Microbial community structure and function on sinking particles in the North Pacific Subtropical Gyre

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Microbial community structure and function on sinking particles in the North Pacific Subtropical Gyre

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Sinking particles mediate the transport of carbon and energy to the deep-sea, yet the specific microbes associated with sedimenting particles in the ocean's interior remain largely uncharacterized. In this study, we used particle interceptor traps (PITs) to assess the nature of particle-associated microbial communities collected at a variety of depths in the North Pacific Subtropical Gyre. Comparative metagenomics was used to assess differences in microbial taxa and functional gene repertoires in PITs containing a preservative (poisoned traps) compared to preservative-free traps where growth was allowed to continue in situ (live traps). Live trap microbial communities shared taxonomic and functional similarities with bacteria previously reported to be enriched in dissolved organic matter (DOM) microcosms (e.g., Alteromonas and Methylophaga), in addition to other particle and eukaryote-associated bacteria (e.g., Flavobacteriales and Pseudoalteromonas). Poisoned trap microbial assemblages were enriched in Vibrio and Campylobacterales likely associated with eukaryotic surfaces and intestinal tracts as symbionts, pathogens, or saprophytes. The functional gene content of microbial assemblages in poisoned traps included a variety of genes involved in virulence, anaerobic metabolism, attachment to chitinaceous surfaces, and chitin degradation. The presence of chitinaceous surfaces was also accompanied by the co-existence of bacteria which encoded the capacity to attach to, transport and metabolize chitin and its derivatives. Distinctly different microbial assemblages predominated in live traps, which were largely represented by copiotrophs and eukaryote-associated bacterial communities. Predominant sediment trap-associated eukaryotic phyla included Dinoflagellata, Metazoa (mostly copepods), Pratalveolata, Retaria, and Stramenopiles. These data indicate the central role of eukaryotic taxa in structuring sinking particle microbial assemblages, as well as the rapid responses of indigenous microbial species in the degradation of marine particulate organic matter (POM) in situ in the ocean's interior.

Keywords: metagenomics, marine particles, sediment trap, biological pump, microbiology
Introduction

Particulate organic matter (POM) generated in the euphotic zone is the major conduit of matter and energy transport to the deep sea and also represents the primary mechanism of carbon removal from surface waters via the biological pump (McCave, 1975; Volk and Hoffert, 1985). POM is operationally defined as particles ranging from 0.1 µm to centimeters in size, and is further qualitatively subcategorized into macroaggregates (marine snow; centimeters to 500 µm in diameter), microaggregates (500–1 µm), and submicron particles (1–0.1 µm) (Simon et al., 2002). Sinking POM can be collected in situ using sediment traps that contain saline solutions slightly denser than seawater that retain sinking particles (Knauer et al., 1979). This broad size spectrum of POM harbors a diverse and complex variety of inorganic as well as living and non-living organic materials (Volkman and Tanoue, 2002; Nebbioso and Piccolo, 2013).

Much of the current knowledge of POM-degrading microbial communities is derived from studies of suspended POM. Analysis of whole seawater segregated into particle-associated (>1 µm) and free-living size fractions has revealed taxonomically and functionally distinct microbial communities in marine anoxic zones (Ganesh et al., 2014), coastal ecosystems (Allen et al., 2012; Smith et al., 2013), estuarine environments (Crump et al., 1999; Waidner and Kirchman, 2007), inland seas (Moeseneder et al., 2001; Fuchsmann et al., 2011, 2012; Crespo et al., 2013), phytoplankton blooms (Riemann et al., 2000; Fandino et al., 2005; Teeling et al., 2012), ocean trenches (Elloe et al., 2011), and the open ocean (Kellogg and Deming, 2009; Allen et al., 2012). These studies have shown that in particular, members of the Bacteroidetes, Planctomycetes, and Deltaproteobacteria are often enriched in larger particle size fractions. Studies of microbial community composition on sinking particles are less extensive than those on suspended particles. Research programs such as the Vertical Transport and Exchange (VERTEX)(Martin et al., 1987) and VERtical Transport In the Global Ocean (VERTIGO) (Buesseler et al., 2008) supported diverse process-oriented studies that revealed the importance of chemolithotrophs like nitrifiers (Karl et al., 1984), organotrophs (Boyd et al., 1999), and exoenzyme-driven degradation on sinking particles (Smith et al., 1992). These findings laid the foundation for phylogenetically-oriented studies that suggested that Bacteroidetes, Planctomycetes, and Roseobacter can act as sinking particle colonizers in the upper water column (DeLong et al., 1993; LeClerc et al., 2013). While sediment traps have proven useful for over 30 years in studies of sinking POM (Karl and Knauer, 1984), to date there exists only one report of the phylogenetic diversity of sediment-trap collected microbes, which grew over 24 h in sediment-trap captured particles from 100 to 120 m (LeClerc et al., 2013).

Given the diverse sources and sinks of sinking particles in the ocean's interior (Honjo et al., 2008), much remains to be learned about the microbes and processes that regulate the degradation of sinking POM. In this study, we sought to examine the nature of sinking particles collected in poisoned traps, which we hypothesized would help preserve sinking materials and allow us to identify (using metagenomics) the sources of larger sinking particulates including larger eukaryotes that are known to aggregate and sink to the deep-sea. We also included paired, un-poisoned traps (live) in our experiments, postulating that these might reveal the nature and identity of microorganisms capable of growth on the collected organic material at the in situ temperatures and pressures of trap deployment. We reasoned that the phylogenetic identity of poisoned vs. live traps would reveal the identity and genomic potential of microbes capable of growth on sinking particulate organic materials in situ in the ocean's interior.

Materials and Methods

Sample Collection

A free-drifting sediment trap array identical to those used in the VERTEX and HOT field programs (Knauer et al., 1979) was deployed at station ALOHA (22.75°N, 158°W) in the North Pacific Subtropical Gyre on July 14, 2012. Each trap tube (cross sectional area of 0.0039 m$^2$) was filled with approximately 1.8 liters of either an 0.2 µm-filtered brine solution (Knauer et al., 1984) (“live“) or an 0.2 µm-filtered RNA later solution (“poisoned”) adjusted to a density of 1.05 g/cc (see Supplementary Material, for further methodological details on trap solutions). Both sets of traps were fitted with a 335 um Nitex screen below the topmost baffle in order to exclude larger zooplankton. The array drifted north-west for 75 nautical miles before recovery on July 26, 2012. Prior to filtration, the 335 µm Nitex screen was removed along with approximately 500 mL of seawater overlying the higher density hypersaline trap solution. Following recovery, particles in the 0.2–335 µm fraction were collected on Sterivex filters (EMD Millipore, Billerica, MA, USA) and preserved with 1.5 mL RNA later (Ambion, Millipore, Billerica, MA, USA). Filters were stored at −80°C prior to nucleic acid isolation.

Microscopy

Epifluorescence and Optical Microscopy

For each depth, 10 mL of fixed sediment trap sample (2% formaldehyde final concentration) was filtered onto black 0.2 µm pore size polycarbonate filters and allowed to dry completely. Ethanol cleansed surgical scissors were used to cut 1/8 pieces from each filter and four pieces were then positioned on a microscope slide (Fisherbrand Superfrost precleaned microscope slides #12-550-143). A 24 x 50 mm coverslip with #1.5 thickness was placed below the slide. Antifade mounting medium (Patel et al., 2007) containing 1 µg mL$^{-1}$ of nucleic acid stain 4′,6-diamidino-2-phenylindole (DAPI) was spotted on the coverslip (15 µL) to align with the filter pieces. The coverslip was inverted onto the slide and the filters were stained for 10 min. Filters were visualized at 1000x total magnification on a Nikon 90i epifluorescence microscope with excitation/emission settings for DAPI, chlorophyll, and phycocerythrin. Images were acquired with a QImaging Retiga EXi camera using optimized exposure times and analyzed with Nikon NIS-Elements software.

For optical microscopy, unmounted filters were visualized through a Nikon AZ100 Multizoom microscope with a 10x objective at 20x and 40x magnification (zoom settings 4 and...
using a Nikon NI-150 illuminator. Images were captured via NIS-Elements software using a Nikon DS-Fi1 camera.

**Library Preparation and Sequencing**

Approximately one-half of each Sterivex filter was used for extraction of total community DNA using the Powerwater DNA isolation kit (Mobio, Carlsbad, CA, USA), with modifications (see Supplementary Material for details). Library preparation followed the Nextera XT DNA sample preparation protocol (Illumina, San Diego, CA, USA). Samples were dual indexed and 10 samples pooled per sequencing run on a MiSeq using MiSeq reagent kit v3 (Illumina, San Diego, CA, USA). Sequencing and quality control followed the manufacturer's recommendations.

**Sequence Analysis and Annotation**

Sequencing and annotation statistics for sediment trap and seawater samples are summarized in Table A1 in Supplementary Material. Metagenomic sequences were filtered with Trimmomatic (Bolger et al., 2014), PandaSeq (Masella et al., 2012), and SortMeRNA to identify rRNA-containing reads (Kopylova et al., 2012) as previously described (Lincoln et al., 2014) with one modification. Unjoined read pairs output from PandaSeq were joined with 6 N's and tracked along with paired reads. The taxonomic origins of rRNA and non-rRNA reads were determined by comparison against SILVA release 115 and the NCBI RefSeq release 61 databases, respectively, using lastal (Kiełbasa et al., 2011). Function of non-rRNA reads was determined by comparison with the September 2013 version of the Kyoto Encyclopedia of Genes and Genomes (KEGG) (Kanehisa and Goto, 2000) database using lastal and the March 22, 2013 version of the Carbohydrate Active Enzyme (CAZy) database (Cantarel et al., 2009) using HMMER3.0 (Eddy, 2011) and hidden Markov models of CAZy signature domains (Yin et al., 2012). See Supplementary Material for further details.

**Statistical Analyses**

All metagenomic sequence counts were normalized and variance stabilized using the regularized log transformation in DESeq2 (Love et al., 2014). Ordination of normalized sequences used principal coordinate analysis with Bray-Curtis distance in the phyloseq R package (McMurdie and Holmes, 2013). Significance \( (p < 0.05) \) of clusters was determined using non-parametric analysis of variance based on dissimilarities in the vegan R package (Dixon, 2003). A negative binomial Wald test in DESeq2 was used to identify statistically significant differences in taxonomic and functional non-normalized gene counts among live traps, poisoned traps, and seawater (data not shown). The presence of copepods in live and poisoned traps was also confirmed by optical microscopy (Figures 1G,H). As replicates of sediment traps at each depth were not available, all four depths belonging to a treatment were modeled as biological replicates. A false discovery rate threshold of 0.01 was used for detecting differentially abundant taxa or functions. For statistical validation of depth-specific taxonomic differences, Fisher’s exact test as implemented in the STAMP v2.01 program (Parks and Beiko, 2010) was used for pairwise comparisons of 150 m vs. 500 m RefSeq-identified non-normalized taxa within treatment types. A false discovery rate threshold of 0.05 and a difference between proportions cutoff of 1 were used to assess statistical and biological significance, respectively.

**Sequence Data**

The sequences reported in this paper have been deposited in the Genbank Short Read Archive (Bioproject PRJNA270248).

**Results and Discussion**

Sinking particles were collected using a free-drifting sediment trap array deployed in the North Pacific Subtropical Gyre, from
the base of the photic zone and into the mesopelagic (150, 200, 300, and 500 m (Figure A1 in Supplementary Material)). The sediment traps included two treatments: a “live” trap which contained a solution of sterile seawater adjusted to a density of 1.05 g/cc with NaCl and a “poisoned” trap which contained a preservative to prevent in situ growth and preserve DNA. Fluorescence microscopy of trap-collected POM revealed bacteria associated with chlorophyll-containing particles in both live and poisoned sediment traps (Figures 1A-D–F). Bacteria in the sediment traps were either unattached, loosely surrounding protists (Figure 1C), or directly associating with amorphous particles (Figure 1D). Unattached bacteria were most abundant in 150 m live traps likely due to the opportunity for enhanced growth during deployment (Figure 1E).

**Domain Level Taxonomic Composition in Live vs. Poisoned Sediment Traps**

DNA was extracted and shotgun sequenced from both live and poisoned sediment trap particulates collected at the base of the photic zone and into the mesopelagic (Figure A1 in Supplementary Material) to determine the taxonomic and functional diversity associated with sinking particles. The diversity of the particle-associated microbes at the domain level indicated the dominance of Bacteria and Eukarya in all traps at all depths sampled (Figure 2). Archaea represented less than 10% of total in both live and poisoned sediment trap microbial assemblages at all depths (Table 1).

Eukaryotic rRNA genes were more highly represented than identifiable protein-encoding sequence reads, likely due to the inherently lower gene density in eukaryotes vs. bacteria, as well as the lack of closely related eukaryotic reference genomes for a number of highly represented taxa. Notably, the percentage of total annotated genes in poisoned traps was consistently lower than that of live traps (Table A1 in Supplementary Material). This result most likely reflects the higher relative abundance of eukaryotic DNA in the poisoned traps compared to live traps. In total, these results are consistent with previous observations that particles are more enriched in eukaryote-associated and unclassified protein-coding genes (Allen et al., 2013; Smith et al., 2013; Ganesh et al., 2014). The decreased ratio of eukaryote-to-bacteria DNA in the live traps was apparently due to in situ microbial growth in the trap during the deployment as POM underwent decomposition, and possibly concomitant degradation of the particle-associated eukaryotic nucleic acids.

Principal coordinate analysis of protein-coding genes indicated the presence of distinctive communities in live traps, poisoned traps, and surrounding seawater (p < 0.001) and a much lesser effect of depth on community composition (Figure 3). We therefore focused subsequent analyses on the taxonomic and functional differences between live and poisoned sediment traps with minor attention given to depth-related differences.

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**Live vs. Poisoned Sediment Trap Bacterial Assemblages**

The most abundant bacterial genera in the live traps were affiliated with the order *Alteromonadales* (*Alteromonas, Marinobacter, Moritella*, and *Pseudoalteromonas*), in contrast to poisoned sediment traps where *Vibrio* was the most highly represented genus (Figure 4A). These trends were consistent across all sampled depths except 500 m, where in the poisoned traps there was a lower more even distribution of different bacterial genera.

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**FIGURE 2** | The relative abundance of Archaea, Bacteria, and Eukarya in live (filled bars) and poisoned (striped bars) sediment traps at the indicated depths as determined by the taxonomic identifications of small subunit ribosomal RNA genes (rDNA) and protein coding genes.
TABLE 1 | The relative abundance of Bacteria, Eukaryota, and Archaea in live (L) and dead (D) sediment traps.

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<td>1.9</td>
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aNormalized relative abundance determined by rDNA, small subunit ribosomal RNA genes.
bNumbers preceding L and D refer to sediment trap depths in meters (150, 200, 300, and 500 m).
cNormalized relative abundance determined by protein-coding genes.

bacterial groups, that included *Pelagibacter* which is typically more abundant in seawater and much less so on particles. The detection of *Prochlorococcus* DNA at 500 m in the poisoned traps may reflect their entrainment on particles and subsequent transport into deeper waters, perhaps reflecting the positive correlation between picophytoplankton productivity and their export to the deep sea (Richardson and Jackson, 2007).

Significant depth-related partitioning in bacterial abundance in the live traps was detected for *Alteromonadales*, with *Alteromonas*, *Moritella*, and *Glaciecola* significantly enriched at 500 m and *Pseudoalteromonas* and *Marinobacter* enriched at 150 m (Figure 5A). In the poisoned traps, *Vibrio* was also enriched at 150 m (Figure 5B). Depth-related partitioning, also observed in seawater (Figure 5C), may be linked to different lifestyles of the bacteria. *Marinobacter* and *Pseudoalteromonas* are known to associate with eukaryotes (Thomas et al., 2008; Gärdes et al., 2010) and their enrichment at 150 m could be due to their attachment to eukaryotic biomass originating from the euphotic zone. Many alteromonads are typical *r*-strategists, capable of multiplying rapidly in response to nutrient-rich particles and *Alteromonas* species have been found in suspended particle fractions in a variety of ocean basins (García-Martínez et al., 2002; López-Pérez et al., 2012). This may explain their overall high abundance in live traps and enrichment on 500 m particles (Figures 4A, 5A) where they outcompete *k*-strategists like *Pelagibacter* which are generally more abundant in seawater at this depth at Station ALOHA (DeLong et al., 2006).

At all depths, the live traps were significantly enriched in bacterial taxa previously implicated with particle-association, hydrocarbon and dissolved organic matter (DOM) degradation, and eukaryote associations. There was a significant enrichment of *Oceanospirillales*, *Flavobacteriales*, and *Alteromonadales* in live traps (Figure 4B and Figure A3 in Supplementary Material). Taxa within the *Bacteroidetes*, including *Cytophaga* and *Flavobacteria*, are often found associated with marine snow and phytoplankton blooms in marine environments (DeLong et al., 1993; Teeling et al., 2012). Many *Flavobacteriales* such as *Zobellia*, *Gramella*, *Formosa*, and *Cellulophaga* specialize in algal-derived organic matter degradation (Bauer et al., 2006; Bowman, 2006; Mann et al., 2013), which may be an ancestral trait of this group (Thomas et al., 2012). *Methylophaga*, *Alteromonas*, *Idiomarina*, *Glaciecola*, *Rheinheimera*, *Polaribacter*, and *Formosa* were also enriched in the live traps, and have also been detected in DOM enrichment events such as phytoplankton blooms and experimental microcosms (Brettar et al., 2006; McCarren et al., 2010; Teeling et al., 2012; von Scheibner et al., 2013). These bacterial types were either growing directly on POM, or on DOM generated in situ from POM.
Genera with at least 2% mean abundance in traps relative to all sequences (including Archaea and Eukarya) in live (L, filled columns) and poisoned (P, striped columns) sediment traps at the indicated depths. Alt, Alteromonas; Pel, Candidatus Pelagibacter; Mar, Marinobacter; Mor, Moritella; Pro, Prochlorococcus; Pse, Pseudoalteromonas; Vib, Vibrio.

The poisoned traps were significantly enriched in chemosynthetic bacterial types and those with eukaryote-associated lifestyles (Figure 4B and Figures A3, A4 in Supplementary Material). These included epsilon-proteobacteria, particularly Campylobacteriales, in the poisoned traps (Figure 4B, Figure A3 in Supplementary Material). Presumptive chemosynthetic sulfur-oxidizing bacteria including Sulfurimonas, Sulfurovum, and Sulfuricurvum (Campbell et al., 2006) were also enriched in the poisoned traps (Figure 4B, Figure A3 in Supplementary Material). Sulfurovum and other epsilon-proteobacterial species have also been found as endosymbionts of marine invertebrates in both hydrothermal vent and coastal environments (Goffredi, 2010; Ruehland and Dubilier, 2010) suggesting a diverse habitat range for these bacteria on metazoan surfaces in niches where both reduced sulfur compounds and oxygen are readily available. Nitratiruptor and Nitratifractor are chemolithotrophic hydrogen-oxidizing denitrifiers (Nakagawa et al., 2005) that were also enriched in the poisoned traps (Figure 4B, Figure A3 in Supplementary Material). Denitrification is not considered to
be a significant process in well-oxygenated seawater; however it may occur in anoxic microniches within large particles (Karl et al., 1984) or more likely within the intestinal tracts of decaying zooplankton carcasses that have been shown to provide anoxic niches for marine bacteria (Tang et al., 2011; Bickel and Tang, 2014).

Several particle-associated taxa enriched in the poisoned traps were closely related to well-described eukaryote-associated groups, that included Enterovibrio, Arcobacter, Wolinella, and Campylobacter (Figure 4B). Their presence in poisoned traps is consistent with the detection of diverse eukaryote-associated microbes in seawater and on marine metazoan surfaces (Gugliandolo et al., 2008; Preheim et al., 2011; Turner et al., 2014). Notably, Vibrionales genera were similarly enriched in both live and poisoned traps, as compared to seawater (Figure A4 in Supplementary Material). Many Vibrionales associate

**FIGURE 5** | Pairwise comparisons of selected Bacterial and Archaeal taxa in 150 m vs. 500 m (A) live sediment traps, (B) poisoned sediment traps, and (C) seawater. Only taxa significantly enriched (FDR < 0.05) at shallow (blue, 150 m) and deep (orange, 500 m) depths with a difference between proportions > 1 are shown. The bar plot displays the mean proportion of sequences assigned to each taxon in each sample.
with diverse eukaryotes and are capable of degrading a wide
variety of abundant marine biopolymers (Thompson et al.,
2004; Takemura et al., 2014). Taken together, these data
indicate that bacteria enriched in poisoned traps were most
likely associated with eukaryotic surfaces or digestive tracts
which is consistent with phyto- and zooplankton detritus
constituting the major fraction of marine POM (Simon et al.,
2002).

**Live vs. Poisoned Sediment Trap Eukaryotic
Assemblages**

The most abundant eukaryotic phyla across all depths in both live
and poisoned sediment traps were *Dinoflagellata, Protalveolata,
Retaria, Metazoa*, and unclassified *Stramenopiles*, which are likely
representative of the composition of sinking particles at Station
ALOHA (Figure A5 in Supplementary Material). Protists in
class Dinophyceae were also identified microscopically in the
200-m poisoned trap (Figure 1B). Dinoflagellates, radiolarians,
and foraminiferan protists, algae, metazoans, and heterotrophic
protists were expected to be captured in live and poisoned
sediment traps at similar rates.

Differences between live and poisoned traps were most
pronounced for *Metazoa* and *Retaria*. The most abundant
metazoa across live and poisoned traps were unclassified
*Maxillopoda* and a variety of copepod genera (data not shown).
Copepod taxa in live traps included *Corycaeus, Claustralum,*
and *Oithona* while *Scolochitrid* and the ostracod *Conchoecia* were
most abundant in poisoned traps (data not shown).

*Retaria* species were slightly more abundant in the poisoned
vs. live traps with the most abundant taxa classified as
*Acantharia* (poisoned vs. live mean abundance; 1.8% vs. 1.6%)
and *Polycystinea* (2.8% vs. 2.4%). A colonial polycystine protist,
*Sphaerozoum*, appeared significantly enriched in the poisoned
trap (Figure A6 in Supplementary Material). The silica skeleton
of polycystines may be solubilized rapidly in the upper water
column at Station ALOHA, with approximately 40% lost
within the mesopelagic (Lamborg et al., 2008). Again, *in situ*
microbial degradation in the live traps are likely responsible
for the differences in eukaryotic taxon abundance between live
and poisoned traps. The detection of *Acantharia*, which have
strontium sulfate skeletons, in both live and poisoned traps is
notable because they are not typically detected in traps without
the addition of strontium to the capture solution to inhibit
their dissolution (Michaels et al., 1995). This suggests that
acantharians detected in the poisoned trap were intact just prior
to capture, and that remnants of their DNA in the live trap
endured during the 12-day deployment.

**Functional Gene Categories Associated with Live
and Poisoned Sediment Trap Assemblages**

KEGG pathways and genes associated with sediment trap DNA
were surveyed to functionally profile live vs. poisoned sediment
trap metagenome content. A large variety of pathways were
significantly enriched in the live vs. poisoned traps, including
those associated with motility, amino acid, carbohydrate, and
energy metabolism, signal transduction, and cofactors and
vitamin biosynthesis (Figure A7 in Supplementary Material).

Notably, only the siderophore biosynthesis pathway was enriched
in poisoned vs. live sediment trap microbial assemblages.
Similarly, siderophore biosynthesis was significantly enriched in
poisoned sediment traps compared to seawater (Figure A7 in
Supplementary Material). In a recent study, a high frequency of
strains containing siderophore biosynthetic genes were linked
to eukaryote-associated lifestyles (Cordero et al., 2012), which
were enriched in our poisoned sediment traps (Figure A4
in Supplementary Material). These siderophore biosynthesis
pathways are likely affiliated with eukaryote-associated taxa
since iron acquisition is important for microbial colonization
of eukaryotes (Miethke and Marahiel, 2007). Pathways for
membrane transport and cell motility were significantly enriched
in both live and poisoned sediment traps, compared to seawater
(Figure A7 in Supplementary Material).

**Bacterial Genes and Pathways Enriched in the
Live Sediment Traps**

To explore the potential functions and metabolism associated with
*in situ* particle degradation, we surveyed genes and
biochemical pathways that were enriched in sediment trap
bacterial DNA. Live sediment traps found to be
significantly enriched in genes for TonB-dependent iron
transporters (TBDTs), assimilatory and dissimilatory single-
carbon compound utilization, and polysaccharide utilization
(Figures 6A,B). The majority of sequences matching TBDTs (*fiu,
*fluE*) were associated with *Alteromonas* spp. (Figures 6A,B).
*Alteromonas*-like gene sequences matching algae- (alginate) and
diatom-derived polysaccharides (fucose) utilization were
also prevalent (Figures 6A,B). Alginate and fucose utilization
genes have been linked to phytoplankton decomposition and
assimilation of diatom exopolysaccharides in previous studies
(Teeling et al., 2012; Smith et al., 2013). *Methylphaga*-like gene
sequences matching key genes of the ribulose monophosphate
RuMP) assimilatory pathway for formaldehyde fixation and
detoxification (hxlA/hxLB) and the tetrahydroxymethanopterin
(TMHP)-dependent dissipatory formaldehyde oxidation
pathway (*FwdABC*, *ftr, mch*) were also prevalent (Figures 6A,B,
Dataset A2 in Supplementary Material). These data are consistent
with previous microcosm experiments that showed the
enrichment of *Alteromonadaceae* and *Methylphaga* phyotypes,
as well as their expressed genes, including *Alteromonadaceae*
TBDTs and *Methylphaga*-like key enzymes of the RuMP
pathway and the TMHP-dependent pathway (Pinhassi et al.,
2004; Neufeld et al., 2008; McCarron et al., 2010). Potential
growth of these groups on DOM generated in the live trap by
POM breakdown is also consistent with previous zooplankton
and phytoplankton degradation studies that have demonstrated
rapid accumulation of DOM over the course of just a few days
during POM diagenesis (Yoshimura et al., 2009; Yoshimura
and Hama, 2012).

Other bacterial genes enriched in the live sediment traps
included those associated with denitrification from nitrate to
*N*$_2$ (nitrate reductase; *narG*, nitrate reductase; *nirS*, nitric
oxide reductase; *norB*, and nitrous oxide reductase; *nosZ*).
(Figures 6A,B, Dataset A2 in Supplementary Material) Genes
encoding a mannose-sensitive hemagglutinin (MSHA) pilus were
found associated with \textit{Marinobacter} at depths between 150–300 m, and \textit{Moritella} at 500 m (Figures 6A,B). The MSHA pilus has been implicated in attachment to animal surfaces in \textit{Vibrio} and \textit{Pseudoalteromonas} (Chiavelli et al., 2001; Dalisay, 2006) and it may also play a role in attachment by \textit{Moritella} and \textit{Marinobacter}, which have previously been shown to associate with eukaryotes (Gärdes et al., 2011; Tunsjø et al., 2011). The \textit{pilA} gene encoding the pilin protein of a novel chitin-regulated pilus (ChRP;K02650) along with a chitin-binding protein gene (CBP;K03933) were also highly enriched in live traps (Dataset A2 in Supplementary Material). In addition, a variety of heavy-metal resistance genes associated with \textit{Alteromonadales} were enriched in the live traps (Figures A8A,B and Dataset A2 in Supplementary Material). The \textit{czcABCD} genes encode a heavy metal efflux pump involved in resistance to cobalt, zinc, and cadmium that were affiliated with \textit{Alteromonas}. \textit{CusAB} and \textit{cusRS} encode copper efflux proteins and copper two-component sensor systems, respectively, that were affiliated with \textit{Alteromonas}, \textit{Glaciecola}, and \textit{Marinobacter}. The genes involved in mercury resistance (\textit{merABR}) and transport (\textit{merTP}) were affiliated with \textit{Alteromonas} and \textit{Marinobacter}. The enrichment of metal resistance genes in the poisoned traps may be linked to their growth on particles, which are known to concentrate heavy metals (Puig et al., 1999).
To further evaluate the potential for carbohydrate degradation capabilities of trap associated microbial assemblages, peptide encoding sequences were compared to a carbohydrate-active enzymes database (CAZymes) (Yin et al., 2012). A large number of glycoside hydrolase (GH) families associated with polysaccharide degradation found in algae and bacterial cell walls including arabinose, pectin, cellulose, and peptidoglycan were significantly enriched in the live traps (Figure 7). Polysaccharide lyase (PL) families 6, 7, and 1 and carbohydrate esterase family 8 complement the degradation potential of GHs (Cantarel et al., 2009) and potentially may enhance the degradation of the algal substrates alginate and pectin in live traps (Figure 7). Several carbohydrate-binding module (CBM) families targeting chitin (e.g., CBM1, CBM14, and CBM18) were also enriched in live traps (Dataset A4 in Supplementary Material).

**Bacterial Genes and Pathways Enriched in the Poisoned Sediment Traps**

Poisoned sediment trap particles were significantly enriched in a variety of iron-scavenging genes and virulence factors, primarily associated with *Vibrio* spp. (Figures 6A–D). *Vibrio* spp. are known to engage in pathogenic, symbiotic, and saprophytic associations with a wide variety of eukaryotes in the marine environment (Takemura et al., 2014). *Vibrio*-like genes for carbohydrate uptake and chemotaxis, supporting eukaryote-associated lifestyles with a wide variety of eukaryotes in the marine environment (Takemura et al., 2014). *Vibrio*-like genes for carbohydrate uptake and chemotaxis, supporting eukaryote-associated lifestyles with a wide variety of eukaryotes in the marine environment (Takemura et al., 2014). *Vibrio*-like genes for carbohydrate uptake and chemotaxis, supporting eukaryote-associated lifestyles with a wide variety of eukaryotes in the marine environment (Takemura et al., 2014). *Vibrio*-like genes for carbohydrate uptake and chemotaxis, supporting 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a wide variety of eukaryotes in the marine environment (Takemura et al., 2014).
and poisoned traps, as compared to seawater (Dataset A4 in Supplementary Material). These data support KEGG functional profiles indicating the potential for chitin degradation in both live and poisoned sediment traps.

### Conclusion

Sinking particles represent the primary vehicles of organic carbon flux from surface waters to the deep ocean (Volk and Hoffert, 1985), yet to date, few data are available on the specific microbes and metabolic pathways responsible for POM degradation throughout the water column. There is a general consensus that particles represent hotspots of microbial activity in the ocean (Karl and Knauer, 1984; Turley and Mackie, 1994; Crump et al., 1999; Bochdansky et al., 2010; Smith et al., 2013), but the nature of those processes and microorganisms responsible still need to be better described.

In this study, sediment trap metagenomic analyses revealed dramatic differences in the taxonomic diversity and functional potential of microbes associated with sinking particles in poisoned sediment traps, compared to those that grew in situ in live traps. Both live and poisoned sediment trap microbial assemblages were distinctly different from those found in seawater, which is consistent with the conclusions of several recent studies (LeCleir et al., 2013; Smith et al., 2013). Live particle-trap assemblages shared many similarities with communities found in microcosm DOM enrichments, with the added dimension of known particle-associated bacteria (e.g., *Flavobacteriales*) and potentially eukaryote-associated bacteria (e.g., *Pseudoalteromonas* and *Marinobacter*). The functional gene content in live traps pointed to the potential for growth by alteromonads on labile DOM produced in situ from sinking POM. Apparently, the contained environment within the sediment trap acted similarly to microcosm enrichment experiments, where fast-growing copiotrophic bacteria out-competed the particle- and eukaryote-associated bacteria for the nutrients available in the trap. The poisoned sediment trap-associated metagenomic analyses provided a clear contrast to live traps and presumably reflected the biological material and microbial assemblages associated with sinking particles. The differences in composition between live and poisoned traps were much greater than depth associated differences, consistent with previous studies of suspended particulates found in oxygen minimum zones (Ganesh et al., 2014).

In total, these findings are consistent with a previous study that suggested initial particle-colonizers are surface-colonizing (or eukaryote-associated) specialists (LeCleir et al., 2013). Our metagenomic data further indicated that microbes in poisoned sediment traps were often associated with eukaryotic surfaces and intestinal tracts as symbionts, pathogens, or saprophytes. Some of these eukaryote-associated bacteria may alternate between symbiotic to pathogenic or saprophytic lifestyles, as has been shown for some phytoplankton symbionts (Grossart, 1999; Seyedsayamdost et al., 2011). The functional gene content in poisoned traps, which included a variety of genes involved in virulence, anaerobic metabolism, attachment to chitinaceous surfaces, and chitin degradation were consistent with this conclusion. Notably, genes for attachment to chitinaceous surfaces and anaerobic metabolism were also detected in live traps, though they were associated with a different set of microbial taxa. Thus, eukaryote-associated communities captured in live and poisoned traps differed, most likely due to bacterial growth in the live trap.

Our data also provide new perspective on the taxonomic identity of the particulate matter itself, namely the eukaryotic taxa that contribute to the complex mixture of detritus and minerals that make up marine particles. While previous studies have reported marine particles as consisting of eukaryote-derived detritus, the analyses we report here suggests that specific interactions between eukaryotes and bacteria may be centrally important in the transport and degradation processes associated with sinking POM. The presence of chitinaceous surfaces provides a habitat for a specialized bacterial community adapted to sense, attach, degrade, and take up chitin derivatives. These same habitats appeared to coincide with the development of copiotrophs known to respond rapidly to labile DOM inputs. The probable sources of labile DOM include turnover of phytoplankton captured in the traps, the excretions of swimming zooplankton, and substrates from the degradation of captured chitinaceous detritus.

This study provides a baseline for understanding microbial community assemblages and metabolic activities associated with the transport and degradation of sinking POM. To date, gene expression associated with POM degradation has not yet been reported, partly due to the technical difficulties associated with preserving RNA in situ. Future metatranscriptomic analyses have the potential to identify those metabolic pathways that are expressed in situ on sinking particles and help to define the processes that actively drive particle degradation in the ocean’s interior. Finer scale studies of particle transport and degradation should also help to define hypothetical successional cascades that reflect sequential processing of POM to DOM during its transport to the deep-sea.

### Author Contributions

KF, ED, DK designed research. JE contributed bioinformatics tools. KF performed research. TS performed microscopy. KF, JE, and ED analyzed data. KF, ED, and DK wrote the paper.

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**Supplementary Material**

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fmicb.2015.00469/abstract

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.