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Stable carbon isotopic composition of amino sugars in heterotrophic bacteria and phytoplankton: Implications for assessment of marine organic matter degradation

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Abstract

Compound-specific isotope analysis has opened up a new realm for resolving the sources and transformation processes of marine organic matter. However, the stable carbon isotope patterns of amino sugars remain unknown. We examined $\delta^{13}C$ of amino sugars in marine phytoplankton and heterotrophic bacteria, and the variations in amino sugar $\delta^{13}C$ during 66-d planktonic organic matter degradation experiments, to investigate the metabolic sources and transformations of amino sugars by bacterial reworking. The $\delta^{13}C$ values of glucosamine (GlcN) and galactosamine (GalN) were comparable in heterotrophic bacteria (difference $\Delta\delta^{13}C_{GlcN-GalN} = 0.4–4.0‰$) but pronouncedly different in phytoplankton ($\Delta\delta^{13}C_{GlcN-GalN} = 4.3–16.6‰$), suggesting similar synthesis pathways of GlcN and GalN in bacteria that differed from phytoplankton. Compared to GlcN and GalN, bacteria preferentially use isotopically light organic compounds for muramic acid (MurA) synthesis. During simulated microbial degradation of organic matter, the $\delta^{13}C$ difference between GlcN and GalN decreased from 5.8‰ on the initial day to 1‰ at a late stage in the experiment, but the difference between GlcN and MurA remained at 5.3‰. This difference is consistent with the pattern in cultured phytoplankton (average $\Delta\delta^{13}C_{GlcN-GalN} = 5.9‰ \pm 1.4‰$) and heterotrophic bacteria (average $\Delta\delta^{13}C_{GlcN-MurA} = 4.6‰ \pm 3.4‰$), indicating enhanced bacterial resynthesis as degradation proceeded. Based on the difference in $\delta^{13}C$ among GlcN, GalN, and MurA, we propose a novel index of variation in amino sugar $\delta^{13}C$, representing amino sugar resynthesis, to describe the diagenetic state of organic matter. Together, these findings suggest that amino sugar $\delta^{13}C$ can be used as a new tool to track heterotrophic processes of marine organic matter.

Marine organic matter plays a key role in ecosystem functioning, the global carbon cycle and regulation of the Earth's climate (Hedges 1992; Hansell and Carlson 2015; Zhang et al. 2018). Most marine organic matter (> 90%) is bio-refractory and can persist in the ocean over thousands of years (Williams and Druffel 1987; Dittmar 2015; Zhang et al. 2018). Only a small fraction (1–10%) of the marine organic matter pool is turned over relatively quickly and is biologically utilized on timescales of days to weeks. The bioavailable fraction supports marine food webs, drives element cycles, and is linked to ocean uptake and release of atmospheric carbon dioxide (CO_2), giving it a disproportionate role in the ocean's carbon reservoir (Hansell 2013). Over the past decades, the sources and fate of marine organic matter have been studied extensively (Hedges et al. 1997; Jiao et al. 2010; Engel et al. 2022),

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but the mechanisms of organic matter degradation and transformation have yet to be fully elucidated due to the complexity of organic matter composition.

Investigations into organic matter decomposition have revealed strong links between chemical characteristics and bioavailability (Amon et al. 2001; Shen and Benner 2020). Some organic compounds (e.g., amino acids and carbohydrates) have a relatively high reactivity and can be preferentially removed during organic matter degradation (Amon et al. 2001; Davis and Benner 2007; Shen et al. 2016). Among carbohydrates, amino sugars are an important class of compounds that are ubiquitous in the marine environment. The sources of amino sugars are diverse (e.g., crustacean zooplankton, phytoplankton and bacteria), and amino sugars occur in the form of biopolymers such as chitin, peptidoglycan, glycoprotein, and lipopolysaccharide (Benner and Kaiser 2003). More than 26 types of amino sugars have been identified in microorganisms, but only 4 have been studied quantitatively, including glucosamine (GlcN), galactosamine (GalN), mannosamine (ManN) and muramic acid (MurA) (Zhang and Amelung 1996). The molecular composition of these compounds provides information on the source and degradation history of organic matter. For instance, the ratio GlcN/GalN is usually >8 in chitin-rich bioactive organic matter, yet decreases as bacterial reworking proceeds. Relatively low GlcN/GalN values (<3) reflect diagenetic alteration and bacterial origin of organic matter (Davis et al. 2009; Lehmann et al. 2020). In comparison, MurA is a component of peptidoglycan in the bacterial cell wall. It is uniquely derived from bacteria, especially Gram-positive bacteria, and can therefore serve as a biomarker for bacterial organic matter (Kaiser and Benner 2008). Studies on the diagenetic state of organic matter based on amino sugars have been widely reported and involve different forms of organic matter in marginal seas and the open ocean (Niggemann and Schubert 2006; Kaiser and Benner 2009; Ren et al. 2020). However, it is difficult to fully resolve the specific mechanisms of metabolic processing of organic matter solely from the analysis of amino sugar concentrations.

In recent years, the application of compound-specific isotope analysis (CSIA) has provided an alternative tool to investigate biogeochemical processing of organic matter (Close 2019). Since CSIA directly determines the isotope ratio of a specific molecule, it addresses the effects of varying compound composition and selective degradation on bulk isotopes. Over the past three decades, the powerful capabilities of CSIA in studies on organic matter sources, metabolism and food webs have been demonstrated (Hayes et al. 1990; Bouillon and Boschker 2006; Kang et al. 2021). Stable isotope analysis of individual compounds has been widely applied to organic molecules, for example, lipids, lignin, carbohydrates, and amino acids (Teece and Fogel 2007; Ohkouchi et al. 2017; Close 2019). These studies indicated that amino acids and carbohydrates generally have more positive $\delta^{13}C$ values than bulk organic matter, with values that vary strongly between organisms. In addition, $\delta^{13}C$ values of amino acids and carbohydrates are typically $\sim$3–4‰ higher than those of lipids due to differences in biosynthesis (Abelson and Hoering 1961; Hayes 2001; van Dongen et al. 2002). To date, only a few studies have reported the $\delta^{13}C$ values of marine amino sugars. Previous studies have mainly involved labeling and amino sugar incubations (Glaser and Gross 2005; Indorf et al. 2015), but the stable carbon isotope characteristics of amino sugars in marine ecosystems are unknown. As important carbohydrates and amino compounds, the CSIA of amino sugars could provide valuable insights into the cycling of marine organic matter. However, major challenges associated with low amino sugar concentrations in marine organic matter may limit the extensive study of amino sugar isotopes. The proportion of amino sugar-derived carbon typically accounts for $\sim$12% of total organic carbon in terrestrial soils (Amelung et al. 1999), while it only comprises $\sim$0.5–2% in marine sediments and suspended particles (Benner and Kaiser 2003; Niggemann and Schubert 2006).

Here, we performed incubation experiments that assessed the variations in $\delta^{13}C$ of individual amino sugars during degradation of particulate organic matter (POM). Culture experiments using different species of heterotrophic bacteria and phytoplankton were conducted to reveal carbon isotopic composition of amino sugars. Our main objective was to unravel the mechanistic links between organic matter remineralization and changes in amino sugar $\delta^{13}C$ values during the transformation of planktonic organic matter to more refractory material. In particular, we examine the potential driving mechanisms of amino sugar $\delta^{13}C$ variations and discuss their use as indicators of organic matter degradation and bacterial resynthesis during early diagenesis.

Materials and methods

Culture experiments

To investigate the stable carbon isotope patterns of amino sugars from photoautotrophic and microbial heterotrophic sources, different species of phytoplankton and heterotrophic bacteria were cultured. All culture experiments were performed in triplicate. Four widely distributed algal species were selected for culture, including the diatom *Skeletonema costatum*, dinoflagellate *Prorocentrum donghaiense*, haptophyte *Phaeocystis globosa* and raphidophyte *Heterosigma akashiwo*. The four algal species were grown in L1 seawater medium (Medhioub et al. 2011) in 100 mL batch cultures at 18°C, and 12:12 h light : dark regime. The phytoplankton were harvested during exponential growth, rinsed three times with sterile seawater and stored at −20°C until analysis of amino sugar $\delta^{13}C$.

Four marine heterotrophic bacteria, namely *Pseudomonas aeruginosa* (Gammaproteobacteria), *Sulfitobacter pontiacus* (Alphaproteobacteria), *Alteromonas marina* (Gammaproteobacteria), and *Vibrio owensii* (Gammaproteobacteria), were cultured

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in the laboratory. The Gram-negative marine bacterium *Ps. aeruginosa* was cultured in 250-mL Erlenmeyer flasks with medium prepared by mixing peptone and yeast (5 : 1, w/w), while the remaining three bacteria were grown in 2216E medium (agar : peptone : yeast = 15 : 5 : 1; w/w). These batch cultures were all maintained at 18°C, harvested in the early stationary phase, and stored at −20°C until isotopic analysis.

Incubation experiments

Plankton tow samples containing a mixture of zooplankton and phytoplankton were collected at 31.65°N, 122.99°E (Fig. 1) onboard the *R/V Runjiang 1* in early July 2020 using a 303-μm pore size tow net. Surface seawater (2 m depth) for incubation experiment was collected with 12-liter Niskin bottles and stored in hydrochloric acid (HCl, 10%) cleaned 25-liter high-density polyethylene containers. Following collection, the net plankton and corresponding surface seawater were taken to the laboratory and processed immediately. Approximately, 100 liters of seawater were mixed thoroughly with ~2.5 liters plankton samples in an acid-cleaned tank. Subsequently, the large volume sample was split into 11 acid-cleaned 20-liter polypropylene carboys, and this was considered as the day 0 sample. All containers were incubated in the dark at a constant temperature (25°C) for 66 d. Each carboy was left with sufficient headspace (~10 liters) and shaken daily to ensure adequate oxygen. Samples were taken on days 1, 2, 3, 5, 7, 10, 14, 20, 26, 46, and 66. One carboy was sacrificed at every sampling timepoint during the degradation experiment. Sampling was conducted as follows: samples for POM were filtered through precombusted (450°C, 5 h) glass fibers filters (Whatman; 0.7 μm nominal pore size) and stored frozen at −20°C. Filters were collected in triplicate for particulate organic carbon (POC), particulate nitrogen (PN), and bulk $\delta^{13}C$ (0.5–1 liter); concentrations of particulate amino sugars (0.5–1 liter); and amino sugar $\delta^{13}C$ (2–4 liters) analysis.

Sampling of suspended particles and sediments

Suspended POM samples were collected during the summer cruise (July 2020) in the Changjiang Estuary (Fig. 1). Surface particles were collected at 2 m depth by filtering 1–3 liters of seawater with precombusted glass fiber filters (Whatman; 0.7 μm nominal pore size). The POM samples were collected in duplicate and stored frozen (−20°C) until laboratory analysis of bulk and amino sugar $\delta^{13}C$. Surface sediments (0–1 cm) were collected in the South Yellow Sea and East China Sea (Fig. 1) using a box corer in November 2019 and July 2020, respectively.

Chemical and biological analyses

Bulk POM characterization

Samples for POC, PN, and bulk $\delta^{13}C$ determination were fumigated with 1 M HCl to remove inorganic carbonate, followed by oven-drying at 60°C for 24 h, and analysis using a Flash EA IsoLink CN elemental analyzer coupled with a MAT 253 plus isotope ratio mass spectrometer (IRMS; Thermo Fisher Scientific). Stable carbon isotope values were corrected for instrumental drift and reported in units of per mil (‰) relative to the Vienna PeeDee Belemnite reference ratio. The analytical precision for six runs of POC, PN, and $\delta^{13}C$ was typically 0.02 wt.%, 0.003 wt.%, and 0.15‰, respectively.

Amino sugar concentrations and stable carbon isotope ratios

Pretreatment of amino sugar samples followed the method of Zhu et al. (2014). Briefly, materials on glass fiber filters or ground sediments were hydrolyzed using 6 M HCl at 105°C for 8 h. The hydrolysates were then neutralized with 6 M potassium hydroxide to pH ~6.8 and centrifuged immediately. The supernatant was taken through a solid phase extraction cartridge (400 mg, 1 mL; Sigma-Aldrich) to remove salts, and then eluted with methanol and dichloromethane. The eluent containing the amino sugars was concentrated under nitrogen gas and then redissolved in deionized water for concentration and stable carbon isotope analysis.

Individual amino sugar concentrations were quantified by external standards (Sigma-Aldrich) using an ion chromatograph (IC, Dionex ICS-5000$^+$ SP) coupled with an electrochemical detector. Compound separation was performed on a PA 20 anion-exchange column (3 × 150 mm; Thermo Fisher Scientific), preceded by a PA 20 guard column (3 × 30 mm; Thermo Fisher Scientific). Elution of the amino sugars included two steps with flow rates of 0.2 mL min^{-1}: GlcN and GalN were eluted with sodium hydroxide (2 mM), while MurA was eluted with sodium hydroxide (2 mM) + sodium nitrate (200 mM) at column temperatures of 25 and 30°C, respectively (Kaiser and Benner 2000).

In this study, the $\delta^{13}C$ values of amino sugars were determined by wet chemical oxidation to avoid the influence of carbon atoms introduced by derivatization (Bode et al. 2009). All individual compounds were separated by ion chromatography (IC, Dionex ICS-5000$^+$ SP), connected directly to an Isolink interface (Thermo Fisher Scientific) and oxidized to CO_2. The oxidizing agent used was a mixture of sodium persulfate and phosphoric acid (4%, w/w) with a flow rate of 40 μL min^{-1}, and the temperature of the oxidation reactor was set at 99°C. The produced CO_2 was stripped with helium and dried, and then entered the IRMS to determine the carbon isotope ratio. To eliminate effects of dissolved CO_2 in solvents on the determination of carbon isotope values, the δ^{13} values of amino sugars were corrected based on the difference (< 0.5‰) between $\delta^{13}C$ values of the same amino sugar standard determined by EA-IRMS and IC-Isolink-IRMS. Standard deviation (SD) values of $\delta^{13}C_{GlcN}$, $\delta^{13}C_{GalN}$, and $\delta^{13}C_{MurA}$ among five injections of a given sample were ± 0.6‰, ± 0.6‰, and ± 0.4‰. The detection limits for $\delta^{13}C_{GlcN}$, $\delta^{13}C_{GalN}$, and $\delta^{13}C_{MurA}$ were 1, 1, and 2 nmol (injection amount).

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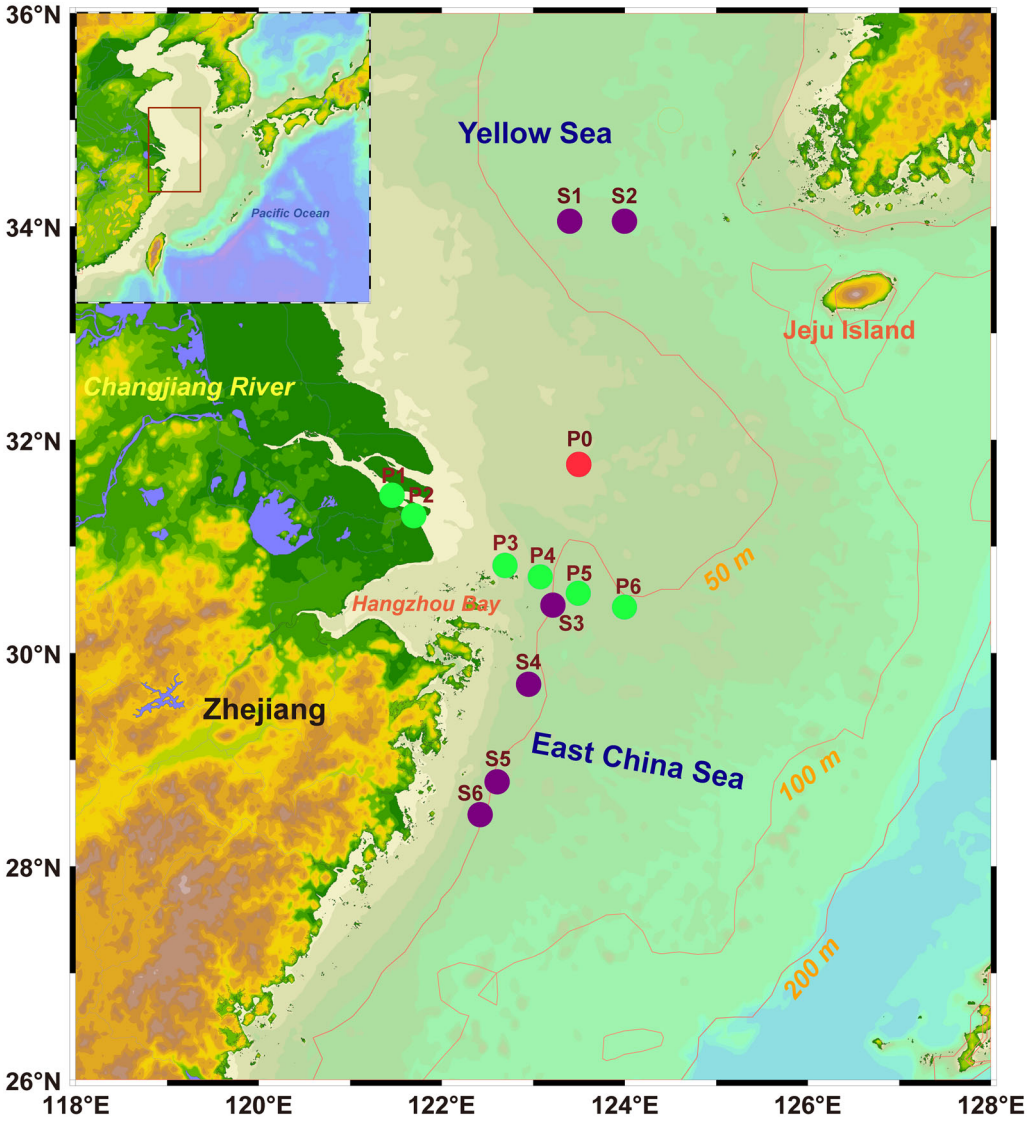

Fig. 1. Study area and sampling locations in the East China Sea and Yellow Sea. Samples for the incubation experiment were collected at Sta. P0 (red dot) in July 2020. Green and purple dots represent sampling sites for suspended particulate matter and sediment, respectively. Samples for suspended particulate matter were collected in July 2020, while surface sediments were sampled in November 2019 and July 2020.

Results

Amino sugar $\delta^{13}C$ in heterotrophic bacteria and phytoplankton

The $\delta^{13}C_{GlcN}$ and $\delta^{13}C_{GalN}$ values of the four heterotrophic bacteria ranged from −27.3‰ to −17.2‰ and −27.7‰ to −13.1‰, respectively. The difference between the $\delta^{13}C_{GlcN}$ and $\delta^{13}C_{GalN}$ $\delta^{13}C$ values for a single bacterial species was small, varying from 0.4‰ to 4.0‰ (Fig. 2). Compared to GlcN and GalN, MurA showed more negative $\delta^{13}C$ values in heterotrophic bacteria, ranging from −30.5‰ to −20.7‰. The amino sugar $\delta^{13}C$ value of *Ps. aeruginosa* exhibited a distinctly different pattern from the other three bacterial taxa, with more negative $\delta^{13}C$ values, probably due to the use of a different culture medium and carbon source. For the cultured phytoplankton, $\delta^{13}C_{GlcN}$ (−15.6‰ to −10.5‰) was higher than $\delta^{13}C_{GalN}$ (−27.2‰ to −18.5‰), and the difference in $\delta^{13}C$ between GlcN and GalN (4.3 to 16.6‰) was significantly larger than for heterotrophic bacteria (Mann–Whitney *U*-test, $p < 0.05$; Fig. 2).

POM concentration and composition during the incubation experiment

Over the 66-d incubation period, POC and PN concentrations decreased by ∼91%, from 318.3 μmol L^{-1} POC and 51.8 μmol L^{-1} PN on day 0 to 29.0 μmol L^{-1} POC and 4.8 μmol L^{-1} PN on day 66 (Fig. 3a,b). Higher POC and PN degradation rates were observed during the first 10 d of incubation, with removal reaching 78% for POC and 76% for

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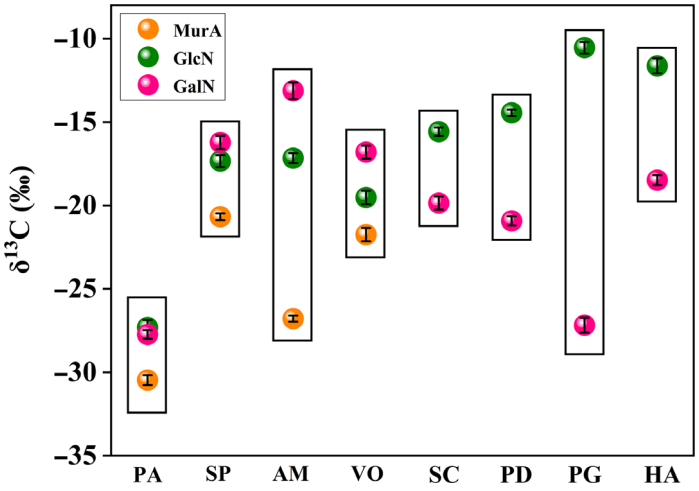

Fig. 2. Patterns of the amino sugar isotopic signature $\delta^{13}C$ in different heterotrophic bacteria and phytoplankton. PA, SP, AM, and VO refer to the bacteria *Pseudomonas aeruginosa*, *Sulfitobacter pontiacus*, *Alteromonas marina*, and *Vibrio owensii*, respectively, while SC, PD, PG, and HA represent the algae *Skeletonema costatum*, *Prorocentrum donghaiense*, *Phaeocystis globosa*, and *Heterosigma akashiwo*, respectively. The error bar represents the standard deviation of triplicate experiments.

PN, and thereafter which POM degradation leveled off. In contrast, amino sugar concentrations peaked on the first day, reaching 2.83 μmol L^{-1}, after which they decreased sharply and reached an equilibrium state after day 3 (Fig. 3c). The ratio of GlcN/GalN showed a pronounced decrease from 12.44 to 4.02 during the first 5 d of the experiment, and subsequently remained ~ 3 (Fig. 3d). Changes in the contributions of particulate amino sugar to POC and PN, PAS (%POC) and PAS (%PN), showed similar patterns, with maxima (6.03% and 5.50%, respectively) observed on day 1 (Fig. 3e,f). After day 1, PAS (%POC) and PAS (%PN) decreased sharply to 0.10% on day 3. However, these values then gradually increased over the next ~ 60 d.

Variations in amino sugar $\delta^{13}C$ during POM degradation

The amino sugar $\delta^{13}C$ values varied considerably during the incubation experiment (Fig. 4). Generally, $\delta^{13}C_{GalN}$ values exhibited a trend towards more negative values during the initial degradation phase (first 3 d), from −24.7‰ to −34.1‰, whereas $\delta^{13}C_{GlcN}$ values declined from −18.9‰ on day 0 to −24.5‰ on day 1 and thereafter stayed essentially invariant until day 3. The average $\delta^{13}C$ difference between GlcN and

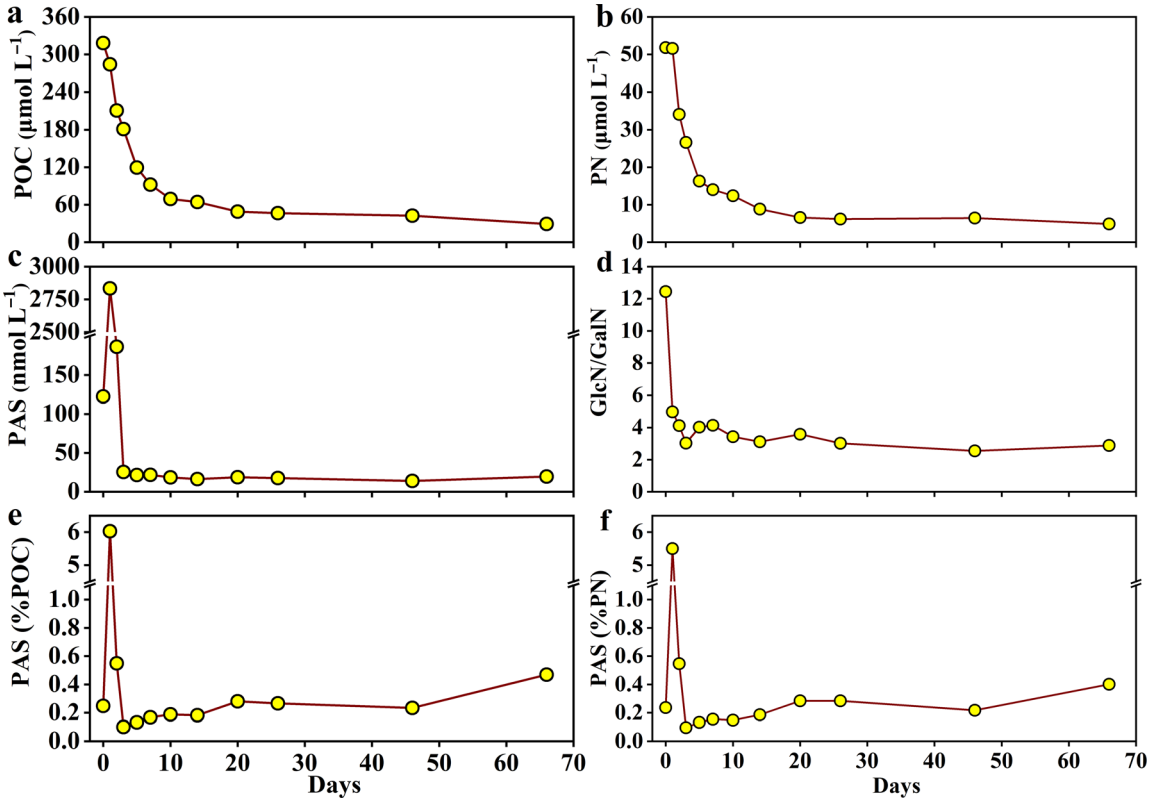

Fig. 3. Changes over the course of the incubation experiment in concentrations of POC (**a**), PN (**b**), and PAS (**c**), GlcN/GalN ratio (**d**), POC normalized yields of PAS (**e**), and PN normalized yields of PAS (**f**). The GlcN/GalN ratio can be used to indicate the diagenetic state of organic matter. Since incubation experiments require large amounts of water, replicate experiments were not performed.

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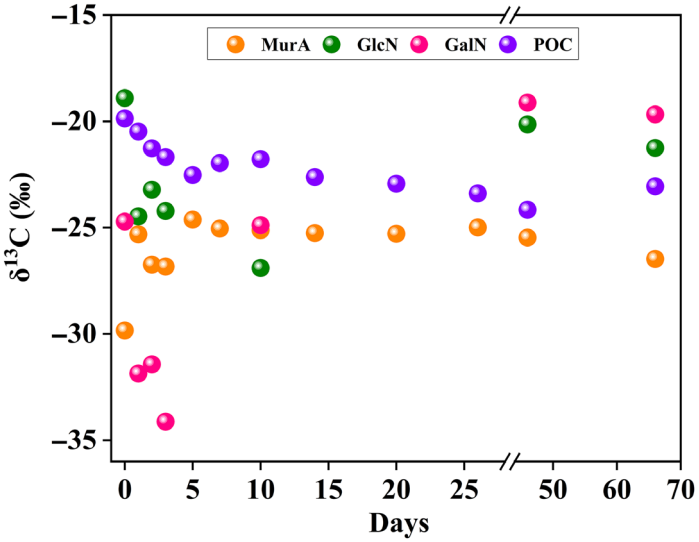

Fig. 4. Changes in $\delta^{13}C$ values of individual amino sugars and POC over the course of the incubation experiment. $\delta^{13}C$ values of GlcN and GalN on days 5, 7, 14, 20, and 26 are missed due to below detection limit. No replicate experiments were performed due to the large amount of water required for the incubation experiments.

GalN ($-7.8‰ \pm 1.7‰$) during the first 3 d was similar to that of phytoplankton ($-8.6‰ \pm 5.5‰$) (Fig. 2). Compared to day 10, the $\delta^{13}C_{GlcN}$ and $\delta^{13}C_{GalN}$ values had increased somewhat on day 46 and then remained more or less constant from days 46 to 66. Compared with the fluctuating pattern during the early incubation stage, the values of $\delta^{13}C_{GlcN}$ and $\delta^{13}C_{GalN}$ at the end of the degradation experiment were closer, a pattern consistent with that of heterotrophic bacteria. In contrast, the $\delta^{13}C$ values of MurA increased rapidly from $-29.8‰$ to $-24.6‰$ during the first 5 d and then stabilized (at $-25.4‰ \pm 0.5‰$) over the following ~ 60 d. The $\delta^{13}C$ values of MurA showed more negative values than those of GlcN and GalN at the end of the incubation period.

Discussion

Carbon isotopic composition of amino sugars in phytoplankton and heterotrophic bacteria

Amino sugars form an important component of marine microbial cells and plankton. Improved qualitative and quantitative understanding of the stable carbon isotope signatures of amino sugars can serve to better understand the marine carbon cycle. The patterns of amino sugar $\delta^{13}C$ in the four heterotrophic bacteria and four phytoplankton species differed markedly (Fig. 2). Specific amino sugar synthesis mechanisms and metabolic processes in organisms shape the differences in carbon isotopic signatures. In microorganisms, GlcN is synthesized by the following pathways: glucose is catalyzed by hexokinase to generate glucose-6-phosphate, and this reacts with glutamine to produce glucosamine-6-phosphate under the action of the Gfa1 protein (Leloir and Cardini 1953; Kottom et al. 2017). Similar pathways were found for the synthesis of GalN (Cardini and Leloir 1957). Moreover, glucose, galactose, and mannose can be interconverted in organisms. Macechko et al. (1992) reported that acetylgalactosamine can be converted from acetylglucosamine by the catalysis of epimerase. The similarity of the synthetic pathways of GlcN and GalN in heterotrophic bacteria is reflected in the small differences between their carbon isotope values (Fig. 2). In comparison, MurA is synthesized by addition of a lactate group after acetylation of GlcN (Reay et al. 2019). An earlier study suggested that phosphoenolpyruvate was the most likely precursor of the MurA side chain (Richmond and Perkins 1963). Specific linking processes of the lactate group to acetylglucosamine in bacteria may contribute to the observed carbon isotope pattern, with MurA $\delta^{13}C$ values lower than that of GlcN by 2.2‰ to 9.6‰ among the four bacteria. Alternatively, the carbon atoms of the lactate group incorporated in MurA may be depleted in ^{13}C. The amino sugar $\delta^{13}C$ of *Ps. aeruginosa* was more negative compared to the other heterotrophic bacteria (Fig. 2), which could be attributed to the difference in the substrate carbon sources (e.g., agar). During culturing, *Ps. aeruginosa* had exclusive access to one type of medium, while the other three bacteria shared the same medium. This result demonstrates the influence of substrate supply on amino sugar isotopes in heterotrophic bacteria.

The initial carbon source of the phytoplankton was dissolved CO_2. Although the $\delta^{13}C_{GlcN}$ values of the four phytoplankton were similar, there were marked differences in $\delta^{13}C_{GalN}$. The $\delta^{13}C$ values of GlcN and GalN were lower compared to dissolved CO_2 ($\sim -8‰$ when in equilibrium with atmospheric CO_2) (Lamb et al. 2006), indicating that the synthetic fractionation process of GlcN and GalN in phytoplankton was enriched in ^{12}C and varied between phytoplankton species. Whilst the specific synthetic pathways of amino sugars in phytoplankton remain unknown, the large differences (4.3–16.6‰) in $\delta^{13}C$ between GlcN and GalN suggest that there are distinct mechanisms. Moreover, phytoplankton culturing conditions may also form an important driver of isotopic changes in their organic compounds. In batch cultures, the increase in phytoplankton abundance during the exponential growth stage can result in an increase in pH (van Dongen et al. 2002). This implies that the dissolved CO_2 is relatively less available and more ^{13}C-enriched inorganic carbon (e.g., HCO_3^-) may be incorporated into the phytoplankton, leading to a relative enrichment of ^{13}C in the phytoplankton-produced amino sugars. van Dongen et al. (2002) compared the carbon isotope values of neutral sugars and lipids in the chlorophyte *Tetraedron minimum* under continuous (constant pH) and batch culture conditions (varying pH). They found that the glucose $\delta^{13}C$ values were $\sim 9‰$ and $\sim 15‰$ more positive compared to bulk material and fatty acids, respectively, in batch culture, while the offsets were $\sim 7‰$ and $\sim 10‰$ in continuous cultures. These results suggest that although culture conditions could influence the $\delta^{13}C$

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values of organic molecules, their impact on carbon isotope patterns appears to be small.

Amino sugar $\delta^{13}C$ variations during POM degradation

Muramic acid is a specific bacterial biomarker, and changes in its carbon isotope directly reflect bacterial metabolism. During the first 5 d of the degradation experiment, $\delta^{13}C_{MurA}$ values showed a rapid increase from −29.8‰ to −24.6‰ (Fig. 4), which coincided with a reduction of the bulk POC $\delta^{13}C$. Among organic compounds, labile amino acids and neutral sugars are usually enriched in ^{13}C. The addition of organic matter derived from fresh plankton rapidly stimulated heterotrophic activity, and part of the ^{13}C of these labile components was incorporated into bacterial MurA (Glaser and Gross 2005; Indorf et al. 2015). However, substantial portions of labile ^{13}C-enriched POM were removed by bacterial respiration, resulting in a decline in bulk POC $\delta^{13}C$ values (Fig. 4). This mechanism has been invoked to explain the decreasing trend of bulk POM $\delta^{13}C$ with depth in the water column (Jeffrey et al. 1983). After 5 d, the $\delta^{13}C_{MurA}$ values remained relatively constant, which may be related to changes in bacterial synthesis strategies. Bacterial recycling of their own cell wall is a common survival feature and was first recognized in the cell division of the Gram-negative bacterium *Escherichia coli* (Goodell 1985). In addition, some bacteria, such as the Gram-negative bacterium *Tannerella forsythia*, lack the MurA synthesis gene and can only build their own cell walls by scavenging the debris of other bacteria (Friedrich et al. 2015). At late stages of degradation, the bioactive organic matter is essentially consumed. Considering the high energy cost of de novo synthesis, it may be reasonable for microbes to use the MurA existing directly by salvage incorporation (Yamaguchi et al. 2017). In this pathway, the carbon skeleton of the MurA remains unchanged and thus has a negligible effect on its carbon isotope fractionation.

The $\delta^{13}C$ patterns of GlcN and GalN at the start (day 0) of the degradation experiment were similar to that of the cultured phytoplankton, with a comparable $\delta^{13}C$ difference ($\Delta\delta^{13}C_{GlcN-GalN} = 5.8‰$) to the average in cultured phytoplankton (5.9‰) except for *Ph. globosa* (16.6‰). *Sk. costatum* and *Pr. donghaiense* are two dominant phytoplankton species at the East China Sea tow station (Guo et al. 2014), and these two species were hence likely dominant sources of amino sugars on day 0. In particular, the $\delta^{13}C_{GlcN}$ (−18.9‰) and $\delta^{13}C_{GalN}$ (−24.7‰) values on day 0 were comparable to those in cultured *Sk. costatum* (−15.6‰ and −19.9‰ for GlcN and GalN, respectively), indicating that phytoplankton were the primary source of amino sugars in labile POM. Compared to day 0, the $\delta^{13}C$ values of GlcN and GalN were relatively low between days 1 and 3 (Fig. 4). In contrast to the unique bacterial origin of MurA, the different sources of GlcN and GalN coupled with the diversity of metabolic processes likely contributed to the variability of their carbon isotopic signatures. During early degradation (< 5 d), the amino sugar sources were dominated by plankton as reflected by the relatively high GlcN/GalN value (> 4; Fig. 3d). Phytoplankton-derived amino sugars were typically characterized by relatively high $\delta^{13}C$ values ("Carbon isotopic composition of amino sugars in phytoplankton and heterotrophic bacteria" section). The relatively low $\delta^{13}C_{GalN}$ and $\delta^{13}C_{GlcN}$ values observed from days 1 to 3, especially the markedly low $\delta^{13}C_{GalN}$, provide a contrast to the amino sugar $\delta^{13}C$ value of cultured phytoplankton. This discrepancy may be caused by (1) degradation of amino sugars and (2) contribution of zooplankton amino sugars. To take up organic molecules, microorganisms must be capable of extracellular enzymatic hydrolysis, a process accompanied by isotopic fractionation. During this process, high-molecular-weight organic matter is enzymatically decomposed into low-molecular-weight organic matter for microbial cell utilization. Recent studies have shown that some bacteria implement a so-called "selfish" strategy to exploit macromolecular substrates (Cuskin et al. 2015; Reintjes et al. 2019). This selfish uptake process does not release low-molecular-weight products into the environment and reduces the availability of small-molecule substrates for scavenging bacteria. In a study on peptide hydrolysis, Silfer et al. (1992) found that bonds with light isotopes are more rapidly broken, while those containing heavy isotopes are more likely to persist in the detrital substrate. This mechanism has been invoked to explain the positive shifts in $\delta^{15}N$ of detrital amino acids as degradation proceeds (Yamaguchi and McCarthy 2018). However, more variable amino acid $\delta^{13}C$ values were observed by Macko et al. (1994). In our incubation experiment, amino sugar concentrations decreased sharply on day 2 compared with day 1 (Fig. 3c; Supporting Information Fig. S1), but the $\delta^{13}C$ values of GlcN and GalN increased only slightly, indicating that degradation was not the dominant explanation for the isotopic changes observed during the first 3 d. Teece and Fogel (2007) reported the $\delta^{13}C$ values of monosaccharide in a series of organisms, and found that the $\delta^{13}C$ values of glucose and galactose in zooplankton at different water depths were ~ 2‰ and ~ 7‰ lower than those of algae, respectively, and the $\delta^{13}C$ values of galactose were about 3‰ to 10‰ lower than those of glucose. We hypothesize that similar fractionation features may also occur in zooplankton amino sugars. Abundant phytoplankton likely stimulated zooplankton growth, leading to depleted $\delta^{13}C_{GlcN}$ and $\delta^{13}C_{GalN}$ excursions and a greater difference between them on days 1 to 3.

During the late phase in the degradation (days 46–66), another distinct pattern of $\delta^{13}C_{GalN}$ and $\delta^{13}C_{GlcN}$ was observed. The $\delta^{13}C_{GalN}$ and $\delta^{13}C_{GlcN}$ values were relatively high and the offset between $\delta^{13}C_{GalN}$ and $\delta^{13}C_{GlcN}$ was minor. However, GalN and GlcN showed greater ^{13}C enrichment than MurA (> 5.3‰). This is consistent with the isotopic signatures identified in bacterial cultures (average $\Delta\delta^{13}C_{GlcN-MurA} = 4.6‰$). Furthermore, the $\delta^{13}C$ values of GlcN and GalN were higher than those of bulk POC during later incubation stages, indicating that bacterial synthesis

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of these two amino sugars may preferentially lead to ^{13}C enrichment. The lack of $\delta^{13}C_{GlcN}$ and $\delta^{13}C_{GalN}$ values from days 14 to 26 was due to measurements below the detection limit and this limited our ability to depict in detail the trends in $\delta^{13}C_{GlcN}$ and $\delta^{13}C_{GalN}$ during POM degradation. Considering the higher $\delta^{13}C_{GlcN}$ and $\delta^{13}C_{GalN}$ values on day 46 relative to day 10, as well as the progressive process of bacterial reworking of organic matter, a trend of gradually increasing in $\delta^{13}C_{GlcN}$ and $\delta^{13}C_{GalN}$ and a smaller offset between them can be expected from days 10 to 46. Nevertheless, from days 46 to 66, the $\delta^{13}C$ values of GlcN and GalN remained constant. At this stage, the organic matter had undergone extensive alteration and was unfavorable for bacteria to perform energy-intensive de novo synthesis (Yamaguchi et al. 2017), implying that bacterial recycling synthesis occurred at low bioavailable organic matter. In short, the patterns of $\delta^{13}C_{GlcN}$, $\delta^{13}C_{GalN}$, and $\delta^{13}C_{MurA}$ during the later incubation stages suggest that under conditions of limited fresh organic matter availability, bacteria adopt extensive metabolic recycling strategies.

Bulk $\delta^{13}C$ reflects the superposition of mole-weighted average carbon isotope ratios of numerous individual organic compounds. van Dongen et al. (2002) found that monosaccharides (e.g., arabinose, xylose, and glucose) were generally enriched in ^{13}C (by 0–9‰) relative to the bulk materials, while lipids (e.g., fatty acids, phytols, sterols, and alkenones) were depleted (by 0–11‰) relative to the bulk biomass. Our results showed that the amino sugar $\delta^{13}C$ values were generally lower than bulk POC $\delta^{13}C$ during the incubation experiment, particularly during the early stages of degradation, indicating that amino sugars do not represent the ^{13}C-enriched component in POM. Considering that neutral sugars and amino acids constitute a substantial fraction of fresh organic matter, the bulk $\delta^{13}C$ signal will be greatly affected by the relative content of these ^{13}C-enriched bioactive components. However, for highly diagenetically altered organic matter, amino sugar $\delta^{13}C$ can be expected to play an important role in the bulk $\delta^{13}C$ due to the accumulation of amino sugars of bacterial origin.

Implications for organic matter degradation

Identifying the diagenetic state of organic matter is important to assess carbon turnover and its response to ecosystem changes. In aquatic environments, a variety of degradation indicators based on amino compounds have been developed, such as carbon normalized yields of amino acids, degradation index based on amino acid composition and the ratio GlcN/GalN (Dauwe and Middelburg 1998; Benner and Kaiser 2003; Davis et al. 2009). As ecosystem decomposers, heterotrophic bacteria are major agents of organic matter decomposition and diagenesis. However, few studies have evaluated bacterial reworking from an isotopic perspective. McCarthy et al. (2007) defined ΣV based on the average deviation of six trophic amino acid $\delta^{15}N$ signatures and used it to reflect the resynthesis by heterotrophic bacteria. Controlled laboratory experiments and systematic data obtained in the present study allow extending the applications to amino sugar $\delta^{13}C$.

Based on the differences in amino sugar $\delta^{13}C$ patterns of heterotrophic bacteria and phytoplankton, we here propose an index of variation in amino sugar $\delta^{13}C$ values (V_{AS}), calculated as follows:

$$V_{AS} = \left|\delta^{13}C_{GalN} - \delta^{13}C_{GlcN}\right| - \left|\delta^{13}C_{MurA} - \delta^{13}C_{GlcN}\right|$$

The establishment of V_{AS} considers the linkage role of GlcN in the synthesis of GalN and MurA. In addition, MurA was incorporated into this expression based on the fact that MurA is exclusively derived from bacteria and that variations in $\delta^{13}C_{MurA}$ are tied directly to heterotrophic metabolism. This index reflects the $\delta^{13}C$ offsets of GalN and MurA relative to GlcN. Although the development of V_{AS} did not involve zooplankton amino sugar $\delta^{13}C$, the results during the early stages of incubation experiments suggest that the amino sugars in fresh organic matter appear to be primarily of phytoplankton origin. Moreover, as discussed in "Amino sugar $\delta^{13}C$ variations during POM degradation" section, amino sugars from zooplankton and phytoplankton probably have similar carbon isotope patterns (i.e., a large difference between $\delta^{13}C_{GlcN}$ and $\delta^{13}C_{GalN}$). With bacterial degradation of organic matter, the difference between $\delta^{13}C_{MurA}$ and $\delta^{13}C_{GalN}$ became gradually larger than that of $\delta^{13}C_{GlcN}$ and $\delta^{13}C_{GalN}$, and the V_{AS} values gradually decreased over the course of the degradation process (Fig. 5). Thus, the proposed V_{AS} index appropriately expresses the view that its reduction in value is linked to progressive heterotrophic resynthesis.

One way to test our interpretation of V_{AS} changes is to consider alternative amino sugar proxies (e.g., GlcN/GalN) for degradation. Decreases in GlcN/GalN are considered to result

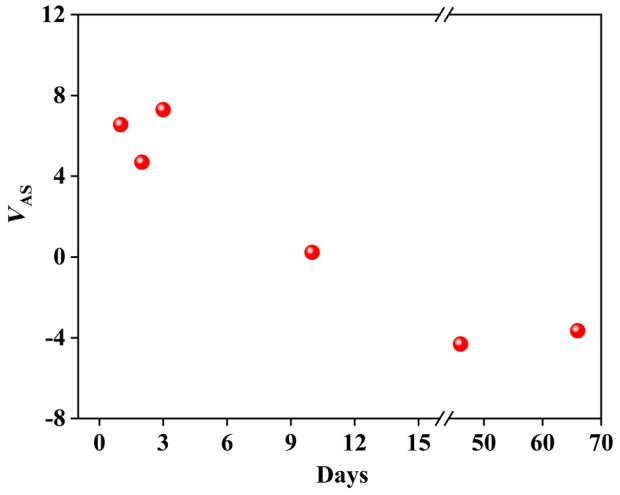

Fig. 5. Index of variation in amino sugar carbon isotopes (V_{AS}) during the incubation experiment.

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from enhanced diagenetic alteration. Selected particulate and sediment samples (Fig. 1) were analyzed to evaluate the utility of V_{AS}. The GlcN/GalN values of these marine organic matter samples ranged from 1.24 to 2.51 (Table 1), indicating differences in their diagenetic state. A strong linear relationship was found between GlcN/GalN and V_{AS} in these samples ($R^2 = 0.82$, $p < 0.01$; Fig. 6), suggesting that V_{AS} is a robust proxy to track heterotrophic resynthesis. Further analysis showed that the linear relationship between GlcN/GalN and V_{AS} was stronger when GlcN/GalN < 2 than when GlcN/GalN > 2. One possible explanation for this observation is that V_{AS} is more sensitive in highly altered organic matter. For organic matter with GlcN/GalN values > 2, the amino sugar components are largely regulated by plankton, thus the amino sugar fractionation is less affected by heterotrophic resynthesis. Although the GlcN/GalN values (< 2) indicate that organic matter has undergone extensive microbial alteration, bacteria may still be undergoing active resynthesis. Compilation of data reported in previous studies reveals that GlcN/GalN values typically exhibit a narrow range (1–3) in marine organic matter samples (Niggemann and Schubert 2006; Kaiser and Benner 2009; Ren et al. 2020). Especially in sediments, the GlcN/GalN values are generally < 2 and vary slightly. Our POM degradation experiments showed that although amino sugar concentrations and GlcN/GalN ratios remained relatively stable after 5 d, the carbon isotope values of GlcN and GlaN had changed considerably on day 46 compared to day 10 (Fig. 4), reflecting ongoing bacterial processes. Thus, characterizing the diagenetic state of organic matter on the basis of molecular concentration and composition may be ambiguous and obscure underlying biogeochemical processes. Despite the demanding analysis of amino sugar $\delta^{13}C$, especially in the open ocean where amino sugar concentrations are very low and need to be preconcentrated from large amounts of water, our results suggest that amino sugar carbon isotopes can provide important insights into metabolic processes of organic matter. Particularly, the proposed V_{AS} allows us to finely unravel bacterial reworking of organic matter when GlcN/GalN < 2. Further work is needed to optimize the carbon isotope determination of amino sugars (e.g., lower detection limits). Overall, the proposed V_{AS} index provides a powerful tool to disentangle complex cycling processes of organic matter, although additional work is required to fully explain our initial findings.

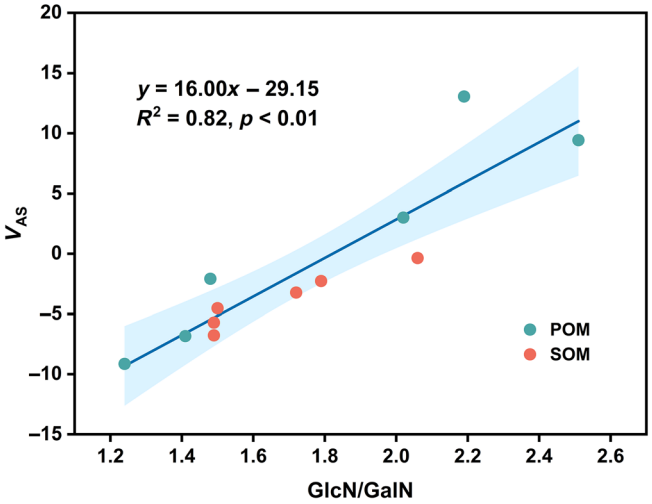

Fig. 6. Relationship between the GlcN/GalN ratio and the index of variation in amino sugar carbon isotopes (V_{AS}) in particulate and sedimentary organic matter samples, POM and SOM, respectively. The fitted regression line, equation, and coefficient of determination (R^2) are shown. Shading indicates the 95% confidence interval.

Table 1. Values of amino sugar carbon isotopes ($\delta^{13}C$-AS) and parameters for POM and sedimentary organic matter (SOM) at different stations in the eastern China marginal sea.

			$\delta^{13}C$ (‰)				
Sample	Station	GlcN/GalN	GlcN	GalN	MurA	Bulk	V_{AS}
POM	P1	2.02	−47.0	−21.8	−44.0	−28.9	3.01
	P2	2.51	−45.2	−26.7	−35.8	−27.9	9.42
	P3	2.19	−41.9	−28.7	−28.5	−23.4	13.06
	P4	1.41	−18.7	−17.2	−25.6	−23.8	−6.83
	P5	1.24	−18.5	−16.0	−27.7	−22.3	−9.14
	P6	1.48	−34.2	−33.4	−30.5	−25.2	−2.09
SOM	S1	1.49	−17.3	−19.5	−25.2	−22.1	−5.71
	S2	1.49	−15.3	−20.1	−26.9	−21.7	−6.77
	S3	1.79	−20.4	−23.7	−26.0	−21.2	−2.27
	S4	1.50	−15.9	−22.0	−26.6	−21.8	−4.53
	S5	1.72	−15.6	−22.5	−25.7	−22.0	−3.23
	S6	2.06	−26.2	−26.1	−25.8	−21.8	−0.36

V_{AS}, index of variation in $\delta^{13}C$-AS.

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Conclusions

The carbon isotope of amino sugars provides an important tool to investigate the bacterial reworking of organic matter. Our findings show that bacteria can rapidly utilize labile organic matter to synthesize their own structures (e.g., peptidoglycan) during early degradation. However, as degradation progresses, bacteria increasingly incorporate recycled compounds to reduce energy expenditure. Results of the culture experiments showed distinct carbon isotope patterns for phytoplankton and heterotrophic bacteria. We found that amino sugar $\delta^{13}C$ in heterotrophic bacteria is controlled by the substrate carbon source, and that bacteria may have similar GlcN and GalN synthesis pathways with comparable $\delta^{13}C_{GlcN}$ and $\delta^{13}C_{GalN}$ values but that these are relatively ^{13}C-depleted for MurA synthesis. In contrast, the synthesis of phytoplankton amino sugars shows greater ^{12}C enrichment than its inorganic carbon source but may perform specific synthesis mechanisms with distinct $\delta^{13}C$ differences between GlcN and GalN. Based on these observations, we propose a V_{AS} index that reflects differences in $\delta^{13}C$ of individual amino sugars. This index decreases with increasing diagenetic state of organic matter, showing that it can be used to characterize heterotrophic resynthesis.

Taken together, these new findings provide insights into bacterial degradation and transformation processes. More importantly, the patterns of amino sugar $\delta^{13}C$ observed in this study provide a novel tool to examine bacterial processes of organic matter, which may be of significance for biogeochemical applications of CSIA and organic geochemical studies.

Data availability statement

Research data associated with this article can be accessed at https://figshare.com/s/bb2297c5ff54871d8160.

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Conflict of Interest

None declared.

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