1 **Microbial community composition and nitrogen availability influence**
2 **DOC remineralization in the South Pacific Gyre**

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Abstract

Many environmental factors are thought to control the bioavailability of marine dissolved organic matter (DOM) for marine microbes including its composition, the microbial community structure, and nutrient availability, yet which factors dominate at the ocean basin scale remains uncertain. Understanding the controls on DOM lability is an important goal given the role of DOM in the marine carbon cycle. We performed DOM lability experiments at two contrasting stations, one oligotrophic and one mesotrophic, in the eastern tropical South Pacific (ETSP) to investigate the controls on microbial remineralization of surface ocean DOM. Surface layer dissolved organic carbon (DOC) and nitrogen (DON) were recalcitrant to remineralization over 9 to 14 days when exposed to the microbial communities from the surface mixed layer, however exposure to microbial communities from the upper mesopelagic (twilight zone) allowed consumption of DOC but not DON. The DOC remineralization response differed between the mesotrophic site (~21 μM consumed), likely experiencing allochthonous inputs of DOM from the adjacent eastern boundary upwelling system, versus the oligotrophic station (~3 μM consumed) further offshore in the South Pacific gyre. DNA fingerprinting of the microbial communities across the ETSP with terminal restriction fragment length polymorphism (T-RFLP) analyses revealed greater differences between microbial communities in surface vs. subsurface (e.g., 100 m) waters at the same station than between surface water microbial communities separated by 1000s of kilometers. The subsurface microbial community at the mesotrophic station responsible for the greatest observed DOC remineralization, with a concomitant consumption of nitrate, consumed DOC to concentrations below that observed *in situ* (at 100 m), suggesting a potential role

for co-metabolism of relatively labile with more recalcitrant DOC or relief from micronutrient limitation, in driving the additional DOC consumption. DOC remineralization by the mesopelagic (200 m) microbial community was much less at the oligotrophic station and similar to previously published results from the Sargasso Sea. Both microbial community composition and nutrient availability contribute to DOM persistence over weekly timescales in the surface mixed layer with varying degrees of DOC lability in the subsurface waters of the ETSP.

Keywords: dissolved organic carbon; microbial communities; C remineralization

Introduction

Estimated at 662 Pg C, DOC contains the largest marine reservoir of reduced carbon and plays a critical role in the global ocean carbon cycle by providing a conduit for 20-25% of annual surface ocean carbon export (Hansell et al., 2009; Letscher et al., 2015). DOM is primarily produced by phytoplankton in the surface ocean and subsequently consumed by heterotrophic processes, however its production and consumption are often temporally and spatially decoupled, allowing it to accumulate in surface waters and be transported away from the site of production by ocean currents (Hansell and Carlson, 1998). Although a portion of the accumulated DOM is removed upon delivery to the mesopelagic ocean (~100 to 1000 m) (Doval and Hansell, 2000; Hansell and Carlson, 2001; Carlson et al., 2010), a small fraction resists degradation and persists within the refractory DOM pool for 1000s of years (Hansell, 2013). The exact mechanisms that allow DOM to escape remineralization and persist in the deep ocean are not known,

however further microbial processing of DOM to a more recalcitrant form has been proposed (Jiao et al., 2010). Thus, shedding light on the conditions under which DOM is consumed or persists is important to understanding the global carbon cycle.

Bottle incubation experiments have been used to investigate the mechanisms and environmental factors that regulate microbial remineralization of DOM (e.g. Carlson et al., 2002; 2004; Lnborg et al., 2009; Letscher et al., 2013). In these experiments, seawater is filtered to remove particulates, primarily leaving DOM as the substrate for an added inoculum of microbial heterotrophs. These experiments indicate that DOM remineralization is influenced by many factors including temperature (Shiah and Ducklow, 1994), abundance of grazers (Thingstad et al., 1997; Caron et al., 2000), DOM composition or quality (Skoog and Benner, 1997; Aluwihare et al., 2005; Goldberg et al., 2011), competition for inorganic nutrients (Cotner et al., 1997; Rivkin and Anderson, 1997), microbial community structure (Giovannoni et al., 1996; DeLong et al., 2006; Treusch et al., 2009; Carlson et al., 2004; 2009), and the presence or specific affinity of microbial cell membrane nutrient transporters (Azam and Malfatti, 2007; Morris et al., 2010). Many of these environmental factors exhibit gradients with depth in the water column, favoring preferential remineralization of surface-accumulated DOM in the upper mesopelagic zone (~100 to 250 m) (Carlson et al., 2004; Letscher et al., 2013).

This depth-dependence of DOM remineralization has been seen at the Bermuda Atlantic Time-series Study (BATS) site in the oligotrophic Sargasso Sea. Early springtime in the Sargasso Sea brings convective overturn of the upper water column that dilutes upper ocean DOC stocks by mixing with lower DOC concentration waters from below the euphotic zone, while simultaneously injecting inorganic nutrients into the

93 upper ocean as the depth of mixing breaches the nutricline (Steinberg et al., 2001). As the
94 upper ocean warms, a shoaling mixed layer combined with the nutrient injection results
95 in a spring bloom that accounts for ~30% of annual net primary productivity at this site
96 (Lohrenz et al., 1992) and produces a net of ~0.4 – 1.0 mol C m⁻² DOC (Hansell and
97 Carlson, 2001). Following the spring bloom, DOC stocks in the upper mixed layer resist
98 microbial remineralization, with elevated DOC concentrations persisting from the end of
99 May through the summer and into the autumn months before dilution by vertical mixing
100 the following winter (Hansell and Carlson, 2001). Subsequent to convective mixing,
101 DOC exported below the euphotic zone is remineralized over the course of months
102 (Hansell and Carlson, 2001), causing a succession of subsurface microbial ‘blooms’ of
103 different heterotrophic bacteria that respond to the inputs of DOC that had accumulated
104 in surface waters (Morris et al., 2005; Carlson et al., 2009; Treusch et al., 2009). Thus,
105 differences in the microbial community structure between the surface mixed layer and the
106 upper mesopelagic, and each community’s ability to consume specific DOM substrates,
107 exert a strong influence on the vertical patterns of DOM remineralization (Carlson et al.,
108 2004).

109 The eastern tropical South Pacific (ETSP, here referring to the region between 10 –
110 20 °S and eastward of 100 °W) is an ideal location to test a number of the hypothesized
111 controls on DOM remineralization. The region includes oligotrophic conditions
112 associated with the edge of the South Pacific subtropical gyre, high nutrient-low
113 chlorophyll conditions along ~10 °S associated with the equatorial upwelling region, as
114 well as waters downstream from the highly productive Peru-Chile eastern boundary
115 upwelling system. Gradients in microbial community structure, nutrient availability, and

DOM lability are expected across this range of biogeochemical conditions. We performed experiments at two stations along 20 °S in the ETSP to compare the environmental controls on DOC remineralization between a mesotrophic station downstream of the eastern boundary upwelling system and at an oligotrophic station located in the South Pacific subtropical gyre. Terminal restriction fragment length polymorphism (T-RFLP) analyses of the 16S rRNA gene are included in this study to investigate potential variations in microbial community composition with differing degrees of DOC remineralization. Small-subunit ribosomal ribonucleic acid (rRNA) 16S rRNA genes have been used extensively as phylogenetic markers for prokaryotic diversity in marine systems (e.g. Giovannoni and Stingl, 2005 and references therein), demonstrating that major prokaryotic groups are highly stratified with depth in the upper ~300 m of the ocean (Giovannoni et al., 1996; DeLong et al., 2006; Treusch et al., 2009), with a marked shift in microbial community structure at the photic/aphotic transition (Giovannoni and Rappé, 2000). T-RFLP data of the 16S rRNA gene have been used previously for ‘DNA fingerprinting’ to distinguish the distinctiveness of environmental microbial communities based on the similarity/dissimilarity of the T-RFLP fragment patterns (Moeseneder et al., 1999; Morris et al., 2005; Nelson et al., 2008; Carlson et al., 2009; Treusch et al., 2009; Hahnke et al., 2013). Our experiments address how differences in microbial community composition, available inorganic nutrients, and DOM lability impact DOC remineralization patterns in both the vertical and horizontal dimensions across the ETSP.

Methods

2.1 Incubation Experiments

Seawater DOM remineralization experiments were carried out at sea aboard the R/V *Melville* during a March to April 2011 cruise to the ETSP (MV1104). Remineralization experiments were performed at Stations 1 (20 °S 80 °W) and 5 (20 °S 100 °W) (Fig. 1). The locations for the experiments were chosen to investigate a hypothesized zonal gradient in DOM lability, contrasting DOM in mesotrophic waters recently advected offshore from the Peru-Chile upwelling system (Station 1) with DOM in waters from the oligotrophic South Pacific subtropical gyre (Station 5). Estimates of ¹⁴C-measured primary productivity are twice as high, and rates of new production gauged from the upward NO₃⁻ flux are five-fold higher, at Station 1 compared with Station 5 at the time of the cruise (Haskell et al., 2015), confirming the mesotrophic vs. oligotrophic status of Stations 1 and 5, respectively. At each station two experimental treatments were performed in triplicate 9 L polycarbonate carboys (pre-cleaned with 10% HCl and then rinsed with 0.2 µm filtered surface seawater): a treatment of surface filtered (0.2 µm) seawater media (8 L) inoculated with 1 L of surface whole (unfiltered) water inoculum, hereafter termed surface-surface, and a second treatment containing the same 8 L of surface filtered media inoculated with 1 L of whole water collected from the upper mesopelagic zone at each station, hereafter termed surface-meso. Both mesopelagic whole water samples were collected from the top of the upper mesopelagic zone (100 m at Station 1, 200 m at Station 5) using Niskin bottles and a CTD/rosette package equipped with a PAR (photosynthetically active radiation) sensor. These mesopelagic depths were located immediately below the base of the euphotic zone (defined as the depth where PAR = 1%), with PAR values of 0.2% at 100 m and 0.5% at 200 m for Stations 1 and 5, respectively. Surface seawater, collected using a trace-metal clean pump fitted with

silicone tubing from 5 m below the hull of the ship at Station 1 and from Niskin bottles at Station 5, was filtered using an inline acid-cleaned Acropak 1000 Supor membrane 0.8/0.2- μ m pore size, large volume cartridge filter. Mesopelagic water was collected directly from Niskin bottles deployed on the CTD/rosette at each station. Following inoculation of the filtered surface (DOM-containing) seawater with the whole water (microbe-containing) sample, each incubation carboy was sampled for biogeochemical concentration analyses at time point 0. Carboys were then incubated in the dark at ambient temperature ($\sim 20^{\circ}\text{C}$) over the course of 9 to 14 days, with samples drawn unfiltered into HDPE bottles for biogeochemical concentration analyses every 2 to 3 days. This experimental scheme is similar to experiments carried out near Bermuda (e.g. Carlson & Ducklow, 1996; Carlson et al., 2004), the Florida Straits (Letscher et al., 2013), and the southern California Current (e.g. Ammerman et al., 1984; Pedler et al., 2014). The dilution culture technique employed in this experimental design releases bacterioplankton from grazer pressure, thus stimulating bacterial growth and substrate (DOM) utilization (Carlson et al., 2004).

2.2 Sample Analyses

2.2.1 TOC and TN concentrations

Samples for total organic carbon concentration, $[\text{TOC}] = \text{DOC} + \text{bacterial biomass C}$, and total nitrogen concentration $[\text{TN}]$ analyses were collected unfiltered into acid-cleaned 60 mL HDPE bottles and immediately frozen upright at -20°C until analysis ashore. TOC was analyzed by high temperature combustion using two Shimadzu TOC- V_{CSH} systems following the procedures for seawater DOM analysis detailed in Dickson et al. (2007). Deep seawater and low carbon reference waters as provided by the Hansell CRM

Program (Hansell, 2005) were measured every sixth analysis to assess day-to-day and instrument-to-instrument variability. Precision for TOC analyses is $\pm 1 \mu\text{M}$ or a CV of 1-2%.

[TN] was measured by persulfate oxidation (Solorzano & Sharp, 1980) using adaptations according to Knapp et al. (2005), with the resulting NO_3^- concentration measured by chemiluminescence as described in the next section. Samples were analyzed in triplicate and corrected for the N blank of the persulfate-oxidizing reagent, yielding a precision of $\pm 0.3 \mu\text{M}$ or a CV of 5-7%.

2.2.2 Nitrate + Nitrite concentrations

Samples for nitrate+nitrite ($\text{NO}_3^- + \text{NO}_2^-$) concentration (hereafter $[\text{NO}_3^-]$) analysis were collected unfiltered into acid-cleaned 60 mL HDPE bottles and immediately frozen upright at -20°C until analysis ashore. $[\text{NO}_3^-]$ was measured by chemiluminescence, following a reduction to NO using heated acidic V(III) (Braman and Hendrix, 1989) in a configuration yielding a detection limit of $0.03 \mu\text{M}$ with a precision of $\pm 0.025 \mu\text{M}$ when nitrate was $< 0.1 \mu\text{M}$ or a detection limit of $0.1 \mu\text{M}$ with a precision of $\pm 0.05 \mu\text{M}$ when nitrate was $> 0.1 \mu\text{M}$.

2.2.3 Ammonium concentrations

Ammonium concentrations ($[\text{NH}_4^+]$) were measured at sea with the orthophthaldialdehyde (OPA) method of Holmes et al. (1999). Samples were reacted for 2 h at room temperature with an OPA-containing solution, with subsequent quantification of fluorescence at an excitation/emission wavelength of 350 nm/410-600 nm. A duplicate $[\text{NH}_4^+]$ standard curve made up using NH_4Cl additions to deep ($> 1000 \text{ m}$) seawater from the same site, ranging from $0.060 \mu\text{M}$ to $0.60 \mu\text{M}$ (Station 1) or $0.030 \mu\text{M}$ to $0.30 \mu\text{M}$

(Station 5) was run with each set of samples. The detection limit was 0.015 μM with a precision of $\pm 0.003 \mu\text{M}$.

2.2.4 TON concentrations

Total organic nitrogen concentrations, $[\text{TON}] = \text{DON} + \text{bacterial biomass N}$, were determined by the difference of the TN concentration measurement and the sum of dissolved inorganic nitrogen species ($\text{NO}_3^- + \text{NO}_2^- + \text{NH}_4^+$) where $\text{TON} = \text{TN} - \text{NO}_3^- - \text{NO}_2^- - \text{NH}_4^+$. Propagation of error yields a precision on $[\text{TON}]$ determinations of $\pm 0.3 \mu\text{M}$ or a CV of $\sim 6\%$. The $[\text{NH}_4^+]$ was not measured at every time point, however the range of measured concentrations in our experiments, 0.015 to 0.040 μM , was one to two orders of magnitude lower than that of $[\text{NO}_3^-]$ or $[\text{TON}]$, thus its omission does not affect the interpretation of our results.

2.3 DNA fingerprinting of microbial communities

Water samples (~ 35) for DNA extraction were collected from multiple depths in the upper ~ 400 m from all stations shown in Fig. 1 and directly from Niskin bottles into acid-washed 4 L polycarbonate bottles using acid-washed silicone tubing. Seawater was pressure-filtered onto 25 mm, 0.2 μm pore size membrane filters (Pall Supor 200) housed in polypropylene filter holders (Swin-Lok, Whatman). Filters were placed into sterile 2 mL gasketed bead beating tubes (Biospec), flash frozen in liquid nitrogen, and stored at -80°C until analysis on land. DNA from size-fractionated filters was extracted according to one of two previously described protocols (Santoro et al., 2010; Moisander et al., 2008). In the method described in Santoro et al. (2010), 850 μL of sucrose EDTA lysis buffer (0.75 M sucrose, 0.05 M Tris-Base, 0.02 M EDTA, 0.4 M NaCl, pH 9.0) and 100 μL of 10% SDS were first UV treated for 30 min and then added to the bead-beating

tubes containing the filters and 30 μ L of 0.1 mm sterile glass beads (Biospec). Bead beating was performed for 1 min and the samples were subsequently heated at 99 °C for 2 min. After heating, 25 μ L of 20 mg mL⁻¹ proteinase K was added and tubes were incubated at 55 °C overnight. DNA was extracted and purified from the lysate using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA, USA). DNA was also extracted from 0.2 μ m filters using the DNeasy kit, with protocol modifications described in Moisander et al., 2008 to optimize cell disruption using multiple freeze/thaw cycles, agitation via bead beating, and proteinase K digestion. The column purification and elution steps were automated using a QIAcube (Qiagen).

Extracted DNA samples were used to perform terminal restriction fragment length polymorphism (T-RFLP) analyses. Polymerase chain reaction, with primers targeting the V1 and V2 hypervariable regions of the 16S rRNA gene, was used to amplify the 16S rRNA gene in a portion of extracted DNA (30 cycles of 94 °C 30s, 57 °C 45s, 72 °C 60s) according to Nelson (2009). Samples were then digested with HaeIII enzyme (Promega) at 37 °C for 2 h followed by enzyme inactivation at 94 °C for 5 min. Fragment analysis of formamide-saturated and heat-denatured samples via capillary sequencer (Applied Biosystems 3730XL) was conducted at the UC Berkeley DNA Sequencing Facility using a custom sizing standard (20 sizes 30-650 bp; Bioventures). Electropherogram peaks in the 30-550 bp range were aligned and analyzed according to Nelson (2009), with peaks <0.5% of total peak area excluded from analysis. Community similarity was then analyzed by comparing the relative abundance of shared to unshared peaks across samples using the Bray-Curtis similarity index (Bray and Curtis, 1957) in PRIMER (Clarke and Gorley, 2006).

3. Results

3.1 Station 5 Incubations

Results for the surface-surface treatment at Station 5 are shown in Fig. 2. Initial [TOC] was between 65 and 67 μM (Fig. 2a), [TN] was 5.0 μM (Fig. 2b), and [TON] was 4.9 μM (Fig. 2c). Initial $[\text{NH}_4^+]$ (Fig. 2d) and $[\text{NO}_3^-]$ (Fig. 2e) were typically $<0.04 \mu\text{M}$ and 0.1 μM , respectively, and exhibited little variability over the duration of the experiment. Delta TOC ($\Delta\text{TOC} = [\text{TOC}]_{\text{final}} - [\text{TOC}]_{\text{initial}}$) and ΔTON values were near zero or below detection (Table 1), indicating little net consumption of TOC or TON across the 9 d experiment. No changes in [TN], [TON], $[\text{NH}_4^+]$, and $[\text{NO}_3^-]$ were observed within the measurement uncertainty (Fig. 2b-e; Table 1).

Surface-meso treatment results from Station 5 are shown in Fig. 3. Initial [TOC] was 62 to 64 μM (Fig. 3a), with net consumption observed after nine days ($\Delta\text{TOC} = -1.5$ to $-3.8 \mu\text{M}$, Table 1), resulting in $[\text{TOC}]_{\text{t_final}}$ values of $\sim 60 \mu\text{M}$. No changes in [TN], [TON], $[\text{NH}_4^+]$, and $[\text{NO}_3^-]$ were observed, which averaged 5.0, 4.4, 0.1, and 0.65 μM , respectively (Fig. 3b-e).

3.2 Station 1 Incubations

Results for the surface-surface treatment at Station 1 are shown in Fig. 4. Initial [TOC] in the incubation carboys was 78 μM , which differed from the [TOC] measured for surface water collected via the CTD/rosette Niskin bottle of 80.5 μM (Fig. 4a). The calculated ΔTOC for each carboy was $\sim 2.5 \mu\text{M}$, indicating a slight net production or contamination of TOC over the course of the experiment (Table 1). However, if the initial [TOC] measured from the surface Niskin bottle is taken to be $[\text{TOC}]_{\text{t_initial}}$ at Station 1,

Δ TOC is near analytical detection for each incubation carboy (Table 1). Regardless, net TOC remineralization was not observed in the surface-surface treatments at either station. Similar to other treatments, changes in [TN], [TON], $[\text{NH}_4^+]$, and $[\text{NO}_3^-]$ were not observed over the 14 d experiment, averaging 5.3, 5.3, 0.01, and 0.1 μM , respectively (Fig. 4b-e).

The surface-meso treatment results from Station 1 are presented in Fig. 5. The initial [TOC] of 76 to 77 μM decreased over the 14 d experiment to $\sim 56 \mu\text{M}$ (Fig. 5a), yielding average Δ TOC of -21 μM (Table 1). The [TN] did not change over the course of the experiment from the initial $\sim 5.5 \mu\text{M}$ (Fig. 5b). Similarly, $[\text{NH}_4^+]$ also exhibited little variability with concentrations generally $< 0.02 \mu\text{M}$ except for an increase to 0.04 μM on day 7 in one of the triplicate incubation carboys (Fig. 5d). Contrary to results from all other treatments, a decrease in $[\text{NO}_3^-]$ (Fig. 5e) and a nearly equivalent increase in [TON] (Fig. 5c) was observed for each of the three replicates of the Station 1 surface-meso treatment. The initial $[\text{NO}_3^-]$ was 0.75 to 0.95 μM , then decreased rapidly between day 0 and the first time point at day 4, remaining at $\sim 0.1 \mu\text{M}$ for the remainder of the experiment (Fig. 5e). This NO_3^- drawdown (average = 0.78 μM , Table 1) was balanced by a nearly equivalent increase in [TON] (average = 0.72 μM , Table 1) from ~ 4.4 to 4.7 μM on day 0 to $\sim 5.5 \mu\text{M}$ thereafter (Fig. 5c).

3.3 DNA fingerprinting of microbial communities

Evaluation of the similarity in the microbial communities present at Stations 1 and 5 as well as the adjacent Stations 3 and 6 (Fig. 1) were investigated by a nonmetric multidimensional scaling (NMS) ordination of the T-RFLP fragments (Fig. 6). Fragments were found to ordinate into two groupings along the y-axis by depth in the water column

for each sample. Euphotic zone (PAR >1%) samples (red triangles) exhibited >40% community similarity across all four stations, except for two deep euphotic zone samples from Station 5, and clustered separately from mesopelagic zone (PAR <1%) communities (blue circles) which also exhibited >40% similarity across all stations. Using a 60% similarity level to define a community, euphotic zone samples from Stations 1 and 5 were distinct. Both surface water inocula for Stations 1 and 5 clustered with the larger set of euphotic zone samples, at >40% and >60% similarity to the *in situ* community at each station, respectively. The fragments from the mesopelagic inoculum (100 m sample) at Station 1 clustered with the euphotic zone community samples at >40% similarity, however, using 60% similarity level to define a community there was a distinct separation of the 100 m inoculum from the surface water inoculum (5 m sample) used for the Station 1 surface-surface experiments. Similarly, the upper mesopelagic inoculum (200 m) at Station 5 exhibited T-RFLP fragments with >40% similarity to other Station 5 mesopelagic zone samples and was distinct from the euphotic zone inoculum.

4. Discussion

The incubation results obtained for oligotrophic Station 5, located in the South Pacific subtropical gyre, are broadly consistent with those obtained from similar experiments conducted in the Sargasso Sea of the North Atlantic subtropical gyre (Carlson et al., 2002; 2004). Initial surface [TOC] was ~65 to 70 μM and surface [TON] was ~4.5 to 5.0 μM in both systems (Hansell and Carlson, 2001; this study) with the surface-surface treatment resulting in negligible net changes in the TOC pool over the course of ~1 week (Fig. 2a) (Carlson et al., 2004). The lack of observed changes in [TOC] and the nitrogen

pools (Fig. 2) point towards the recalcitrance of surface DOM over the timescale of our experiments (days/weeks) and the lack of a surface microbial community response to surface mixed layer DOM. However, we observed a small but detectable remineralization of TOC ($\sim 3 \mu\text{M}$, Table 1; Fig. 3a), or $\sim 4\%$ of the initial TOC pool, for the surface-meso treatment at this station, which is consistent with the results of Carlson et al. (2004) from the Sargasso Sea. Because samples were unfiltered, measurements of [TOC] contain the contribution from bacterial biomass at all times points, thus net TOC remineralization is equivalent to microbial respiration. We observed no net resolvable changes in the nitrogen pools in the surface-meso treatment (Fig. 3b-e), although we cannot rule out fluxes through the nitrogen pools on timescales shorter than our sampling resolution.

Results from the surface-surface treatment at the mesotrophic Station 1 are consistent with those found at Station 5 located in the oligotrophic gyre. Net changes in [TOC] (Table 1) and nitrogen pools (Fig. 4) were negligible, indicating surface-accumulated DOM was mostly recalcitrant to the surface mixed layer microbial community over the weekly timescale investigated. These results for the surface-surface treatment at Station 1 contrast with the large changes in [TOC], [TON], and $[\text{NO}_3^-]$ observed for the surface-meso treatment at the same station (Fig. 5). Exposure of surface ocean DOM to the upper mesopelagic microbial community resulted in net remineralization of $\sim 21 \mu\text{M}$ TOC (Table 1; Fig. 5a), or $\sim 27\%$ of the initial TOC pool. This large TOC consumption signal was accompanied by a drawdown in $[\text{NO}_3^-]$ of $\sim 0.8 \mu\text{M}$ (Fig. 5e), which was not observed for the Station 5 surface-meso treatment that also initially contained $\sim 0.6 \mu\text{M}$ $[\text{NO}_3^-]$ (Fig. 3e). Genes encoding for bacterial assimilatory nitrate reductase (i.e. *nasA*) are ubiquitous in the marine environment (Jiang et al., 2015) and their abundance has

346 been found to positively correlate with NO_3^- concentrations (Allen et al., 2005). Therefore
347 we hypothesize that both surface-meso microbial communities harbored the capacity for
348 NO_3^- uptake, suggesting some other environmental factor such as nitrogen limitation or
349 the availability of an energy source, i.e. labile DOM, is responsible for the differing
350 microbial NO_3^- uptake response between the experiments at Stations 1 and 5. Assuming
351 all of the NO_3^- assimilation accumulated as microbial biomass N, and a C:N ratio of 5 for
352 bacteria, we estimate a bacterial growth efficiency (BGE) of 33-46% for the observed
353 TOC consumption between day 0 and day 4 in the surface-meso treatments at Station 1.
354 These BGE estimates are near the upper limit of those reported previously for aquatic
355 systems (Del Giorgio and Cole, 2000), and we cannot rule out flaws in our assumptions
356 such as some small amount of NO_3^- assimilation accumulating as DON that is not
357 analytically resolved. Direct measures of the bacterial response are needed to verify these
358 estimates, however, the BGE we calculate are comparable in magnitude to those reported
359 by Pedler et al., (2015) (i.e. 27-52%) for microbial DOC consumption in a similar eastern
360 boundary upwelling system in the North Pacific.

361 The lack of a measurable change in [TOC] for the surface-surface treatments at either
362 station in the ETSP is consistent with previous results from the Sargasso Sea, where the
363 lack of DOM consumption by surface microbial communities has been attributed to the
364 relatively aged and degraded DOM left to accumulate in the surface mixed layer of
365 oligotrophic gyres (Carlson et al., 2002; 2004; Letscher et al., 2013). The results
366 presented here are consistent with the hypothesis that the relative ‘freshness’ of DOM,
367 and thus its attractiveness as a microbial substrate, is one control on surface ocean DOM
368 concentrations and lability. Station 1 is located downstream from the Peru-Chile eastern

boundary upwelling system (Fig. 1). As a result, the [TOC] in surface waters at Station 1 is elevated, ~80 μM (Fig. 4), compared to ~65 μM at Station 5 in the oligotrophic South Pacific gyre (Fig. 2). Across the global ocean, subtropical surface ocean [DOC] is typically in the range of 60 to 70 μM (Hansell et al., 2009). Thus the higher surface ocean [TOC] of ~80 μM at Station 1 in the ETSP indicates this site recently received an input of DOM from the highly productive, upstream eastern boundary system. Eastern boundary upwelling systems are known to export up to 35-60% of net community production as DOM transported laterally offshore (Álvarez-Salgado et al., 2007). Therefore, we hypothesize that the surface mixed layer DOM at Station 1 is ‘fresher’ and more bioavailable than the DOM in the surface waters at Station 5 in the South Pacific gyre.

Microbial communities are known to be vertically stratified in the upper ocean (Giovannoni et al., 1996; DeLong et al., 2006), which may contribute to the differing DOC remineralization responses observed between incubation treatments inoculated with surface mixed layer versus upper mesopelagic microbial communities. Ordination plots of the T-RFLP fragments collected from the microbial communities in the ETSP confirm that they are vertically segregated, as expected. However, there is no analogous zonal variation in the community structure within the same depth horizon across the cruise transect, in spite of a separation of >2000 km between some stations (Fig. 6). T-RFLP fragments clustered separately into two distinct groups based on depth in the water column that were common to all stations sampled: samples from ≤ 100 m (euphotic zone) versus between 100 m to 400 m (mesopelagic). Results from the T-RFLP analysis (Fig. 6) indicate that the inoculum from 200 m at Station 5 came from the ‘true’ mesopelagic community whereas the inoculum from 100 m at Station 1 was more closely related to

the euphotic zone community. Thus it appears that dissimilarity of the upper mesopelagic microbial community inoculum and the surface mixed layer community at each station offers a better explanation for the large difference in DOC consumption between Station 1 and Station 5 than the differing response being driven by distinct microbial communities present at the two stations. However, the surface and mesopelagic microbial populations were distinct at each station, suggesting that microbial community composition may have played a role in the different magnitude of TOC consumption at each station.

Limitation of DOM consumption by the availability of inorganic nutrients to bacteria [e.g. NO_3^- and phosphate (PO_4^{3-})] has been reported in previous studies (e.g. Zweifel et al., 1993; Kirchman, 1994; Cotner et al., 1997; Rivkin and Anderson, 1997; Caron et al., 2000; Hoch and Bronk, 2007). However, just as numerous are reports where inorganic nutrient amendments failed to stimulate bacterial production and DOM remineralization (e.g. Kirchman, 1990; Rivkin and Anderson, 1997; Carlson et al., 2002, Church, 2008). This discord likely points to variability in both time and space with respect to the microbial communities present and their nutritional status. The combination of low inorganic nutrient concentrations and the microbes adapted to those conditions in the mixed layer of the tropics and subtropics throughout most of the year may limit DOM remineralization and allow its surface accumulation. Because both micro- and macronutrient concentrations increase below the euphotic zone, export of DOM from surface to subsurface waters and observations of its subsequent remineralization there (e.g. Hansell and Carlson, 2001; Carlson et al., 2010) support arguments that increased availability of inorganic nutrients to the microbial community supports DOM

remineralization. Our results from Station 1 are consistent with this interpretation, suggesting that TOC remineralization was in part limited by the availability of nitrogen to the surface microbial community (as is typical of the ETSP, concentrations of PO_4^{3-} were $>0.3 \mu\text{M}$ in surface waters at both stations, indicating microbes were not limited by PO_4^{3-}). While the surface microbial community at Station 1 was unable to consume surface TOC (Fig. 4a), this same TOC was bioavailable to the microbial community at 100 m, which also consumed nearly all the NO_3^- in the incubation media (Fig. 5e). Using 60% similarity to define a community our data show that the microbial community at 100 m was distinct from the surface mixed layer inoculum microbial community (Fig. 6), thus the differing TOC remineralization responses can best be attributed to both differences in microbial community composition and NO_3^- availability. That TOC consumption is in part limited by NO_3^- in the ETSP surface waters is consistent with reports of nitrogen-limitation of primary production in the subtropical Pacific Ocean (e.g. Karl et al., 1997; Moore et al., 2004; Behrenfeld et al., 2006), suggesting heterotrophic production may also be NO_3^- -limited in this region.

We observed a small but measurable TOC consumption, $\sim 3 \mu\text{M}$ (Table 1), at Station 5 for the surface-meso treatments, however the question of nitrogen limitation of TOC remineralization at this station is enigmatic given the lack of observed changes in inorganic N (Fig. 3d, 3e). Thus it is unclear whether the smaller TOC consumption signal at Station 5 versus Station 1 is due to differences in the microbial community inoculum, nitrogen limitation, or both. One explanation for the observed gradient in TOC consumption between the two stations relates to the mesotrophic-to-oligotrophic gradient in DOM lability argument provided earlier. At both stations, TOC was consumed to a

similar final concentration, ~58-60 μM (Fig. 3a, 5a). From this observation, we hypothesize that this TOC concentration quantifies the recalcitrant fraction that is not immediately biologically available with concentrations above this value representing the labile TOC component in the ETSP system, which is remineralized over timescales of days to weeks. Surface TOC concentrations were ~80 μM and ~65 μM at Stations 1 and 5, respectively, thus the ~21 μM (~27% of pool) and ~3 μM (~4% of pool) TOC remineralization signals we observed are consistent with a mesotrophic-to-oligotrophic gradient in the amount of surface-accumulated labile TOC and near complete remineralization of this component when incubated with subsurface microbes at both stations after 1 to 2 weeks.

The large ~21 μM TOC remineralization observed for the surface-meso treatment at Station 1 is unique in that the 100 m microbial community was able to consume TOC to concentrations below that observed *in situ* at the same depth (Table 1). This additional TOC remineralization averaged ~2.5 μM ($[\text{TOC}]_{\text{t_final}} = \sim 55.5 \mu\text{M}$ in the seawater cultures (Fig. 5a); $[\text{TOC}]_{100\text{m_in situ}} = 58 \mu\text{M}$ (Table 1)). This outcome may reflect recent advective delivery of labile TOC to 100 m at Station 1 above the ~55.5 μM concentration that has yet to be consumed *in situ*, however subduction of the mixed layer with its burden of semilabile TOC is not expected until the colder months of austral winter at this site in the ETSP (Wong and Johnson, 2003), such that the *in situ* microbial community has likely had sufficient time (days/weeks) to consume the *in situ* TOC at our time of sampling. One possible explanation for this additional TOC consumption lies in the co-metabolism hypothesis (Madigan et al., 1997; Williams, 2000), in which bacteria utilize the energy gained from remineralization of labile OM to break down more recalcitrant

OM, which has been observed previously in marine systems (Carlson et al., 2002; Bianchi, 2011). Carlson et al. (2002) found that the addition of glucose, NH_4^+ , and PO_4^{3-} stimulated the co-metabolism of an additional 1 to 5 μM semilabile DOC by the *in situ* microbial community in the surface mixed layer of the Sargasso Sea. Both the stimulation of TOC remineralization by nutrient (NO_3^-) availability and the magnitude of additional semilabile TOC consumption at Station 1 in the ETSP are consistent with the co-metabolism response observed in the Sargasso Sea. Co-limitation of the 100 m microbial community at Station 1 in the ETSP by a micronutrient, e.g. iron (Church et al., 2000; Kirchman et al., 2000; Cochlan, 2001), reduced sulfur (Tripp et al., 2008), or B vitamins (Dupont et al., 2011; Sañudo-Wilhelmy et al., 2012), may provide an alternative explanation for the additional TOC remineralization. Such a mechanism would require elevated concentrations of the micronutrient within the surface mixed layer incubation media, such that when mixed with the upper mesopelagic microbial community, the seawater culture was relieved from micronutrient limitation to consume additional DOC below the *in situ* concentration. Dissolved iron [dFe] was not measured during the 2011 cruise to the ETSP, however observations of [dFe] from a 2010 cruise to Station 1 indicate a surface mixed layer [dFe] enrichment of ~ 0.2 nM above that measured in the upper mesopelagic (100 to 150 m) (Kondo & Moffett, 2015). If this pattern is assumed to be representative of the mean state of [dFe] near Station 1 in the ETSP, it is possible that relief from iron and NO_3^- co-limitation played a role in the large observed magnitude of TOC remineralization below *in situ* [TOC] in the Station 1 surface-meso experiments. Abundant marine heterotrophic bacterial lineages such as SAR11 and SAR86 lack the metabolic pathways for methionine and certain B vitamin synthesis (Tripp et al., 2008;

Dupont et al., 2011), making these groups auxotrophic for these necessary growth factors. However, concentrations of these species measured in the eastern North Pacific exhibit surface depletion and an upper mesopelagic maximum (Sañudo-Wilhelmy et al., 2012), opposite the pattern required to explain our results from Station 1.

Summary

We performed bottle incubation experiments to investigate environmental controls on DOM remineralization in the ETSP, comparing a mesotrophic station likely receiving fresh organic matter from the adjacent eastern boundary upwelling system with an offshore, oligotrophic station located in the South Pacific gyre. Surface-accumulated TON was largely recalcitrant to microbial remineralization by either surface or upper mesopelagic microbial communities at both stations. Surface-accumulated TOC was also recalcitrant to remineralization by surface communities at both stations over daily/weekly timescales, however subsurface microbial communities could consume TOC, albeit in differing quantities, and with or without concomitant drawdown of ambient NO_3^- . The microbial community found at 100 m at the mesotrophic station was able to consume ~21 μM of surface TOC and most of the NO_3^- present ($<1 \mu\text{M}$), while a smaller but significant amount of TOC (~3 μM) was consumed by the microbial community from 200 m at the oligotrophic station with no accompanied NO_3^- utilization. Distinct microbial communities in the surface mixed layer and the upper mesopelagic zone were apparent when comparing the DNA fingerprints from T-RFLP fragments, with smaller differences amongst communities within the same depth horizon across the zonal ETSP transect.

The lack of TOC remineralization when incubated with surface mixed layer microbial communities and the observed TOC remineralization when incubated with distinct communities from the upper mesopelagic support a microbial community composition control on TOC remineralization in the ETSP. DOM ‘freshness’ and nutrient stimulation also contributed to the observed gradient in the magnitude of TOC remineralization, with both a higher initial concentration of [TOC] (~80 μM vs. ~65 μM) and NO_3^- consumption contributing to significantly more TOC remineralization at the mesotrophic vs. oligotrophic station in the ETSP. Our experiments presented here suggest that co-metabolism could be a possible mechanism to explain the enhanced removal of recalcitrant TOC in the presence of more labile TOC, however additional work will be necessary to test this hypothesis. Relief of the microbial community from dFe limitation may have also played a role in explaining the additional TOC consumption in the seawater culture beyond *in situ* [TOC], however this hypothesis too remains to be tested. If either co-metabolism or nutrient (NO_3^- , dFe, etc.) stimulation of DOM consumption is determined to be widespread in the marine environment, then the potential for carbon to be sequestered via the microbial carbon pump, whereby microbial remineralization processes produce refractory DOM, is limited. Our results demonstrate how gradients in microbial community composition, DOM ‘freshness’, and nutrient availability can influence the bioavailability of DOM both vertically in the water column and zonally across ocean basins. Future efforts should examine the specific genomic and functional characteristics of microbial communities that allow for the differing magnitudes of DOM and nutrient substrate utilization.

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821 Figure 1. Map of the eastern tropical South Pacific showing station locations for the
 822 bottle incubation experiments (white markers) and adjacent stations where additional T-
 823 RFLP samples were collected (black markers). Map colors are sea surface chlorophyll *a*
 824 concentrations (mg m⁻³) at 9 km resolution as detected by the MODIS-Aqua satellite
 825 averaged over the months of March and April 2011 (data downloaded from:
 826 <http://disc.sci.gsfc.nasa.gov/giovanni/>). Arrow denotes the generalized surface ocean
 827 circulation with flow to the north and west along the Chilean eastern boundary upwelling
 828 system.

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831 Figure 2. Results from the surface-surface treatments at Station 5 (oligotrophic):

832 concentration (μM) vs. incubation duration (days) plots for (a) [TOC], (b) [TN], (c)

833 [TON], (d) [NH_4^+], and (e) [NO_3^-]. Solid, dashed, and dotted lines indicate triplicate

834 incubation carboys. Error bars are ± 1 S.D. of the analytical error of each measurement.

835 The X at time point 0 in (a) represents the TOC concentration measured in surface (5 m)

836 water collected via Niskin bottle.

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Figure 3. Results from the surface-meso treatments at Station 5 (oligotrophic): concentration (μM) vs. incubation duration (days) plots for (a) [TOC], (b) [TN], (c) [TON], (d) [NH₄⁺], and (e) [NO₃⁻]. Solid, dashed, and dotted lines indicate triplicate incubation carboys. Error bars are ± 1 S.D. of the analytical error of each measurement. The X at time point 0 in (a) represents the expected initial TOC concentration based on mixing of surface (5 m) media and subsurface inoculum (200 m) water collected via Niskin bottle.

Figure 4. Results from the surface-surface treatments at Station 1 (mesotrophic): concentration (μM) vs. incubation duration (days) plots for (a) [TOC], (b) [TN], (c) [TON], (d) [NH_4^+], and (e) [NO_3^-]. Solid, dashed, and dotted lines indicate triplicate incubation carboys. Error bars are ± 1 S.D. of the analytical error of each measurement. The X at time point 0 in (a) represents the TOC concentration measured in surface (5 m) water collected via Niskin bottle.

Figure 5. Results from the surface-meso treatments at Station 1 (mesotrophic): concentration (μM) vs. incubation duration (days) plots for (a) [TOC], (b) [TN], (c) [TON], (d) $[\text{NH}_4^+]$, and (e) $[\text{NO}_3^-]$. Solid, dashed, and dotted lines indicate triplicate incubation carboys. Error bars are ± 1 S.D. of the analytical error of each measurement. The X at time point 0 in (a) represents the expected initial TOC concentration based on mixing of surface (5 m) media and subsurface inoculum (100 m) water collected via Niskin bottle.

Figure 6. Nonmetric multidimensional scaling (NMS) ordination plot of T-RFLP fragments generated from community 16S rRNA at the four stations in the ETSP (see Fig. 1 for reference). Sample nomenclature reads station #_sample depth (m). Euphotic zone samples ($\text{PAR} > 1\%$) are denoted by red triangles, with open triangles defining the surface inocula. Mesopelagic zone samples ($\text{PAR} < 1\%$) are denoted by blue circles, with open

870 circles defining the mesopelagic inocula. Samples with >40% and >60% similarity (Bray-
871 Curtis) are encircled by solid and dashed lines, respectively. Based on the depth of each
872 sample, T-RFLP fragments partition by depth along the y-axis of the ordination plot.
873 |

Station 5	[TOC] _{in_situ}	[TOC] _{t_initial}	[TOC] _{t_final}	ΔTOC	ΔTN	ΔTON	ΔNO ₃ ⁻
surface- surface	5 m						
A		65.0	65.4	BD ^a	BD ^a	BD ^a	BD ^a
B		64.8	65.9	1.1	BD	BD	BD
C		66.9	64.7	-2.2	BD	BD	BD
mean	66.3	65.6	65.3	-0.4	--	--	--
surface-meso	200 m						
A		61.9	60.4	-1.5	BD	BD	BD
B		63.8	60.0	-3.8	BD	BD	BD
C		63.1	60.0	-3.1	BD	BD	BD
mean	55.6	62.9	60.1	-2.8	--	--	--
Station 1							
surface- surface	5 m						
A		78.9	81.5	2.6 (1.0) ^b	BD	BD	BD
B		77.8	81.0	3.2 (0.5) ^b	BD	BD	BD
C		77.6	79.5	1.9 (-1.0) ^b	BD	BD	BD
mean	80.5	78.1	80.7	2.6 (BD) ^b	--	--	--
surface-meso	100 m						
A		76.9	55.5	-21.4	BD	0.73	-0.77
B		76.7	56.6	-20.1	BD	0.78	-0.69
C		76.1	54.8	-21.3	BD	0.66	-0.87
mean	58.1	76.6	55.6	-20.9	--	0.72	-0.78

874

875 ^aBD = Below Detection where the detection limit is taken as the analytical uncertainty of
876 the nutrient measurement: 1 μM for ΔTOC, 0.3 μM for ΔTN and ΔTON, and 0.05 μM for
877 ΔNO₃⁻.

878 ^bΔ if calculated using initial [TOC] as measured from *in situ* (Niskin) samples at this
879 station

880

881 Table 1. Net changes (deltas; Δ) for the biogeochemical concentrations in each incubation
882 carboy for the four experiments. Letters A, B, and C denote each of three replicate

883 carboys for each treatment with the mean reported. Negative values indicate net
884 remineralization and positive values are net production at the end of the 9 day (Station 5)
885 or 14 day (Station 1) incubation experiment.
886