# A Deep-Sea Sponge Loop? Sponges Transfer Dissolved and Particulate Organic Carbon and Nitrogen to Associated Fauna

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Cold-water coral reefs and sponge grounds are deep-sea biological hotspots, equivalent to shallow-water tropical coral reefs. In tropical ecosystems, biodiversity and productivity are maintained through efficient recycling pathways, such as the sponge loop. In this pathway, encrusting sponges recycle dissolved organic matter (DOM) into particulate detritus. Subsequently, the sponge-produced detritus serves as a food source for other organisms on the reef. Alternatively, the DOM stored in massive sponges was recently hypothesized to be transferred to higher trophic levels through predation of these sponges, instead of detritus production. However, for deep-sea sponges, the existence of all prerequisite, consecutive steps of the sponge loop have not yet been established. Here, we tested whether cold-water deep-sea sponges, similar to their tropical shallow-water counterparts, take up DOM and transfer assimilated DOM to associated fauna via either detritus production or predation. We traced the fate of $^{13}$carbon (C)- and $^{15}$nitrogen (N)-enriched DOM and particulate organic matter (POM) in time using a pulse-chase approach. During the 24-h pulse, the uptake of $^{13}$C/$^{15}$N-enriched DOM and POM by two deep-sea sponge species, the massive species *Geodia barretti* and the encrusting species *Hymedesmia* sp., was assessed. During the subsequent 9-day chase in label-free seawater, we investigated the transfer of the consumed food by sponges into brittle stars via two possible scenarios: (1) the production and subsequent consumption of detrital waste or (2) direct feeding on sponge tissue. We found that particulate detritus released by both sponge species contained C from the previously consumed tracer DOM and POM, and, after 9-day exposure to the labeled sponges and detritus, enrichment of $^{13}$C and $^{15}$N was also detected in the tissue of the brittle stars. These results therefore provide the first evidence of all consecutive steps of a sponge loop pathway via deep-sea sponges. We cannot distinguish at present whether the deep-sea sponge loop is acting through a detrital or predatory pathway, but conclude that both scenarios are feasible. We conclude that sponges could play an important role in the recycling of DOM in the many deep-sea ecosystems where they are abundant, although *in situ* measurements are needed to confirm this hypothesis.

**Keywords:** deep-sea sponge loop, brittle star, carbon, nitrogen, transfer, detritus, predation, DOM

# INTRODUCTION

In the deep-sea, sponges and cold-water corals (CWC) form complex reef structures, which support rich communities of suspension-feeding fauna and play crucial roles as habitat and feeding grounds for motile taxa, including commercial fish species (Miller et al., 2012). These ecosystems are amongst the most productive deep-sea habitats and they are responsible for significant carbon (C) and nitrogen (N) cycling (van Oevelen et al., 2009; Radax et al., 2012; Kutti et al., 2013; Cathalot et al., 2015). In fact, CWC reefs and sponge grounds have been identified as benthic biodiversity hotspots, even comparable to tropical coral reefs in terms of grams organic C m$^{-2}$ and kg dry weight km$^{-2}$ (Polovina, 1984; van Oevelen et al., 2009; Grebmeier et al., 2015; Maldonado et al., 2017).

Paramount to the productivity of benthic ecosystems in oligotrophic waters is their capacity to efficiently retain and recycle resources. The largest organic resource in the oceans is dissolved organic matter (DOM) (Benner et al., 1992), but this complex mixture of polysaccharides, proteins, and lipids is deemed biologically unavailable to most heterotrophic organisms (Carlson, 2002). However, DOM is known to be processed by bacterioplankton and then returned to the classic food chain through planktonic grazing, a pathway termed the microbial loop (Azam et al., 1983). Essentially, by consuming DOM, bacteria remineralize nutrients that would otherwise be lost to the environment (Fenchel, 2008).

Within shallow-water tropical coral reefs, an additional DOM recycling pathway has been established: the sponge loop (de Goeij et al., 2013). In this pathway, encrusting sponges that dominate the surface of cryptic habitats (e.g., crevices, cavities), but also occupy exposed reef surfaces, assimilate DOM and produce significant amounts of particulate detritus (de Goeij et al., 2013; Alexander et al., 2014; Rix et al., 2016, 2017; Lesser et al., 2020). This detritus subsequently feeds the detrital food chain (de Goeij et al., 2013; Rix et al., 2018). Carbon fluxes through these ubiquitous, but largely hidden and thus usually "overlooked" sponges can amount to daily gross primary production rates of the entire reef (de Goeij et al., 2013, 2017). However, no detritus production was found for several sponges with a non-encrusting, but massive, emergent growth form, that generally occur on the exposed reef (McMurray et al., 2018; Wooster et al., 2019). Therefore, a complementary sponge-loop pathway was hypothesized, in which sponge-assimilated DOM is transferred to higher trophic levels via direct predation on sponge tissue (McMurray et al., 2018; Pawlik and McMurray, 2020). To date, this predatory sponge loop has not yet been confirmed. Whether via detritus production or predation, the sponge loop, together with the microbial loop, helps to explain how tropical shallow-water coral reefs maintain a high productivity and biodiversity in otherwise oligotrophic marine environments (de Goeij et al., 2013; Rix et al., 2016; Pawlik and McMurray, 2020).

The (re)cycling and transfer of DOM could be of particular importance for benthic deep-sea ecosystems as, for large parts of the year, particulate phytodetritus transported from the ocean surface cannot fulfill the carbon demands of these systems (Gooday, 2002; Duineveld et al., 2004, 2007;

van Oevelen et al., 2009; Kahn et al., 2018). Recently, first evidence was found that the sponge-loop pathway may not just operate on tropical shallow-water coral reefs, but also in the deep-sea (Rix et al., 2016; Bart et al., 2020a,b; Maier et al., 2020). The capacity to take up DOM represents the first step of the sponge loop (de Goeij et al., 2013), and both encrusting and massive deep-sea sponges have been shown to utilize DOM as a food source (Rix et al., 2016; Bart et al., 2020a,b; Maier et al., 2020). The second step of the sponge loop is the assimilation of DOM into particulate organic matter (POM), leading to either the release of detritus (including pseudo faeces) or an increase in sponge biomass. Using stable isotope tracers, multiple studies have shown that deep-sea sponges are capable of assimilating DOM into biomass (Rix et al., 2016; Kazanidis et al., 2018; Bart et al., 2020a; Maier et al., 2020). Additionally, the encrusting deep-sea sponge *Hymedesmia coriacea* was found to convert 39% of the organic C derived from coral mucus into detritus (Rix et al., 2016), and two massive deep-sea sponge species, *Geodia barretti* and *Mycale lingua*, were found to take up DOM and produce POM, although at much lower rates (0.03 and 3%, respectively) as percentage of their biomass (Maier et al., 2020). However, the third—ecologically critical, but most difficult to experimentally identify—step of the sponge loop has not been established in the deep-sea to date: the transfer of assimilated DOM by sponges to higher trophic levels. Note also that all the aforementioned studies on DOM cycling by deep-sea sponges are based on *ex situ* measurements in controlled laboratory settings. The existence and ecological relevance of a deep-sea sponge loop has therefore not been established to date.

Dissolved organic carbon (DOC) may represent more than 90% of the daily carbon intake of sponges (de Goeij et al., 2017), including deep-sea species (Bart et al., 2020b), but DOM is clearly not the only food source of sponges. In fact, they are established as very efficient filter-feeders of organic particles, such as bacterio-, and phytoplankton (e.g., Reiswig, 1971; Pile et al., 1996; Leys et al., 2018). Food bacteria, as part of their POM diet, were found to be assimilated more efficiently into sponge tissue compared to DOM (Kazanidis et al., 2018; Bart et al., 2020a), implying that bacteria are a high quality and therefore crucial food source to sponges. Currently, it is unknown how the processing of DOM and POM by encrusting and massive sponges affects the subsequent steps of the sponge loop.

The present study aims to test the hypothesis that, similar to their tropical counterparts, deep-sea sponges transfer assimilated DOM and POM to associated fauna, following the prerequisite, consecutive steps of the sponge loop. Therefore, we qualitatively investigated the potential retention and subsequent transfer of C and N derived from DOM and POM (i.e., bacterioplankton) via two species of deep-sea sponges, the massive species *Geodia barretti* and the encrusting species *Hymedesmia* sp., to sponge-associated fauna (i.e., brittle stars). Brittle stars (Echinodermata: Ophiuroidea) are a predominant member of deep-sea ecosystems (Stöhr et al., 2012) and sponge infauna (Clark, 1933; Duarte and Nalesso, 1996). They are known to feed on (pseudo-)fecal droppings of bivalves (Maier et al., 2020), and, in tropical shallow-water ecosystems, on sponge detritus (Rix et al., 2018). Brittle stars may also directly feed on sponge tissue

(Morison, 1979; McClintock, 1994). We traced the fate of $^{13}$C- and $^{15}$N-enriched DOM and POM in time using an *ex situ* pulse-chase experiment. The assimilation of $^{13}$C/$^{15}$N-enriched DOM and POM into the two sponge species was assessed during the 24-h pulse. The transfer of assimilated DOM and POM by the two sponges (via either detritus or direct predation) into brittle stars was investigated during the subsequent 9-day chase, after transfer of the pulse-labeled sponges to label-free running seawater aquaria containing brittle stars.

## MATERIALS AND METHODS

### Study Areas, Sponge Collection, and Maintenance

This study investigated the nutritional relationship between two North-Atlantic deep-sea sponge species, the massive species *Geodia barretti* and the encrusting species *Hymedesmia* sp., and one deep-sea brittle star species, *Ophiura* sp. (**Figure 1A** and **Supplementary Table 1**).

Whole, intact *Geodia barretti* individuals were carefully collected by ROV (Remotely Operated Vehicle), during the G.O. Sars cruise 2018108 (August 2018) at a sponge ground on the Tromsøflaket, a sea bank in the western Barents Sea (70°47′13.9″N 18°03′23.8″E). The Barents Sea is a shelf sea with an average depth of 230 m (Sundfjord et al., 2007). The benthic community at the Tromsøflaket is primarily dominated by scattered large specimens of *G. barretti*, which account for approximately 90% of the benthic biomass (Buhl-Mortensen et al., 2009; Jørgensen et al., 2015). In addition, Actinaria, Bryozoa, Crustacea, Echinodermata, Echiura, and Hydrozoa are part of the benthic community (Kędra et al., 2017). *Hymedesmia* sp. and *Ophiura* sp. were carefully collected attached to their rocky substrate by ROV at Stjernsund reef (70°30′N, 22°30′E), a 30 km long, 3.5 km wide, > 400 m deep glacial sound in northern Norway that connects the Altafjord to North-Atlantic waters (Rüggeberg et al., 2011; Rovelli et al., 2015). This cold-water coral reef is characterized by the presence of the reef framework-forming scleractinian coral *Lophelia pertusa* and reef-associated fauna such as sponges, crustaceans, and other corals (Rovelli et al., 2015).

Sponges and brittle stars were kept on board the research vessel in the dark in 20-L flow-through tanks in a climate room at 6°C. North-Atlantic seawater was pumped in from 6 m water depth at 30 L h$^{-1}$. All individuals were transported without air-exposure to the laboratory facilities at the University of Bergen, Norway, where the experiments took place. In Bergen, sponges and brittle stars were kept in a dark climate room (8°C) in multiple 20-L flow-through aquaria systems. Each holding tank contained a maximum of five sponge individuals and five brittle stars. Flow originated from unfiltered water, pumped from 200 m depth from the outer fjord near Bergen at ∼ 50 L h$^{-1}$ with a temperature ranging from 6 to 8°C. All sponges were acclimatized for a minimum of 1 week prior to the incubation experiments and all sponges and their attached rocky substrates were cleared from epibionts prior to incubations.

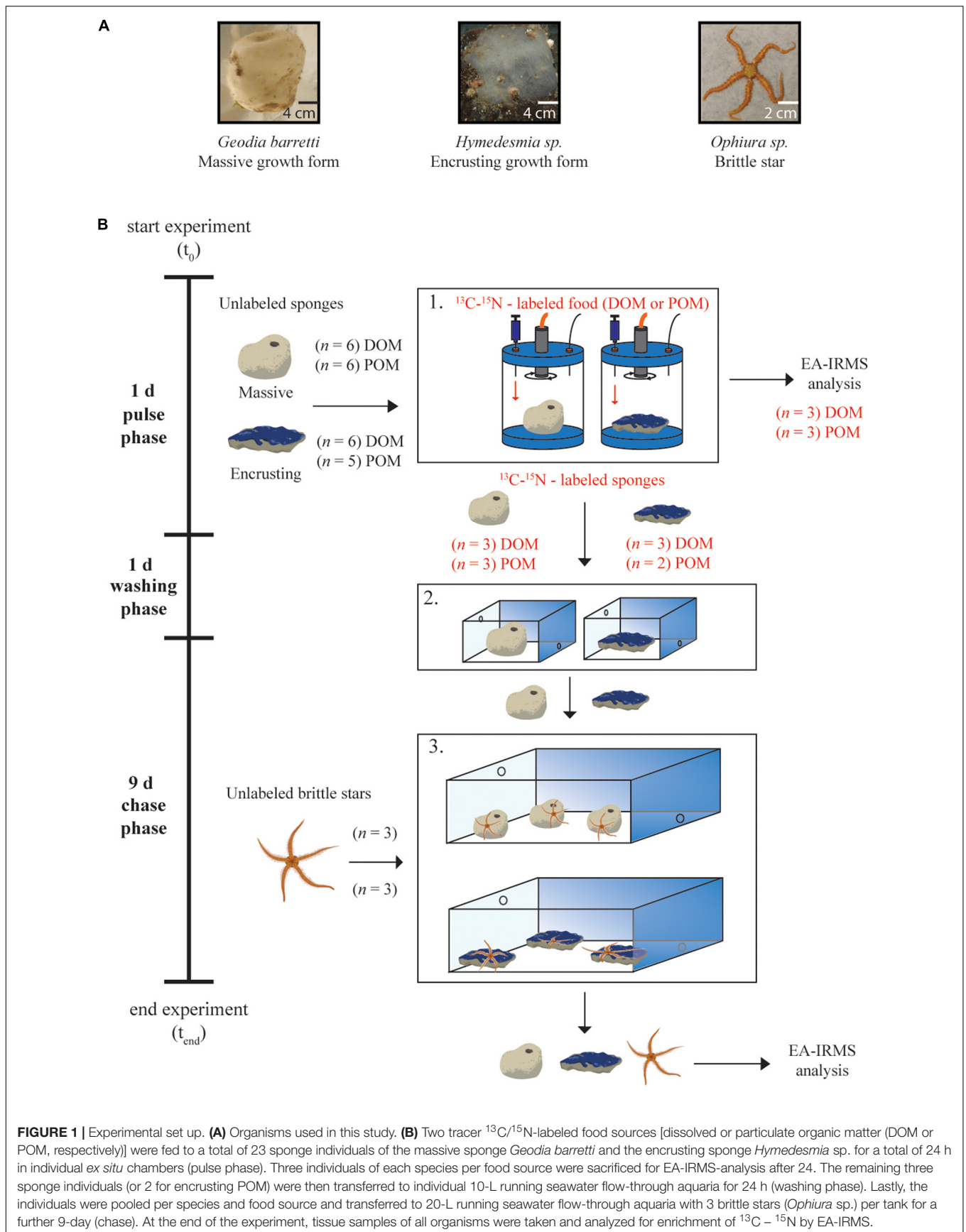## Preparation of $^{13}$C/$^{15}$N-Labeled DOM and POM

Isotopically enriched DOM was prepared by axenically culturing the marine diatom *Phaeodactylum tricornutum* in multiple 2 L Fernbach flasks on F/2 medium amended with $^{15}$N (80%)-NaNO$_3$ (*Eurisotop*, CLM-157) and $^{13}$C (99%)-NaHCO$_3$ (*Eurisotop*, CLM-441) (de Goeij et al., 2008; Bart et al., 2020a). Non-labeled axenic *P. tricornutum* pre-cultures were added (60 mL) to 1 L of sterile labeled F/2 medium in a flow cabinet. The diatoms were grown at 20°C on a 12:12 day:night cycle. After 10 day, diatoms were concentrated on a 0.45 μm filter (147 mm ø) and carefully flushed from the filter with sterile artificial seawater. Subsequently, the collected diatoms were centrifuged for 10 min at 750 × *g*, the supernatant was removed and the pellet was frozen at -20°C. To lyse the cells and release $^{13}$C/$^{15}$N-labeled DOM, the frozen diatoms were lyophilized in a FD5515 *Ilchin Biobase* freeze-drier, after which MilliQ water was added and the solution was placed in an ultrasonic bath for 10 min. Lastly, the DOM solution was filtered over a 0.7 μm GF/F filter and subsequently over a 0.2 μm polycarbonate filter. The filtrate was collected, lyophilized, and analyzed for C and N content and isotopic composition. Before adding DOM to the incubations, aliquots of 5 mL were made by dissolving the lyophilized DOM in MilliQ to a final concentration of 80 μmol L$^{-1}$ DOC or 15 μmol L$^{-1}$ DON added to each incubation.

Isotopically-enriched POM was prepared by labeling ambient seawater bacterioplankton (de Goeij et al., 2008; Bart et al., 2020a). Briefly, seawater bacteria were concentrated by prefiltering natural seawater over a 0.7 μm GF/F filter and subsequent ultrafiltration (0.2 μm; Vivaflow). The inoculum was added to M63 medium (Miller, 1972), amended with thiamine (0.00001%) and MgSO$_4$ (67 μmol L$^{-1}$). Labeled bacterial cultures were amended with $^{13}$C (99%)-glucose (1 g L$^{-1}$, glucose D U-13C6, *Euriso-Top*, CLM-1396) and $^{15}$N (99%)-NH$_4$Cl (1.2 g L$^{-1}$, *Euriso-Top*, NLM-467-5). The culture was grown for 48 h in the dark at 25°C and labeled bacteria were isolated by centrifuging (5 min, 10,000 × *g*), rinsing the pellet in 0.2 μm filtered seawater, centrifuging again, and resuspending the pellet in 0.2 μm filtered seawater before dividing in aliquots to a final concentration of 0.5 × 10$^6$ bacteria mL$^{-1}$ (approximately 12 μmol L$^{-1}$ bacterial carbon (BC) and 1 μmol L$^{-1}$ bacterial nitrogen (BN) added to each incubation) and storing at 4°C.

### Experimental Set-Up

A schematic of the pulse-chase experimental set-up is shown in **Figure 1B** and comprised three phases: a pulse, a washing, and a chase phase. From here on, for simplicity and to distinguish between the two sponge loop pathways scenarios—i.e., the hypothesis that massive sponges are predominantly cycling resources through the predatory pathway and encrusting sponges predominantly through the detrital pathway—we will refer to *G. barretti* as "massive," *Hymedesmia.* sp. as "encrusting" and *Ophiura* sp. as "brittle star."

*Phase 1: pulse*—During the pulse, we continuously administered two tracer $^{13}$C/$^{15}$N-labeled food sources (DOM and POM) to individuals of both species using air-tight 3-L

**FIGURE 1 |** Experimental set up. **(A)** Organisms used in this study. **(B)** Two tracer $^{13}$C/$^{15}$N-labeled food sources [dissolved or particulate organic matter (DOM or POM, respectively)] were fed to a total of 23 sponge individuals of the massive sponge *Geodia barretti* and the encrusting sponge *Hymedesmia* sp. for a total of 24 h in individual *ex situ* chambers (pulse phase). Three individuals of each species per food source were sacrificed for EA-IRMS-analysis after 24. The remaining three sponge individuals (or 2 for encrusting POM) were then transferred to individual 10-L running seawater flow-through aquaria for 24 h (washing phase). Lastly, the individuals were pooled per species and food source and transferred to 20-L running seawater flow-through aquaria with 3 brittle stars (*Ophiura* sp.) per tank for a further 9-day (chase). At the end of the experiment, tissue samples of all organisms were taken and analyzed for enrichment of $^{13}$C – $^{15}$N by EA-IRMS.

**FIGURE 2 |** *Geodia barretti* and *Ophiura* sp. After placing them at random position in the aquaria, brittle stars settled themselves on the surface of the sponges.

incubation flow chambers (Bart et al., 2020a,b). Six individuals of both species were fed DOM, six individuals of the massive sponge were fed POM and five individuals of the encrusting sponge were fed POM. Prior to the incubations, chambers were acid washed (0.4 mol $L^{-1}$ HCl) overnight. During the experiment, the chambers were kept in a water bath to maintain a constant seawater temperature (ranging from 6 to 8°C depending on the incubation). Incubation water was replenished every 8 h (24 h total incubation time) to ensure that sponge individuals were regularly receiving fresh seawater after which new, labeled substrate was added. At the end of the incubation, sponges were removed from the chamber and rinsed in 0.2 μm filtered unlabeled seawater. This experiment was part of a larger isotope-tracer study conducted simultaneously and the uptake rates of DOM and POM by massive and encrusting sponges ($n = 3$ per species, per food source) were taken from already published data (**Figure 3**, gray marked area) (Bart et al., 2020a). To test the direct uptake rates of DOM and POM by brittle stars, separate incubations ($n = 3$ per food source) were performed according to the aforementioned protocol. After the 24-h incubation period, brittle stars were directly sacrificed and placed in a drying oven for 48 h at 60°C. After drying, tissue was homogenized with mortar and pestle and stored in a desiccator until further analysis by EA-IRMS.
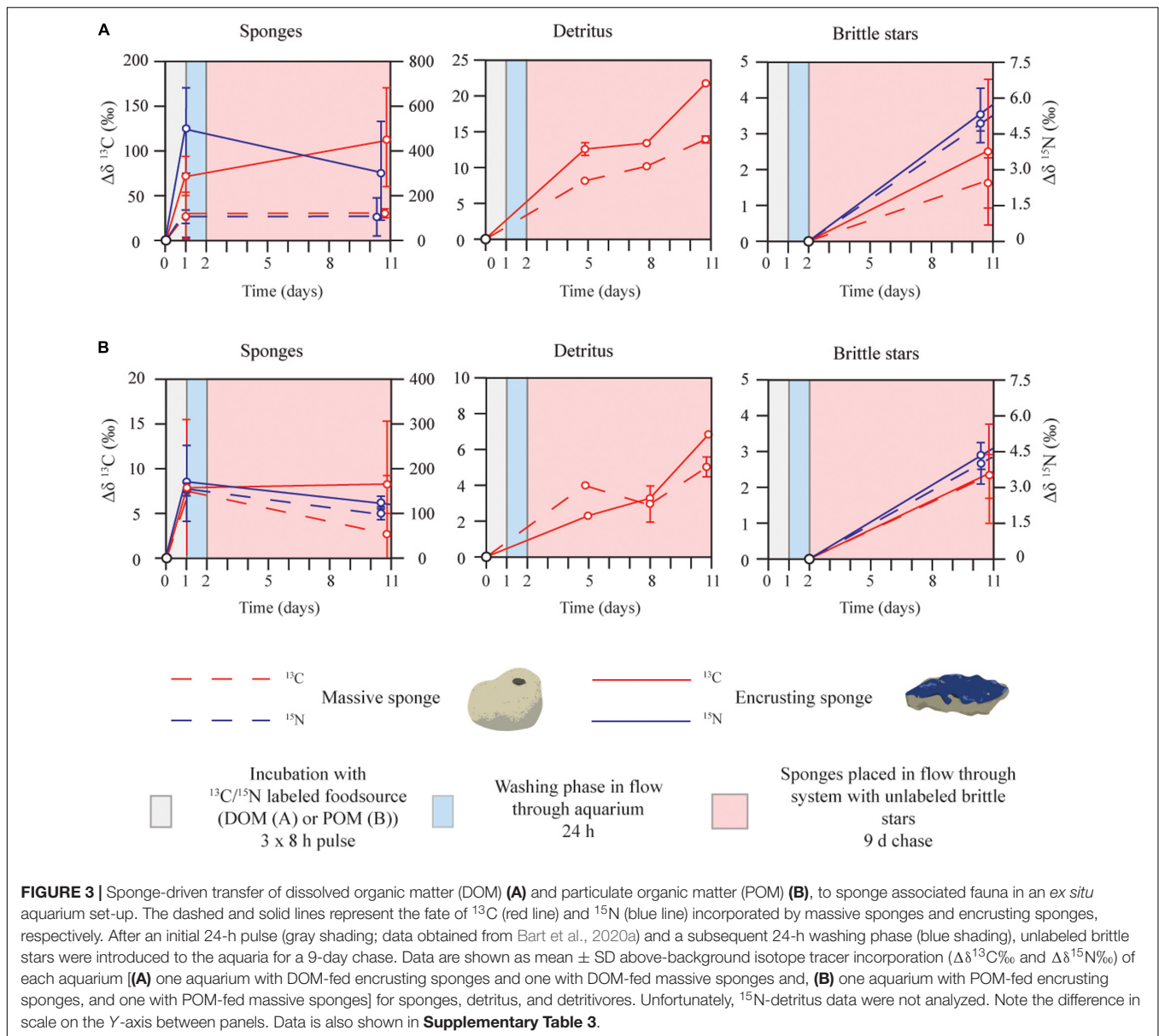
*Phase 2: washing*—Post-labeling, sponge individuals were transferred to individual 10-L label-free running seawater flow through aquaria for 24 h to ensure no residual $^{13}$C- and $^{15}$N-labeled substrate remained on the (inner and outer) surface of the sponges.

*Phase 3: chase*—Post-washing, DOM-fed and POM-fed massive and encrusting sponges were placed in four 20-L label-free running seawater flow-through aquaria together with 3 unlabeled brittle stars per tank: one tank with three DOM-fed massive sponges plus brittle stars, one with three DOM-fed

encrusting sponges plus brittle stars, one with three POM-fed massive sponge plus brittle stars, and one with two POM-fed encrusting sponges plus brittle stars. After placing the brittle stars at random positions in each aquarium, they settled themselves on the surface of the sponges (**Figure 2**). Detritus was collected every 3-day during the chase from each tank with a sterile plastic pipette, pooled per timepoint and frozen at -80°C for later isotopic analysis (Rix et al., 2018). After 9-day, all sponges and brittle stars were rinsed in 0.2 μm filtered, unlabeled seawater, shortly dipped in ultrapure water to remove salts, and carefully removed from their rocky substrate using a sterile scalpel blade (i.e., sponges) and oven-dried (48 h at 60°C). Then, tissue was homogenized with mortar and pestle and stored in a desiccator until further analysis for C and N content and stable isotope enrichment ($^{13}$C and $^{15}$N) by EA-IRMS (see below sections for details on flux calculations). Background detritus samples and tissue samples from each species were collected prior to the pulse-chase experiment and served as non-labeled controls.

## DOM and POM Tracer Incorporation Rate Calculations

DOM and POM substrates, labeled and non-labeled sponge tissue, brittle star, and detritus samples were analyzed for organic C and total N content on an elemental analyzer [Elementar Isotope cube (*Elementar GmbH*, Langenselbold, Germany)] coupled to a BioVision isotope ratio mass spectrometer (*Elementar ltd*, Manchester, United Kingdom) for simultaneous measurement of organic carbon and nitrogen content and $^{13}$C:$^{12}$C and $^{15}$N:$^{14}$N ratios. Before analysis, samples were lyophilized for 24 h in a FD5515 *Ilchin Biobase* freeze drier. After freeze-drying, approximately 10 mg per sample was weighed out into separate tin capsules and acidified. The peak area (from the elemental analyzer) to content ratio was calculated with respect

**FIGURE 3 |** Sponge-driven transfer of dissolved organic matter (DOM) **(A)** and particulate organic matter (POM) **(B)**, to sponge associated fauna in an *ex situ* aquarium set-up. The dashed and solid lines represent the fate of $^{13}C$ (red line) and $^{15}N$ (blue line) incorporated by massive sponges and encrusting sponges, respectively. After an initial 24-h pulse (gray shading; data obtained from Bart et al., 2020a) and a subsequent 24-h washing phase (blue shading), unlabeled brittle stars were introduced to the aquaria for a 9-day chase. Data are shown as mean ± SD above-background isotope tracer incorporation ($\Delta\delta^{13}C‰$ and $\Delta\delta^{15}N‰$) of each aquarium [**(A)** one aquarium with DOM-fed encrusting sponges and one with DOM-fed massive sponges and, **(B)** one aquarium with POM-fed encrusting sponges, and one with POM-fed massive sponges] for sponges, detritus, and detritivores. Unfortunately, $^{15}N$-detritus data were not analyzed. Note the difference in scale on the *Y*-axis between panels. Data is also shown in **Supplementary Table 3**.

to several replicates of a standard (acetanilide) of known C and N content. To calculate the $^{13}C{:}^{12}C$ and $^{15}N{:}^{14}N$ ratios, C and N stable isotope ratios are expressed in standard delta notation as:

$$\delta^{13}C \; or \; \delta^{15}N \; (\%) \;\; = \left( \frac{R_{sample}}{R_{ref} - 1} \right) \; \times \; 1000$$

where R is the ratio of $^{13}C{:}^{12}C$ or $^{15}N{:}^{14}N$ in the sample ($R_{sample}$; e.g., sponge tissue, detritus) or reference material: Vienna Pee Dee Belemnite for C ($R_{ref}$ = 0.01118) and atmospheric nitrogen for N ($R_{ref}$ = 0.00368). The above background enrichment of $^{13}C$ or $^{15}N$ in the samples was calculated as the excess fractional abundance of $^{13}C$ or $^{15}N$ in the samples compared with the background (i.e., non-enriched) values of non-labeled control samples ($n$ = 3 for massive sponges, $n$ = 4 for encrusting sponges, $n$ = 2 for detritus). Unfortunately, the $^{15}N$-detritus background

measurements could not be analyzed and therefore no $^{15}N$-enrichment of detritus samples was calculated.

$$E_{sample} \;\; = \; F_{sample} \; - \; F_{background}$$

where F is the fractional abundance of heavy isotope ($^{13}C$ or $^{15}N$) in the sample calculated as:

$$F_{sample \; or \; background} \;\; = \; \frac{\delta^{13}C}{\delta^{13}C + \delta^{12}C} \; or \; \frac{\delta^{15}N}{\delta^{15}N + \delta^{14}N}$$

and

$$F_{sample \; or \; background} \;\; = \left( \frac{\delta^{13}C \; or \delta^{15}N}{1000 + 1} \right) \; \times \; R_{ref}$$

Total tracer incorporation was calculated by multiplying the excess fractional abundance ($E_{sample}$) by the total $C_{org}$ or N

content (μmol) of the tissue, divided by the labeling efficiency (as atom%) of the food source. Rates were then normalized to time and tissue C or N content of the sponges or detritivores.

Both detritus (detrital pathway) and the sponge tissue itself (predatory pathway) are considered stable-isotope-enriched food sources to brittle stars during the label-free chase. We calculated brittle star tracer uptake rates for two hypothetical scenarios: in scenario 1 with detritus as food source (using the labeling efficiency of detritus), and in scenario 2 with sponge tissue as food source (using the labeling efficiency of the sponge tissue).

The C:N ratios of DOM- and POM-derived transfer to brittle stars were calculated by dividing the organic C tracer incorporation rate by the total N tracer incorporation rate.

## Statistics

Statistical analysis was performed in Primer V7 (Clarke and Gorley, 2015) with the add-on PERMANOVA+ (Anderson et al., 2008). Permutational multivariate analysis of variance (PERMANOVA) with Monte Carlo tests were used as this method is robust for low sample replication and non-normally distributed data. PERMANOVAs were run using Type III sum of squares and unrestricted permutation of raw data (9999 permutations); resemblance matrices were conducted using Euclidean distances. Individual one-way PERMANOVAs were conducted to test (1) the effect of treatment timepoint (24 h vs. 11-day) on stable isotope enrichment ($\delta^{13}$C and $\delta^{15}$N) of sponge tissue after incubation with isotopically labeled DOM or POM, and (2) differences between brittle star tracer incorporation rates when sponges were fed DOM or POM. These incorporation rates were calculated assuming transfer from sponge to brittle star via detritus or via direct predation. Adjustments for multiple tests were made using the Bonferroni procedure. Full statistical output is available in **Supplementary Table 2**.

## RESULTS

### Incorporation of Tracer-Isotope Food Sources

The massive and encrusting sponge species assimilated DOM- and POM-derived $^{13}$C and $^{15}$N during the 24-h pulse (data obtained from Bart et al., 2020a). Stable isotope enrichment of sponge tissue did not significantly change between the end of the pulse and end of the chase phase (**Figures 3A,B**, left panels; **Supplementary Tables 2, 3**) for both sponge species, except for POM-fed massive sponges, where tissue isotopic enrichment decreased significantly during the chase for $^{13}$C.

Released detritus showed a continuous increase in above-background $^{13}$C-enrichment (no $^{15}$N data available) during the chase (**Figures 3A,B**, middle panels), demonstrating turnover of DOM and POM by sponges. After 9-day exposure to the labeled sponges and detritus, above-background enrichment of $^{13}$C and $^{15}$N was also detected in the tissue of the brittle stars (**Figures 3A,B**, right panels). This demonstrates transfer of sponge-assimilated DOM and POM to brittle stars.

## Detrital or Predatory Transfer of DOM and POM to Brittle Stars

Both detritus and the sponge tissue itself are possible sources of enrichment found in brittle stars after the label-free chase phase. Thus, transfer of DOM and POM via sponges to brittle stars is possible via detritus feeding (scenario 1) and/or by predation on sponge tissue (scenario 2).

In scenario 1 (**Figure 4A** and **Supplementary Table 4**), brittle star uptake rates of detrital-C from POM-fed massive and encrusting sponges were higher compared to DOM-fed sponges, however, these differences were not significant (**Supplementary Table 2**).

In scenario 2 (**Figure 4B** and **Supplementary Table 4**), brittle star uptake rates of sponge tissue-C from POM-fed massive sponges were significantly higher than for DOM-fed massive sponges, while a similar, but non-significant trend was found between $C_{DOM}$ and $C_{POM}$ transfer for encrusting sponges (**Supplementary Table 2**).

For the transfer of tissue-N via predation, no significant differences were found in brittle star uptake rates between DOM- and POM-fed sponges, for both massive and encrusting sponges (**Supplementary Tables 2, 4**).

The C:N ratios of uptake by brittle stars under scenario 2 (direct tissue predation) were lower for DOM-fed sponges (1.3 for massive and encrusting) than for POM-fed sponges (15 and 8 for massive and encrusting, respectively).
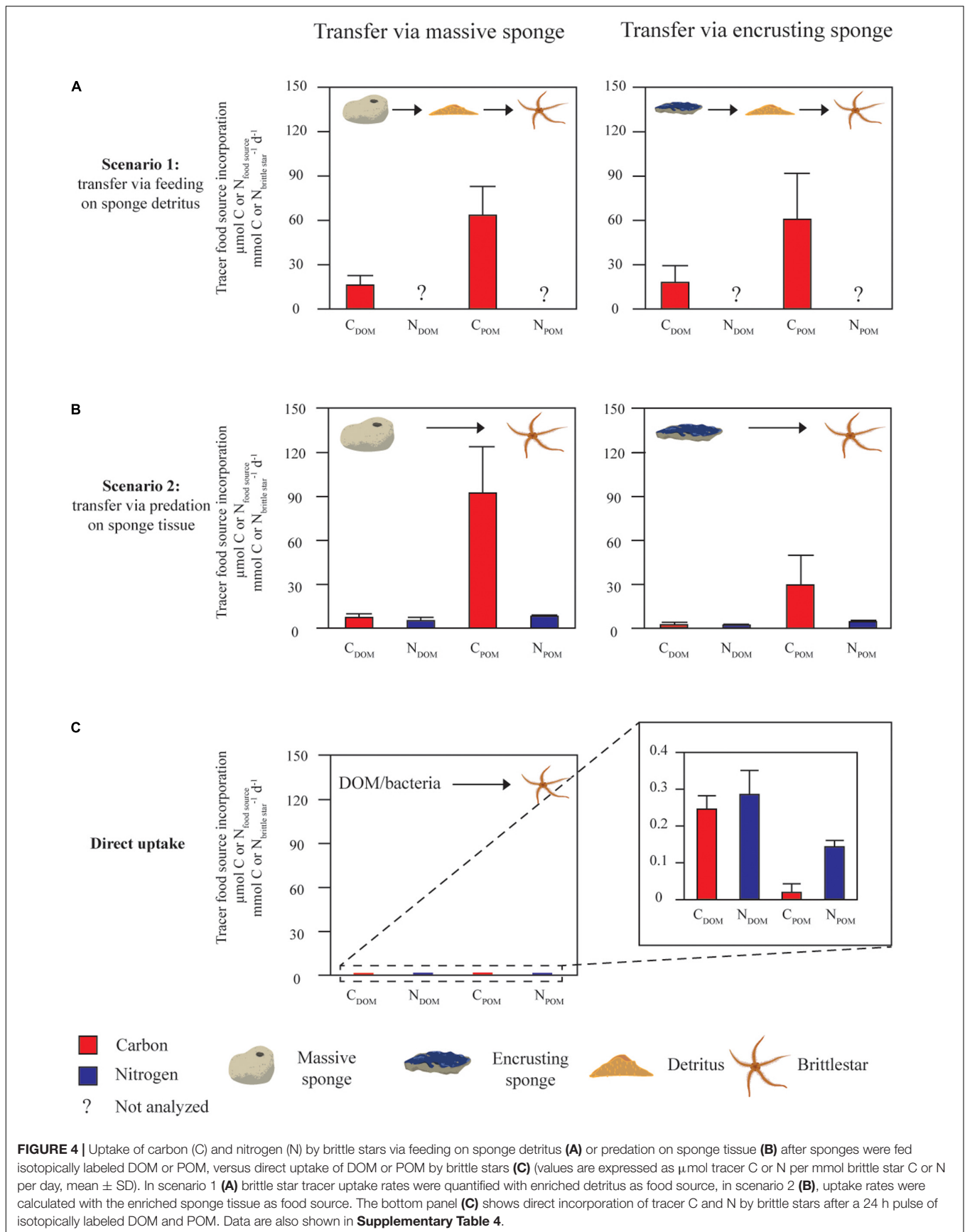
Direct uptake of DOM and POM by brittle stars (**Figure 4C**) was one to three orders of magnitude lower than transfer through scenario 1 or 2 (**Supplementary Table 4**).

## DISCUSSION

This study provides the first evidence of all three consecutive steps of the sponge loop in deep-sea sponges. The two investigated deep-sea sponge species take up and assimilate DOM and POM, subsequently turn sponge-assimilated DOM and POM into detritus, and transfer carbon and nitrogen derived from both food sources to associated fauna. Transfer of assimilated food to associated fauna by sponges is possible via two scenarios: (1) via the production of detrital waste or (2) via direct predation on sponge tissue. The plausibility of both scenarios, and their potential ecological relevance for deep-sea ecosystems, are discussed below.

### Transfer of DOM via Detrital and/or Predatory Pathway?

At present, DOM cycling by various types of sponges and its relevance for marine ecosystems, is a heavily debated topic (e.g., de Goeij et al., 2017; Leys et al., 2018; Pawlik and McMurray, 2020; Rix et al., 2020). The original sponge-loop hypothesis proposed that coral reef sponges recycle DOM by converting it into particulate detritus, which is then used by various detritivorous organisms and thereby re-enters the classical food chain (de Goeij et al., 2013). This pathway was tested on sponge species with mm-thin sheet to cm-thick

**FIGURE 4 |** Uptake of carbon (C) and nitrogen (N) by brittle stars via feeding on sponge detritus **(A)** or predation on sponge tissue **(B)** after sponges were fed isotopically labeled DOM or POM, versus direct uptake of DOM or POM by brittle stars **(C)** (values are expressed as $\mu$mol tracer C or N per mmol brittle star C or N per day, mean $\pm$ SD). In scenario 1 **(A)** brittle star tracer uptake rates were quantified with enriched detritus as food source, in scenario 2 **(B)**, uptake rates were calculated with the enriched sponge tissue as food source. The bottom panel **(C)** shows direct incorporation of tracer C and N by brittle stars after a 24 h pulse of isotopically labeled DOM and POM. Data are also shown in **Supplementary Table 4**.

(e.g., conulose, lobate, ficiform) encrusting growth forms—i.e. following the contours of the surface rather than growing upward—typically inhabiting crevices of the reef framework, but also appearing on the open reef. Based on encrusting sponges alone, sponge-loop carbon cycling is estimated to amount to the gross primary production rates of an entire coral reef ecosystem (de Goeij et al., 2013). Interestingly, massive upright growing sponges, living on the exposed parts of the reef, were not found to produce significant quantities of detritus (McMurray et al., 2018; Wooster et al., 2019). Massive sponges may allocate the majority of assimilated C in three-dimensional (upward) tissue growth, while (mm to cm-thin) encrusting species are restricted to space-limited, two-dimensional growth. Consequently, encrusting sponges may invest relatively more carbon in cell turnover, shedding and detritus production compared to massive species. This hypothesis was strengthened by recent work of Maier et al. (2020), who showed that deep-sea sponge detritus production rates of the lobate encrusting species *Mycale lingua,* exceeded detritus production rates of the globular massive species *G. barretti* by two orders of magnitude. McMurray et al. (2018) therefore proposed an alternative, predatory sponge-loop pathway for massive, non-space limited sponge species. Based on the limited number of replicates used in our study due to the difficulties obtaining and experimenting with live deep-sea organisms, we cannot rule out one of these scenarios, and will discuss both.

*Deep-sea detrital sponge loop*—Multiple studies have shown that sponge species from various deep-sea ecosystems produce particulate waste material in the form of detritus or fecal pellets (Witte and Graf, 1996; Rix et al., 2016; Kahn et al., 2018; Maier et al., 2020). Only few studies have quantified the release of detritus. The encrusting sponge *Hymedesmia coriacea* was found to convert 39% of ingested coral-derived DOM (i.e. coral mucus) into detritus (Rix et al., 2016), and five species of tropical shallow-water encrusting sponges released on average 12% of their biomass daily (Alexander et al., 2014). In comparison, the massive sponge *G. barretti* released very small amounts of detritus (0.03% of their biomass daily) (Maier et al., 2020). Yet, from an ecological perspective, detritus production by massive sponges can still have a significant effect on C turnover in marine ecosystems. The average total organic C content ($C_{org}$) of the massive *G. barretti* species in our experiments outweighed the organic C content of our encrusting sponges by three orders of magnitude (on average 6.5 g $C_{org}$ (*G. barretti*) versus 0.008 g $C_{org}$ (*Hymedesmia* sp.) per individual). Consequently, even when the relatively small *G. barretti* individuals used in our study release only 0.03% of its $C_{org}$ as particulate detritus, detritus release rates would amount to 2 mg C $d^{-1}$ per sponge individual. In contrast, a 12–39% $C_{org}$ release by the much smaller encrusting sponges would mean a release of 1–3 mg C $d^{-1}$ per sponge individual, which is similar to the absolute amount of detritus production by the massive sponge. Although little is known about the relative contribution of encrusting versus massive sponges in deep-sea ecosystems, the carbon standing stock of *G. barretti* in deep-sea ecosystems, such as the Western Barents Sea, can add up to 200 g C $m^{-2}$ (Klitgaard and Tendal, 2004, converted to g C via Bart et al., 2020b). *Geodia barretti* can thus

potentially produce 66 mg detritus per $m^{-2}$ $d^{-1}$, which amounts to an abundant supply of particulate organic carbon to the local deep-sea benthos. For example, Piepenburg and Schmid (1996) estimated a mineralization rate of 21.9 mg C $m^{-2}$ $d^{-1}$ for benthic brittle stars on the North-East Greenland shelf even though this area is extremely rich in epifauna (30–340 individuals $m^{-2}$), and strongly dominated (80–98% of the total amount of organisms) by brittle stars.

*Deep-sea predatory sponge loop*—Spongivory is a strategy performed by various animals, including echinoderms (Randall and Hartman, 1968; Pawlik, 2011), and sponge spicules have been found in the stomachs of various brittle star species (Pearson and Gage, 1984). A study on 1165 deep-sea ophiuroid individuals of six different species by Pearson and Gage (1984) showed that deep-sea brittle stars are trophic generalists lacking dietary specialization. Feeding strategies ranged from detritivory to scavenging and even suspension feeding. Indeed, we found brittle stars to be capable of directly feeding on small amounts of our tracer DOM and POM during incubations (**Figure 4C**). Yet, rates of direct tracer incorporation were up to three orders of magnitude lower compared to the incorporation of sponge-derived C and N. Some massive sponge species are known to produce metabolites that deter predation (Chanas et al., 1997; Lindel et al., 2000) and thus spongivory is mostly seen on species that lack chemical defenses and have faster growth and reproduction rates (Pawlik, 2011). In contrast, chemically defended individuals that grow relatively slowly may be long lived, and therefore important for the sequestration and storage of C as biomass (McMurray et al., 2018). The massive HMA sponge used in our study, *G. barretti*, is known to produce various metabolites that inhibit biofouling (Sjögren et al., 2004; Hedner et al., 2008), but their effect on possible predators is unknown. Furthermore, it is difficult to make a genuine comparison with spongivory in shallow-water tropical systems, as deep-sea sponge metabolism is much slower due to lower ambient temperatures (Bart et al., 2020b), and deep-sea sponges are generally known for their longevity and C sequestration (Fallon et al., 2010; Kahn et al., 2015).

## Ecological Relevance of DOM and POM Cycling Through a Sponge Loop

A comparison of potential deep-sea sponge-loop scenarios and comparing dissolved and particulate food sources reveals two interesting trends: when sponges feed on POM, relatively more C is transferred to associated fauna, compared to DOM. Secondly, relatively more DOM-derived C appears to be transferred via detritus compared to predation. This implies that POM (i.e. microbial plankton) is rather stored in sponge biomass, whereas DOM is mostly respired. This corresponds with the observations made by Kazanidis et al. (2018) and Bart et al. (2020a) that sponges use DOM for maintenance metabolism and turnover, while bacteria serve as an important food source for anabolic processes and are preferably incorporated into tissue. This is further reflected in the C:N ratios of uptake under the predatory sponge loop by brittle stars after sponge-feeding on DOM or

POM. The drastically decreased C:N after DOM-feeding (1.3) compared to POM-feeding (8–15) does not reflect that brittle stars obtain more N from DOM, but rather that the sponges *lost* more C from the isotopically enriched source, likely through respiration. Deep-sea sponges are indeed found to assimilate POM at very high assimilation efficiencies (up to 97%) compared to DOM (32–77%) (Bart et al., 2020a). This strengthens our hypothesis that DOM and POM are both essential parts of deep-sea sponge diet, as source of maintenance and as building blocks, respectively. This could also have ecological consequences for the wider associated ecosystem and food web, since both C and N may transfer at different rates according to the diet of sponges and the chosen feeding strategy of associated fauna.

It is important to note that both the detrital and predatory scenario are plausible for both types of sponges (i.e. massive and encrusting) and for both food sources (i.e. DOM and POM), and we cannot distinguish between the two scenarios under the current experimental approach. Moreover, our limited replication, in both sponge species and species of associated fauna, and the effect of concentration and lability of ambient DOM in time and space available to sponges living in different deep-sea ecosystems, make it very difficult to extrapolate our data to deep-sea benthic ecosystem-wide processes. The main aim of our study was to establish whether (common) deep-sea sponges possess all the consecutive steps of a sponge loop, rather than to show its potential ecological importance. To assess the full importance of sponge-loop pathways in the deep-sea, both qualitatively and quantitatively—many questions remain to be answered. For example, the relative importance of a deep-sea sponge loop may vary with the abundance and feeding activity of other benthic suspension feeders in deep-sea benthic ecosystems, such as CWC reefs and sponge grounds, that could participate in the cycling of DOM and POM. A recent extensive isotope tracer study by Maier et al. (2020) showed a four time higher production of detritus after DOM- and POM-feeding by the bivalve *Acesta excavate* compared to the sponge *G. barretti*. This detritus was further incorporated by brittle stars, suggesting that bivalves may be the main recyclers of organic matter within the CWC reefs under study, and the sponge loop in the deep-sea may in fact be better qualified as a "suspension feeder" loop. Interestingly, this was not found for shallow water tropical coral reefs, where other suspension feeders did not take part in a DOM-metazoan-detritus-detritivore pathway, but was restricted to sponges (de Goeij et al., 2013). Note, however, that Maier et al. (2020) did not test the recycling of sponge-produced detritus to associated fauna and that sponge *M. lingua* was the highest producer of detritus, i.e., 25 times higher production of detritus compared to the bivalve *A. excavata*. To estimate each organisms' role in the recycling of DOM and POM in deep-sea benthic ecosystems, resource fluxes need to be extrapolated to respective biomass estimates within these ecosystems. Furthermore, there is a lack of *in situ* studies to verify *ex situ* rates presented to date. *In situ* studies to determine uptake rates of ambient DOM and the use of stable-isotope tracer food sources on deep-sea benthic communities, including multiple sponge- and other suspension-feeding species and associated fauna, will shed light on how sponges drive food webs in the dark deep-sea.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

## AUTHOR CONTRIBUTIONS

JG and HR acquired the funding. MB and JG designed the experiments. JG, HR, and MB collected the animals. MB conducted the incubations experiments. MB, JG, and MH performed the data analyses and designed the tables and figures. MB, JG, MH, and PV wrote the manuscript. All co-authors approved the manuscript. All authors contributed to the article and approved the submitted version.

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