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RESEARCH ARTICLE

Limited microbial degradation of elevated concentrations of dissolved organic carbon in the deep ocean

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Abstract

Understanding the ocean's capacity potential to store dissolved organic carbon (DOC) is essential for predicting its role in long-term carbon sequestration and climate regulation. This capacity hinges on the behavior of DOC at elevated concentrations, a critical yet unresolved question that has produced mixed results due to narrow concentration ranges tested previously and limited molecular insights. This study addresses these gaps by investigating microbial degradation of DOC across a broad concentration range (2- to 55-fold) in year-long bioassay experiments using solid-phase extracted DOC (SPE-DOC) from 2000-m-deep waters. Specific SPE-DOC compounds (combined amino acids) were analyzed to provide a molecular-level understanding of DOC reactivity at varying concentrations. Our results show that microbial communities rapidly proliferated and became more uniform following SPE-DOC amendments, with *Nitrosococcales*, *Flavobacteriales*, and *Alteromonadales* dominating. Despite these shifts, microbial utilization of SPE-DOC was constrained, exhibiting a nonlinear relationship with concentration, from < 3% in the control to a maximum of 9% in DOC-enriched groups. Degradation was predominantly confined to the initial 28 d, with negligible additional removal (0–2%) thereafter. Compound-specific analysis showed only moderate utilization (7–11%) of amino acid compounds within the first 3 d, indicating restricted microbial access even when these individual compounds were concentrated. These results indicate that a fraction of deep-sea DOC molecules can persist for long at elevated concentrations. Our study demonstrates the ocean's substantial potential for DOC storage and suggests that modern ocean is capable of accommodating a larger DOC reservoir than is currently present.

Earth's oceans hold a vast reservoir of dissolved organic carbon (DOC) comparable in magnitude to the atmospheric CO_2 inventory, playing a crucial role in the global carbon cycle and the regulation of atmospheric CO_2 levels (Hedges 2002; Hansell et al. 2009; Ridgwell and Arndt 2015). Despite this importance, the capacity of the ocean to store DOC, defined here as the maximum limit or potential of the ocean to accommodate DOC over long timescales, remains an unresolved question of critical significance. Paleoceanographic evidence derived from isotopic records in marine sediment cores reveals that during the Neoproterozoic period (1000–540 million years ago), the DOC reservoir in the ocean was up to 1000 times greater than it is today (Rothman et al. 2003; Ridgwell and Arndt 2015; Mitchell et al. 2023). These records suggest that the ancient ocean had an exceptional ability to store DOC, raising the question of whether the modern ocean could similarly possess the potential to accommodate an expanded DOC reservoir under suitable conditions. However, contemporary measurements of DOC concentrations, constrained to observational periods spanning only a few decades, are insufficient to assess the ocean's long-term DOC storage potential (Hansell 2002; Wagner et al. 2020; Baltar et al. 2021).

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One approach to addressing this question involves bioassay experiments that test microbial utilization of DOC at elevated concentrations (e.g., Barber 1968; Arrieta et al. 2015a; Shen and Benner 2018). However, previous studies have used variable experimental conditions (e.g., different concentration factors or/and incubation durations), leading to inconsistent results (Barber 1968; Arrieta et al. 2015a; Jiao et al. 2015; Shen and Benner 2018; Bercovici et al. 2021; Zheng et al. 2022). For example, studies using a single concentration factor have shown that deep-sea DOC concentrated up to 10-fold remained largely resistant to microbial degradation during months-long incubations (Barber 1968; Shen and Benner 2018; Zheng et al. 2022). In contrast, experiments utilizing concentration gradients have suggested that deep-sea DOC at elevated concentrations (ranging from 2- to 19-fold) could undergo microbial degradation within weeks (Arrieta et al. 2015a; Jiao et al. 2015; Bercovici et al. 2021). The extent of degradation in these studies was relatively low (< 6%) and did not consistently increase with concentration factors. This low degradation was attributed to the short incubation duration used (Arrieta et al. 2015b), although the effects of longer incubation periods on DOC utilization might be limited (Zheng et al. 2022).

These inconsistencies point to a potential nonlinear microbial response to DOC concentration. Marine DOC is a complex mixture of molecules with highly variable reactivities (Hertkorn et al. 2013; Benner and Amon 2015). The divergent outcomes of previous studies likely reflect the intricate interplay between microbial utilization and the chemical diversity of DOC. The fates of DOC molecules at elevated concentrations, particularly those with potential bioactivity (e.g., amino acids), remain largely unknown. The limited compositional data, narrow concentration ranges tested, and variable incubation durations in prior work have left some aspects of DOC behavior at elevated concentrations not fully clarified.

In this study, we aim to address these uncertainties and explore the ocean's DOC storage capacity by investigating the microbial response and degradation of DOC and its potentially bioactive components (amino acids) across a broad range of concentration factors up to 55-fold DOC. DOC was isolated and concentrated using solid-phase extraction (SPE) from the deep ocean (2000 m), then exposed to a natural microbial community collected at the same depth over a 1-yr period. Changes in microbial abundance, microbial community composition, DOC, and a series of hydrolysable amino acid compounds were monitored during the incubation. Our results indicate a nonlinear microbial response to concentrated SPE-DOC, revealing an upper limit of SPE-DOC degradation regardless of concentration. These findings suggest that the modern ocean has a significant potential for additional DOC storage.

Materials and methods

Sample collection

Seawater samples used in this study were collected in the South China Sea onboard the R/V *Tan Kah Kee* in October 2022 (Supporting Information Fig. S1). A total of 1100 L of seawater was collected at a depth of 2000 m using Niskin bottles mounted on a CTD rosette sampler. Seawaters were drained directly into acid-cleaned polypropylene carboys and stored at 4°C until DOC extraction. An additional 10 L of seawater was collected from the same depth and filtered through a pre-combusted (450°C, 4.5 h) 0.7-μm GF/F filter for biodegradation experiments.

Solid-phase extraction of deep-sea dissolved organic carbon

Deep-sea DOC was extracted onboard using a solid-phase extraction technique within 48 h of collection. Seawater samples were acidified to pH 2–3 using 6 mol L^{-1} H_2SO_4. The acidified samples were passed through C-18 cartridges (Agilent Bond Elut, 5 g) at a flow rate of 30 mL min^{-1}, with approximately 15 L of seawater processed per cartridge. The cartridge was pre-conditioned with methanol and H_2SO_4-acidified Milli-Q water prior to sample loading. After extraction, the cartridge was rinsed with H_2SO_4-acidified Milli-Q water to remove salts and then stored at 4°C until use. The extracted DOC was eluted from each cartridge with 40 mL of methanol, dried under a stream of N_2 gas, redissolved in 200 mL of Milli-Q water, and filtered through a pre-combusted 0.7-μm GF/F filter. The C-18 extraction recovered 18% of bulk DOC and 12% of total hydrolysable amino acids, respectively.

Bioassay experiments

Bioassay experiments were conducted to evaluate microbial utilization of DOC at varying concentrations. Dissolved organic carbon extracted at 2000 m was added at ambient, 2-, 4-, 8-, 38-, and 55-fold concentrations to microbial inocula that were collected at the same depth. The experiments were performed in pre-combusted (450°C, 4.5 h) 1 L glass bottles in triplicate, maintained at 4°C, under atmospheric pressure, and in the dark for 365 d.

Subsamples were taken from each bottle on days 0, 3, 7, 14, 28, 60, 120, 180, 240, and 365 to determine prokaryotic abundance, DOC concentration, and dissolved organic matter composition (combined amino acids). Samples for prokaryotic abundance were fixed with glutaraldehyde (0.5% final concentration) for 15 min in the dark, flash-frozen in liquid nitrogen, and stored at −80°C until analysis. To gain further insights into the microbial community dynamics, additional water samples were collected from the 8-, 38-, and 55-fold DOC treatments on day 0 and on days 60, 120, 180, 240, and 365 for analysis of microbial community composition. Samples collected after day 60 were combined to ensure sufficient material for the analysis. All

microbial community samples were filtered using 0.2-μm pore size polycarbonate filters (Millipore, USA). The filters were flash-frozen in liquid nitrogen and kept at −80°C until DNA extraction. DOC and amino acids samples were stored at −20°C until analysis.

Analysis of prokaryotic abundance and microbial community composition

Prokaryotic abundance was measured using a flow cytometer (BD Accuri C6). Samples were stained with the fluorescent dye SYBR Green I (Invitrogen) for 15 min in the dark prior to analysis (Marie et al. 1997). Prokaryotes were identified on the two-parameter dot-plot of green fluorescence against sideward scatter.

Microbial community composition was determined by 16S ribosomal RNA (rRNA) gene sequencing. The total DNA of microorganisms collected on polycarbonate membranes was extracted using HiPure Soil DNA Kits (Magen, China) according to the manufacturer's protocols. The V4-V5 region of microbial 16S rRNA gene was amplified with bacterial/archaeal primers 515F 5′-GTGYCAGCMGCCGCGGTAA-3′ and 926R 5′-CCGYCAATTYMTTTRAGTTT-3′ (Parada et al. 2016). The amplified products were sequenced using the Illumina MiSeq platform (Illumina, USA). Sequencing data were quality-filtered using R package DADA2, denoised, and chimera-checked to obtain amplicon sequence variants (ASVs) (Callahan et al. 2016).

Analysis of dissolved organic carbon and amino acids

DOC concentrations were measured by high-temperature catalytic oxidation method using a Shimadzu Total Organic Carbon analyzer (TOC L-CPN). Samples were thawed at room temperature and acidified to pH 2–3 with 2 mol L^{-1} hydrochloric acid prior to injection. Hydrochloric acid-acidified Milli-Q waters and deep-sea DOC reference materials (collected from the Northwestern Pacific Ocean at a depth of 4000 m) were analyzed along with seawater samples to check the instrumental blanks and performance (Benner and Strom 1993). The blanks were negligible and the coefficient of variation of all measured references was 3% (± 1.3 μmol C L^{-1}, $n = 21$).

Concentrations of total hydrolysable amino acids (THAA) were determined using an ultrahigh performance liquid chromatography instrument (Agilent 1260) equipped with a fluorescence detector (Shen et al. 2017). Water samples were hydrolyzed and derivatized following protocols described previously (Kaiser and Benner 2005). Briefly, 100 μL of water sample was transferred into a pre-combusted 400 μL micro insert and dried under N_2. Dried samples were acid-hydrolyzed at 150°C for 32.5 min using a vapor-phase hydrolysis method in a CEM microwave digestion system. A total of 16 amino acids were derivatized with a reagent mixture of *o*-phthaldialdehyde and *N*-isobutyryl-L-cysteine, followed by separation on an Agilent Poroshell 120 EC-C18 column. The following individual amino acids were quantified in the analysis: asparagine + aspartic acid (Asx), glutamine + glutamic acid (Glx), serine (Ser), histidine (His), glycine (Gly), threonine (Thr), β-alanine (β-Ala), arginine (Arg), alanine (Ala), γ-aminobutyric acid (γ-Aba), tyrosine (Tyr), valine (Val), phenylalanine (Phe), isoleucine (Ileu), leucine (Leu), and lysine (Lys). The performance of the analysis was monitored throughout sample measurements by injecting reference materials (0.2-μm filtered seawater collected at 100 m in the South China Sea) that were hydrolyzed in the same way as samples. The relative standard deviation of reference materials was 6%.

Parameter calculation and statistical analysis

Prokaryotic specific growth rates were calculated as the slope of the natural logarithm of prokaryotic abundance against time (Baranyi and Pin 1999) (Eq. 1):

$$\text{specific growth rate}\left(\text{d}^{-1}\right) = \frac{\ln N_i - \ln N_j}{\Delta t} \quad (1)$$

where N_i and N_j are the cell numbers on days i and j, respectively, and Δt is the time interval between days i and j.

DOC removal percentage was calculated as the difference in DOC concentration between the initial and final time points, divided by the initial DOC concentration (Eq. 2):

$$\text{DOC removal}\,(\%) = \frac{\text{DOC}_{\text{initial}} - \text{DOC}_{\text{final}}}{\text{DOC}_{\text{initial}}} \times 100\% \quad (2)$$

where $\text{DOC}_{\text{initial}}$ and $\text{DOC}_{\text{final}}$ are the DOC concentrations at the initial and final time points of incubation, respectively.

The relationship between DOC removal percentage and initial DOC concentration was fitted using a Michaelis–Menten-like function (Eq. 3):

$$\text{DOC removal}\,(\%) = \frac{V_{\max} \times \text{DOC}_0}{K_{\text{m}} + \text{DOC}_0} \quad (3)$$

where $V_{\max}$ is the maximum DOC removal percentage, K_{m} is the apparent half-saturation constant (DOC concentration value when the DOC removal is half of $V_{\max}$), and DOC_0 is the initial DOC concentration on day 0.

All statistical analyses, including non-linear regression analysis, correlation analysis (Pearson correlation or Spearman correlation for variables that were normally or not normally distributed, respectively), principal coordinates analysis (PCoA), and statistic differences (Mann–Whitney U test, $a = 0.05$), were carried out using R software.

Results

Microbial response to elevated concentrations of deep-sea dissolved organic carbon

Prokaryotic abundance in the original deep-sea waters was $1.2 \pm 0.1 \times 10^4$ cells mL^{-1} and showed minimal changes throughout the incubation period in the control group

(Fig. 1). The addition of concentrated SPE-DOC led to a rapid increase in prokaryotic abundance during the first 14 d across all treatments, followed by a slow and minor decline in cell numbers during the rest of the incubations (Fig. 1). The sustained high prokaryotic abundance during the mid-late phases of the incubations was likely attributable to a combination of reduced grazing pressure (due to large removal of grazers by 0.7-μm filtration) and a minor contribution from chemoautotrophic production (discussed below). Maximum prokaryotic abundances observed in the control (i.e., ambient bulk DOC concentration) and the treatments (2-, 4-, 8-, 38-, and 55-fold DOC concentrations) were 0.1×10^6, 0.5×10^6, 0.8×10^6, 1.0×10^6, 3.1×10^6, and 4.5×10^6 cells mL^{-1}, respectively.

Microbial community structures were analyzed in selected treatment groups with notable increases in prokaryotic abundance following SPE-DOC additions (8-, 38-, and 55-fold concentrations) (Fig. 2). At the start of incubation, the microbial community was dominated by two bacterial groups (*Oceanospirillales* and *Alteromonadales*) and an archaeal group (*Nitrosopumilales*) (Fig. 2). By days 60–365, the relative abundance of these initially dominant groups declined, while the previously rare bacterial groups such as *Nitrosococcales*, *Flavobacteriales*, and *Rhodobacterales* increased, leading to a more uniform microbial community composition (Fig. 2). During this period, the five dominant prokaryotic groups were *Nitrosococcales*, *Flavobacteriales*, *Alteromonadales*, *Oceanospirillales*, and *Rhodobacterales*, which together accounted for 87–95% of the total microbial community. Principal coordinates analysis (PCoA) of microbial community composition revealed a broadly similar community structure across DOC-amended groups, with an exception of the 8-fold DOC group (Supporting Information Fig. S2).

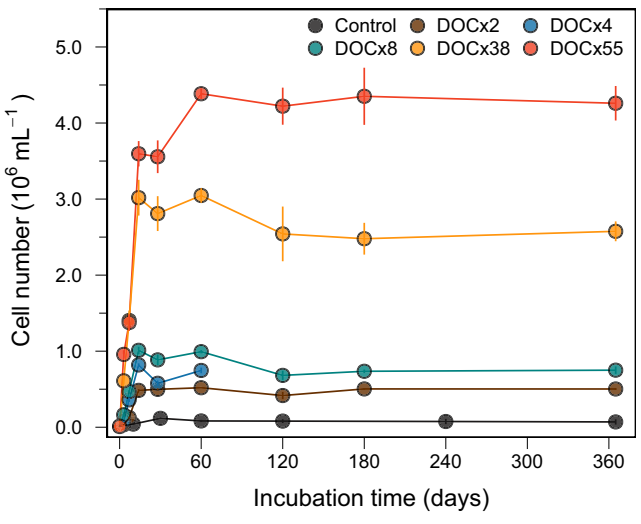

Fig. 1. Changes in prokaryotic abundance during the 365-d incubations with deep-sea DOC at original DOC concentrations (control) and at 2-, 4-, 8-, 38-, 55-fold DOC concentrations. Error bars represent one standard deviation of triplicate.

Several prokaryotic taxa with chemoautotrophic ability (e.g., ammonium-oxidation, sulfur-oxidation) were identified during the bioassay experiments (Supporting Information Table S1). At the start of the incubation, chemoautotrophic archaea *Nitrosopumilales*, *Woesearchaeales*, and *Thermoplasmata* constituted 13%, 0.25%, 0.20% of the microbial community, respectively. Chemoautotrophic bacteria, such as ammonium-oxidizing *Nitrosococcales* and sulfur-oxidizing *Rhodospirillales* and *Burkholderiales*, collectively accounted for 2% of the initial microbial community. By days 60–365, the relative abundance of chemoautotrophic archaea *Nitrosopumilales*, *Woesearchaeales*, and *Thermoplasmata* had decreased to 0.03–0.16%, 0.0%, and 0.0%, respectively. In contrast, the relative abundance of bacterial taxa increased, with *Nitrosococcales* comprising 18–45%, *Rhodospirillales* 1–3%, and *Burkholderiales* 0.04–0.70% of the total microbial community (Supporting Information Table S1).

Microbial degradation of dissolved organic carbon at increasing concentrations

The concentration of DOC in the control group with original deep-sea water was relatively low (43 μmol L^{-1}) and showed only a minor decrease ($\sim$ 1 μmol L^{-1}) over the 365-d incubations (Fig. 3). The addition of concentrated SPE-DOC to the amended groups increased the initial concentrations of DOC by a factor of 2- (78 μmol L^{-1}), 4- (160 μmol L^{-1}), 8- (347 μmol L^{-1}), 38- (1605 μmol L^{-1}), and 55-fold (2323 μmol L^{-1}), respectively. Removal of DOC was observed in all amended groups, with the majority of the removal occurring during the first 28 d (Fig. 3). The total removal of DOC concentrations over the course of 1-yr incubation in the amended groups (2-, 4-, 8-, 38-, and 55-fold concentration) was 5, 13, 23, 137, and 220 μmol L^{-1}, respectively.

The percentage removal (%) of DOC was calculated to evaluate the extent of DOC degradation and its relationship with concentration factors (Fig. 4). The total percentage removal of DOC ranged from 2.7% $\pm$ 0.3% in the control, to 5.5% $\pm$ 1.3%, 7.4% $\pm$ 0.8%, 6.4% $\pm$ 0.2%, 8.5% $\pm$ 0.7%, and 9.4% $\pm$ 1.2% in the 2-, 4-, 8-, 38-, and 55-fold DOC groups. Despite these removals, over 90% of the concentrated DOC remained resistant to microbial utilization regardless of the DOC concentration factor. The percentage removal of DOC showed a nonlinear relationship with initial DOC concentration, showing an increase at lower concentrations ($\leq$ 8-fold DOC; Pearson's $r = 0.6$, $p < 0.05$) and leveling off at higher concentrations (38- and 55-fold DOC; Mann–Whitney U test, $p > 0.1$). The relationship between percentage removal of DOC and initial DOC concentration was best described by a Michaelis–Menten-like function (Fig. 4). This function predicts a maximum DOC removal of 9.1% at higher concentrations.

Considering the observed varying DOC degradation and prokaryotic growth over time (Figs. 1, 3), the incubation period was further divided into two stages: days 0–28 (early

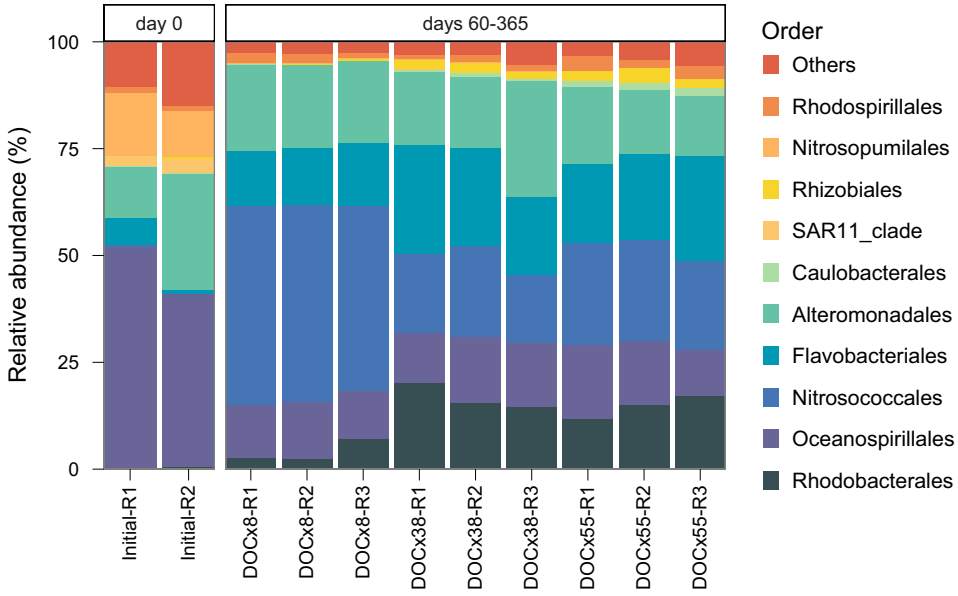

Fig. 2. Changes in microbial community composition from day 0 to days 60–365 in the 8-, 38-, and 55-fold DOC groups, depicted at the order level. The horizontal axis label represents the replicate groups (R1, R2, and R3).

phase) and days 28–365 (mid-late phase). Percentage removal of DOC and specific growth rate of prokaryotes were calculated and compared between these two stages (Fig. 5). During the early phase (days 0–28), DOC removal (%) varied from 2.7% in the control to a maximum of 7.8% in the treatments, with the highest removal rate reaching 6.0 μmol C $L^{-1}d^{-1}$ (Fig. 5a). This phase accounted for most of the total DOC removal observed over the year in all groups. The specific growth rates of microorganisms during the early phase mirrored the pattern of DOC removal, peaking at about 0.20 d^{-1} in the highest DOC amendment (Fig. 5c). In the mid-late phase (days 28–365), the percentage of DOC removal (0–2.2%) and DOC removal rate (< 0.1 μmol C $L^{-1}d^{-1}$) in all groups were significantly lower than those in the early phase (Mann–Whitney *U* test, $p < 0.05$) and showed no clear pattern with DOC concentration factors (Spearman's $\rho = 0.34$, $p > 0.1$, Fig. 5b). The specific growth rates during this phase approximated zero and did not vary with DOC concentrations (Fig. 5d).

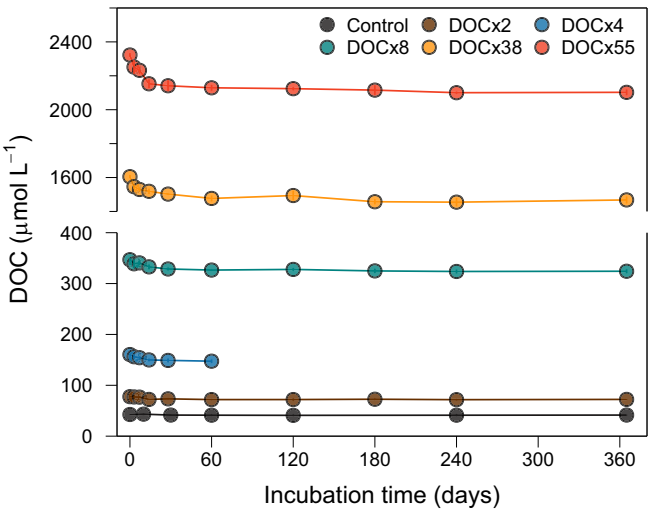

Fig. 3. Changes in DOC concentrations during the 365-d incubations. Data are reported as the mean ± standard deviation of triplicate experiments. Error bars are not visible as their sizes are smaller than the symbols.

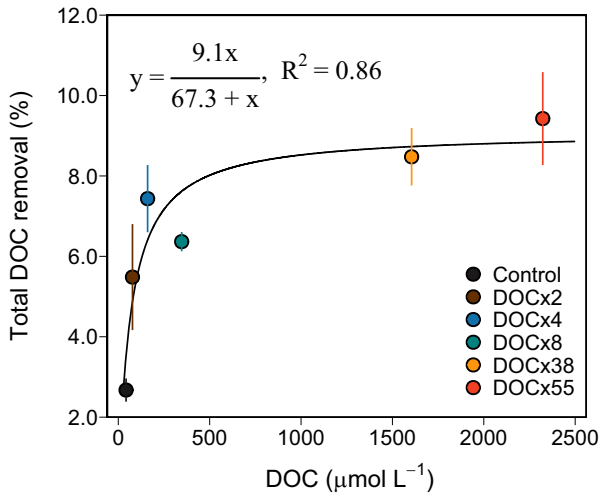

Fig. 4. Percentage removal (%) of DOC during the 365-d incubations with original (control) and 2-, 4-, 8-, 38-, and 55-fold DOC concentrations. Data were fitted using a Michaelis–Menten-like function, as described by the equation in the figure. Data are reported as the mean and standard deviation of triplicate experiments.

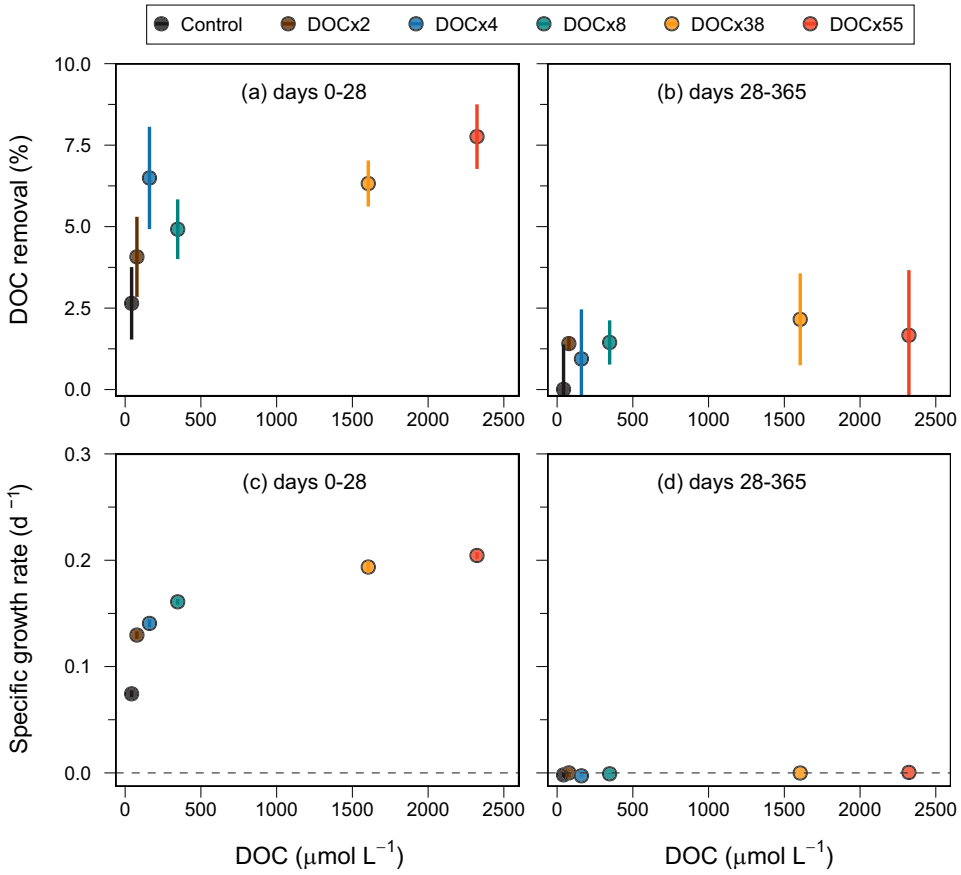

Fig. 5. DOC removal percentages (**a**, **b**) and specific growth rates (**c**, **d**) during days 0–28 and days 28–365 of the incubations for different DOC concentrations. Error bars represent one standard deviation of triplicate.

Changes in dissolved organic matter composition

The concentration of THAA in the control group was relatively low, with an initial concentration of 94 nmol L^{-1}, representing 0.8% of the bulk DOC. The addition of SPE-DOC to the amended groups increased the initial THAA concentrations to 178 nmol L^{-1}, 328 nmol L^{-1}, 615 nmol L^{-1}, 2986 nmol L^{-1} and 4015 nmol L^{-1} in the 2-, 4-, 8-, 38-, and 55-fold DOC groups, respectively (Fig. 6). These concentrations corresponded to 0.6–0.8% of the SPE-DOC. Notable removal of THAA was observed across all groups but only within the first 3 d, with losses ranging from 8 to 330 nmol L^{-1} (7–11% of the initial THAA). The extent of this decrease, however, was not significantly correlated with the initial THAA concentrations (Spearman's $\rho = -0.26$, $p > 0.1$; Supporting Information Fig. S3). Following this initial decrease, THAA concentrations increased between days 3 and 14, with increments ranging from 14 to 654 nmol L^{-1}. After day 14, THAA levels plateaued and remained stable for the remainder of the 365-d incubations (Fig. 6). By the end of the incubation, the final concentrations of THAA in DOC-amended groups were either comparable or higher than their initial values (Fig. 6).

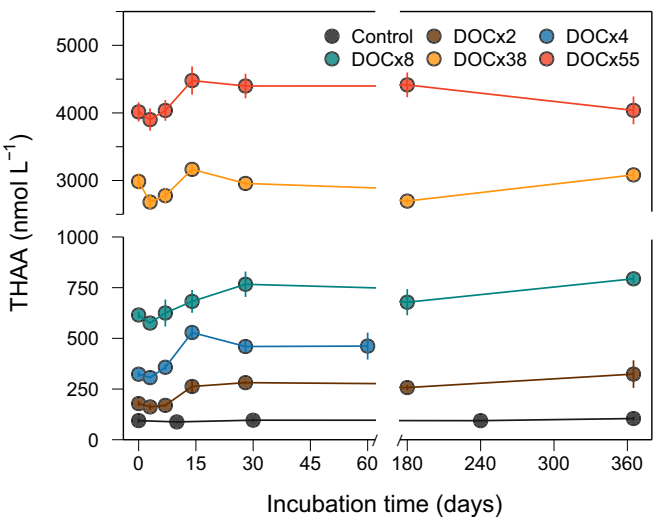

Fig. 6. Changes in total hydrolysable amino acids (THAA) concentrations during the 365-d incubations. Error bars denote one standard deviation of triplicate.

The composition of THAA was dominated by Asx, Glx, Gly, and Ala across all experimental groups, collectively accounting for 63–67% of THAA (Fig. 7). The amino acid composition remained relatively stable throughout the incubation period and across all groups (Fig. 7). While individual amino acids exhibited some variability, no consistent patterns of degradation were observed during the 365-d incubations (Supporting Information Fig. S4).

Discussion

Limited microbial degradation of concentrated deep-sea dissolved organic carbon

Evaluating the fate of marine DOC at increasing concentrations provides valuable insights into the ocean's capacity for carbon storage (e.g., Barber 1968; Arrieta et al. 2015a; Zheng et al. 2022). In this study, 'capacity' is referred to as the theoretical potential of the ocean to store DOC, rather than the actual change in DOC reservoir size. Previous bioassay experiments examining microbial degradation of concentrated DOC have reported mixed results (Barber 1968; Arrieta et al. 2015a, 2015b; Jiao et al. 2015; Shen and Benner 2018; Bercovici et al. 2021). These inconsistencies arise partially from the limited range of concentration factors tested (2–19 folds) and/or the short duration (weeks) of the experiments (e.g., Arrieta et al. 2015b; Jiao et al. 2015). In this study, we tested a total of 6 concentration factors (up to 55-fold) and extended the duration of the experiments to over a year with multiple sampling points to address these limitations. Our results showed that the extent of DOC degradation did not linearly increase with initial DOC concentrations, peaking at a maximum removal of 9.1% at higher concentration factors. Additionally, most DOC degradation occurred within the first 28 d, with only marginal increases in degradation (an additional 0–2%) observed over the extended period. These new results suggest that microbial degradation of deep-sea DOC is limited, even under scenarios of increasing concentrations and extended degradation durations.

The monitoring of multiple time points throughout the 1-yr incubations reveals the presence of different DOC pools with varying levels of bioreactivity: labile, semi-labile, and refractory (Hansell 2013). A small fraction (4–8%) of the concentrated DOC was rapidly utilized (up to 6.0 μmol C L^{-1} d^{-1}) within the first 28 d. DOC with a turnover time of days to weeks is generally considered labile (Ogura 1972; Carlson and Hansell 2015). The observed labile DOC after concentration may consist of labile molecules present in the original seawater DOC and concentrated during extraction, or other molecules that were too diluted to be utilized and became bioavailable after enrichment (Arrieta et al. 2015a). While both sources could contribute to the labile DOC observed, the former appears to be the primary contributor. Increasing the bulk DOC concentrations from 2- to 55-fold did not result in a coherent increase in the percentage of labile DOC, which would be expected if dilution were the limiting factor (Jiao et al. 2015). Consistent with the above assertation, the original deep-sea DOC used in this study was found to contain a small fraction (1 μmol C L^{-1} or 2.7%) of labile DOC (Fig. 4). Regardless, the relatively low abundance of labile DOC observed in all amended groups was sufficient to support rapid prokaryotic growth (Fig. 1).

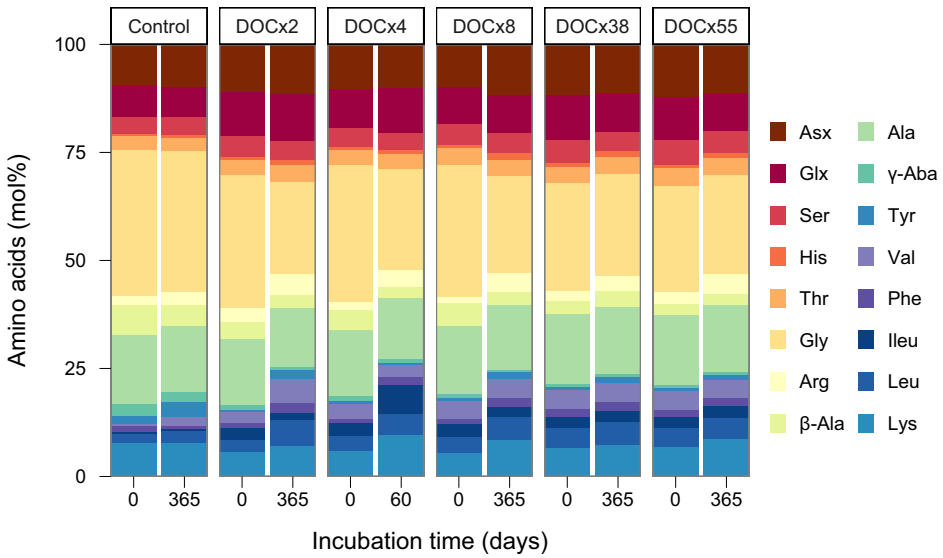

Fig. 7. Changes in composition of total hydrolysable amino acids at the start and end of the incubations. Each colored bar represents the average mole percentage of individual amino acids from triplicate samples. Abbreviations: asparagine + aspartic acid (Asx), glutamine + glutamic acid (Glx), serine (Ser), histidine (His), glycine (Gly), threonine (Thr), β-alanine (β-Ala), arginine (Arg), alanine (Ala), γ-aminobutyric acid (γ-Aba), tyrosine (Tyr), valine (Val), phenylalanine (Phe), isoleucine (Ileu), leucine (Leu), and lysine (Lys).

An additional but very minor (0–2%) removal of concentrated DOC was observed from days 28 through the rest of the 1-yr incubations. DOC utilized on a timescale of months to years is considered semi-labile (Carlson and Hansell 2015). In contrast to labile DOC, the semi-labile DOC was detected at much lower concentrations with significantly slower removal rates ($< 0.1\ \mu$mol C L^{-1} d^{-1}). During this phase, specific microbial growth rates declined to nearly zero, suggesting that the slowly degraded semi-labile DOC was primarily used to maintain prokaryotic metabolic demands rather than growth. Potential sources of this fraction include original seawater DOC, bacterial transformation on the pre-existing labile DOC (Ogawa et al. 2001; Lechtenfeld et al. 2015), or minor chemoautotrophic production (Bayer et al. 2019). Several chemoautotrophic archaea and bacterial groups (e.g., ammonium-oxidizing *Nitrosopumilales* and *Nitrososcoccales*) were detected between days 60–365 in the experiments (Supporting Information Table S1). Regardless, the minor additional removal of concentrated SPE-DOC over extended periods suggests that prolonging incubation does not lead to substantial further degradation of DOC. This experimental observation contrasts with a previous study that used a simulation approach to predict a linearly increasing removal (up to 60%) of concentrated DOC when extending the experiment from 40 d to 1 yr (Arrieta et al. 2015b). Our results illustrate the complexity of DOC reactivity that cannot be fully captured by DOC concentration or incubation duration alone.

Refractory DOC, operationally defined as DOC that resists microbial degradation over the course of incubation, comprised over 90% of the concentrated SPE-DOC in all amended groups. The relationship between DOC removal percentage and initial DOC concentration was found to be best described by a Michaelis–Menten-like function (Fig. 4). This function suggests that no additional DOC removal would occur even with further increases in concentration. These findings indicate limited microbial degradation of concentrated DOC in the deep ocean. The consistent predominance of refractory DOC across varying concentration levels supports previous experimental and modeling observations indicating that molecular properties primarily control the microbial utilization of DOC in the ocean (Shen and Benner 2020; Zakem et al. 2021; Zheng et al. 2022).

The responses of microbial community to elevated DOC concentrations provide new insights about the influence of microbial community structure on DOC utilization. Our results indicate that while the amendment of concentrated SPE-DOC induced a compositional shift in the microbial community, the overall microbial community composition remained similar across treatments with different DOC concentrations (Fig. 2). It has been shown that different substrates could lead to distinct microbial communities (Nelson and Carlson 2012; Lian et al. 2021; Xu et al. 2022). As a consequence, the observed microbial similarity across our study groups suggests that the DOC being degraded by these microbial assemblages at varying concentrations shared similar molecular properties.

Several bacterial groups dominant in our experiments (e.g., *Flavobacteriales*, *Alteromonadales*, and *Rhodobacterales*) are known for their specialization in degrading different components of DOC (Buchan et al. 2014; Ferrer-González et al. 2021; Xu et al. 2022). Despite this specialization, the extent of DOC degradation was found to reach a maximum of 9.1%, suggesting a constrained metabolic capacity within the microbial community under the conditions of these bioassay experiments. Our experiments were conducted under depressurized conditions. This may have enhanced the activity of some piezo-sensitive prokaryotes (e.g., *Flavobacteriales*, one of the dominant prokaryotes in our DOC-amended groups), compared to in situ conditions with high hydrostatic pressure (Amano et al. 2022). As such, the observed extent of DOC degradation might be overestimated to some extent. The limited ability to degrade the remaining refractory DOC fractions implies that the deep-sea microbial communities residing in a single location may not have the full metabolic repertoire required to process the diverse molecules that comprise the bulk of deep-sea DOC (Sala et al. 2020; Zakem et al. 2021). Degrading the remaining fractions of DOC likely necessitates a more diverse array of environmental conditions and microbial taxa (Shen and Benner 2018), which may not be adequately represented in the current experiment setup. These observations highlight the complexity of DOC degradation in the deep ocean and suggest that a broader range of environmental factors and microbial diversity are essential for the complete processing of deep-sea DOC.

Compositional insight into the persistence of concentrated deep-sea dissolved organic carbon

Hydrolysable amino acids, typically considered more bioavailable than bulk DOC (Amon et al. 2001; Davis and Benner 2007; Davis et al. 2009), were analyzed to evaluate the degradation of DOC molecules at varying concentrations. Our compositional analysis showed that SPE-DOC extracted from the deep ocean was depleted in hydrolysable combined amino acids, comprising only 0.6–0.8% of the SPE-DOC. Amino acids are abundant in freshly plankton-derived organic matter (> 20% of the DOC) and become rapidly depleted as the organic matter degrades (Amon et al. 2001; Cherrier and Bauer 2004; Shen et al. 2016). The remarkably low relative abundance of amino acids in the extracted deep-sea SPE-DOC suggests its extensive degradation state.

In DOC-amended groups, THAA exhibited moderate degradation (7–11% of the initial THAA) only during the first 3 d of incubation, followed by an increase at comparable levels between days 3–14, and then a long-term plateau during the remainder of the incubation (Fig. 6). The unexpectedly short period of amino acid removal contrasts with the continuous degradation of bulk DOC observed during the first 28 d, suggesting that labile DOC utilized in the early stage consists of compounds other than amino acids. Previous studies on deep-sea DOC recovered via solid-phase extraction have

identified minor fractions of carbohydrates and unsaturated aliphatic compounds (Zheng et al. 2022), which are enriched in surface waters with elevated chlorophyll levels and are potentially bioavailable substrates (Medeiros et al. 2015; Li et al. 2019).

The lack of substantial utilization of amino acids differs from the conventional perception of amino acids as readily bioavailable compounds (Benner 2003; Carlson and Hansell 2015). The extent of THAA removal during the first 3 d did not correlate with their initial concentrations (Supporting Information Fig. S3), suggesting that the utilization of amino acid compounds was not solely limited by concentration. Hydrolysable amino acids measured in this study occurred exclusively in combined forms, potentially derived from structure-modified proteins or non-proteinaceous materials that are largely resistant to microbial degradation (Keil and Kirchman 1993; Ianiri et al. 2022). This resistance associated with the composition and structure may explain the limited microbial utilization observed.

A subsequent increase in THAA was observed between days 3–14, coinciding with rapid microbial growth. This pattern reflects the temporary production of amino acid-containing byproducts during the degradation of other DOC compounds. Microbes supplied with labile organics can transform substrates into various metabolites, including peptides (Ogawa et al. 2001; Lechtenfeld et al. 2015). These metabolic byproducts were found to be resistant to further microbial degradation. The bioresistant nature of newly produced amino acids was evidenced by the minimal removal observed after day 14. By the end of the 1-yr incubations, the final concentrations of THAA were either similar to or higher than the initial values across all DOC-amended groups. Neither THAA nor individual amino acids exhibited clear degradation or production patterns in relation to concentration factors (Fig. 6; Supporting Information Fig. S3). These molecular observations provide new insights into the compositional factors that influence DOC reactivity, indicating that the persistence of DOC compounds is largely dictated by their molecular properties (Shen and Benner 2020). They also highlight the critical role of molecular composition in shaping the long-term stability and fate of organic carbon in marine environments.

Implications for understanding the storage capacity of marine dissolved organic carbon

Understanding the ocean's capacity for DOC storage has been a longstanding challenge in marine and climate science, primarily due to uncertainties surrounding the mechanisms that drive DOC persistence. If DOC molecules become bioavailable at elevated concentrations (Arrieta et al. 2015a; Mentges et al. 2019), a dynamic balance between supply and removal would establish a stable DOC pool size over extended timescales. In contrast, if DOC molecules can persist at elevated concentrations (Barber 1968; Shen and Benner 2018; Bercovici et al. 2021), the ocean may possess great potential to store additional organic carbon under favorable conditions.

Results of this study provide experimental evidence supporting the latter scenario, revealing a considerable capacity of the ocean for long-term DOC storage. Less than 10% of the deep-sea SPE-DOC was degraded over the course of a year, and neither increasing the concentrations nor extending incubation periods led to significantly greater degradation. It is important to point out that the solid-phase extraction method used in this study recovered ~ 20% of the bulk deep-sea DOC. It is uncertain whether the unextracted fraction would exhibit similar persistence patterns as the extracted SPE-DOC. Future research employing complementary sorbents or alternative extraction techniques could further explore the behavior of the unextracted DOC fraction. Despite these uncertainties, the robust persistence observed in the extracted fraction suggests that the marine DOC pool contains components capable of resisting degradation at elevated concentrations over extended periods. These results indicate that the modern ocean, much like its ancient state, has the ability to accommodate substantial quantities of DOC over prolonged timescales.

While the ocean's potential for long-term DOC storage is considerable, future changes in marine DOC reservoir size are unclear. Key uncertainties include the effects of global warming on marine primary production (Laufkötter et al. 2015; Kwon et al. 2022) and the associated production rate of refractory DOC. The removal rate of refractory DOC is anticipated to decline in response to the observed slowdown in overturning circulation in the modern ocean (Shen and Benner 2018). If assuming stable production rates of refractory DOC, reductions in its degradation under altered circulation patterns would allow the ocean to store larger quantities of DOC. A comprehensive understanding of the factors governing refractory DOC dynamics is essential for accurately predicting how the marine carbon reservoir will respond to ongoing climate change.

In recent years, various strategies have been proposed to enhance the ocean's carbon sink (National Academies of Sciences, Engineering and Medicine 2022; Zhang et al. 2022; Jiao et al. 2023). These include minimizing the loss of refractory DOC by reducing land-based chemical fertilization (Jiao et al. 2011) and improving wastewater treatment technologies (Lv et al. 2022, 2024), as well as increasing the sequestration of refractory DOC through large-scale seaweed farming (Krause-Jensen and Duarte 2016; Zhang et al. 2017; Li et al. 2022) and implementing techniques such as artificial upwelling and alkalinity enhancement (Pan et al. 2015; Gómez-Letona et al. 2022; Jiao et al. 2023). Our results suggest that the ocean would be able to store additional organic carbon introduced through these methods, potentially enhancing its role as a long-term carbon sink.

Overall, the results of this study contribute to a more solid understanding of the mechanisms behind DOC persistence and highlight the significant storage potential of marine DOC. These findings reinforce the critical role of marine DOC in the global carbon cycle and its potential as a long-term carbon reservoir, with implications for climate regulation and carbon sequestration strategies.

Author Contributions

Yuan Shen conceived the project. Yixian Li and Yuan Shen collected water samples. Tao Liu conducted the bioassay experiments and chemical analyses with assistance from Yixian Li. Tao Liu and Yuan Shen analyzed the data and wrote the manuscript together.

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Conflicts of Interest

None declared.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

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