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Relating sinking and suspended microbial communities in the California Current Ecosystem: digestion resistance and the contributions of phytoplankton taxa to export

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Summary

We used 16S, 18S, plastid and internal transcribed spacer (for *Synechococcus* strains) sequencing to quantify relative microbial abundances in water-column samples and on sediment-trap-collected particles across an environmental gradient in the California Current Ecosystem (CCE) spanning a > 60-fold range of surface chlorophyll. Most mixed-layer dominant eukaryotes and prokaryotes were consistently underrepresented on sinking particles. Diatoms were the only phototrophic taxa consistently overrepresented. Even within this class, however, one genus (*Thalassiosira*) was a particle-enriched dominant, while a similarly abundant species was poorly represented. *Synechococcus* was significantly enriched on sinking particles at only one of four sites, but clade I was disproportionately abundant on sinking particles throughout the region compared with clade IV, the euphotic-zone co-dominant. The most abundant microbes on particles across the CCE were organisms with distributional maxima close to the sediment-trap depth (rhizarians), microbes associated with metazoans or sinking particles as a nutritional habitat (certain alveolates, Gammaproteobacteria) and organisms that resist digestive degradation of their DNA (*Thalassiosira*, *Synechococcus*). For assessing taxon contributions of phytoplankton to carbon export, our results highlight the need for sequence-based quantitative approaches that can be used to integrate euphotic-zone abundances, compute rates and account for taxon differences in preservation of sequence markers through trophic processing.

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Introduction

The biological pump includes all of the processes that fix organic carbon production and transform and transport it to the deep sea (Longhurst and Harrison, 1989; Ducklow *et al*., 2001). The pump magnitude and efficiency are affected by the structure and trophic interactions of plankton communities in the euphotic zone and by the activities of microbial and metazoan consumers that attenuate sinking particle flux in the water column (Longhurst and Harrison, 1989; Ducklow *et al*., 2001; Buesseler *et al*., 2007; Stukel *et al*., 2011). Large phytoplankton with mineral tests, such as diatoms, are conventionally considered responsible for a disproportionate amount of the ~10% (Siegel *et al*., 2014) of global ocean productivity exported out of the euphotic zone (Michaels and Silver, 1988). More recently, however, metabarcoding characterization of microbial communities on sinking particles has challenged this view, with sequences of smaller forms, such as *Synechococcus*, prasinophytes and dinoflagellates, now associated with or considered to have dominant roles in particulate carbon export in open-ocean systems (e.g. Amacher *et al*., 2013; Guidi *et al*., 2016). Rhizarians, Flavobacteria and parasitic taxa (e.g. Syndiniales) have also been found to be enriched in sinking material (Fontanez *et al*., 2015; Guidi *et al*., 2016; Boeuf *et al*., 2019; Gutierrez-Rodriguez *et al*., 2019). Despite these new insights, the relative importance of different microbes to carbon fluxes under varying environmental conditions and the mechanisms driving their export remain poorly known (Richardson and Jackson, 2007; Stukel and Landry, 2010). A particularly important interpretative question is whether metabarcoding results reflect the relative contributions of source microbial populations to the export process, as opposed to revealing possible biases.

Process cruise studies of the California Current Ecosystem – Long-Term Ecological Research (CCE-LTER) Program provide an opportunity to evaluate how microbial assemblages vary in their associations to particle flux in an environment with contrasting physical and ecological conditions (Stukel and Barbeau, 2020). The inshore region of the CCE is a highly productive coastal upwelling habitat characterized by high biomass and dominance of larger primary producers, typically diatoms and dinoflagellates (Venrick, 2002; Taylor *et al*., 2015). In contrast, the offshore open-ocean region resembles the oligotrophic central gyres in low biomass and dominance of small cells (Taylor and Landry, 2018). These differences also translate to the properties of exported particles, with mesozooplankton faecal pellets accounting for most export in the inshore region and amorphous aggregates the dominant transport mechanism offshore (Knauer *et al*., 1979; Stukel *et al*., 2013a; Morrow *et al*., 2018).

In the present study, we use metabarcoding to characterize microbial assemblages in the water column and on sinking particles across the gradient of CCE environmental conditions. In addition to distinguishing eukaryote and prokaryote assemblages separately with standard 18S and 16S rRNA gene markers, we use plastid sequences to compare the relative abundances of cyanobacteria and eukaryotic phytoplankton directly and internal transcribed spacer (ITS) sequencing to distinguish *Synechococcus* strains. We focus on taxa that are consistently over- or underrepresented on sinking particles (relative to the water column) and address the following questions: How do the relative sequence abundances of microbial taxa primarily present in the mixed layer differ in their representation on sinking particulate matter? What are the common characteristics of taxa that are consistently enriched on sinking particles? Our results suggest that taxon differences in DNA survival through food web interactions is a significant issue for using relative sequence abundances to infer phytoplankton contributions to carbon export.

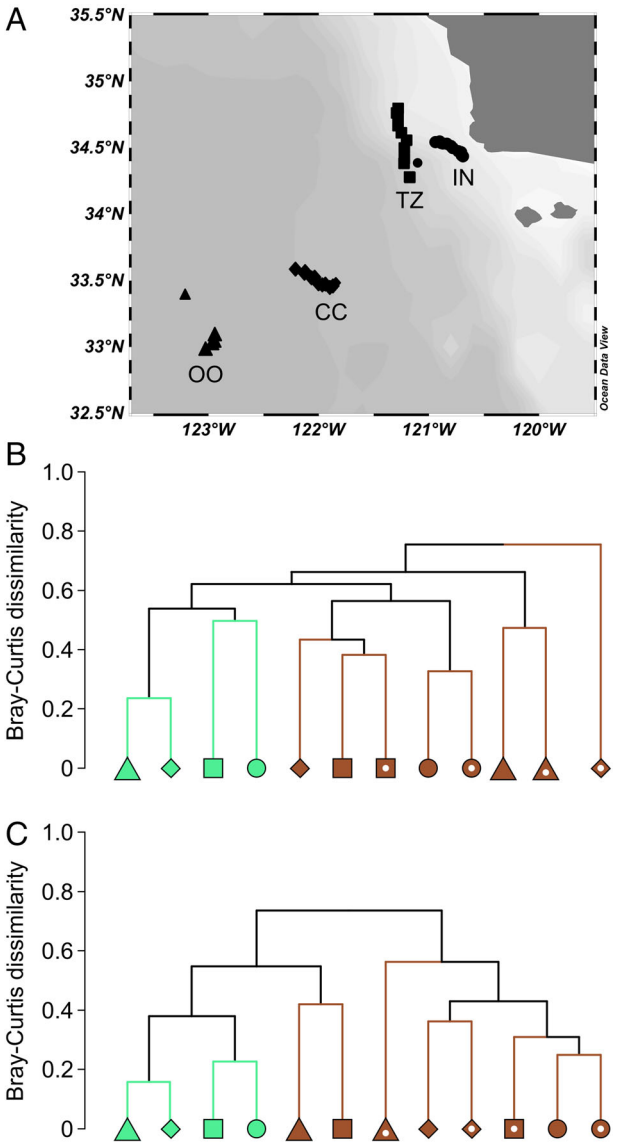

Fig. 1. Microbial communities in the water column and sinking particles spanning an inshore-offshore gradient in environmental conditions across the California Current Ecosystem.
A. Map showing the sampling locations. Dendrogram showing the differences in the structure of microbial communities between water column (green) and sinking particles (brown) for (B) protists (18S) and (C) bacteria (16S). Sinking particles were collected in live and fixed sediment traps (symbols with a dot).

Results

Mixed-layer microbes on sinking particles

Despite a 60-fold range in chlorophyll *a* and > 100-fold variability in nitrate concentration (Valencia *et al*., 2021), mixed-layer microbial communities across the CCE environmental gradient were more similar to each other than to the communities collected on sinking particles directly below them (Fig. 1B and C, Table S1). The assemblages on sinking particles differ significantly from water-column communities for both eukaryotes and prokaryotes (Simprof, $p < 0.05$; Fig. 1B and C). The samples also group into two clusters, differentiating protistan and bacterial communities of the richer and poorer CCE waters [Inshore (IN) and Transition Zone (TZ) versus California Current (CC) and Oligotrophic Offshore (OO)]. Protists on sinking particles group mainly by site, independent of live or fixed trap treatment (Fig. 1B). For particle-associated bacteria, two groups contain sequences of the IN and CC sites, whereas the TZ and OO site sequences group together for live but not fixed traps (Fig. 1C).

Of the 451 protistan taxa from 18S sequencing, only 10 showed > 5% relative abundance in at least one mixed-layer sample (Fig. S1). Four of these dominant mixed-layer taxa, the chlorophytes *Ostreococcus* and *Micromonas* and unidentified Cryptomonadales and Bacillariophyceae, were unenriched or significantly underrepresented on sinking particles at all of the CCE sites (Fig. 2). The diatom *Thalassiosira* stands out as the only taxon significantly overrepresented on sinking particles at all sites, with trap enrichment factors ranging from 2.4 (IN live) to 1163 (CC live) higher than relative mixed-layer sequence abundances, and averaging 192 $\pm$ 140 (SEM, standard error of the mean) times higher for live and fixed traps at all sites. Dinoflagellate sequences for MALV-I clade I and *Lepidodinium* are also disproportionately abundant on trap particles by mean relative enrichment factors of 4.9 $\pm$ 2.7 and 2.5 $\pm$ 0.8, respectively, but their patterns differ spatially, with the highest values for *Lepidodinium* (3.8X – live; 6.5X – fixed) at IN and the highest enrichment for MALV-I (12.6X – live; 21.0X – fixed) at TZ. In contrast, the dinoflagellates *Karlodinium*, *Glenodiniopsis* and unidentified Dinophyceae are underrepresented on sinking particles on average across all sites, despite each being significantly enriched in one trap at one site (Fig. 2).

Of the 319 bacteria taxa identified from 16S sequencing, only 11 had > 5% relative abundance in a mixed-layer sample (Fig. S2). All water-column dominants were significantly underrepresented in the prokaryote trap samples at IN and TZ sites, and most taxa, including SAR 11, *Prochlorococcus*, *Synechococcus*, *Fluvicola* and *Formosa*, were significantly underrepresented in trap particles at all sites (Fig. 3). Only unidentified Bacteroidetes Flavobacteriaceae was consistently overrepresented in sinking particles from the CC and OO traps, while relative sequences of Alphaproteobacteria *Roseobacter* and Gammaproteobacteria OM60(NOR5) were significantly higher only in OO live traps.

The plastid analyses allow comparison of the relative abundances of cyanobacteria *Prochlorococcus* and *Synechococcus* to dominant eukaryotic classes in water-column and trap samples, which cannot be done from the separate 16S and 18S analyses (Fig. S3). Among these, diatoms (Bacillariophyceae) are significantly overrepresented in all traps and sites except CC (Fig. 4), with an overall mean trap enrichment factor of 19.8 $\pm$ 6.6 compared with relative plasmid sequences in the water column. *Synechococcus* are significantly overrepresented on sinking particles in CC trap samples, but, on average, have relatively similar relative abundances in water-column and trap samples (mean 1.1 $\pm$ 0.3 enrichment) with respect to other phototrophs. At the most oligotrophic OO site, relative sequences of cyanobacteria and eukaryotes separate cleanly, with the former significantly underrepresented in trap samples and the much rarer diatom, chlorophyte (Mamiellophyceae) and prymnesiophyte sequences significantly overrepresented on sinking particles (Fig. 4).

ITS sequencing revealed 14 *Synechococcus* strains, with all strains occurring at the OO site and the number decreasing shoreward (Fig. S4). Most sequences were assigned to clades I and IV. Regardless of whether clade abundances are similar (IN and OO) or clade IV dominated (TZ and CC) (Fig. S4), relative abundances of clade I are consistently overrepresented on trap particles, and clade IV sequences are consistently underrepresented (Fig. 5; mean enrichment factors of 2.0 $\pm$ 0.2 and 0.4 $\pm$ 0.1 respectively).

Taxa common in sediment trap samples

Protistan sequences on sinking particles were dominated by alveolates and rhizarians (Fig. S1). Among the alveolates, the ciliates *Strombidium* and Scuticociliatia show significantly higher relative sequence abundances in live compared with fixed traps, which may indicate active feeding and growth on the unfixed particles during trap deployment. In contrast, *Pseudotontonia*, *Lepidodinium*, *Karlodinium*, the syndiniales MALV-I clade I and MALV-III, and an uncultured dinoflagellate have similar relative sequence proportions in live and fixed traps (Fig. 6). Sequences of the parasite Cephaloidophoroidea were the most abundant of all protists (Fig. 6) and were disproportionately important in fixed traps at the TZ and CC sites (Fig. S1).

Rhizarians had high relative abundances in trap particles, mainly at the TZ and OO sites (Fig. S1). Considering all locations, the polycystines *Tetrapyle* and Spumellaria and the Chaunacanthida *Helkesimastix* are significantly overrepresented in live traps (Fig. 6). In contrast, Phaeodaria *Aulosphaera* has significantly higher relative abundances in fixed traps (Fig. 6). *Thalassiosira* shows high relative abundances on trap particles at the IN, TZ and CC sites (Fig. S1) and are significantly overrepresented in fixed traps ($p < 0.01$ FET) (Fig. 6).

Bacterial sequences in trap particles were largely dominated by the Gammaproteobacteria *Pseudoalteromonas* and *Vibrio* (Fig. S2). Both taxa were significantly overrepresented in fixed traps at all sampling locations (Fig. 6). In contrast, relative sequence abundances of other Proteobacteria (*Roseobacter*, BD1-7 and *Colwellia*) were significantly higher in live traps (Fig. 6). *Synechococcus*, the dominant phototrophic bacteria in trap particles (Fig. S2), have similar sequence proportions in live and fixed traps (Fig. 6).

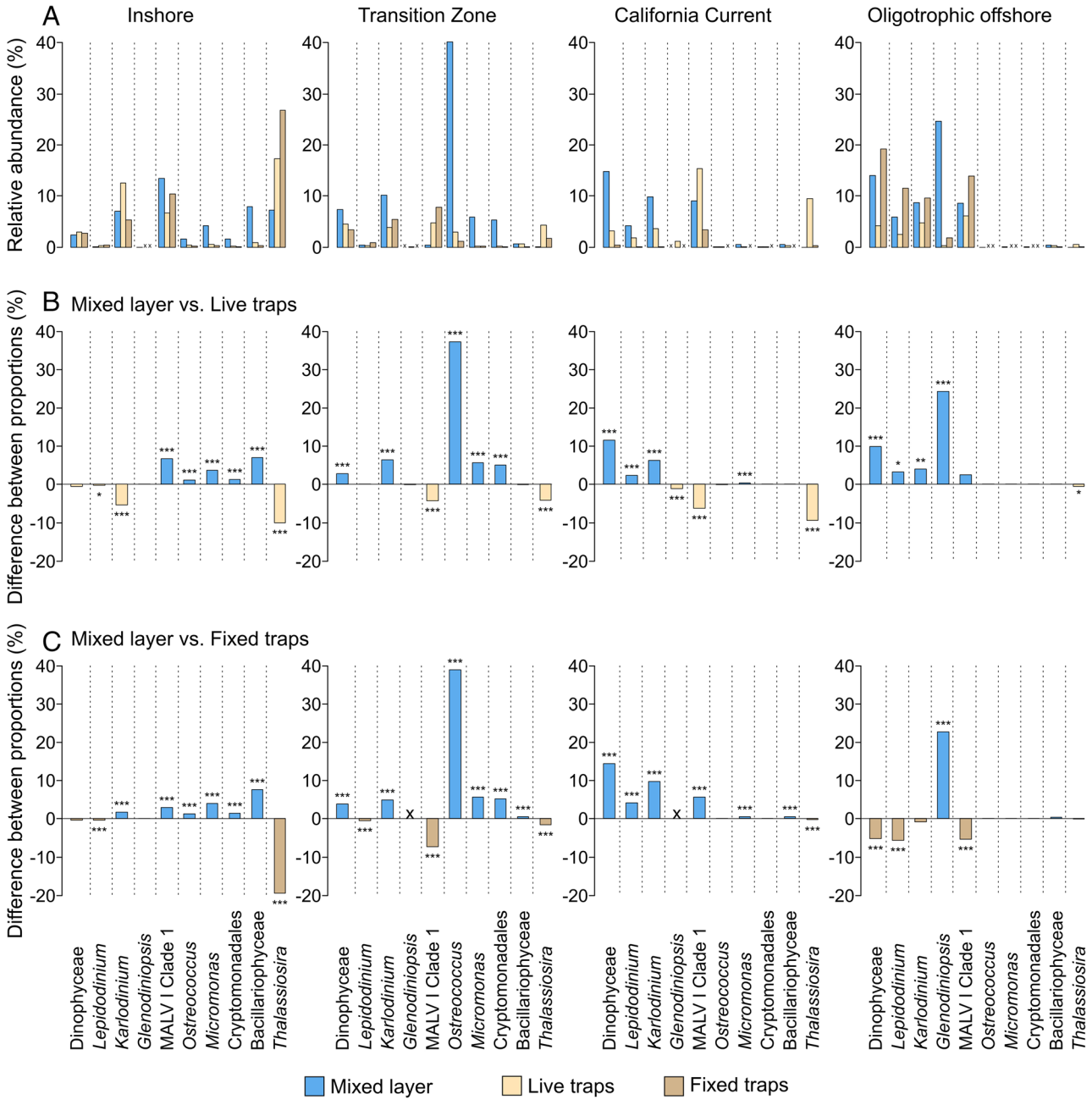

Fig. 2. Comparisons of protist sequences in the water column to sinking particles collected below the euphotic zone.
A. Relative abundances of 18S protist sequences in the mixed layer and sinking particles collected in live and fixed sediment traps. Differences in proportions of 18S protistan sequences between (B) the mixed layer and live traps and (C) the mixed layer and fixed traps. Negative values indicate the protists that were over-represented in the traps. Differences in proportions were evaluated for protists with a relative abundance > 5% in the mixed layer using the Fisher's exact test. Significant differences are indicated as follows: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Discussion

Metabarcoding analyses of samples collected across the CCE environmental gradient allow an unprecedented characterization of the microbial taxa in the water column and on sinking particles from coastal upwelling to stratified oligotrophic conditions. As expected, the water-column community transitioned sharply from strong diatom dominance in the coastal area to mostly *Prochlorococcus* and *Synechococcus* in the offshore waters, but that was not the primary determinant of the taxa found in trap samples. Overall, the similarities among water-column samples across stations were much greater than between water-column and sinking particles at any location, suggesting that specific taxa are more

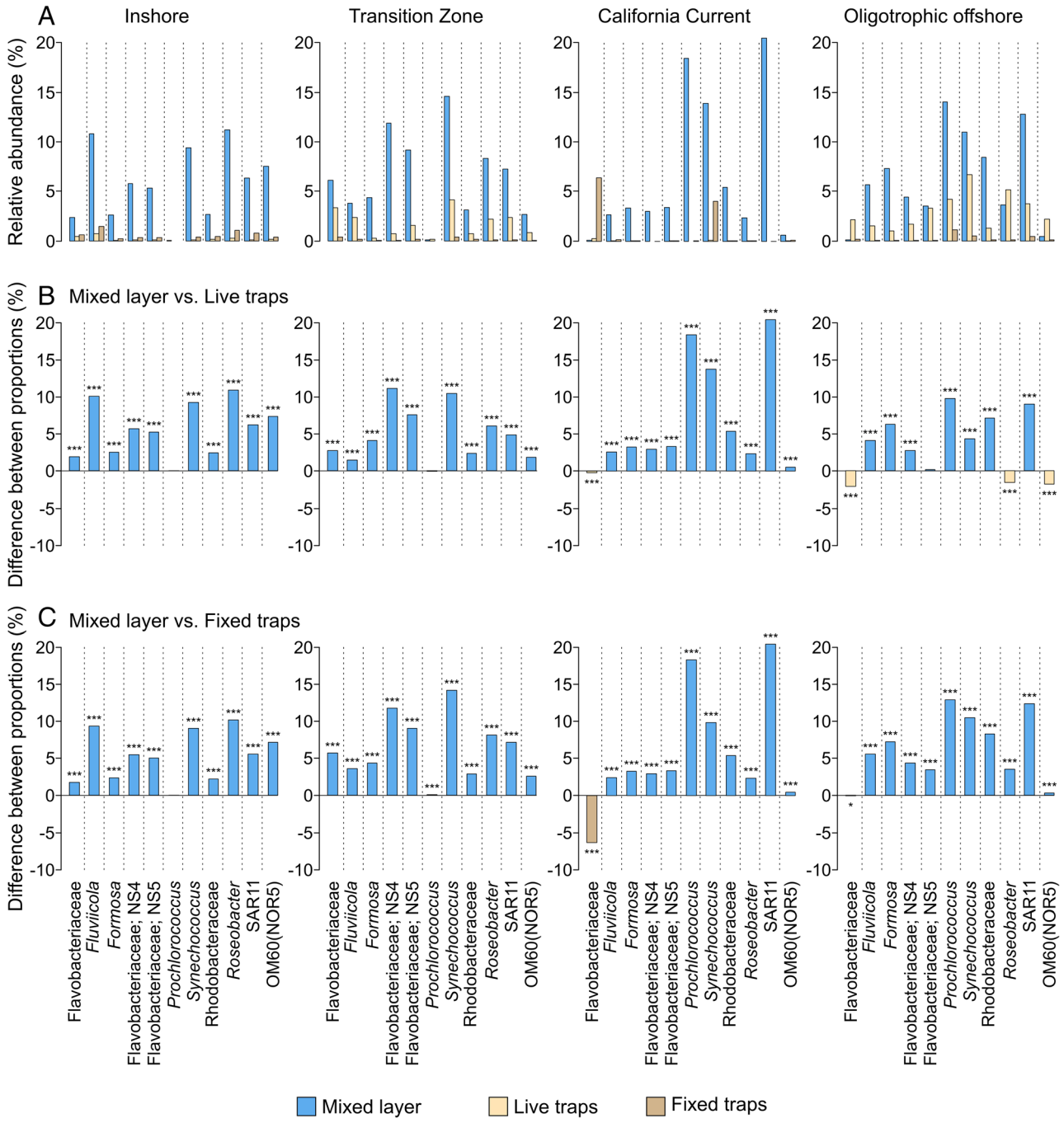

Fig. 3. Comparisons of bacteria sequences in the water column to sinking particles collected below the euphotic zone.
A. Relative abundances of 16S bacteria sequences in the mixed layer and sinking particles collected in live and fixed sediment traps. Differences in proportions of 16S bacteria sequences between (B) the mixed layer and live traps and (C) the mixed layer and fixed traps. Negative values indicate the bacteria that were over-represented in the traps. Differences in proportions were evaluated for bacteria with a relative abundance > 5% in the mixed layer using the Fisher's exact test. Significant differences are indicated as follows: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

likely to be overrepresented in trap sequences regardless of the physical, ecological and biogeochemical processes driving variability of the epipelagic ecosystem.

Assessing microbial community composition from relative sequence abundances is known to be affected by methodological limitations associated with DNA extraction and amplification, the presence of extracellular DNA, and organismal variability in rDNA copy number that tends to overrepresent certain groups, such as dinoflagellates, ciliates and rhizarians (Not *et al*., 2009; Wang

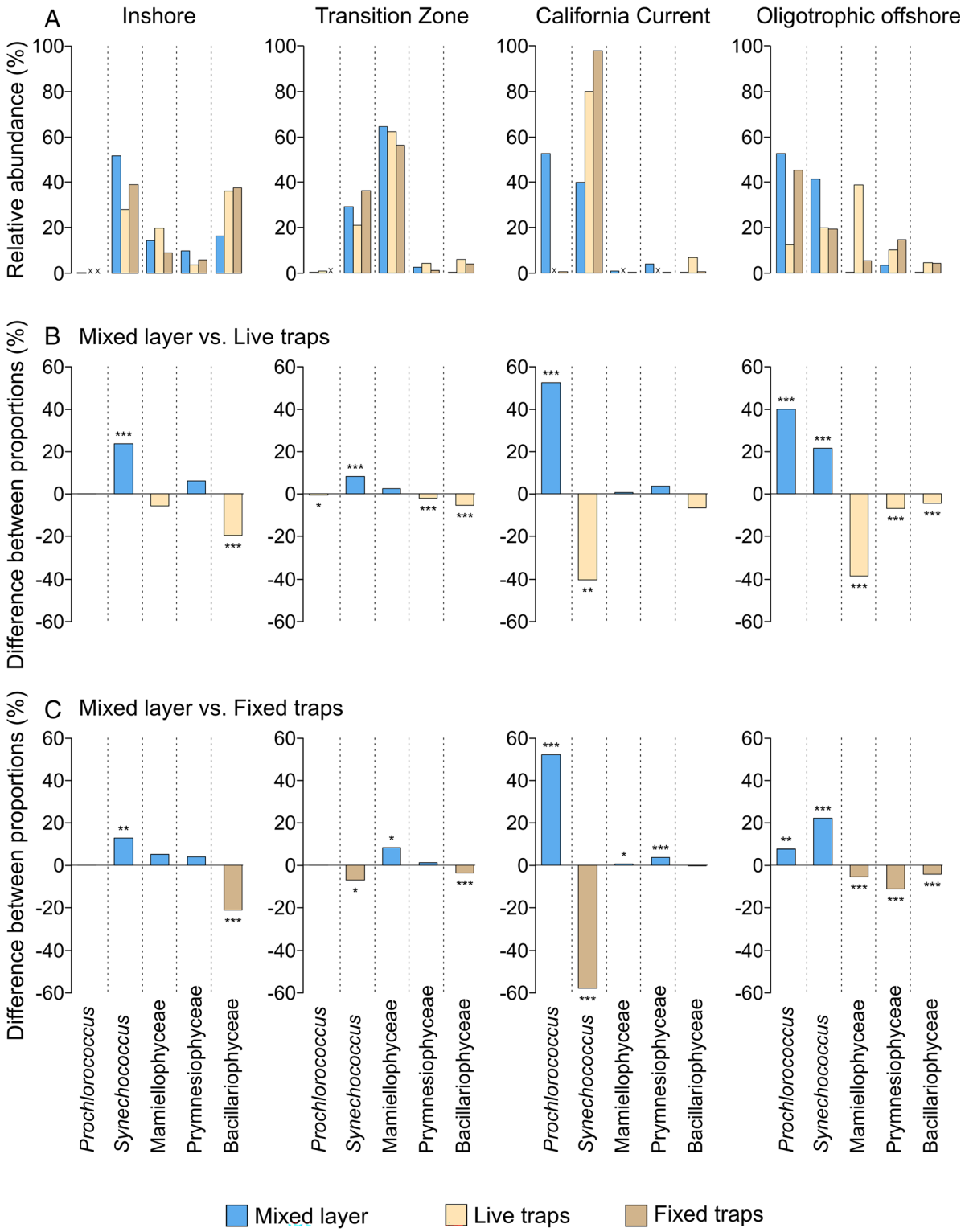

Fig. 4. Comparisons of phytoplankton sequences in the water column to sinking particles collected below the euphotic zone.
A. Relative abundances of 16S plastid sequences in the mixed layer and sinking particles collected in live and fixed sediment traps. Differences in proportions of 16S plastid sequences between (B) the mixed layer and live traps and (C) the mixed layer and fixed traps. Negative values indicate phytoplankton that were overrepresented in the traps. Difference in proportions were evaluated for plastid sequences with a relative abundance > 5% in the mixed layer using the Fisher's exact test. Significant differences are indicated as follows: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

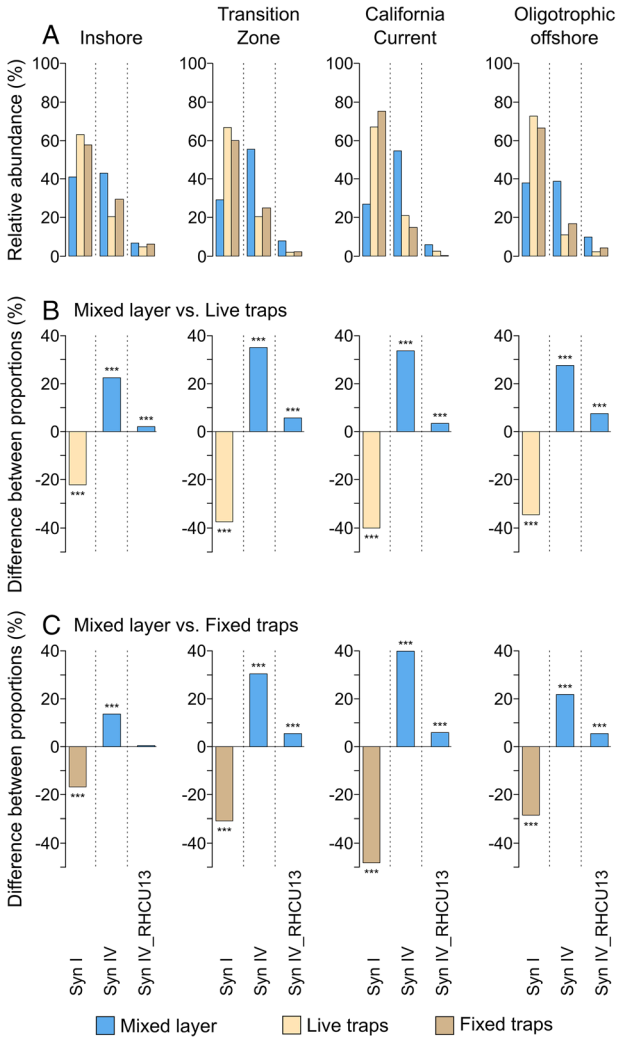

Fig. 5. Comparisons of *Synechococcus* strain sequences present in the water column to sinking particles collected below the euphotic zone.
A. Relative abundances of *Synechococcus* ITS sequences in the mixed layer and sinking particles collected in live and fixed sediment traps. Differences in proportions of *Synechococcus* sequences between (B) the mixed layer and live traps and (C) the mixed layer and fixed traps. Negative values indicate the *Synechococcus* strains that were over-represented in the traps. Differences in proportions were evaluated for strains with a relative abundance > 5% in the mixed layer using the Fisher's exact test. Significant differences are indicated as follows: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

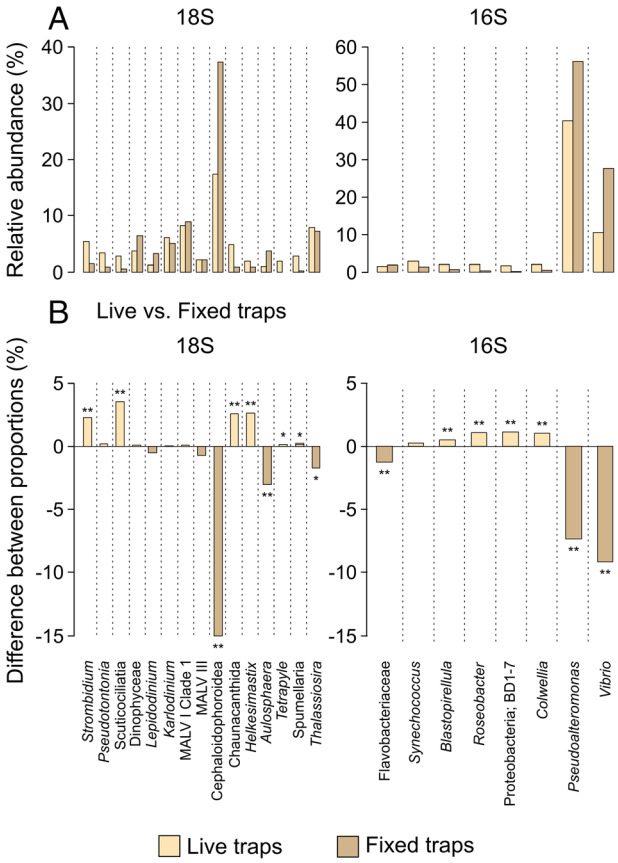

Fig. 6. Comparisons of protistan and bacterial sequences in sinking particles collected in live versus fixed sediment traps.
A. Relative abundances of 18S protistan sequences and 16S bacteria sequences in live and fixed sediment traps.
B. Differences in proportions of 18S protist sequences and 16S bacteria sequences between live and fixed traps. Negative values indicate the protists and bacteria that were over-represented in fixed traps. Differences in proportions were evaluated for protists and bacteria with a relative abundance > 5% in the mixed layer using the Fisher's exact test. Significant differences are indicated as follows: *$p < 0.05$, **$p < 0.001$.

et al., 2019). Beyond these, using relative sequence abundances to compare microbial contributions to export should also carefully consider how the interpretations can be affected by specific biases, such as: (i) population depth distributions, (ii) microbial associations with metazoans or sinking particles as a nutritional habitat and (iii) varying degrees of food-web digestion resistance. In the sections below, we use the full CCE water-column and trap sample data (Figs S1–S4) to illustrate these general principles and their implications for evaluating the relative contributions of phytoplankton taxa to carbon export from the euphotic zone.

Populations with deep distributions

Rhizarians are generally among the dominant protists on sinking particles across the CCE (Gutierrez-Rodriguez *et al*., 2019; Preston *et al*., 2020) and in oligotrophic areas of the North Atlantic and North Pacific (Amacher *et al*., 2013; Fontanez *et al*., 2015). Video profiles in the CCE show that they occur in highest abundance beneath the euphotic zone, where they are major contributors to flux attenuation of sinking particles and the transport of biogenic silica to deeper depths (Biard *et al*., 2016, 2018; Stukel *et al*., 2018a; Biard and Ohman, 2020). The deeper distributions of rhizarians are also evident in

the relative sequence abundances from our water-column sampling (Fig. S1), which we use here to illustrate a potential bias associated with assessing the export contributions of populations that reside in low numbers in the mixed layer or euphotic zone. For example, if the relative sequences of Spumellaria, RAD-A and the Acantharia Chaunacanthida in 150 m traps are compared with their low relative sequence abundances in the mixed layer, they appear to be greatly enriched in the trap samples, by an average factor of 74. However, when compared with relative sequence abundances of these taxa in the water column at the trap depth of 150 m, they are actually underrepresented in the trap samples (mean trap enrichment factor = 0.3). Thus, while these deep-living, mineral-dense taxa have relatively short distances to sink into the traps from their depth distribution maxima, our results suggest that they are less likely to appear in traps than other particles that pass through their depth stratum. That sequences of Chaunacanthida, *Helkesimastix*, *Tetrapyle* and Spumellaria were all significantly enriched in live traps compared with formalin-fixed traps might also indicate that they are able to grow actively or experience lower consumption/degradation in the live traps compared with other components of the sinking particles.

Although less is known about their specific ecologies, similarities to the rhizarian sequence distributions noted above suggest that other protistan and prokaryotic groups might also be better viewed as originating closer to the trap depth than from the shallow euphotic zone. For example, Scuticociliata and MALV-1 clades II and VII, the proteobacteria *Vibrio*, *Colwellia*, ZD0405 and SAR11Deep, Thaumarchaeota MGI and Deferribacteres SAR406 all have consistently higher relative sequence abundances at 150 m depth than in the mixed layer (Figs S1 and S2). Among these, however, only *Vibrio* and *Colwellia* show evidence of trap enrichment relative to the sequence abundances at 150 m (mean enrichment factors of 163 and 10, respectively), which would be even more exaggerated (mean enrichment of 2310 and 92-fold) in comparison to the mixed-layer relative sequences. As discussed further below, we attribute the unusually high trap enrichment of these two bacterial taxa to their likely arrival in metazoan hosts rather than as surviving food-web processing.

Lastly, depth distribution might also be a relevant factor for interpreting the relative associations of *Synechococcus* clades with export. The mixed-layer dominance of clades I and IV at all CCE sites is consistent with upwelling regions of the North and South Pacific (Zwirglmaier *et al*., 2008; Sohm *et al*., 2016; Nagarkar *et al*., 2021). In the Sargasso Sea, however, clades II and III dominate the water column, with clade III more strongly associated with export (De Martini *et al*., 2018). In our data, direct comparisons of relative ITS trap sequences to mixed-layer clade distributions indicate that clade I sequences are five times more likely to appear in the traps than clade IV sequences. However, the relative clade sequences in the water column at 150 m are roughly proportional to those in the trap samples. This implies a mechanism – possibly digestion resistance (Zwirglmaier *et al*., 2009) or aggregation properties (Deng *et al*., 2015, 2016) – that selectively transports clade I over clade IV sequences to depth, but once there, the deep water and the traps have similar clade compositions, likely resulting from the disintegration of faecal pellets or aggregates at depth.

Metazoan- and particle-associated microbes

Previous studies comparing 'live' and 'fixed' sediment trap samples found that preservation differences were a major determinant of variability (Fontanez *et al*., 2015; Gutierrez-Rodriguez *et al*., 2019), implying that microbial community succession on sinking particles may be rapid and selective. Contrary to this expectation and despite following similar protocols, we did not find consistent differences between the two treatments, which could be due to sampling a broader range of environmental conditions. Among alveolates, however, ciliates were generally more enriched in live trap samples (Silver *et al*., 1984), suggesting that they colonize and actively graze and grow on the unfixed particles during trap deployment and likely play an important role in transforming particles during sinking. In contrast, the sequences of parasitic alveolates such as syndiniales (MALV-I clade I and MALV-III) and Cephaloidophoroidea were either enriched in fixed traps or similar in both treatments, suggesting that they sank within hosts and did not grow afterwards. Together, these protistan groups, which we attribute to arriving in sediment traps via mechanisms that did not involve prior consumption in the food web, comprised 44%–53% of all Operational Taxonomic Units (OTUs) (55%–59% of the dominant OTUs) in the live and fixed traps, respectively, across all CCE conditions. As a consequence, the majority of the protistan sequences in the trap samples cannot be viewed as having survived trophic processing.

Similarly, among the prokaryotes, two eukaryote-associated Gammaproteobacteria, *Vibrio* and *Pseudoalteromonas*, account for the majority of the total OTUs (51% live, 83% fixed) and the dominant OTUs (59% and 94%, respectively) in trap samples. Fontanez *et al*. (2015) similarly found that *Vibrio* was significantly enriched in fixed traps along with the genes for carbohydrate uptake, chemotaxis and chitin utilization, consistent with the presence of copepods. Fontanez *et al*. (2015) further suggested that *Pseudoalteromonas* may be able to grow on their hosts in live traps. In our case, however,

it appears that even if *Pseudoalteromonas* and *Vibrio* were able to grow in the CCE live traps, they likely fell behind other prokaryote taxa in growth rate and subsequently decreased in relative sequence abundance over the duration of trap deployment.

Variability in food-web digestion resistance

Recognizable sequences from mixed-layer microbes can sink to sediment traps in aggregates or faecal pellets either as intact cells or as the surviving products of food-web processing (Alldredge and Gotschalk, 1989; Bauerfeind *et al*., 1994; Ebersbach and Troll, 2008; Stukel *et al*., 2013a). For considering taxonomic variability in food-web survival, our premise is that the vast majority of phytoplankton production is consumed by grazers before leaving the euphotic zone. On a global basis, protistan microzooplankton consume about two-thirds of phytoplankton biomass production, with the proportion varying modestly, on average, between coastal and open ocean habitats and tropical to polar regions (Calbet and Landry, 2004; Steinberg and Landry, 2017). More specific to our study area, experimental studies have demonstrated that the combined grazing of protists and metazooplankton strongly determine the measured net rates of change of phytoplankton biomass in the ambient water-column as well as the export ratios across the CCE coastal to oceanic environmental gradient, with generally negligible evidence of direct phytoplankton sinking measured as chlorophyll *a* flux to sediment traps (Landry *et al*., 2009; Stukel and Landry, 2010).

One implication of pervasive grazing pressure is that organisms of similar size with common consumers should be utilized at roughly comparable rates. For example, biomass turnover rates of picophytoplankton (*Prochlorococcus*, *Synechococcus* and < 2 μm eukaryotic taxa), mainly driven by predation of small protists, are typically within a factor of two in a given system (Brown *et al*., 1999; Landry *et al*., 2011). Larger phytoplankton, notably diatoms, are often able to grow 2–3 times faster than picoplankton under nutrient replete conditions but typically outstrip the grazing potential of their metazoan and larger protistan consumers, so that their rates of grazing losses can be more like those of smaller cells. Some taxonomic differences are thus expected if the phytoplankton sequence compositions in traps reflect their relative utilization rates within pelagic food webs, but very large differences are inconsistent with our general understanding of how these food webs function. In the present study, one mixed-layer dominant, the diatom *Thalassiosira*, stands out as having consistently high relative enrichment factors in traps, suggesting a preservational bias in passage through the food web.

While *Thalassiosira* is strongly grazed by mesozooplankton, it appears to have some ability to pass through the ingestion and digestion process at least partially intact. In the Baltic Sea, for example, Schrader (1971) found high densities of *Thalassiosira baltica* in faecal pellets of the dominant copepod grazer, *Calanus finmarchicus*, that were visually similar to living cells, whereas the thecae of ingested co-occurring diatoms (*Chaetoceros* sp.) were crushed and broken. In the Mediterranean Sea, Fowler and Fisher (1983) demonstrated that viable cultures of *Thalassiosira* spp. could be grown from faecal pellets that passed through zooplankton digestion in lab experiments and, more importantly, from the faecal pellets of field-collected zooplankton feeding at low ambient food conditions that precluded superfluous feeding or inefficient digestion. In the CCE study region, Preston *et al*. (2020) recently reported that *Thalassiosira* accounted for > 70% of the photosynthetic sequences surviving an episodic export pulse that reached abyssal depth (Station M, sediment trap at 4100 m). Although the mechanism of transport out of the euphotic zone was not specifically investigated, correlation with increased sequences of metazoan consumers during this time suggested efficient transport in fast-sinking faecal pellets (Preston *et al*., 2020).

In the present study, the case for *Thalassiosira* exiting the euphotic zone as consumed cells inside faecal pellets is strongest for the IN station, where > 2 μM nitrate concentration in the mixed layer remained sufficient for phytoplankton growth (i.e. not a senescent bloom) and where 98% of the organic material measured in traps directly below the euphotic zone at 50 m was in the form of recognizable faecal pellets (Morrow *et al*., 2018; Valencia *et al*., 2021). At this station, *Thalassiosira* sequences were enriched 94-fold in preserved trap samples compared with a mixed-layer co-dominant diatom (unidentified bacillariophyte), much too high a difference to be explained by relative grazing rates. While we cannot rule out that cell aggregates may have contributed to the *Thalassiosira* sequences collected in traps at other stations, senescent mono-specific sinking aggregates also would have been unlikely at the > 5 μM nitrate concentration in the TZ mixed layer and at the much lower cell densities (encounter frequencies) of more offshore waters. The relative enrichment factors of *Thalassiosira* sequences in preserved trap samples were notably highest compared with other mixed-layer dominants (> 1600, > 1330, 400, 85 and 19 times higher than *Ostreococcus*, *Micromonas*, unidentified bacillariophyte, *Karlodinium* and *Lepidodinium*, respectively) at the TZ site, where the pelagic tunicate *Doliolum denticulatum* was a major mesozooplankton grazer. While such differences highlight *Thalassiosira*, they also suggest that the sequences of other taxa vary substantially, perhaps by

orders of magnitude, in their abilities to survive food-web processing.

Another common taxon with substantial evidence of digestion resistance is *Synechococcus*. In early studies predating the microbial loop (Azam *et al*., 1983), Johnson *et al*. (1982) observed that *Synechococcus* were fully degraded and digested in the feeding vacuoles of protistan consumers but persisted intact in the guts and faecal pellets of copepods, thereby establishing a dichotomy on the fate of *Synechococcus* – support of microbial growth versus export to the deep ocean – depending on its primary consumer. Subsequent studies have confirmed that substantial numbers of *Synechococcus* survive ingestion by copepods, appendicularians and salps and are transported to the deep sea in faecal pellets (Pfannkuche and Lochte, 1993; Gorsky *et al*., 1999; Wilson and Steinberg, 2010; Stukel *et al*., 2013b), but aggregate formation has also been hypothesized as a mechanism that would facilitate *Synechococcus* sinking as well as increase their availability to mesozooplankton grazing (Stukel *et al*., 2013b; Agusti *et al*., 2015; Deng *et al*., 2016). Based on these findings, we expected to see large and consistent differences in the relative sequences of *Synechococcus* compared with other picophytoplankton, *Prochlorococcus* and *Ostreococcus*, which are regarded to be more digestible (e.g. Gorsky *et al*., 1999). However, large differences were only measured at the CC site, where the relative sequences of *Synechococcus* in preserved 150 m traps were enriched 332-fold compared with *Prochlorococcus* (both 16S and plastids) and 10-fold compared with Mamiellophyceae (plastids). At other sites where *Prochlorococcus* (TZ, OO) and *Ostreococcus* (IN, TZ) sequences could be compared with *Synechococcus*, the relative abundances of all three taxa in 150 m trap samples were within a factor of two of their relative abundances in mixed-layer samples, suggesting little, if any, differential preservation. Among these, it is notable that the site with the large abundance of mucus-net feeding *Doliolum* and the very large differences in trap enrichment factors among eukaryotic taxa showed low relative sequences and no trap enrichment differences among picophytoplankton populations. This might be indicative of the picophytoplankters being mainly consumed and digested by protistan grazers, whereas *Doliolum* was more selective for larger taxa (Frischer *et al*., 2021).

Methodological considerations for assessing phytoplankton contributions to carbon export

By revealing species presence and hidden diversity, molecular approaches have established themselves as powerful tools for analysing the microbial consortia on sinking particles in the oceans and their variability and transformations with depth (DeLong *et al*., 1993; Amacher *et al*., 2013; Fontanez *et al*., 2015; Boeuf *et al*., 2019; Gutierrez-Rodriguez *et al*., 2019; Preston *et al*., 2020). One way that large metagenomic databases have been used to good effect is network correlation analysis, which has shed new light on taxonomic associations with export proxies (Guidi *et al*., 2016), on productivity-diversity relationships and their trends with depth (Baumas *et al*., 2021) and on the metabolic pathways and traits of microbes connecting surface productivity to the deep sea (Poff *et al*., 2021). However, what would seem to be a simpler goal of using sequence abundances to assess the relative contributions of euphotic-zone primary producers to the export/particle flux process (e.g. Amacher *et al*., 2009) remains a difficult challenge.

As the present study indicates, resolving the production sources to export is complicated in part by the dominance of sinking particle sequences by taxa that arise predominantly below the euphotic zone or as particle colonizers, consumers or their associated flora. A more focused analysis on the question-relevant taxa (e.g. primary producers) would also greatly benefit from the development and widespread adoption of rigorous internal standards that allow metabarcoding results to be interpreted as absolute, rather than relative, sequence abundances (Satinsky *et al*., 2013; Hardwick *et al*., 2018; Crossette *et al*., 2021; Shen *et al*., 2021). While not perfect, adequate methods do exist that can be employed in combination with metabarcoding to measure and compare taxon-specific differences in growth and turnover rates of primary producers in the euphotic zone (e.g. Landry *et al*., 2011). What is missing is the ability to use results from separate metabarcoding analyses in absolute terms to compute rates from incubation experiments, to integrate and compare sequence standing stocks of microbes with different depth distributions over the full euphotic zone, to assess sediment trap collections as rates of sinking sequences and to compare fractions associated with different mechanisms (e.g. faecal pellets versus amorphous aggregates). Combining integrated euphotic-zone sequence stocks and turnover rates with quantitative export flux estimates would provide a first-order constraint on the export contribution problem and be an important step in assessing the magnitude of sequence losses and biases due to trophic processing.

A more difficult problem is relating sequences to their carbon equivalents through the full carbon export process. In principle, a source carbon-sequence relationship can be determined for any phytoplankton cell type that can be recognized, enumerated and sized by imaging techniques, converted to carbon by well-used relationships (e.g. Garrison *et al*., 2000) and divided by the

quantitative abundances of its sequences per sample volume. However, the subsequent loss of the sequence marker due to consumer digestion or microbial degradation does not proportionally reduce carbon flux. For example, if two phytoplankton taxa differ in digestion resistance such that the DNA of one passes intact into a consumer's faecal pellets (or sinks whole in aggregates) while the other's DNA is fully destroyed during digestion, they will be grossly different in terms of relative sequence reads in sediment traps where the sinking pellets are collected but differ by only by a factor of 3.3 in their contribution to carbon export (assuming an ~70% absorption efficiency for zooplankton gut passage; Steinberg and Landry, 2017). Because intermediate steps of protistan consumers also reduce carbon export contribution only by about a factor of 3 relative to direct sinking, the 100-to-1000s fold differences in sequence enrichment factors that we observe in some CCE traps are clearly better explained by taxon differences in DNA digestive degradation than by carbon losses in microbial food chains averaging 4–7 consumer steps (Landry and Décima, 2017).

Experimental studies have demonstrated 10-fold lower estimates of grazing rates based on qPCR sequences retained in copepod gut contents relative to direct microscopical measurements of prey ingestion rates (Nejstgaard *et al*., 2008), implying, at least for some prey taxa, a 90% loss of sequence identity through partial gut digestion. Corrections for taxonomic biases in sequence recovery thus require quantitative experimental studies with natural prey assemblages and representative major consumers that compare the recovery rate of sequences in faecal pellets to sequence-based estimates of ingestion rates. Should such studies reveal clear and consistent patterns of DNA degradation and carbon relationships, comparing sequences on water-column and sinking particles might remain a viable approach for inferring export contributions. More likely, answering this simple but important question will require that metabarcoding analyses be integrated within more complex process studies designed to use interdependent system constraints to estimate and partition mean carbon-based flows and their uncertainties in pelagic food webs (e.g. Stukel *et al*., 2018b; Landry *et al*., 2020).

Experimental procedures

Cruise plan

Samples and environmental data were collected in the southern CCE on CCE-LTER process cruise P1604 (April–May 2016). Four experimental sites with homogeneous conditions were selected based on satellite images of sea surface temperature and chlorophyll and following surveys with a Moving Vessel Profiler (e.g. Ohman *et al*., 2013). The locations were representative of (i) mesotrophic, nutrient-rich waters influenced by seasonal upwelling (IN); (ii) the transition between the California Current and inshore-mesotrophic waters (TZ); (iii) core water of the CC and (iv) offshore oligotrophic open-ocean waters (OO) (Fig. 1). Cruise data and detailed protocols for collection and processing of environmental samples are available at http://oceaninformatics.ucsd.edu/datazoo/catalogs/ccelter/datasets

Sample collection for metabarcoding

At each site, sinking particles were collected over 3–5 day periods with a sediment trap array of 12 VERTEX-style particle interceptor traps (Knauer *et al*., 1979; Stukel *et al*., 2013a) at each of two depths, the base of the euphotic zone (50–100 m, varying with location) and at 150 m (Table S1). Here, we focus on the sinking particles collected at 150 m in two tubes assigned for metabarcoding analyses. One 'live' trap tube was filled with 2.2 l of a brine solution to allow the succession of microbial communities on degrading particles, and one 'fixed' trap tube was filled with RNA later solution to preserve DNA on the particles. Upon recovery, the upper water was removed from each trap tube, and the remaining water was filtered onto a 200 μm Nitex screen and inspected by stereomicroscope to remove zooplankton swimmers. Non-swimmer > 200 μm particles were frozen in liquid nitrogen on the Nitex screen and stored at −80°C. Remaining trap particles were concentrated onto 0.2 μm Sterivex filters, flash frozen and stored at −80°C.

Samples from the mixed layer (upper 12–50 m, varying with location) were collected by CTD casts adjacent to the trap array to compare to trap-collected particles (Table S1). Samples of the water-column microbial assemblages were also collected from the lower euphotic zone (50–100 m) and from the 150 m trap depth to assess water column-trap relationships that might arise from deeper living populations (Table S1). The samples were collected on 0.2 μm Supor membrane filters (Pall) after pre-screening through 200 μm (280 ml) or 500 μm Nitex screens (650 ml) to remove zooplankton, then flash-frozen and stored at −80°C.

DNA from water-column and trap samples was extracted using the NucleoMag 96 Plant kit (Macherey Nagel) following the manufacturer's instructions; < 200 and > 200 μm particles were extracted separately but pooled for subsequent analysis. DNA was amplified by Polymerase Chain Reaction using the Q5 high-fidelity PCR kit (New England Biolabs). Prokaryotes were characterized by amplification of the V4-V5 regions of the

16S small subunit ribosomal RNA gene (SSU-rRNA) using primers 515F and 926R (Table S2). Eukaryotes were characterized by amplifying the V9 region of the 18S rRNA gene using primers 1389F and 1510R (Table S2). *Synechococcus* strains were characterized by amplifying the 16S-23S rRNA ITS using primers ITS1F and ITS4R (Table S2). For trap samples that did not amplify during PCR, we used the OneStep™ PCR Inhibitor Removal Kit (Zymo Research) to remove inhibitory substances.

PCR products were pooled in equimolar amounts (~10 ng μl^{-1}) and sequenced using a dual-barcode index on an Illumina MiSeq platform at the Institute for Genomic Medicine (IGM, University of California, San Diego). Initial quality control of the raw sequence reads was done using the workflow for read filtering, swarm OTU clustering and SSU-rRNA taxonomic classification (JP McCrow; https://github.com/allenlab/rRNA_pipeline). Amplification, sequencing and bioinformatics processing (denoising, chimera detection and OTU clustering) of *Synechococcus* sequences was carried out at RTL Genomics (Lubbock, Texas). Demultiplexed raw reads provided by IGM have been deposited in NCBI under BioProject PRJNA445287 and Biosample Accession Nos SAMN08784582–SAMN08784552 for 18SrRNA, and under BioProject PRJNA422420 and Biosample Accession Nos SAMN08784494–SAMN08784464 for 16S rRNA. See Supporting Information for processing details and Table S3 for average sequence reads and OTUs for each primer set.

Statistical analyses

Prior to analyses, relative abundances were calculated from the sequence reads of each genus (319 prokaryotes, 451 eukaryotes) and the data were square-root transformed to reduce effects of the more abundant genera. Dissimilarity of communities sampled was evaluated by hierarchical cluster analysis with a group-average linkage, with cluster significance defined by Simprof analysis (alpha < 0.05). Both analyses are based on the Bray–Curtis dissimilarity index, comparing composition and relative abundance of genera among samples. Multivariate analyses and Simprof were done in R using 'vegan' (Oksanen *et al*., 2017) and 'clustsig' (Whitaker and Christman, 2015). Pairwise comparisons of each microbial group (protists, prokaryotes and *Synechococcus* strains) and of 16S plastids (prokaryotic and eukaryotic phytoplankton) were done to assess the relative proportions of mixed-layer taxa on sinking particles, focusing on the dominant water-column microbes (> 5% relative abundance in any mixed-layer sample). A false discovery rate threshold of 0.05 was considered significant.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1: Supporting Information