Metabolic control of nitrogen isotope composition of amino acids in macroalgae and gastropods: Implications for aquatic food web studies. *Marine Ecology Progress Series*, *342*, 85–90.

Chikaraishi, Y., Ogawa, N. O., Kashiyama, Y., Takano, Y., Suga, H., Tomitani, A., Miyashita, H., Kitazato, H., & Ohkouchi, N. (2009). Determination of aquatic food-web structure based on

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compound-specific nitrogen isotopic composition of amino acids. *Limnology and Oceanography: Methods*, *7*(11), 740–750.

Colaço, A., Desbruyères, D., & Guezennec, J. (2007). Polar lipid fatty acids as indicators of trophic associations in a deep-sea vent system community. *Marine Ecology*, *28*(1), 15–24.

Dalsgaard, J., St John, M., Kattner, G., Muller-Navarra, D., & Hagen, W. (2003). Fatty acid trophic markers in the pelagic marine environment. *Advances in Marine Biology*, *46*, 225–340.

de Goeij, J. M., Lesser, M. P., & Pawlik, J. R. (2017). Nutrient fluxes and ecological functions of coral reef sponges in a changing ocean. In J. Carballo & J. Bell (Eds.), *Climate change, ocean acidification and sponges* (pp. 373–410). Springer.

de Goeij, J. M., van den Berg, H., van Oostveen, M. M., Epping, E. H., & Van Duyl, F. C. (2008). Major bulk dissolved organic carbon (DOC) removal by encrusting coral reef cavity sponges. *Marine Ecology Progress Series*, *357*, 139–151.

de Goeij, J. M., Van Oevelen, D., Vermeij, M. J., Osinga, R., Middelburg, J. J., de Goeij, A. F., & Admiraal, W. (2013). Surviving in a marine desert: The sponge loop retains resources within coral reefs. *Science*, *342*(6154), 108–110.

de Kluijver, A. (2020). Fatty acid analysis sponges. *Digital Repository for Research Protocols, protocols.Io*.

de Kluijver, A., Bart, M. C., Oevelen, D. V., de Goeij, J. M., Leys, S., Maier, S. R., Maldonado, M., Soetaert, K., Verbiest, S., & Middelburg, J. J. (2021). An integrative model of carbon and nitrogen metabolism in a common deep-sea sponge (*Geodia barretti*). *Frontiers in Marine Science*, *7*, 1131.

de Kluijver, A., Nierop, K. G., Morganti, T. M., Bart, M. C., Slaby, B. M., Hanz, U., de Goeij, J. M., Mienis, F., & Middelburg, J. J. (2021). Bacterial precursors and unsaturated long-chain fatty acids are biomarkers of North-Atlantic deep-sea demosponges. *PLoS ONE*, *16*(1), e0241095.

Evans-White, M. A., & Halvorson, H. M. (2017). Comparing the ecological stoichiometry in green and brown food webs—A review and meta-analysis of freshwater food webs. *Frontiers in Microbiology*, *8*, 1184.

Freeman, C. J., Easson, C. G., & Baker, D. M. (2014). Metabolic diversity and niche structure in sponges from the Miskito cays, Honduras. *PeerJ*, *2*, e695.

Gillan, F. T., Stoilov, I. L., Thompson, J. E., Hogg, R. W., Wilkinson, C. R., & Djerassi, C. (1988). Fatty acids as biological markers for bacterial symbionts in sponges. *Lipids*, *23*, 1139–1145.

Griffith, D. R., McNichol, A. P., Xu, L., McLaughlin, F. A., Macdonald, R. W., Brown, K. A., & Eglinton, T. I. (2012). Carbon dynamics in the western Arctic Ocean: Insights from full-depth carbon isotope profiles of DIC, DOC, and POC. *Biogeosciences*, *9*, 1217–1224.

Hansell, D. A., & Carlson, C. A. (2014). *Biogeochemistry of marine dissolved organic matter*. Academic Press.

Hanz, U. (2021). *Biological hotspots in the deep sea: Environmental controls and interactions in deep-sea sponge and coral assemblages* (Dissertation). Utrecht University. https://doi.org/10.33540/967.

Hanz, U., Beazley, L., Kenchington, E., Duineveld, G., Rapp, H. T., & Mienis, F. (2021). Seasonal variability in near-bed environmental conditions in the *Vazella pourtalesii* glass sponge grounds of the Scotian shelf. *Frontiers in Marine Science*, *7*, 597682.

Hanz, U., Mienis, F., & Riekenberg, P. (2020a). Isotope data of deep sea fauna, organic matter and sediment of a sponge ground on an Arctic North Atlantic seamount. *Pangaea Repository*, https://doi.org/10.1594/PANGAEA.923765

Hanz, U., Mienis, F., & Riekenberg, P. (2020b). Amino acid isotope data of deep sea fauna, organic matter and sediment of a sponge ground on an Arctic North Atlantic seamount. *Pangaea Repository*, https://doi.org/10.1594/PANGAEA.923764

Hanz, U., Mienis, F., & Riekenberg, P. (2020c). Bulk isotope data of deep sea fauna, organic matter and sediment of a sponge ground on an Arctic North Atlantic seamount. *Pangaea Repository*, https://doi.org/10.1594/PANGAEA.923749

Hanz, U., Roberts, E. M., Duineveld, G., Davies, A., van Haren, H., Rapp, H. T., Reichart, G. J., & Mienis, F. (2021). Long-term observations reveal environmental conditions and food supply mechanisms at an Arctic deep-sea sponge ground. *Journal of Geophysical Research: Oceans*, *126*(3), e2020JC016776.

Hentschel, U., Fieseler, L., Wehrl, M., Gernert, C., Steinert, M., Hacker, J., & Horn, M. (2003). Microbial diversity of marine sponges. In W. E. G. Müller (Ed.), *Sponges (Porifera)* (Vol. 37, pp. 59–88). Springer.

Hentschel, U., Piel, J., Degnan, S. M., & Taylor, M. W. (2012). Genomic insights into the marine sponge microbiome. *Nature Reviews Microbiology*, *10*, 641–654.

Hentschel, U., Usher, K. M., & Taylor, M. W. (2006). Marine sponges as microbial fermenters. *FEMS Microbiology Ecology*, *55*, 167–177.

Hobson, K. A., & Welch, H. E. (1995). Cannibalism and trophic structure in a high Arctic lake: Insights from stable-isotope analysis. *Canadian Journal of Fisheries and Aquatic Sciences*, *52*, 1195–1201.

Hoffmann, F., Radax, R., Woebken, D., Holtappels, M., Lavik, G., Rapp, H. T., Schläppy, M. L., Schleper, C., & Kuypers, M. M. (2009). Complex nitrogen cycling in the sponge *Geodia barretti*. *Environmental Microbiology*, *11*, 2228–2243.

Iken, K., Brey, T., Wand, U., Voigt, J., & Junghans, P. (2001). Food web structure of the benthic community at the porcupine abyssal plain (NE Atlantic): A stable isotope analysis. *Progress in Oceanography*, *50*, 383–405.

Imbs, A. B., Demidkova, D. A., & Dautova, T. N. (2016). Lipids and fatty acids of cold-water soft corals and hydrocorals: A comparison with tropical species and implications for coral nutrition. *Marine Biology*, *163*, 202.

Kahn, A. S., Chu, J. W., & Leys, S. P. (2018). Trophic ecology of glass sponge reefs in the strait of Georgia, British Columbia. *Scientific Reports*, *8*, 756.

Kahn, A. S., Yahel, G., Chu, J. W., Tunnicliffe, V., & Leys, S. P. (2015). Benthic grazing and carbon sequestration by deep-water glass sponge reefs. *Limnology and Oceanography*, *60*(1), 78–88.

Keesing, J. K., Strzelecki, J., Fromont, J., & Thomson, D. (2013). Sponges as important sources of nitrate on an oligotrophic continental shelf. *Limnology and Oceanography*, *58*(6), 1947–1958.

Kelly, J. R., & Scheibling, R. E. (2012). Fatty acids as dietary tracers in benthic food webs. *Marine Ecology Progress Series*, *446*, 1–22.

Kiran, G. S., Sekar, S., Ramasamy, P., Thinesh, T., Hassan, S., Lipton, A. N., Ninawe, A., & Selvin, J. (2018). Marine sponge microbial association: Towards disclosing unique symbiotic interactions. *Marine Environmental Research*, *140*, 169–179.

Koga, Y., & Morii, H. (2007). Biosynthesis of ether-type polar lipids in archaea and evolutionary considerations. *Microbiology and Molecular Biology Reviews*, *71*, 97–120.

Maier, S. R., Kutti, T., Bannister, R. J., Fang, J. K.-H., van Breugel, P., van Rijswijk, P., & van Oevelen, D. (2020). Recycling pathways in cold-water coral reefs: Use of dissolved organic matter and bacteria by key suspension feeding taxa. *Scientific Reports*, *10*, 1–13.

Maldonado, M., Aguilar, R., Bannister, R. J., Bell, J. J., Conway, K. W., Dayton, P. K., Díaz, C., Gutt, J., Kelly, M., Kenchington, E. L., Leys, S. P., Pomponi, S., Rapp, H. T., Rützler, K., Tendal, O. S., Vacelet, J., & Young, C. M. (2017). Sponge grounds as key marine habitats: A synthetic review of types, structure, functional roles, and conservation concerns. In S. Rossi, L. Bramanti, A. Gori, & C. O. Saco del Valle (Eds.), *Marine animal forests: The ecology of benthic biodiversity hotspots* (pp. 145–183). International Publishing.

Maldonado, M., Ribes, M., & van Duyl, F. C. (2012). Nutrient fluxes through sponges: Biology, budgets, and ecological implications. *Advances in Marine Biology*, *62*, 113–182.

McCarthy, M. D., Benner, R., Lee, C., & Fogel, M. L. (2007). Amino acid nitrogen isotopic fractionation patterns as indicators of heterotrophy in plankton, particulate, and dissolved organic matter. *Geochimica et Cosmochimica Acta*, *71*, 4727–4744.

McClelland, J. W., & Montoya, J. P. (2002). Trophic relationships and the nitrogen isotopic composition of amino acids in plankton. *Ecology*, *83*(8), 2173–2180.

13652435, 2022, 9, Downloaded from https://besjournals.onlinelibrary.wiley.com/doi/10.1111/1365-2435.14117 by Universitetsbiblioteket I, Wiley Online Library on [10/02/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

McMahon, K. W., & McCarthy, M. D. (2016). Embracing variability in amino acid δ^{15}N fractionation: Mechanisms, implications, and applications for trophic ecology. *Ecosphere*, *7*, e01511.
Mehl, S. (1991). The Northeast Arctic cod stock's place in the Barents Sea ecosystem in the 1980s: An overview. *Polar Research*, *10*, 525–534.
Meyer, H., Roberts, E., Rapp, H., & Davies, A. (2019). Spatial patterns of arctic sponge ground fauna and demersal fish are detectable in autonomous underwater vehicle (AUV) imagery. *Deep Sea Research Part I: Oceanographic Research Papers*, *153*, 103137.
Middelburg, J. (2014). Stable isotopes dissect aquatic food webs from the top to the bottom. *Biogeosciences*, *11*, 2357–2371.
Middelburg, J. J., Mueller, C. E., Veuger, B., Larsson, A. I., Form, A., & Van Oevelen, D. (2015). Discovery of symbiotic nitrogen fixation and chemoautotrophy in cold-water corals. *Scientific Reports*, *5*, 17962.
Nielsen, J. M., Popp, B. N., & Winder, M. (2015). Meta-analysis of amino acid stable nitrogen isotope ratios for estimating trophic position in marine organisms. *Oecologia*, *178*, 631–642.
O'Connell, T. C. (2017). 'Trophic' and 'source' amino acids in trophic estimation: A likely metabolic explanation. *Oecologia*, *184*, 317–326.
Parzanini, C., Parrish, C. C., Hamel, J. F., & Mercier, A. (2019). Reviews and syntheses: Insights into deep-sea food webs and global environmental gradients revealed by stable isotope (δ^{15}N, δ^{13}C) and fatty acid trophic biomarkers. *Biogeosciences*, *16*, 2837–2856.
Pawlik, J. R., & McMurray, S. E. (2020). The emerging ecological and biogeochemical importance of sponges on coral reefs. *Annual Review of Marine Science*, *12*, 315–337.
Peterson, B. J., & Fry, B. (1987). Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics*, *18*, 293–320.
Pham, C. K., Murillo, F. J., Lirette, C., Maldonado, M., Colaço, A., Ottaviani, D., & Kenchington, E. (2019). Removal of deep-sea sponges by bottom trawling in the Flemish cap area: Conservation, ecology and economic assessment. *Scientific Reports*, *9*, 1–13.
Polunin, N., Morales-Nin, B., Pawsey, W., Cartes, J. E., Pinnegar, J. K., & Moranta, J. (2001). Feeding relationships in Mediterranean bathyal assemblages elucidated by stable nitrogen and carbon isotope data. *Marine Ecology Progress Series*, *220*, 13–23.
Randall, J. E., & Hartman, W. (1968). Sponge-feeding fishes of the West Indies. *Marine Biology*, *1*, 216–225.
Reiswig, H. M. (1971). Particle feeding in natural populations of three marine demosponges. *The Biological Bulletin*, *141*, 568–591.
Riekenberg, P. M., van der Meer, M., & Schouten, S. (2020). Practical considerations for improved reliability and precision during determination of δ^{15}N values in amino acids using a single combined oxidation–reduction reactor. *Rapid Communications in Mass Spectrometry*, *34*, e8797.
Rix, L., de Goeij, J.M., Mueller, C.E., Struck,U., Middelburg, J.J., van Duyl, F.C., Al-Horani, F.A., Wild, C., Naumann, M.S. & van Oevelen, D. (2016). Coral mucus fuels the sponge loop in warm- and cold- water coral reef ecosystems. *Scientific Reports 6*, 18715.
Roberts, E., Mienis, F., Rapp, H., Hanz, U., Meyer, H., & Davies, A. (2018). Oceanographic setting and short-timescale environmental variability at an Arctic seamount sponge ground. *Deep Sea Research Part I: Oceanographic Research Papers*, *138*, 98–113.
Rooks, C., Fang, J. K.-H., Mørkved, P. T., Zhao, R., Rapp, H. T., Xavier, J. R., & Hoffmann, F. (2020). Deep-sea sponge grounds as nutrient sinks: Denitrification is common in boreo-Arctic sponges. *Biogeosciences*, *17*, 1231–1245.
Siegl, A., Kamke, J., Hochmuth, T., Piel, J., Richter, M., Liang, C., Dandekar, T., & Hentschel, U. (2011). Single-cell genomics reveals the lifestyle of Poribacteria, a candidate phylum symbiotically associated with marine sponges. *The ISME Journal*, *5*, 61–70.
Suess, E. (1980). Particulate organic carbon flux in the oceans—Surface productivity and oxygen utilization. *Nature*, *288*, 260–263.
Tabachnick, K., Van Soest, R., van Kempen Th, M., & Braekamn, J. (1994). Distribution of recent Hexactinellida. In R. W. M. van Soest, T. M. G. van Kempen, & L. C. Braekman (Eds.), *Sponges in time and space* (pp. 213–223). Balkema Press.
Taylor, M. W., Hill, R. T., Piel, J., Thacker, R. W., & Hentschel, U. (2007). Soaking it up: The complex lives of marine sponges and their microbial associates. *The ISME Journal*, *1*, 187–190.
Thiel, V., Blumenberg, M., Hefter, J., Pape, T., Pomponi, S., Reed, J., Reitner, J., Wörheide, G., & Michaelis, W. (2002). A chemical view of the most ancient metazoa–biomarker chemotaxonomy of hexactinellid sponges. *Naturwissenschaften*, *89*, 60–66.
Thiel, V., Jenisch, A., Wörheide, G., Löwenberg, A., Reitner, J., & Michaelis, W. (1999). Mid-chain branched alkanoic acids from "living fossil" demosponges: A link to ancient sedimentary lipids? *Organic Geochemistry*, *30*, 1–14.
Vacelet, J., & Donadey, C. (1977). Electron microscope study of the association between some sponges and bacteria. *Journal of Experimental Marine Biology and Ecology*, *30*, 301–314.
van Duyl, F. C., Hegeman, J., Hoogstraten, A., & Maier, C. (2008). Dissolved carbon fixation by sponge–microbe consortia of deep water coral mounds in the northeastern Atlantic Ocean. *Marine Ecology Progress Series*, *358*, 137–150.
Vander Zanden, M. J. V., & Rasmussen, J. B. (2001). Variation in δ^{15}N and δ^{13}C trophic fractionation: Implications for aquatic food web studies. *Limnology and Oceanography*, *46*, 2061–2066.
von Bodungen, B., Antia, A., Bauerfeind, E., Haupt, O., Koeve, W., Machado, E., Peeken, I., Peinert, R., Reitmeier, S., & Thomsen, C. (1995). Pelagic processes and vertical flux of particles: An overview of a long-term comparative study in the Norwegian Sea and Greenland Sea. *Geologische Rundschau*, *84*, 11–27.
Webster, N. S., & Taylor, M. W. (2012). Marine sponges and their microbial symbionts: Love and other relationships. *Environmental Microbiology*, *14*, 335–346.
Wuchter, C., Abbas, B., Coolen, M. J., Herfort, L., van Bleijswijk, J., Timmers, P., Strous, M., Teira, E., Herndl, G. J., Middelburg, J. J., Schouten, S., & Sinninghe Damste, J. S. (2006). Archaeal nitrification in the ocean. *Proceedings of the National Academy of Sciences of the United States of America*, *103*, 12317–12322.
Yahel, G., Sharp, J. H., Marie, D., Häse, C., & Genin, A. (2003). In situ feeding and element removal in the symbiont-bearing sponge *Theonella swinhoei*: Bulk DOC is the major source for carbon. *Limnology and Oceanography*, *48*, 141–149.
Yahel, G., Whitney, F., Reiswig, H. M., Eerkes-Medrano, D. I., & Leys, S. P. (2007). In situ feeding and metabolism of glass sponges (Hexactinellida, porifera) studied in a deep temperate fjord with a remotely operated submersible. *Limnology and Oceanography*, *52*, 428–440.

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How to cite this article: Hanz, U., Riekenberg, P., de Kluijver, A., van der Meer, M., Middelburg, J. J., de Goeij, J. M., Bart, M. C., Wurz, E., Colaço, A., Duineveld, G. C. A., Reichart, G-J, Rapp, H-T, & Mienis, F. (2022). The important role of sponges in carbon and nitrogen cycling in a deep-sea biological hotspot. *Functional Ecology*, *36*, 2188–2199. https://doi.org/10.1111/1365-2435.14117

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