

Production and diversity of microorganisms associated with sinking particles in the subtropical North Pacific Ocean

Matthew J. Church¹,^{*} Eint Kyi,² Robert O. Hall Jr.,¹ David M. Karl,² Markus Lindh,³ Alexa Nelson,² Emma K. Wear¹

¹Flathead Lake Biological Station, University of Montana, Polson, Montana

²Daniel K. Inouye Center for Microbial Oceanography: Research and Education, University of Hawaii at Manoa, Honolulu, Hawaii

³Swedish Meteorological and Hydrological Institute, Gothenburg, Sweden

Abstract

Sinking particulate organic matter controls the flux of carbon (C) from the surface ocean to the deep sea. Microorganisms actively colonize particles, but the extent to which microbial metabolism influences particle export remains uncertain. We conducted experiments to quantify rates of bacterial production (derived based on ³H-leucine incorporation) and dark C-fixation (based on ¹⁴C-bicarbonate assimilation) associated with sinking particles collected from the base of the euphotic zone (175 m) in the subtropical North Pacific Ocean. Seawater was amended with sinking particles and rates of filter size-fractionated (0.2, 2, and 20 μm) bacterial production and dark C-fixation were measured. Sequencing of 16S ribosomal RNA (rRNA) gene amplicons revealed that microorganisms in the particle-amended treatments differed from those in the unamended seawater controls, with the particle treatments enriched in putative copiotrophic bacteria. The addition of sinking particles increased rates of bacterial production (by 6- to 9-fold), and to a lesser extent dark C-fixation (by 1.7- to 4.6-fold), relative to unamended controls, with most of the production associated with filter pore sizes < 20 μm. Normalizing production to concentrations of particulate C yielded rates that were statistically indistinguishable between particle-amended treatments and unamended controls. We then examined possible relationships between sinking particulate C flux attenuation and its supply to the mesopelagic waters, revealing that flux attenuation was positively related to increases in particulate C supply. Together with results from our experiments, we suggest processes that contribute to sinking particle disaggregation both increase flux attenuation and favor microbial mineralization of particle-derived organic matter.

Downward export of organic matter drives movement of carbon (C) from the atmosphere into the interior waters of the sea, accounting globally for an estimated 5–12 Pg C yr⁻¹ (Volk and Hoffert 1985; Siegel et al. 2014). Gravitational settling of particulate C constitutes most of this downward export (Buesseler and Boyd 2009; Buesseler et al. 2020), with numerous biological and physical processes controlling the

magnitude of particulate matter flux into the interior waters of the ocean (Turner 2015; Boyd et al. 2019; Omand et al. 2020). As particles settle out of upper ocean, they undergo intensive transformation through the lower euphotic zone and upper mesopelagic waters (150–500 m); such transformations include mineralization of organic material by microorganisms and animals (Steinberg et al. 2008; Giering et al. 2014), changes in particle size via fragmentation or aggregation (Briggs et al. 2020; Omand et al. 2020), and solubilization (Smith et al. 1992). Together, these processes result in strong vertical attenuation of sinking particulate matter (Martin et al. 1987). Quantifying processes that define flux attenuation are of particular interest for establishing the degree to which settling particles remove C from the actively cycling global pool.

Planktonic microorganisms (defined here to include Bacteria and Archaea) are considered major controls on attenuation of particle flux (Cho and Azam 1988). Sinking particles provide habitats where concentrations of reduced chemical

*Correspondence: matt.church@umontana.edu

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substrates such as organic matter, ammonium (NH_4^+), and nitrite (NO_2^-) can be enriched relative to the surrounding seawater (Shanks and Trent 1979), fueling diverse microbial metabolisms (Karl et al. 1988). Moreover, sinking particles support high microbial cell densities and elevated enzymatic activities (Smith et al. 1992; Simon et al. 2002), and these particle microenvironments often harbor distinct communities of microorganisms (DeLong et al. 1993; Fontanez et al. 2015). Extracellular enzymatic activities can attenuate sinking flux through reduction in particle size and via transformation of particulate organic matter to the dissolved phase (Smith et al. 1992; Datta et al. 2016). Hence, particle-associated microbial metabolic interactions can be closely coupled in space and time. To date, however, the extent to which particle decomposition supports different forms of microbial metabolism (e.g., chemoheterotrophy and chemoautotrophy) remains largely unknown.

Since 1988 the Hawaii Ocean Time-series (HOT) program has measured particulate matter flux at the open ocean field site Sta. ALOHA (22.75°N, 158°W), providing time-resolved information on particle export in this oligotrophic habitat. Despite persistently low upper ocean nutrient concentrations and dominance of small (<20 μm equivalent spherical diameter) suspended and sinking particles (Barone et al. 2015; White et al. 2015), particulate C flux out of the upper ocean (150 m) varies more than five-fold (Karl and Church 2017). Small particles (typically <50 μm) are increasingly recognized as substantial contributors to C export in the open ocean (Trull et al. 2008; Durkin et al. 2015; Richardson 2019), and such particles can support a large fraction of mesopelagic animal production (Romero-Romero et al. 2020). Moreover, particle size emerges as a key determinant of the attenuation of particle flux through the mesopelagic waters in ocean biogeochemical models (DeVries et al. 2014; Weber et al. 2016; Omand et al. 2020).

Using a series of particle-amendment experiments, we quantified rates of microbial production associated with freshly collected sinking particles in the subtropical North Pacific Ocean. We size-fractionated (>0.2 μm , >2.0 μm , and >20 μm) rates of bacterial production (based on ^3H -leucine incorporation) and dark C-fixation (based on assimilation of ^{14}C -bicarbonate) from experiments where sinking particles, collected from the base of the euphotic zone, were added to whole seawater. We also assessed the phylogenetic diversity of both Bacteria and Archaea through sequencing of 16S ribosomal RNA (rRNA) gene amplicons in particle-amended treatments and unamended controls. Based on results from these experiments, we analyzed HOT program sinking particulate C fluxes at Sta. ALOHA for a period (1989–1995) when particle fluxes were measured at three discrete depths in the lower euphotic zone and into the upper mesopelagic waters (150, 300, and 500 m), to examine temporal variability in particulate C flux attenuation. Results from our analyses of these

time series sediment trap data indicated flux attenuation was positively related to increases in sinking particulate C flux.

Methods

Sinking particle amendment experiments

We conducted experiments in March and April 2017 during three research cruises to Sta. ALOHA (Table 1). Sinking particulate matter was collected using a large diameter (1 m), surface-tethered, 50 μm nylon mesh net trap equipped with a 15 cm diameter $\times$ 45 cm long cod-end (Peterson et al. 2005). The net trap was hung at 175 m to collect sinking particles for 24 h. The open end of the net was closed prior to recovery using an acoustic release secured to array float lines (Peterson et al. 2005). On recovery of the net trap, particles that were concentrated in the net's cod-end were subsampled using a Folsom plankton splitter, and these sample splits were subsequently screened through a 335- μm mesh to remove large zooplankton. The screened splits were then further divided into ten 100 mL aliquots using a McLane Wet Sample Divider (McLane Research Laboratories). Triplicate splits were pooled, and 300 mL of the pooled splits were added to triplicate 10-liter carboys containing seawater collected from the same depth as the net trap deployment (175 m). Unamended seawater controls (no particle amendments) consisted of triplicate 10-liter carboys filled with seawater collected from 175 m from the same cast as the seawater used for the particle-amended treatments. Carboys were subsampled at the beginning of each experiment for subsequent measurements of bacterial production, dark C-fixation, and rRNA gene diversity.

Bacterial production and dark C-fixation

Rates of bacterial production from the particle-amended experiments were estimated based on the incorporation of ^3H -leucine into protein (Kirchman et al. 1985) into filter size fractionated plankton samples. From each carboy (triplicate particle-amended and triplicate controls), six individual 40 mL polycarbonate centrifuge tubes were filled and amended with 20 nmol L^{-1} ^3H -leucine (PerkinElmer; specific activity of the stock ^3H -leucine was 455 GBq mmol $^{-1}$). Three of these 40 mL incubation tubes from each carboy served as time zero blanks and were immediately parallel filtered onto 25 mm diameter polycarbonate filters of varying pore sizes (0.2 μm , 2 μm , and 20 μm , respectively) and subsequently processed as described below. The remaining triplicate samples from each carboy were incubated in the dark at in situ temperatures for 4 h. Incubations were terminated by parallel filtration (one 40 mL tube per filter per carboy) onto the same filter pore sizes used for the time zero blanks. Filters were rinsed three times with 5 mL of cold 5% trichloroacetic acid, followed by three rinses (5 mL each) with cold 80% ethanol. Filters were removed from the vacuum filtration manifold and stored frozen in glass scintillation vials. In the shore-based laboratory, 10 mL of PerkinElmer UltimaGold™ scintillation cocktail was added to

Table 1. Summary of cruises, particulate C flux, and mean (± 1 SD) based on triplicate measurements of particulate C and total organic C concentrations from unamended controls and particle-amended treatments.

Cruise	Dates	150 m mean particulate C flux $\pm$ SD (mmol C m ⁻² d ⁻¹)	Treatment	Mean particulate C $\pm$ SD (μ mol C L ⁻¹)	Mean total organic C $\pm$ SD (μ mol C L ⁻¹)
KOK 1703	08 Mar 2017	N/A	Control	1.2 $\pm$ 0.3	63.4 $\pm$ 0.4
			Particle-amended	10.5 $\pm$ 6.1	66.4 $\pm$ 0.7
KOK 1703	10 Mar 2017	N/A	Control	1.1 $\pm$ 0.1	63.9 $\pm$ 0.8
			Particle-amended	5.9 $\pm$ 3.6	68.3 $\pm$ 0.8
HOT 291 (KOK 1705)	29 Mar 2017	1.8 $\pm$ 0.21	Control	3.3 $\pm$ 2.1	57.2 $\pm$ 0.7
			Particle-amended	8.3 $\pm$ 0.4	62.9 $\pm$ 0.8
HOT 292 (KOK 1707)	26 Apr 2017	2.4 $\pm$ 0.19	Control	1.0 $\pm$ 0.01	57.0 $\pm$ 0.7
			Particle-amended	8.7 $\pm$ 0.8	61.7 $\pm$ 0.5

each scintillation vial containing the filters and radioactivity on each filter was measured by liquid scintillation counting (PerkinElmer Tri-Carb 2800TR). Rates of bacterial production (nmol C L⁻¹ d⁻¹) were estimated from the measured ³H-leucine incorporation (after subtracting the time zero blanks) based on a C conversion factor of 0.44 kg C mol⁻¹ leucine incorporated (Giering et al. 2014).

Dark C-fixation was estimated based on ¹⁴C-bicarbonate assimilation into filter size fractionated plankton biomass. Each of the particle-amended and control carboys were subsampled into six 40 mL polycarbonate centrifuge tubes which were amended with ~ 0.05 MBq mL⁻¹ (final activity) ¹⁴C-bicarbonate (PerkinElmer). Three tubes from each carboy were parallel filtered immediately onto the same filter sizes used for the ³H-leucine incorporation measurements; these samples served as time zero blanks. The remaining triplicate tubes were incubated for 24 h at in situ temperatures in the dark. At the end of the incubation period, a 25 μ L subsample of the ¹⁴C-amended seawater was removed from each tube and added to a 20 mL scintillation vial containing 500 μ L β -phenylethylamine; these samples enabled quantification of the total amount of radioactivity added to each sample. The remaining sample volume was immediately parallel filtered onto the same filter types previously described (one tube per filter per carboy). The filters were removed from the filtration manifold and stored frozen in 20 mL glass scintillation vials until processing. In the laboratory, filters were acidified to remove inorganic C with 1 mL of 2 mol L⁻¹ hydrochloric acid and allowed to vent, uncapped, for 24 h in a fume hood. After acidification, 10 mL of PerkinElmer UltimaGold™ scintillation cocktail was added to scintillation vials containing the filters, and the resulting radioactivity was quantified by liquid scintillation counting. Rates of dark C-fixation were calculated, following subtraction of the time zero blanks, using HOT

program measurements of dissolved inorganic C concentrations at 175 m in March and April of 2017 (2060 μ mol C L⁻¹).

Total organic C and particulate C

Carboys were also subsampled for subsequent measurements of total organic C and particulate C concentrations. For total organic C analyses, seawater from each carboy was subsampled into acid-cleaned 125 mL polyethylene bottles and frozen at -20°C . Total organic C concentrations were measured by high temperature combustion using a Shimadzu TOC-V. For particulate C determinations, 2–4 L of seawater was subsampled from each carboy and filtered onto precombusted glass fiber filters (Whatman GF/F) using positive pressure filtration. Filters were analyzed by high temperature combustion using an Exeter CE-440 Elemental Analyzer (Grabowski et al. 2019).

To facilitate comparison between experiments where the addition of sinking particles resulted in differences in final concentrations of particulate C added to treatments, rates of bacterial production and dark C-fixation were normalized to the measured particulate C concentrations for both treatments and controls (yielding units of d⁻¹). Since measurements for particulate C were not filter size-fractionated (particulate C concentrations were measured from filters with a nominal pore size of 0.7 μ m), only the cumulative rates (based on the >0.2 μ m filter fraction) of bacterial production and C-fixation were normalized to particulate C.

DNA sample collection, rRNA gene amplification, and sequence analyses

Seawater (3 L) was subsampled from each carboy for filter size-fractionated collections of plankton biomass for subsequent extraction of DNA. From these subsamples, 1 L of seawater was parallel filtered onto 25 mm diameter 0.2 μ m, 2 μ m,

and 20 μm pore-size polycarbonate filters, respectively, using a peristaltic pump. Filters were placed into 2 mL microcentrifuge tubes containing 400 μL of lysis buffer AP1 (Qiagen DNeasy Plant Mini Kit) and 0.2 g of 100 μm diameter zirconium beads, immediately flash-frozen in liquid nitrogen, and stored at -80°C until processed. In the laboratory, DNA was extracted following a modified version of the Qiagen DNeasy Plant Mini Kit. Briefly, plankton cells concentrated onto filters were subjected to a freeze/thaw cycle in lysis buffer AP1, then physically disrupted via bead beating for 2 min, followed by addition of proteinase K. Samples were incubated at 55°C for 1 h, vortexing the microcentrifuge tubes every 15 min. Following this incubation, DNA was extracted and purified following the manufacturer's instructions. An extraction blank (an unused filter) was processed alongside samples. DNA extracts were stored at -80°C for subsequent analyses.

DNA extracts were polymerase chain reaction (PCR)-amplified using multiplexed index primers (forward primer: 515F-Y and reverse primer: 926R) targeting the V4-V5 hypervariable regions of the 16S rRNA gene (Parada et al. 2016). Samples, filter blanks, and a PCR blank (no template control) were amplified in triplicate. Each reaction included: 9 μL PCR-grade water, 12 μL PCR master mix, 1 μL forward primer (10 $\mu\text{mol L}^{-1}$ stock concentration), 1 μL reverse primer (10 $\mu\text{mol L}^{-1}$ stock concentration), and 2 μL DNA for a total reaction volume of 25 μL . Thermal cycling conditions were as follows: 3 min initial denaturation at 94°C , followed by 35 cycles of denaturation at 94°C for 45 s, annealing at 55°C for 60 s, and elongation at 72°C for 90 s; followed by a final 10 min extension at 72°C . PCR products from triplicate reactions were combined, cleaned, quantified, and pooled at equimolar proportions. The pooled library was sequenced on a MiSeq System (Illumina) using paired-end 300 v3 chemistry. DNA sequences have been archived with the Sequence Read Archive (<https://www.ncbi.nlm.nih.gov/sra>) under Project Number PRJNA478094.

Amplicon sequence variants were generated in DADA2 v1.14 (Callahan et al. 2016) and classified using the SILVA v138 database (Quast et al. 2013); informatic workflow parameters are available at <https://github.com/ekwear/ALOHAParticles16S>. Sequences identified as plastids, mitochondria, and eukaryotes, as well as those not classified at the domain level, were removed. Samples were subsampled to 8950 sequences using the "rarefy" function in the R package vegan (Oksanen et al. 2016). Sequences were aligned using the R package DECIPHER (Wright 2016); aligned sequences were used to generate a phylogenetic tree using the R package phangorn (Schliep 2010). A weighted UniFrac distance matrix (Lozupone and Knight 2005) was calculated using the R package phyloseq (McMurdie and Holmes 2013). We retained replicate samples for multivariate analyses of amplicons, except where specified; for statistical analyses such as *t*-tests, we used mean values within size-treatment-cruise categories to avoid pseudo-replication.

Because rate measurement determinations and samples for subsequent 16S rRNA gene amplicon analyses were parallel

filtered onto separate filters of differing pore size, we consider the $>0.2 \mu\text{m}$ filter fraction to represent the cumulative rates and microbial community, inclusive of plankton caught by the larger filter size fractions ($>2 \mu\text{m}$ and $>20 \mu\text{m}$). Similarly, the $>2 \mu\text{m}$ size class should be inclusive of rates and microorganisms found in the $>20 \mu\text{m}$ size fraction.

Particulate C export and vertical attenuation

Using HOT program measurements of sinking particulate C flux measured at three discrete depths (150, 300, 500 m) from near-monthly collections between 1989 and 1995 (Karl et al. 1996), depth-dependent attenuation in particle flux was computed. Our analyses were restricted to this initial period of HOT observations because after 1995 the quantification of sinking particulate C flux has been limited to a single discrete depth (150 m). Unfortunately, there were no coincident measurements of bacterial production conducted during this period. We estimated the individual (k_j) and mean (k) sinking flux attenuation coefficients (km^{-1}) by fitting exponential decay functions in Bayesian multilevel fashion to the particulate C fluxes.

$$F_{j,z} = F_{j,150} e^{-k_j(z-150)} \quad (1)$$

where $F_{j,z}$ is the particulate C flux at depth z for sampling date j , $F_{j,150}$ is the particulate C flux measured at 150 m on sampling date j , k_j is the flux attenuation (km^{-1}) derived for a sampling date j , and z is the depth (m). For derivation of k_j , we first normalized the particulate C flux measurements at 300 and 500 m to the flux at 150 m ($F_{j,z}$ as $F_{j,z} = \frac{F_{j,z}}{F_{j,150}}$), yielding the simplified flux attenuation model:

$$F'_{j,z} = e^{-k_j(z-150)} \quad (2)$$

To evaluate how $F_{j,150}$ influences variation in k , we used a Bayesian framework to estimate k_j and regressed k_j vs. $F_{j,150}$. We assumed normally distributed errors on particle fluxes such that

$$F'_{j,z} \sim N(e^{-k_j}, \sigma_F) \quad (3)$$

where prior probability on k_j estimated as

$$k_j \sim N(\alpha + \gamma F_{j,150}, \sigma_k) \quad (4)$$

implying the mean of all k_j is a linear function of $F_{j,150}$ with an intercept of α and a slope of γ with residual standard deviation σ_k . Priors for α and γ were weakly informative with $\alpha \sim N(0, 5)$ and $\gamma \sim N(0, 2)$. Prior probability for σ_F (residual standard deviation of $F_{j,z}$) was half normal, $\sigma_F \sim |N(0, 1)|$ as was σ_k , $\sigma_F \sim |N(0, 3)|$. The benefits of a Bayesian multilevel approach are twofold: it reduces uncertainty in k_j compared to individual fits due to the borrowing strength in the estimate of a single k_j from all of the other

estimates, and the estimates of α and γ accounts for error within and among sampling date estimates of k_j .

We fit this model to the particle flux data by simulating posterior probability distributions using Stan (Stan Development Team 2018) via the RStan package in R, which uses a Hamiltonian Monte Carlo method to simulate posterior distributions. For each parameter, we ran four chains with 500 steps for burn-in and 1000 for sampling. Chain convergence was checked visually and by using the Gelman-Rubin diagnostic $\hat{R} < 1.1$.

Results

Biomass production in particle-amendment experiments

HOT program estimates of particulate C flux at 150 m during March and April of 2017 were $1.8 \text{ mmol C m}^{-2} \text{ d}^{-1}$ and $2.4 \text{ mmol C m}^{-2} \text{ d}^{-1}$, respectively (Table 1), similar to the HOT program long-term mean flux ($2.3 \pm 0.39 \text{ mmol C m}^{-2} \text{ d}^{-1}$) for this depth (Karl et al. 2021). Concentrations of particulate C in the unamended controls ranged $1.0\text{--}3.3 \mu\text{mol C L}^{-1}$, also consistent with HOT program measurements of these C pools at 175 m. The addition of particles to the experimental treatments increased concentrations of particulate C by $5\text{--}9 \mu\text{mol C L}^{-1}$ relative to controls (Table 1). Concentrations of dissolved organic C (calculated as total organic C – particulate C) in the particle-amended treatments and the controls were not clearly different (one-way ANOVA, $p = 0.13$), with concentrations ranging $53\text{--}63 \mu\text{mol C L}^{-1}$.

Bacterial production in the unamended controls was always lower than in the particle-enriched treatments. In the unamended seawater controls, bacterial production averaged ($\pm$ standard deviation [SD]) $0.47 \pm 0.63 \text{ nmol C L}^{-1} \text{ d}^{-1}$, $0.09 \pm 0.04 \text{ nmol C L}^{-1} \text{ d}^{-1}$, and $0.05 \pm 0.03 \text{ nmol C L}^{-1} \text{ d}^{-1}$ for the $> 0.2 \mu\text{m}$, $> 2 \mu\text{m}$, and $> 20 \mu\text{m}$ size fractions, respectively (Fig. 1). In comparison, for these same filter fractions, bacterial production in the particle-amended experiments averaged 2.9 ± 0.63 , 0.84 ± 0.17 , and $0.36 \pm 0.14 \text{ nmol C L}^{-1} \text{ d}^{-1}$ (Fig. 1). Treatment-specific changes in bacterial production (i.e., treatment/control) were similar across filter size fractions, with particle-amended treatments 4.4- to 17-fold greater than the controls. Subtraction of the controls from the particle-amended treatments revealed that $> 2 \mu\text{m}$ and $> 20 \mu\text{m}$ fractions accounted for between 24–37% and 11–15%, respectively, of the cumulative ($> 0.2 \mu\text{m}$) sinking particle-associated bacterial production (PA_{BP} ; Table 2).

For all filter size fractions, and irrespective of controls or treatments, rates of dark C-fixation were consistently greater than coincident measurements of bacterial production. Rates of dark C-fixation in the controls averaged $21 \pm 12 \text{ nmol C L}^{-1} \text{ d}^{-1}$, $16 \pm 5.8 \text{ nmol C L}^{-1} \text{ d}^{-1}$, and $14 \pm 4.9 \text{ nmol C L}^{-1} \text{ d}^{-1}$ for the $> 0.2 \mu\text{m}$, $> 2 \mu\text{m}$, and $> 20 \mu\text{m}$ filter fractions, respectively (Fig. 1), while C-fixation in the particle-amended treatments averaged $98 \pm 30 \text{ nmol C L}^{-1} \text{ d}^{-1}$, $51 \pm 19 \text{ nmol C L}^{-1} \text{ d}^{-1}$, and $23 \pm 9.2 \text{ nmol C L}^{-1} \text{ d}^{-1}$ for

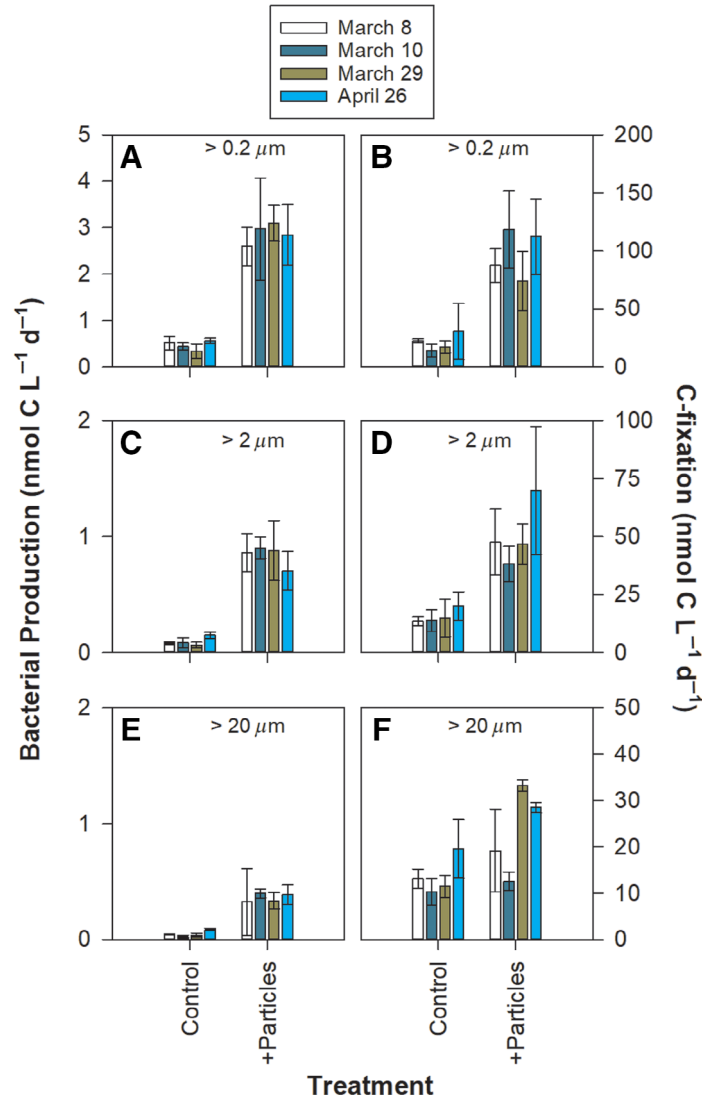


Fig 1. Mean rates of bacterial production and C-fixation in particle-amended treatments and unamended controls. Panels (A), (C), and (E) are rates of bacterial production from $> 0.2 \mu\text{m}$, $> 2 \mu\text{m}$, and $> 20 \mu\text{m}$ filter size fractions, respectively. Panels (B), (D), and (F) are rates of C-fixation from $> 0.2 \mu\text{m}$, $> 2 \mu\text{m}$, and $> 20 \mu\text{m}$ filter size fractions, respectively. Error bars are ± 1 SD of the mean rates derived from triplicate carboys. Note differences in Y-axis scaling.

these same filter fractions (Fig. 1). C-fixation in the $> 0.2 \mu\text{m}$ and $> 2 \mu\text{m}$ size fractions was 4.6- and 3.2-fold greater (on average) in the particle-amended treatments than in the controls (one-way ANOVA, values of p ranged 0.001–0.04). In contrast, with one exception (HOT 291; one-way ANOVA, $p = 0.0001$), there were no clear differences in dark C-fixation in the $> 20 \mu\text{m}$ filter fraction between the particle-amended treatments and controls (one-way ANOVA, values of p ranged from 0.07 to 0.32). On average, relative stimulation (treatment/control) in dark C-fixation was greater in both the $> 0.2 \mu\text{m}$ and $> 2 \mu\text{m}$ than in the $> 20 \mu\text{m}$ filter fractions

Table 2. Mean (± 1 SD) rates of bacterial production and C-fixation associated with sinking particles (PA_{BP} and PA_{C-fix} , respectively). Also depicted are relative (%) contributions by larger filter size fractions ($> 2 \mu m$ and $> 20 \mu m$ filter fractions) to the cumulative ($> 0.2 \mu m$) PA_{BP} and PA_{C-fix} . Mean and SDs computed based on triplicate rate measurements.

Date (cruise)	PA_{BP} (nmol C L ⁻¹ d ⁻¹) > 0.2 μm	PA_{BP} (nmol C L ⁻¹ d ⁻¹) > 2 μm	PA_{BP} (nmol C L ⁻¹ d ⁻¹) > 20 μm	PA_{C-fix} (nmol C L ⁻¹ d ⁻¹) > 0.2 μm	PA_{C-fix} (nmol C L ⁻¹ d ⁻¹) > 2 μm	PA_{C-fix} (nmol C L ⁻¹ d ⁻¹) > 20 μm
08 Mar 2017 (KOK1703)	2.1 $\pm$ 0.44	0.78 $\pm$ 0.16 (37% $\pm$ 30%)	0.28 $\pm$ 0.29 (13% $\pm$ 107%)	65 $\pm$ 14	34 $\pm$ 14 (52% $\pm$ 48%)	6.0 $\pm$ 9.1 (9.3% $\pm$ 153%)
10 Mar 2017 (KOK1703)	2.5 $\pm$ 1.1	0.82 $\pm$ 0.10 (32% $\pm$ 45%)	0.37 $\pm$ 0.041 (15% $\pm$ 45%)	104 $\pm$ 34	24 $\pm$ 8.9 (23% $\pm$ 49%)	2.3 $\pm$ 3.5 (2.2% $\pm$ 157%)
29 Mar 2017 (HOT 291)	2.8 $\pm$ 0.42	0.82 $\pm$ 0.26 (29% $\pm$ 35%)	0.30 $\pm$ 0.07 (11% $\pm$ 28%)	57 $\pm$ 26	32 $\pm$ 12 (55% $\pm$ 59%)	22 $\pm$ 2.7 (38% $\pm$ 47%)
26 Apr 2017 (HOT 292)	2.3 $\pm$ 0.66	0.56 $\pm$ 0.17 (24% $\pm$ 42%)	0.30 $\pm$ 0.08 (13% $\pm$ 40%)	82 $\pm$ 40	50 $\pm$ 28 (61% $\pm$ 75%)	8.9 $\pm$ 6.4 (11% $\pm$ 87%)

Rates of particle-associated bacterial production (PA_{BP}) and particle-associated dark C-fixation (PA_{C-fix}) calculated as mean rate measured in particle-amended treatment – mean rate measured in control; errors represent propagated SDs of the mean rate.

(one-way ANOVA, $p = 0.02$). Subtracting dark C-fixation measured in the controls from the particle-amended treatments (i.e., particle-associated C-fixation, PA_{C-fix}) demonstrated that larger filter fractions ($> 2 \mu m$ and $> 20 \mu m$, respectively) accounted for 23–61% and 2.2–38% of the cumulative ($> 0.2 \mu m$) PA_{C-fix} (Table 2).

Particulate C normalization of rates of bacterial production and dark C-fixation provided information on C-specific production rate constants (units of d⁻¹) in both treatments and controls. The resulting bacterial production rate constants were similar among the controls and particle-amended treatments (one-way ANOVA, values of p ranged 0.07–0.42), with rate constants averaging 4.4×10^{-4} d⁻¹ (Fig. 2), and varying little (less than twofold) between experiments. Similarly, PC-normalized rates of dark C-fixation also did not differ much among the controls and the particle-amended treatments, ranging 2.8×10^{-3} to 8.2×10^{-2} d⁻¹ across all experiments (Fig. 2; one-way ANOVA, values of p ranged 0.07–0.36).

Analyses of rRNA gene amplicon sequences

Estimates of richness, Pielou's evenness (J'), and Shannon diversity (H') based on amplicon sequence variants revealed that diversity in the controls varied less than the particle-amended treatments, particularly in the two smallest filter size fractions (Fig. 3). The total number of amplicon sequence variants identified for all samples was 8942, with each sample containing between 229 and 2331 amplicon sequence variants (Fig. 3A). Richness was 30–70% greater in the controls than the particle-amended treatments for the $> 0.2 \mu m$ and $> 2 \mu m$ size fractions, respectively (one-way t -test, $p = 4.8 \times 10^{-3}$ and 1.2×10^{-5} , respectively), while evenness (Pielou's J') and Shannon diversity (H') were greater in controls than particle-amended treatments for all three filter size classes (one-way ANOVA, p values ranging from 1.1×10^{-5} to 1.9×10^{-3}).

Microorganisms in the particle-amended treatments differed from those in the controls, whether examined across the data set as a whole (analysis of similarity, $R = 0.61$, $p = 0.001$) or within filter size classes ($> 20 \mu m$: $R = 0.742$; $> 2 \mu m$: $R = 0.768$; $> 0.2 \mu m$: $R = 0.588$; $p = 0.001$; Fig. 4A). Particle-amended treatments were strongly enriched in *Gammaproteobacteria*, notably *Vibrio* and *Pseudoalteromonas*, with a single *Vibrio* amplicon sequence variant constituting as much as 47% of an individual sample (Fig. 4B). In both controls and particle-amended treatments, microorganisms in both the $> 2 \mu m$ and $> 20 \mu m$ filter size fractions were phylogenetically distinct from the $> 0.2 \mu m$ filter fraction (Fig. 4). The $> 0.2 \mu m$ samples in both controls and particle-amended treatments exhibited elevated relative abundances of microorganisms commonly observed in this region of the water column at Sta. ALOHA, including SAR11 Clades 1a and 1b, SAR324, and the archaeon *Candidatus Nitrosopelagicus*.

Particulate C flux attenuation

Based on ~ 6.5 yr (1989–1995) of HOT program sediment trap measurements at 150, 300, and 500 m, particle flux was both time-variable and underwent rapid attenuation through the upper mesopelagic zone. Particulate C flux measured at 150 m varied between 0.89 and 4.7 mmol C m⁻² d⁻¹ (Fig. 5), consistent with the long-term (1989–2018) range in particulate C flux measured at this depth (ranging 0.89–5.1 mmol C m⁻² d⁻¹; Karl et al. 2021). On average, particulate C fluxes at 300 m and 500 m were 45% and 61%, respectively, lower than the flux at 150 m (Fig. 5). There was considerable temporal variability associated with flux attenuation, with values of k_j ranging between 0.2 and 5.2 km⁻¹, averaging 3.1 km⁻¹. The resulting mineralization length scale (defined as $1/k_j$) averaged 0.32 km (ranging 0.2–5.2 km). Particulate C flux at 150 m was positively related to k_j [slope = 6.6×10^{-4} m d (mmol C)⁻¹; 95% credible interval: 2.3×10^{-4} , 11.3×10^{-4} m d (mmol C)⁻¹; Fig. 5C], suggesting mesopelagic particulate C flux decay depended, in part, on variation in the sinking flux of particulate C with time.

Discussion

The downward transport of particles is a central conduit for the transfer of energy and material into the deep sea (Martin et al. 1987; Boyd et al. 2019; Buesseler et al. 2020), and this export of chemically reduced material into the ocean's interior waters fuels diverse microbial metabolisms (Karl et al. 1984). The metabolism of organisms in the dimly lit lower euphotic zone and upper mesopelagic waters consume a large fraction of sinking particulate matter flux, coincident with mineralization of bioelements and reduction in the sinking flux energy content (Martin et al. 1987; Karl et al. 1988; Grabowski et al. 2019). Here, we conducted a series of experiments aimed at quantifying rates of bacterial production and dark C-fixation associated with sinking particles collected from the lower euphotic zone (175 m) waters of the subtropical North Pacific. In addition, we examined the phylogenetic identity of microorganisms associated with these sinking particles. Finally, we examined possible dependence in the rate of particulate C flux decay on particulate C flux supplied to the mesopelagic waters at Sta. ALOHA. Three general findings emerged from this work:

1. The addition of sinking particles increased both bacterial production and dark C-fixation relative to unamended seawater controls. Although relative changes (treatment/control) in microorganism production did not appear dependent on particle size, rates of bacterial production and dark C-fixation were greatest in the two smallest filter size fractions. When normalized to concentrations of particulate C, the resulting production rate constants were low and similar among the controls and particle-amended treatments. These results suggest that when scaled to concentrations of particulate C, rates of production by

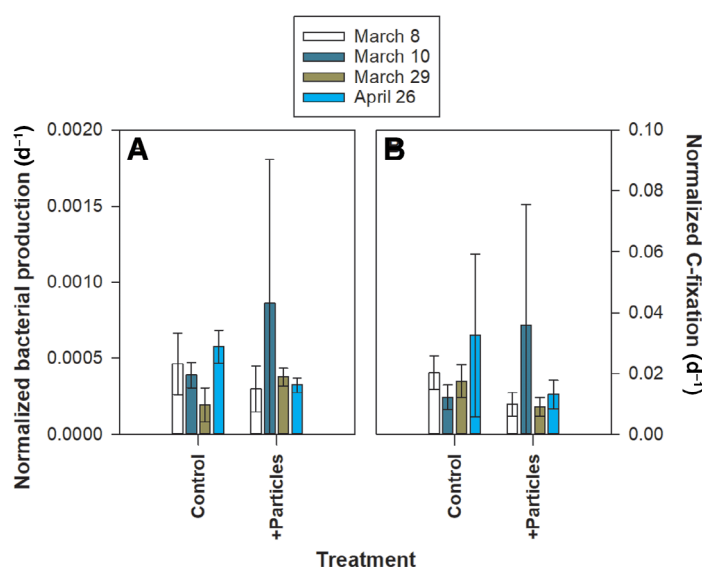


Fig 2. Particulate C-normalized rates (d⁻¹) of bacterial production (**A**) and C-fixation (**B**) for experiments conducted as part of this study. Mean normalized rate measurements from triplicate $>0.2 \mu\text{m}$ filter size fractions are depicted. Error bars are ± 1 SD of the mean normalized rates from triplicate carboys.

microorganisms associated with sinking particles are similar to production by “free-living” microorganisms together with those attached to suspended and slowly sinking particles in the surrounding waters.

2. Particle-amended treatments, particularly the larger filter size fractions ($> 2 \mu\text{m}$ and $> 20 \mu\text{m}$), were relatively enriched in putative chemoheterotrophic copiotrophs, including members of the *Gammaproteobacteria* (e.g., *Vibrio* and *Pseudoalteromonas*).
3. Leveraging historical HOT program measurements of particulate C flux in the lower euphotic zone and upper mesopelagic waters allowed us to examine temporal variability in particulate C flux attenuation with depth, revealing that the attenuation coefficients of particulate C flux decay (k) were positively related to particulate C supply to these waters. These results imply that increases in particulate C flux accelerate the vertical decay of this flux. When considered together with results from our experiments, these results point to processes other than microorganism production in shaping particle flux attenuation through the mesopelagic waters.

Microorganism production and community composition

In nearly all experiments conducted as part of this study, bacterial production and, to a lesser extent, dark C-fixation, were greater in the particle-amended treatments relative to unamended seawater controls. Rates of both processes were greatest in the smallest filter size fraction ($> 0.2 \mu\text{m}$) in both particle-amended treatments and controls. These results are

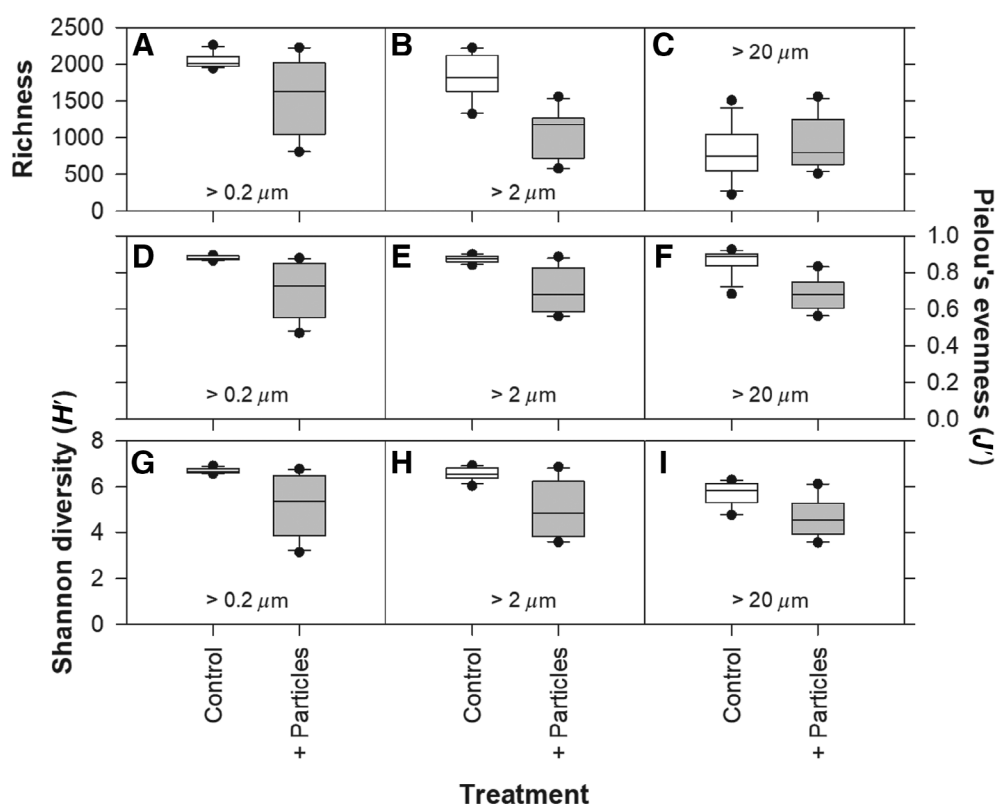


Fig 3. Box and whisker plots depicting richness (A–C), evenness (D–F), and Shannon diversity (G–I) of amplicon sequence variants in size fractionated particle-amended treatments and controls from all experiments. Indices of microorganism diversity from the >0.2 μm filter size fraction (A, D, G), the >2 μm size fraction (B, E, H), and the >20 μm size fraction (C, F, I). Edges of boxes depict upper and lower bounds 75% and 25% percentiles, whiskers depict 5% and 95% percentiles, and median values are shown by solid lines.

consistent with the parallel filtration procedures used for this study, where the > 0.2 μm filter fraction reflects cumulative rates of production, inclusive of the larger filter fractions. However, we also found that small particles account for a large fraction of particle-associated microorganism production; particle-associated rates of bacterial production and C-fixation in the > 2 μm filter fraction accounted for 31% and 40% of the cumulative production (> 0.2 μm), while rates in the > 20 μm filter fraction averaged only 13% and 15% of the cumulative bacterial production and C-fixation. Hence, although sinking particles of varying sizes (for this study > 0.2 μm to < 335 μm) appear to actively support microorganism production, small (< 20 μm), presumably slowly settling particles disproportionately supported microorganism production.

Small particles are increasingly recognized as important contributors to vertical export (Richardson and Jackson 2007; Alonso-González et al. 2010; Durkin et al. 2015). Although models generally predict small particles sink more slowly than large particles, several factors, including variation in particle density and morphology, can result in elevated sinking speeds by small particles (Stemmann et al. 2004; McDonnell and Buesseler 2010). Phytoplankton biomass at Sta. ALOHA is

dominated by small (< 1 μm diameter) picoplankton, specifically *Prochlorococcus* (Campbell et al. 1994; Rii et al. 2016), and small particles (< 10 μm in diameter) are abundant components of the suspended particle inventory (Barone et al. 2015) and comprise a substantial fraction of the sinking flux in the subtropical North Pacific Ocean (Close et al. 2013; Cael and White 2020). In addition to the size structure of the plankton community, numerous physical and biological processes can also reduce sinking particle size, including turbulent shear, zooplankton grazing, and microorganism enzymatic hydrolysis (Lam and Marchal 2015; Briggs et al. 2020). In the North Atlantic, these processes are hypothesized to be equal, or greater than, respiratory demand in controlling particle flux attenuation (Collins et al. 2015).

We used a net trap to collect sinking particles, followed by subsequent screening of particles through a 335- μm mesh to remove large zooplankton. We did not evaluate possible changes in particle size structure resulting from these methods to collect and concentrate particles; however, these sampling approaches could have physically fragmented or aggregated particles relative to natural particle size distribution. Nonetheless, we suggest that small, presumably slowly sinking particles support a large fraction of particle-associated microorganism

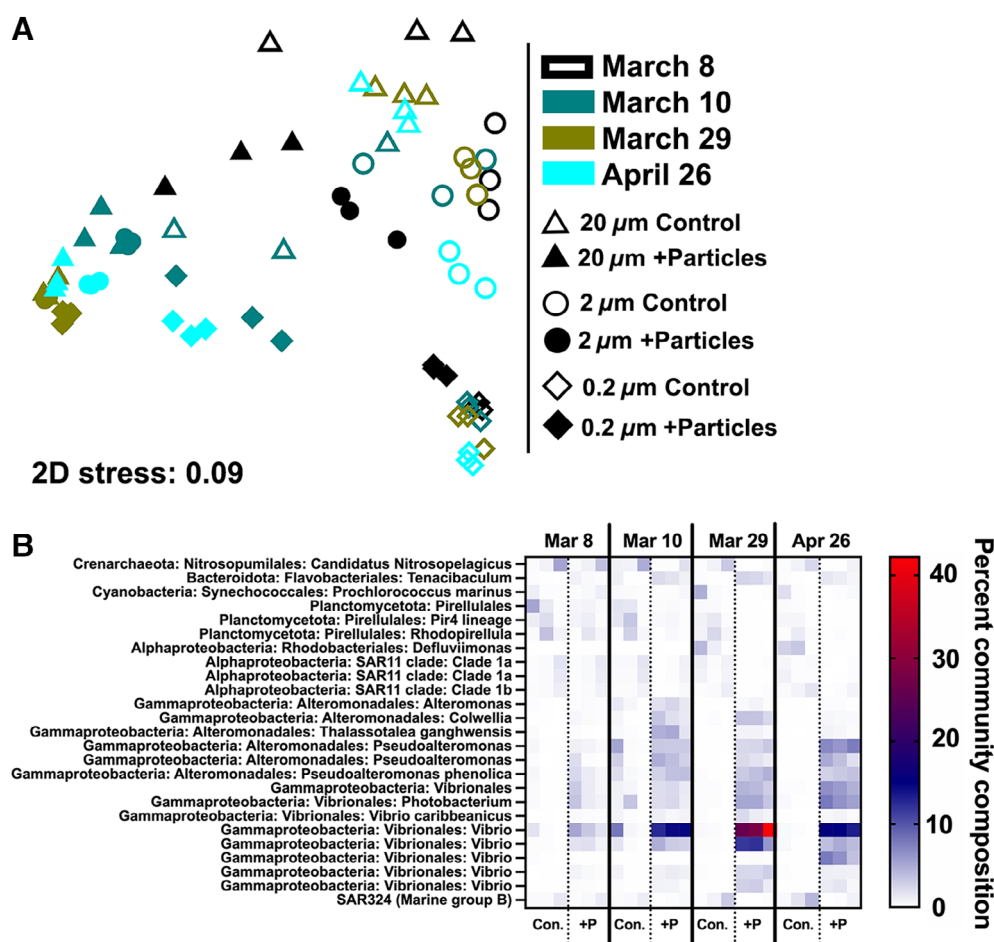


Fig 4. Patterns in microbial community composition. **(A)** Nonmetric multidimensional scaling ordination of all replicate samples, based on weighted UniFrac distance. **(B)** Heatmap of the relative abundance of the 25 overall most abundant amplicon sequence variants, averaged across triplicate samples. Reported taxonomy is phylum, order (class for *Proteobacteria*), genus, and species where determined. Within experiment and treatment groupings, filter sizes classes are in order: > 20 μm , > 2 μm , and > 0.2 μm from left to right.

production in the lower euphotic zone of the central North Pacific Ocean. Such findings are consistent with a study in the North Atlantic (conducted between 5°N and 35°N), where suspended particles were suggested to constitute an important source of C supporting mesopelagic bacterial metabolism (Baltar et al. 2009). However, at higher latitudes in the North Atlantic (49°N), microorganism growth rates associated with rapidly sinking particles were found to exceed growth rates of microorganisms associated with slowly or nonsettling particles (Baumas et al. 2021). We were unable to determine the extent to which small and large particles were colonized by microorganisms in our experiments because we did not measure abundances of microorganisms attached to sinking particles; however, when we normalized our rate measurements to measured particulate C concentrations (which would include the biomass contributions of particle-associated microorganisms), production rate constants in the particle-enriched treatments were similar to those in the unamended controls. Hence, our

results suggest comparable production rate constants (d^{-1}) among sinking particle-associated and free-living microorganisms, which in this study would likely include those associated with suspended or slowly sinking particles.

Microorganism assemblages differed in the particle-amended treatments relative to the controls, particularly among the larger filter size fractions. Such analyses provided insight into the organisms catalyzing measured rates of bacterial production and dark C-fixation in our experiments. The particle-amended treatments, particularly the larger filter size fractions, tended to be enriched in bacteria known for rapid growth, including putative copiotrophic heterotrophs belonging to the *Vibrionales*, *Alteromonadales*, and members of the *Flavobacteriales*. Such microorganisms occur in environments where concentrations of inorganic nutrients and organic C are enriched, including those associated with marine particles (Pedler et al. 2014). Moreover, such taxa can contain genomic features thought to facilitate growth on particles, including

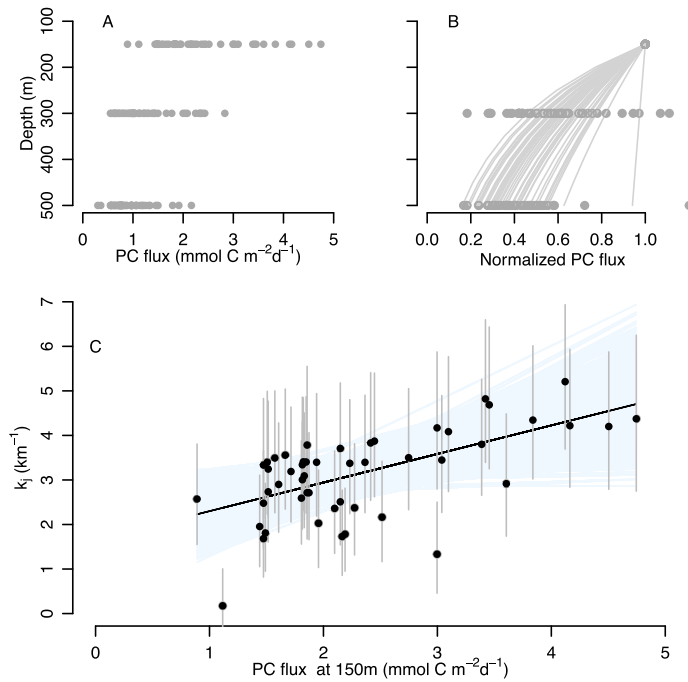


Fig 5. (A) HOTS program (December 1998–May 1995) measurements of sinking particulate C flux at three discrete depths (150 m, 300 m, 500 m). (B) Particulate C (PC) flux normalized to fluxes measured at 150 m, with each line representing an exponential decay model fit from Eq. 2. (C) Flux attenuation rates (k_j , km^{-1}) increased with increasing particulate C flux at 150 m for the time-series measurements. Points are estimates of k_j . Gray lines are the 95% credible intervals for each estimate of k_j . Line depicts linear model, $k_j = 0.64 (\text{PC flux}_{150\text{m}}) + 1.67$. Blue lines represent the uncertainty in the fit of this linear model to the estimates of k_j ; 1000 lines were derived from the joint probability distribution of the slope and intercept. Credible interval for the slope parameter, γ was -1.1 to 0.2 and the intercept a was -2.7 to 0.6 , indicating high confidence for a positive relation between PC flux at 150 m and the attenuation rate coefficients k_j .

genes specific to motility, surface adhesion, and enzymes specific to degradation of polysaccharides like chitin (López-Pérez et al. 2012; Datta et al. 2016; Bižić-Ionescu et al. 2018).

Previous studies examining particle-attached microorganisms, including those at Sta. ALOHA, have found elevated relative abundances of many of the same copiotrophic genera we describe in our particle-amended treatments (Fontanez et al. 2015; Farnelid et al. 2019; Baumas et al. 2021). Intriguingly, although sinking particles were selectively enriched in these putative copiotrophs, in our study, the particulate C normalized rates of production from our particle-amended treatments were similar to controls. We expected to observe elevated rates of production in the larger filter size fractions that were often enriched in putative copiotrophs; however, the parallel filtration approach used for this study may have obscured biomass production by such microorganisms. Normalizing to particulate C concentrations assumes that the concentration of particles, rather than concentrations of dissolved substrates (e.g., dissolved organic C), regulates variability in

microorganism production in both the treatments and controls; however, in these experiments, we did not observe large changes in dissolved organic C concentrations following the addition of sinking particles.

The finding that most bacterial production was associated with the smallest filter size fraction suggests “free-living” bacteria, inclusive of those associated with suspended and slowly settling particles, were important contributors to productivity in this ecosystem. Hence, our experimental results highlight the importance of processes that catalyze the transfer of organic matter from larger sinking particles to dissolved phase or more slowly settling and suspended particles. Microorganism colonization and initial particle solubilization and disaggregation may have occurred during the period when the net trap sampler was deployed (24 h), thereby reducing the availability of labile substrates supporting production by the copiotrophic bacteria. We do not know the time history of particles collected and used in our incubations, and microorganisms associated with particles appear to undergo successional patterns, often linked to coupled metabolic interactions (Datta et al. 2016; Bižić-Ionescu et al. 2018). Hence, the particles used in these experiments could have been produced several days to weeks prior to our sampling; as such, these particles may have previously been colonized by rapidly growing copiotrophic microorganisms in the upper euphotic zone, but by the time (i.e., depth) we sampled these particles production by these microorganisms had slowed due to removal of labile components of the particle flux.

Our motivation for quantifying both bacterial production and dark C-fixation stemmed from interests in assessing the extent to which rapidly sinking particles contributed to different forms of microorganism production in the mesopelagic waters. The transition from the well lit upper ocean to the dimly lit deeper waters coincides with vertical shifts in microbial metabolism and community structure, including increased prevalence of chemoautotrophic metabolisms (DeLong et al. 2006; Swan et al. 2011). Chemoautotrophic microorganisms in the dimly lit regions of the ocean appear to derive energy from oxidation of diverse reduced inorganic substrates (e.g., NH_4^+ , NO_2^- , SO_3^{2-}), presumably released as by-products of chemoheterotrophic catabolism (Swan et al. 2011; Pachiadaki et al. 2017). Given the short time scales (< 24 h) of our experiments, accumulation of catabolic by-products derived from organic matter decomposition would likely be low, although such degradation by-products could have been present at the onset of our experiments. Nonetheless, we observed low relative abundances of putative obligate or facultative chemoautotrophic microorganisms (e.g., *Candidatus Nitrosopelagicus*, members of the SAR324 clade) in the particle-amended treatments relative to the controls. Dark $^{14}\text{CO}_2$ fixation includes both autotrophic and heterotrophic assimilatory synthesis and anaplerotic uptake pathways, and diverse and abundant lineages of putative chemoheterotrophs appear to fix CO_2 (Herndl et al. 2005; Pachiadaki et al. 2017), including

some of the microorganisms enriched in the particle-amended treatments in the current study (e.g., *Gammaproteobacteria*, *Flavobacteriales*).

In our experiments, rates of dark C-fixation were consistently greater (by 20- to 95-fold) than coincident measurements of bacterial production in both the unamended controls and particle-amended experiments. Rates of dark C-fixation can rival or exceed bacterial production (Alonso-Sáez et al. 2010; Baltar et al. 2016). The derived rates of bacterial production estimated from the unamended controls are similar to previous measurements of bacterial production at Sta. ALOHA (Viviani and Church 2017). Rates of dark C-fixation in the controls (ranging 11–58 nmol C L⁻¹ d⁻¹) were somewhat greater than historical (1989–2000) HOT program dark C-fixation determinations (ranging 3–39 nmol C L⁻¹ d⁻¹) measured at 175 m (Laws et al. 2014). However, there are several method differences between the dark C-fixation measurements in our experiments and those conducted by the HOT program. Specifically, for our experiments, we incubated samples for 24 h in temperature-controlled shipboard incubators, while the HOT program dark C-fixation measurements utilized dawn to dusk (~12 h) incubations conducted in situ on a free-drifting array (Karl et al. 1996).

The large differences between bacterial production and dark C-fixation might reflect methodological uncertainties, in particular, those associated with determinations of bacterial production based on measurements of ³H-leucine incorporation (e.g., isotope dilution or appropriate C conversion factors). We assumed 0.44 kg C mol⁻¹ leucine incorporated, a value used in other studies of open ocean mesopelagic zone bacterial production (Giering et al. 2014). Empirically determined C conversion factors for mesopelagic waters are highly variable, but the factor we selected is consistent with factors obtained from the mesopelagic waters of the North Atlantic (Gasol et al. 2009; Baltar et al. 2010). Differences in the duration of incubations used to derive rates of bacterial production and dark C-fixation in our study also complicate direct comparison between these rates. Bacterial production incubations lasted 4 h, while dark C-fixation incubations were 24 h in duration. Longer incubation times might allow for greater trophodynamic transfer of fixed C into the food web; however, respiratory demands associated with such dynamics would be expected to reduce the measured C-fixation. Another factor complicating comparison of the rates is the potential contribution of phytoplankton to C-fixation. Our experiments were conducted near the base of the euphotic zone (175 m), so phytoplankton likely contributed to dark C-fixation (Morris et al. 1971); not knowing relative contribution of this process makes attribution of specific processes to the determinations of dark C-fixation difficult.

Our finding that production rate constants in particle-amended treatments were similar to those derived from the unamended controls suggests C-specific microorganism production rates associated with sinking particles were similar

those of “free-living” microorganisms (inclusive of those associated with suspended and slowly sinking particles). Such findings point to processes that fragment sinking particles (e.g., turbulent shear, zooplankton grazing) and facilitate the solubilization of particulate matter, generating diffusible dissolved substrates to fuel the metabolism of microorganisms in the surrounding waters (Smith et al. 1992; Azam and Malfatti 2007). Results from the current study suggest that small particles actively support microorganism production, consistent with the view that small particles fuel the metabolism of microorganisms in the dimly lit lower euphotic zone.

We also found that the particulate C-specific rates of bacterial production, inclusive of both controls and particle-amended treatments, were relatively invariant between experiments, averaging 4.4×10^{-4} d⁻¹, equivalent to a mean turnover time (the time for complete consumption of particles) of nearly 2300 d. However, this turnover time includes only C assimilated into microorganism biomass and does not include respiratory consumption of organic C. With some assumptions of bacterial growth efficiency (i.e., bacterial production/[bacterial production + bacterial respiration]), we can estimate total bacterial C demand (bacterial production + bacterial respiration). Bacterial growth efficiencies in mesopelagic waters are highly uncertain, with published estimates typically ranging between 0.1% and 13% (Carlson et al. 2004; Reinthaler et al. 2006; Collins et al. 2015). By applying a bacterial growth efficiency of 5% to the measured bacterial production in this study, a value similar to empirically derived bacterial growth efficiency obtained in the North Atlantic Ocean (Baltar et al. 2010), we obtain a C-normalized rate of bacterial C demand of 8×10^{-3} d⁻¹ (on average), equivalent to a mean turnover time of ~120 d. This estimate assumes particle consumption occurs exclusively via bacterial metabolism and ignores the contributions of respiratory activities deriving from other members of the plankton community (i.e., protistan grazers, metazoans). Previous studies, based on measurements of microbial respiration associated with sinking particles or particle-aggregates, have reported C-specific particle turnover times that range from <1 d to >1000 d (Smith et al. 1992; Ploug and Grossart 2000; Collins et al. 2015). In the North Atlantic, C-normalized rates of particle-associated microbial respiration have been estimated between 0.02 d⁻¹ and 0.058 d⁻¹, equivalent to particle turnover times of approximately 17–50 d (Collins et al. 2015; Belcher et al. 2016). In the Southern California Bight, Smith et al. (1992) found particle aggregates were sites of rapid bacterial enzymatic activity; however, similar to our findings, these authors also noted that particle-associated bacteria demonstrated low C demand, with aggregate turnover times ranging between 403 and 1429 d (Smith et al. 1992). The relatively long turnover times estimated as part of the current study may reflect uncertainties associated with the leucine to C conversion factors and our estimates of bacterial growth efficiency, or both.

The relatively low particle-associated production rate constants derived from our study point to processes other than microbial metabolism as potentially important controls on particle flux attenuation. Such processes could include those that solubilize organic matter (e.g., extracellular enzymes which can transform particles to dissolved phases) or processes that result in reduction of particle size and result in transforming particles from actively sinking to suspended (e.g., zooplankton metabolism and fragmentation; Steinberg et al. 2008).

Relationship between particle flux attenuation and supply

The finding that both bacterial production and dark C-fixation scaled proportionally with the amount of particulate C added to the experimental treatments suggests that production rate constants of microorganisms associated with particles do not depend on the amount of particulate C available. This observation led us to predict that if microorganism production was a key control on rates of sinking particle decomposition, attenuation coefficients (k_j ; km^{-1}) describing particle flux decay would also not depend on variations in particulate C flux. Using analyses of sediment trap-derived particulate C fluxes at Sta. ALOHA, we found considerable temporal variability in particulate C flux attenuation, with k_j varying between 0.2 and 5.2 km^{-1} , and a mean attenuation length scale ($1/k_j$) of 0.33 km. These length scales are similar to those for the subtropical and high-latitude North Atlantic based on neutrally buoyant sediment trap measurements (ranging between 0.14 and 0.26 km; Marsay et al. 2015). The same study estimated attenuation length scales between 0.18 and 0.22 km for Sta. ALOHA. Importantly, contrary to our expectation, k_j positively covaried with particulate C flux at 150 m, implying flux attenuation increased (i.e., the attenuation length scale decreased) with increases in the supply of sinking particulate C into these waters. Such results suggest that the magnitude of sinking particle flux influences the efficiency of flux attenuation in the upper mesopelagic waters. A synthesis of particulate C export measurements from various regions across the global ocean (Arabian Sea, Southern Ocean, Equatorial Pacific, and North Atlantic) observed a similar positive correlation between export measured at 100 m and the derived “ b ” value from the empirical Martin et al. (1987) power-law formulation (Berelson 2001). Hence, despite our experimental results indicating that particle-associated microorganism production rate constants (d^{-1}) were largely invariant to changes in the amount of particulate C added to each experiment, sediment trap measurements revealed that particle attenuation through the mesopelagic zone appears to depend, in part, on the supply of material to these waters.

It is not clear which mechanisms explain the positive relationship between particulate C flux and k at Sta. ALOHA. This relationship could reflect microorganism colonization of larger particles facilitating particle disaggregation or solubilization, thereby reducing particle sinking speeds and increasing the

decay rate of sinking particulate C flux. Alternatively, these results could reflect time-varying changes in the lability of sinking organic matter supplied under periods of high and low flux. High flux periods might be expected to coincide with delivery of more labile material, due to faster sinking particles or through increased particle concentrations. Such conditions could be associated with phytoplankton blooms, which can introduce recently produced, presumably labile particles to the mesopelagic waters (Lam et al. 2011). Increased flux attenuation during periods of elevated sinking flux could reflect priming of organic matter mineralization, a process where labile organic matter stimulates microorganism decomposition of older, more recalcitrant organic matter (Fontaine et al. 2007; Guenet et al. 2018). Organic matter priming has been intensively studied in soils, but its importance in the decomposition of marine organic matter is less well known (Bianchi 2011).

We suggest our results point to microorganisms playing central roles in mineralization of small, slowly settling particulate material, with microbial growth associated with larger, sinking particles linked to disaggregation processes. Models describing the transfer of organic matter into the interior waters of the ocean highlight the importance of particle size as a key control on flux attenuation through the mesopelagic zone (DeVries et al. 2014; Cram et al. 2018; Omand et al. 2020). The overall low particulate C-specific production rate constants derived from our measurements hint that whatever processes contribute to production of small particles, such particles appear nutritionally or energetically impoverished. Together, our observations suggest that during times of increased particulate C flux, particle attenuation increases, potentially transforming larger, sinking particles into slowly sinking or suspended particles, facilitating microorganism growth and intensifying flux attenuation.

Although the ~ 6.5 yr of near-monthly observations from which we derived information on flux attenuation appear representative of the dynamic range of particulate C export for this ecosystem, there is evidence, based on near-continuous sampling by bottom-moored sediment traps in the bathypelagic waters (2000 and 4000 m), that rapidly sinking particles undergo much less mineralization on their transit through the mesopelagic waters at Sta. ALOHA (Karl et al. 2012; Grabowski et al. 2019). These export events appear limited to a relatively short period during the mid-to-late summer; however, such events do not appear well represented by the near-monthly scale upper ocean (150 m) sediment trap collections of the HOT program (Karl et al. 2012). Such export events likely are not well represented in the time-series measurements we used to derive flux decay estimates, and such events would likely not demonstrate patterns consistent with the observed relationship between particle decay rates and export flux through the mesopelagic waters. In a study quantifying vertical changes in energy content and elemental content of sinking particles at Sta. ALOHA, Grabowski et al. (2019) concluded

that sinking particles can generally be divided into two types: those that sink rapidly and escape mineralization through the meso- and bathypelagic waters, and those that sink slowly and undergo intensive transformation and mineralization in the mesopelagic waters. We suspect our results are specific to this latter class of sinking particles.

Relative controls on particulate C flux

We found that sinking particles supported active rates of both bacterial production and C-fixation, and based on sequencing of 16S rRNA genes, these particles were inhabited by putative copiotrophic microorganisms that are distinct from the surrounding seawater. Moreover, in our experiments, most of the particle-associated microorganism production was associated with small particles ($<20\ \mu\text{m}$). Notably, when normalized to concentrations of particulate C, rate constants associated with microorganism production were invariant, implying increases in the particle production scaled proportionately with the concentration of particulate C. These findings led us to expect that if microorganisms were the primary agents responsible for catalyzing the decay of sinking particulate C flux, flux decay rate constants (k_j) would be independent of the particulate C flux supplied to the mesopelagic waters. In contrast to our expectations, we found that through the upper mesopelagic waters (150–500 m), k_j increased with the particulate C flux measured at 150 m. Such results could reflect a priming-like dynamic where increased organic matter supply leads to increased flux attenuation; alternatively, these results may suggest that processes other than production of microorganism biomass regulate variability in depth-dependent changes in particulate C flux attenuation. Our findings point to particle-associated microorganism growth playing a key role in mineralization of small, slowly settling particles, with rates of production that scale proportionally with particulate C flux supply. However, during periods of elevated flux, where larger, faster sinking particles presumably constitute a greater fraction of the sinking flux, particle consumption and disaggregation catalyzed by zooplankton, together with copiotrophic microorganisms, act to accelerate the attenuation of sinking flux.

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Conflict of Interest

None declared.

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