Sinking particles promote vertical connectivity in the ocean microbiome

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The sinking of organic particles formed in the photic layer is a main vector of carbon export into the deep ocean. Although sinking particles are heavily colonized by microbes, so far it has not been explored whether this process plays a role in transferring prokaryotic diversity from surface to deep oceanic layers. Using Illumina sequencing of the 16S rRNA gene, we explore here the vertical connectivity of the ocean microbiome by characterizing marine prokaryotic communities associated with five different size fractions and examining their compositional variability from surface down to 4,000 m across eight stations sampled in the Atlantic, Pacific, and Indian Oceans during the Malaspina 2010 Expedition. Our results show that the most abundant prokaryotes in the deep ocean are also present in surface waters. This vertical community connectivity seems to occur predominantly through the largest particles because communities in the largest size fractions showed the highest taxonomic similarity throughout the water column, whereas free-living communities were more isolated vertically. Our results further suggest that particle colonization processes occurring in surface waters determine to some extent the composition and biogeography of bathypelagic communities. Overall, we postulate that sinking particles function as vectors that inoculate viable particle-attached surface microbes into the deep-sea realm, determining to a considerable extent the structure, functioning, and biogeography of deep ocean communities.

particle sinking | deep ocean | marine prokaryotic metacommunities | dispersion | connectivity

A significant fraction of the organic carbon produced in the surface sunlit ocean through photosynthesis is exported into the deep ocean as sinking particles (1, 2), supporting a globally relevant carbon flux fueling the generally starved bathypelagic microbial communities (3, 4). Although the carbon reaching the dark ocean is ultimately controlled by the activity of the prokaryotic communities that attach to these particles (5, 6), so far no study has investigated whether sinking particles may also constitute dispersion vectors of viable prokaryotic diversity into the deep ocean.

Particles formed in surface waters are rapidly colonized by prokaryotes, and particle-attached communities are often more metabolically active (7–9) and phylogenetically diverse (e.g., refs. 10–12) than suspended (free-living) assemblages. Although it has been shown that particle-attached prokaryotic communities change compositionally with depth (e.g., refs. 13–15), it is not clear whether these changes are due to an ecological succession of taxa on the degrading particle (16) or continuous colonization of the particles during sinking. Assemblages attached to sinking particles could influence the structure of deeper prokaryotic communities if dormant or slow-growing surface taxa thrive when reaching a certain depth or as the nature of the particle changes. In this case, sinking particles would provide a continuous supply of viable immigrants to the deep ocean.

Assessing the relevance of such dispersal process would require comparing particle-attached communities between surface and bathypelagic waters, yet this has never been attempted, not even at local scales. Indeed, the vertical connectivity between communities from surface to bathypelagic waters remains poorly understood because spatial surveys focusing in the vertical dimension often describe the communities from each depth without assessing their potential linkages throughout the water column (e.g., refs. 17–19). Only a few recent studies have shown that communities from surface and deep waters may be connected through advection or sinking of water masses (20–22), but they did not consider the role of sinking particles as a vector supporting vertical connectivity. In addition, because large particles sink faster than small particles—which might even remain buoyant (23–25)—communities associated with larger, fast-sinking particles are likely to have greater chances to reach the deepest layers due to higher dispersal rates. To date, however, most literature on particle-attached prokaryotes has been restricted to the dichotomous exploration of free-living versus attached populations (e.g., refs. 26–28), yet it is known that particles occur along a continuum of sizes that can be colonized by different microbial populations (29). Furthermore, this continuum of sizes is strongly related to the composition of the particle, which also indicates the particle origin: larger particles are younger and more labile

Significance

Prokaryotes dominate the living biomass and the biological diversity of the ocean, one of the largest ecosystems on earth. The sinking of particles is a widespread mechanism that transports materials to the deep ocean, with a significant role in the global carbon cycle. Whether this process constitutes a global dispersal pathway for prokaryotic diversity connecting surface communities to those in the dark ocean has never been tested. Here we show that surface and deep-sea prokaryotic communities are strongly connected, constituting a vast oceanic metacommunity where local assemblages are linked through the transport of sinking particles. This vertical dispersal, mediated mainly by the largest sinking particles, emerges as a fundamental process shaping the assembly and biogeography of deep ocean prokaryotic communities.

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and are originated in the surface (30), whereas smaller particles tend to be older and more recalcitrant (31), originating from the degradation of larger particles (32).

Given that the origin and composition of particles in surface waters vary spatially (25, 33–35), it could be hypothesized that the microbial diversity patterns in the deep ocean mirror to some extent such surface heterogeneity. In fact, it was recently shown that deep-sea particle-attached communities present a much clearer biogeography than their free-living counterparts (36), but whether these patterns are established locally in the deep sea or transferred from surface via sinking particles remains unexplored. Although the importance of hydrologically mediated microbial dispersal for shaping local assemblages has been clearly demonstrated in freshwater ecosystems (37–39) and more recently also in oceanic waters through movement of water masses (20–22, 40), the role of particle-driven vertical dispersion in explaining the biogeographic patterns of deep-sea prokaryotes has not yet been assessed.

Here we explore whether sinking particles represent a dispersal vector of prokaryotes into the deep ocean, contributing to the vertical connectivity of the marine microbiome, and test the hypothesis that particle size influences vertical connectivity. We investigated the composition of free-living prokaryotic communities as well as that of those attached to particles of different sizes (ranging from 0.8 to 200 μm) in eight stations across the global tropical and subtropical ocean, assessing changes in their composition from surface (3 m) to bathypelagic waters (4,000 m). Specifically, we test the hypothesis that communities attached to the largest particles show a strong vertical similarity due to their assumed faster sinking rates (i.e., higher dispersal) and that free-living prokaryotic assemblages are more different across the water column than their vertically connected particle-attached counterparts. Moreover, if part of the sinking diversity were to detach from particles when reaching the bathypelagic layer, thus becoming part of the free-living community, a percentage of the prokaryotes attached to particles in surface waters should also be present as free-living in bathypelagic communities. Finally, we test the hypothesis that if vertical connectivity is a globally relevant phenomenon, then deep ocean biogeographic patterns should resemble those of the surface particle-attached communities.

Results

Taxonomic Composition of Prokaryotic Assemblages. We studied stations spanning a broad longitudinal gradient across the tropical and subtropical Pacific, Atlantic, and Indian Oceans (*SI Appendix*, Fig. S1*A*). The depths sampled within each station presented pronounced vertical physicochemical and biological variation (*SI Appendix*, Fig. S1*B*). Accordingly, the studied prokaryotic communities were clearly structured along the water column, differing mostly between the photic versus the aphotic realms, and presented also distinct biogeographic signatures (Fig. 1 *B* and *C* and *SI Appendix*, Table S1). Whereas communities from the largest particles (≥3.0 μm) clustered together regardless of depth, communities from the two smallest fractions differed greatly between surface and deeper layers (Fig. 1 *A* and *B*). Consequently, vertical community differences were higher for the smallest size fraction (PERMANOVA $R^2 = 0.37$, $P < 0.001$) than for the largest size fractions ($R^2 = 0.09$, $P = 0.597$) (*SI Appendix*, Table S2), whereas horizontal differences, i.e., between stations, were higher in the largest size fractions ($R^2 = 0.54$, $P < 0.001$) than in the smallest size fraction ($R^2 = 0.21$, $P < 0.753$) (*SI Appendix*, Table S2). As indicated by the RC_{Bray} (Raup-Crick metric based on Bray Curtis distances), the observed beta diversity patterns differed significantly from those expected by chance (i.e., ecological drift) in ∼92% of cases [following Stegen et al. (41)], and this suggests that other processes (like selection and limited or high dispersal) rather than random community assembly (drift) generated the observed beta diversity patterns.

Operational Taxonomic Units (OTUs) richness was highly variable among size fractions and depths (range 136–1,044 OTUs per sample, average 580), but in general, richness decreased toward larger size fractions in all depths (*SI Appendix*, Fig. S2). In terms of taxonomic composition at the phylum or class level, the three fractions larger than 3.0 μm were in general more similar among each other than they were to the smallest size fractions (*SI Appendix*, Fig. S3). The distribution of Particle-Association Niche Index (PAN-Index) values, used to identify preferences of OTUs for particular size fractions, showed two modes around values 1.5 and 3.5 (*SI Appendix*, Fig. S4). We, therefore, differentiated OTUs enriched in small size fractions (PAN-Index < 2.7) from those enriched in large size fractions (PAN-Index ≥ 2.7). We found that the preference for one lifestyle or the other seemed to be phylogenetically conserved at the order level (but in some cases not at the class nor at the phylum level). Whereas some orders such as SAR11, SAR324, or Acidimicrobiales showed preference for small size fractions, other orders like Rhizobiales, Alteromonadales, or Planctomycetales were preferentially enriched in large size fractions (PAN-Indexes ≥ 2.7; *SI Appendix*, Fig. S5).

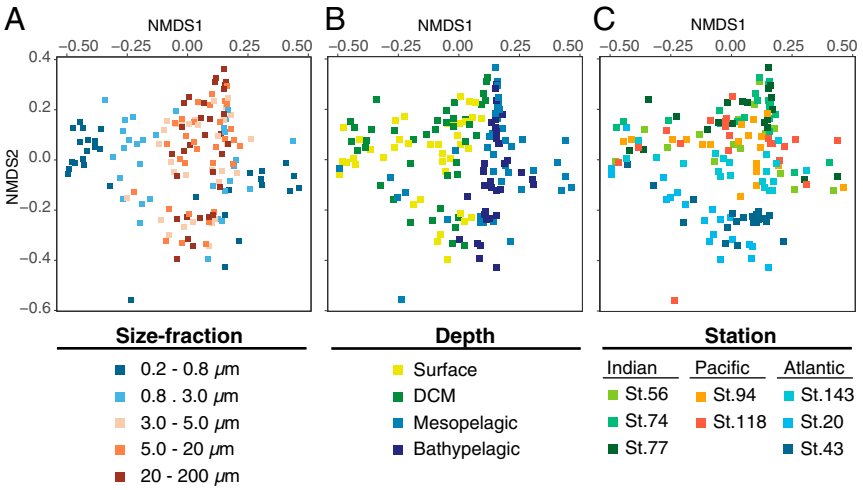

Fig. 1. nMDS ordinations representing spatially the Bray–Curtis distances between the prokaryotic communities studied. Distances were calculated using the rarefied OTU table. Samples are color-coded depending on (*A*) size fraction, (*B*) depth, and (*C*) sampling station.

Vertical Connectivity Between Oceanic Prokaryotic Communities. To determine the vertical connectivity between prokaryotic communities, we explored whether OTUs present at one depth could be detected in the other depths. To do so, all OTUs were categorized into four depth groups: surface (SFC), deep-chlorophyll maximum (DCM), mesopelagic (MESO), and bathypelagic (BATHY), defined by the depth where they were first detected, assuming a directionality from surface to bathypelagic waters and considering all stations together. For example, if an OTU was detected in any of the surface samples, it was categorized as SFC, but if an OTU was first detected in mesopelagic waters but not in the previous depths (surface and DCM), it was categorized as MESO, and so on. For this categorization, the nonrarefied OTU table was used, so that we could detect the largest number of OTUs per sample. This analysis showed that even though new OTUs appeared continuously when moving from one depth to the next one (Fig. 2), communities from all depths and size fractions were largely dominated by OTUs present in surface waters (SFC OTUs). When this categorization of OTUs was done considering each station separately, we observed a similar pattern, but in some stations, there was a larger contribution of OTUs not present in surface waters in deep layers, particularly in the free-living fraction (*SI Appendix*, Fig. S6). This indicates that some of the DCM, MESO, or BATHY OTUs in some stations were not present at the surface of these particular stations but were present in surface waters at other sites. In any case, bathypelagic communities of all stations were still numerically dominated by surface sequences, pointing to a high vertical connectivity of the open ocean microbial communities. This implies that community changes across depths (Fig. 1*B*) are to a large extent due to shifts in the relative abundances of taxa present throughout the water column (e.g., rare surface taxa that become abundant in deeper layers).

The vertical differentiation among communities of a given size fraction varied gradually from the free-living prokaryotic communities toward those in the largest particles (Fig. 3), and differences from the small to the largest size fraction were statistically significant (Wilcoxon test, P value < 0.001). We found the highest beta diversity and OTU turnover among depths in the 0.2–0.8 μm fraction (Fig. 3 *A* and *B*), indicating a higher replacement of OTUs within the free-living communities across depths compared with communities attached to the largest particles. Conversely, communities from the largest size fraction showed higher nestedness (Fig. 3*C*), and OTUs were present across more depths (Fig. 3*D*) compared with those in smaller size fractions, suggesting that communities attached to larger particles are more connected throughout the water column than those free-living or attached to small particles. We then divided the SFC OTUs (i.e., OTUs detected in any of the surface stations; see above) into those enriched in small size fractions (PAN-Index < 2.7) and those enriched in large size fractions (PAN-Index ≥ 2.7) and compared their distribution along the water column in small (and suspended, i.e., <3.0 μm) and large (and sinking, i.e., ≥3.0 μm) size fractions (Fig. 4). We found that surface OTUs present in the smallest surface size fractions (SFC OTUs with PAN-Index < 2.7) accounted for a decreasing proportion of the communities toward deeper waters in the small suspended size fractions (Fig. 4*A*) and composed around 25% of abundance in the largest size fractions along the water column (Fig. 4*B*). Conversely, most bathypelagic communities were composed by OTUs present in the surface in association to large particles (SFC OTUs with PAN-Index ≥ 2.7), which composed about 80% of bathypelagic sequences in the largest size fractions (Fig. 4*B*) and more than 50% of bathypelagic sequences in the small suspended size fractions (Fig. 4*A*). This suggests that deep-sea communities are largely populated by surface microbes arriving via the largest particles and that this dispersal influences mostly the bathypelagic particle-attached communities (Fig. 4*B*) but also the deep-sea free-living assemblages (Fig. 4*A*). The colonization of the bathypelagic free-living communities by sinking particles is also supported by the observation that free-living communities from the bathypelagic are more similar to attached communities from the bathypelagic than to any other communities (Fig. 1). On the contrary, surface free-living communities contribute much less to the bathypelagic diversity likely due to their more limited vertical dispersal.

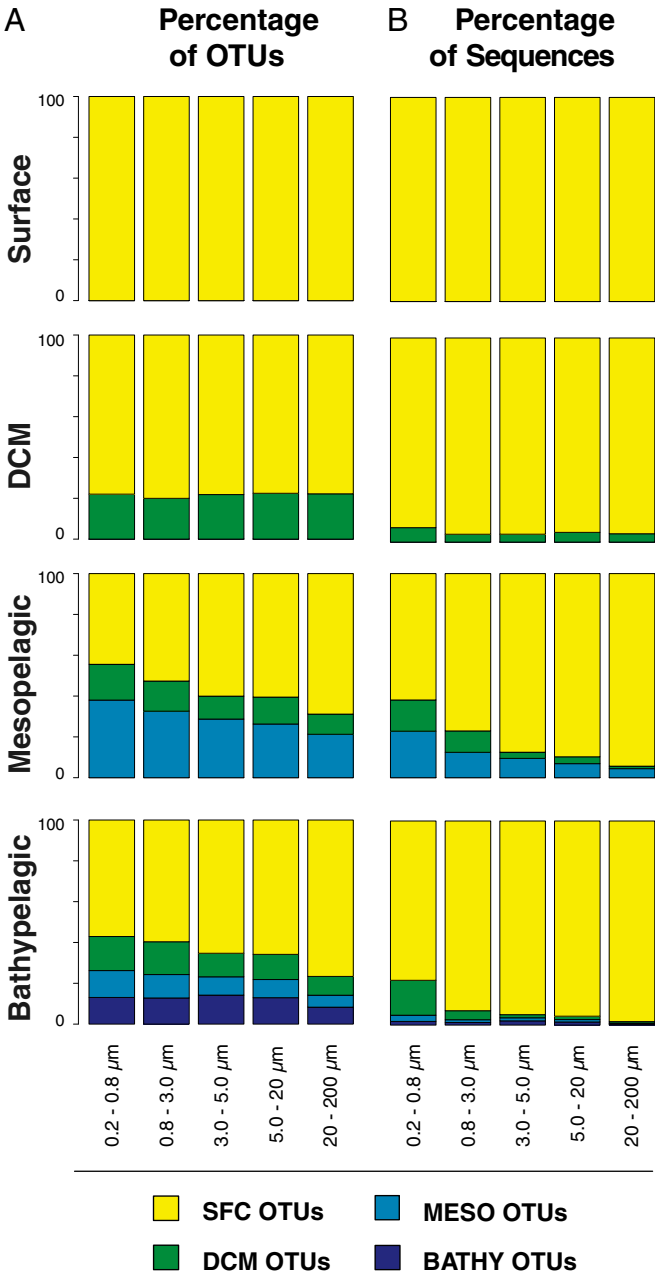

Fig. 2. Contribution of the OTUs categorized as surface (SFC), deep-chlorophyll maximum (DCM), mesopelagic (MESO), and bathypelagic (BATHY), in each depth and size fraction, expressed as (*A*) percentage of OTUs and (*B*) percentage of sequences. The category of each OTU was defined as the depth where they were first detected, considering a directionality from surface to bathypelagic waters, and considering all stations together (see *Materials and Methods* for details).

Transfer of Biogeographic Patterns from Surface to the Deep Sea. Given the higher vertical transport of the microbes associated to larger particles, we would expect that spatial differences (i.e., differences between stations) among communities from the largest particles are maintained vertically, whereas suspended communities are expected to be more vertically isolated, and thus, their surface biogeographic patterns are not expected to be

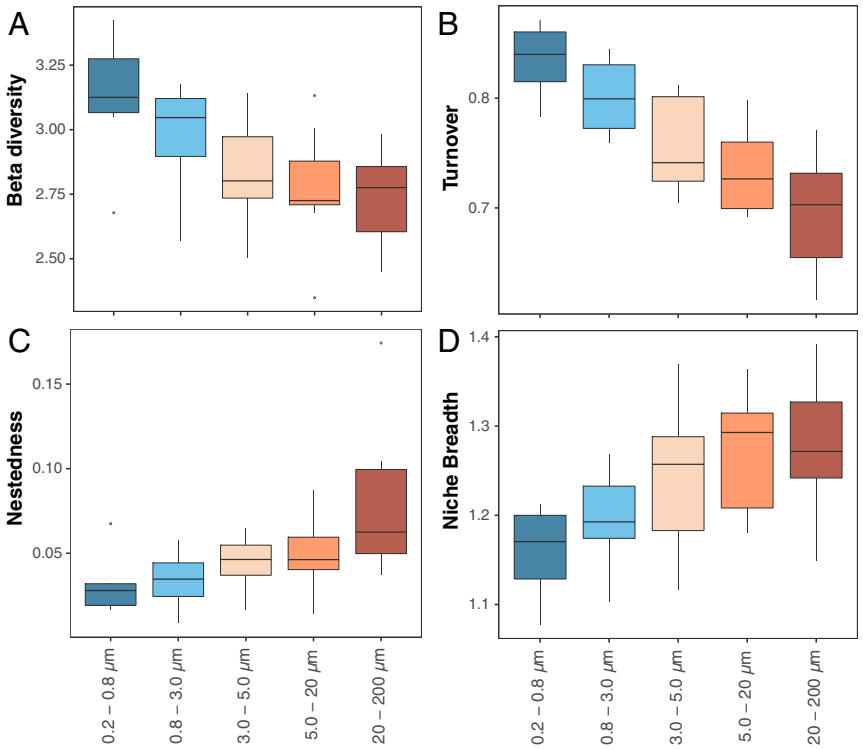

Fig. 3. Vertical variation in (*A*) beta diversity (i.e., community differentiation), (*B*) spatial OTU turnover (i.e., species replacement), (*C*) nestedness (species loss), and (*D*) niche breadth (i.e., habitat specialization of the OTUs based on the number of depths where an OTU was detected) for each size fraction. Values were calculated among depths, for each size fraction, and separately for each station. Boxplots summarize the data from the eight stations. Beta diversity comprised values from 1 to 4, where 1 indicates that all OTUs from the four depths are the same and 4 indicates that OTUs from the four depths are completely different. Beta diversity can be partitioned into two components: turnover and nestedness. Turnover indicates the replacement of some OTUs by others from depth to depth, and nestedness indicates the subset of OTUs from one depth to the other. Both turnover and nestedness comprised values from 0 to 1, indicating the level of contribution to beta diversity. Niche breadth was calculated for each OTU, indicating the number of depths where an OTU was found. Values ranged from 1 to 4, where 4 indicates that a given OTU is present at the four depths (i.e., higher values of the boxplot indicate that the OTUs of a given size fraction were present across more depths). See *Materials and Methods* for more details.

transferred across depths. We tested this inference by comparing, for each size fraction, spatial differences between surface communities and mesopelagic or bathypelagic communities using Mantel tests (Table 1). We found that the dissimilarities between suspended communities from the surface and the mesopelagic or bathypelagic waters were not significantly correlated. In contrast, the dissimilarities between particle-attached communities from the surface and the deep waters presented a high significant correlation (Table 1), suggesting that the compositional differences among stations of deep-sea particle-attached communities were caused at least partially by the biogeographic patterns of surface particle-attached assemblages.

The results presented support the hypothesis that the biogeography of surface particle-attached prokaryotes is transferred to deeper waters via particle sinking, but the fact that communities from the photic and aphotic realms were very different in terms of taxonomic composition (*SI Appendix*, Fig. S3) suggests that this vertical dispersal of particle-attached microbes must be accompanied by large changes in their abundances during sinking. For example, the growth of taxa that were rare (and perhaps dormant) in surface waters but can thrive in deeper depths could explain such compositional shifts. We identified at each station the OTUs that potentially grew during particle sinking by choosing the surface OTUs prevalent in the larger size fractions (SFC OTUs with PAN-Index $\geq$ 2.7) that increased in relative abundance (dominance) toward deeper waters, named here as "seed" OTUs (see details in *Materials and Methods*). We identified 90 seed OTUs in total, which contributed to ~6% of sequences at the surface, and showed clear increases in relative abundance toward deeper waters with up to 55% (average ~35%) of the total abundance in bathypelagic particle-attached communities (Fig. 5). The pool of taxa behaving as seeds differed between stations, and different dominant seed groups were found in each station (e.g., *Oceanospirillales* dominate at station 20, *Sphingobacteriales* at station 77, and *Corynebacteriales* at station 94) (Fig. 5), Moreover, when we inspected the community structure of only these seed OTUs, we observed that they clustered according to the eight stations (*SI Appendix*, Fig. S7). This geographic signature was less clear for the smallest size fractions (smaller symbols) in some cases, but still it points to the high relevance of the surface particle colonization processes in determining the structure of communities from deeper layers.

Discussion

Our results support the hypothesis of strong particle-mediated prokaryotic connectivity between the surface and the deep ocean, a hypothesis suggested earlier (e.g., refs. 42 and 43) and tested here. Most of the dominant prokaryotes from the deep-ocean can also be detected in surface waters, and this vertical connectivity is higher in communities associated with the larger size fractions (i.e., larger particles), likely due to their higher sinking rates. Our results demonstrate that particle sinking constitutes a dispersal vector of viable microbial diversity from surface waters to the bathypelagic zone that ultimately determines the biogeography of deep-sea prokaryotes. They also suggest that sinking particles may indeed be a relevant seed bank of viable taxa for the deep ocean, in line with previous studies suggesting the existence of an oceanic reservoir of dormant diversity (44–46).

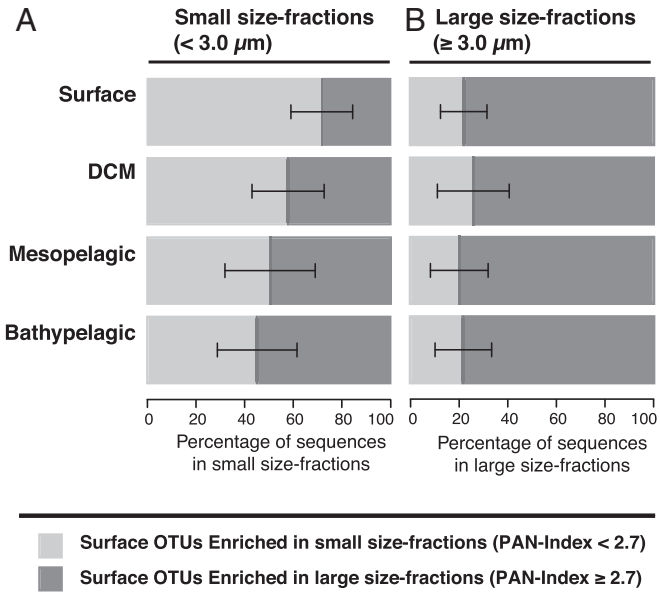

Fig. 4. Vertical variation of the contribution (in percentage of sequences) of surface OTUs enriched in small size fractions (PAN-Index < 2.7) (light gray) and surface OTUs enriched in large size fractions (PAN-Index ≥ 2.7) (dark gray) to communities present in (*A*) fractions <3.0 μm and (*B*) fractions ≥3.0 μm and at each depth. The boxplots summarize the data from the eight stations. See *Materials and Methods* for further details.

Although the vertical differentiation of communities from photic to aphotic realms is well documented (e.g., refs. 47–49), we show here that this difference varies with particle size, being greatest among free-living assemblages and prokaryotic communities associated to the smallest size fractions (Fig. 1 and *SI Appendix*, Fig. S3 and Table S1). Our results also concur with previous studies indicating that prokaryotic communities strongly differ between the free-living (suspended) and attached fractions in epipelagic (29, 43, 50) and also in bathypelagic (10, 51, 52) waters, supporting that niches present on particles are distinct from the dissolved phase. Others have evidenced that the composition of the larger particles is different from that of the smaller particles (and also from the dissolved phase), with larger particles being younger and more labile and the smaller particles being older and more recalcitrant (31, 32).

The finding that all communities, including those inhabiting the bathypelagic layer, were numerically dominated by OTUs present in surface waters supports a strong vertical connection between surface and bathypelagic communities at the local (*SI Appendix*, Fig. S6) and the global (Fig. 2) scales. Given the variability across depths in the structure of the communities (Fig. 1 and *SI Appendix*, Fig. S3), the vertical changes in prokaryotic assemblages are likely driven by changes in the relative abundance of taxa present through several depths during sinking, i.e., rare surface taxa that become abundant in deeper waters. Only a few recent studies have assessed the vertical connectivity of marine prokaryotic communities along the water column, indicating that advection and convection of water masses can shape the structure of surface and deep microbial communities by promoting their transport and new habitat colonization (20–22). However, these studies have focused only on the free-living assemblages and vertical water mass transport processes as the driving mechanism. Here we provide evidence that the export of sinking particles represents an important dispersal pathway of diversity from surface to bathypelagic waters. Although this process could be considered as unidirectional from surface to deep waters, supported by evidence of rapid-sinking particles from surface to the deep ocean along the stations sampled by the Malaspina expedition (53), it is also possible that upwelling events of deep-water masses transport viable deep-sea prokaryotes back into surface waters. This possibility should be tested in future research in upwelling zones.

The hypothesis that vertical prokaryotic connectivity is due to the dispersion of taxa from surface to deeper waters driven by sinking particles is further supported by the observation that the connectivity throughout the vertical column is higher for communities associated to the largest particles than for those attached to the smaller ones, as expected from the faster sinking of larger particles (e.g., sinking rates of <1 $m \cdot d^{-1}$ for small particles and >1,000 $m \cdot d^{-1}$ for large aggregates, reviewed in ref. 3). For example, the community composition of the smaller size fractions was more variable between depths (i.e., higher beta diversity) than those of the largest size fractions (Fig. 3*A* and *SI Appendix*, Table S2). Also, the highest OTU replacement (turnover) across depths was found between communities from the smallest particles, and OTUs from communities associated with largest particles were the most ubiquitous across depths (Fig. 3*C*). Altogether, this points to a more intense vertical dispersal of taxa between communities from the largest size fractions, whereas prokaryotic communities from the smallest size fractions present more restricted depth distributions, i.e., are more isolated vertically, due to their more limited connectivity.

In addition, we observed that the OTUs with preference for large particles in surface waters also dominate the mesopelagic and bathypelagic communities (Fig. 4). The fact that the deep communities in the small size fractions were also composed to a large extent of surface particle-attached prokaryotes (Fig. 4*A*), and that free-living communities from the bathypelagic layer are more similar to attached communities from the bathypelagic layer than to any others (Fig. 1), suggests that large particles are indeed vectors transporting viable prokaryotes from the surface to the bathypelagic, some of which can thrive in the free-living fraction of the bathypelagic realm. Thus, the transport of prokaryotes from surface to deep waters occurs mostly via large particles, which act as a source of potential immigrants (or inoculum) to the suspended community living in the deep ocean. These results agree with a previous study indicating that particular taxa can change their preference from large particles to small particles through depth (51), probably responding to environmental conditions.

Particles have a highly heterogeneous organic and inorganic composition that is altered during sinking but that is mainly determined by the environmental conditions of the surface waters where they formed (25, 34, 35). Such particle composition determines the colonization and the structure of the initial microbial community (reviewed in ref. 54). It follows from this that the pool

Table 1. Comparisons of community structure between depths

	Surface vs. mesopelagic		Surface vs. bathypelagic	
Size fraction	R	*P* value	R	*P* value
0.2–0.8 μm	−0.18	0.752	−0.18	0.829
0.8–3.0 μm	−0.14	0.675	0.29	0.11
3.0–5.0 μm	0.58	**	0.73	**
5.0–20 μm	0.82	**	0.73	**
20–200 μm	0.87	***	0.64	**

R coefficients of the Mantel correlations between the taxonomic dissimilarity matrices from surface and mesopelagic communities and surface and bathypelagic communities, for each of the five size fractions. Higher R values mean that the compositional differences between communities at a given depth were highly correlated (and thus were similar) to the differences between communities from a different depth. Significance of the correlations is stated as follows: ***$P < 0.001$, **$P < 0.01$, and *$P < 0.05$.

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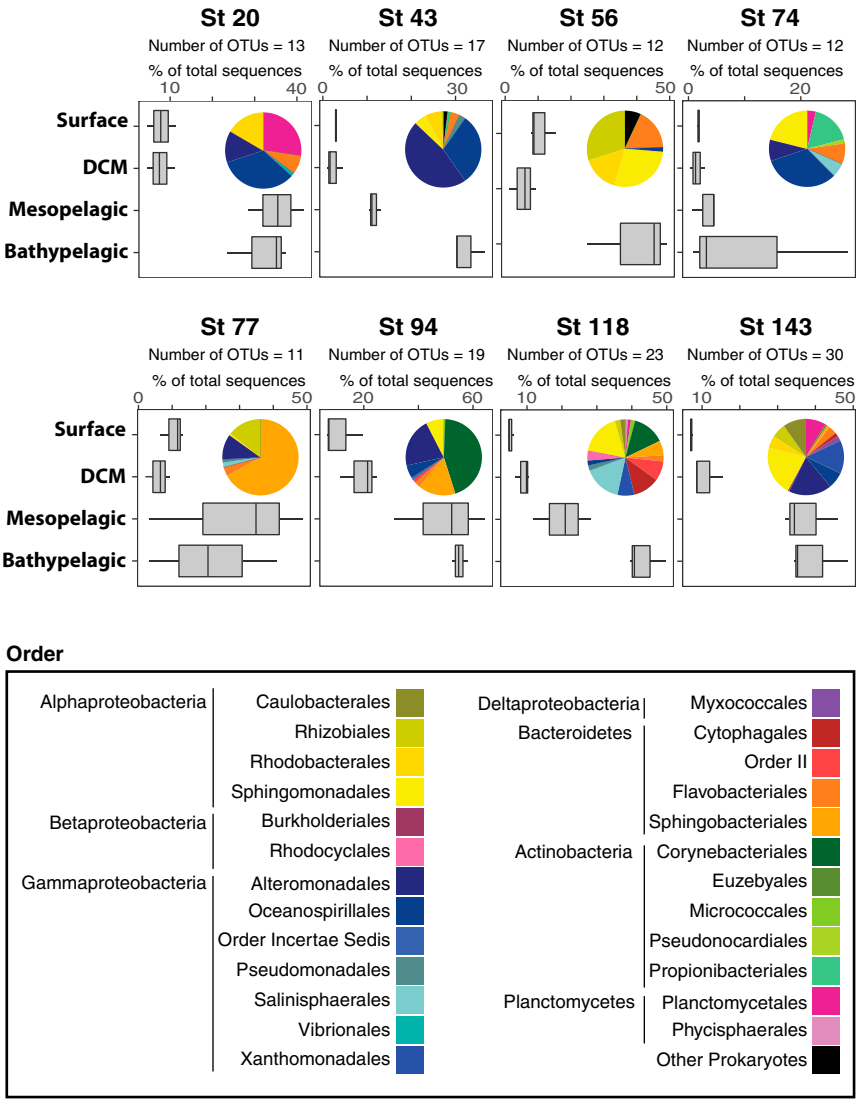

Fig. 5. Dynamics of seed OTUs at each station and across depths. Seed OTUs are the surface OTUs enriched in the larger size fractions that increase in relative abundance with depth. Data represent the contribution of the OTUs categorized as seeds to the total sequences of communities associated to the largest size fractions (≥3.0 μm; see *Results* for details). Pie charts indicate the taxonomic composition at the order level (in percentage of sequences) of the seed OTUs at each station. Note the different scales in the graphs.

of particle-dispersed taxa that sinks and has the potential to thrive when arriving to deeper layers will depend on local conditions and particle origin in surface waters and should differ across stations. We tested this hypothesis by identifying the pool of surface particle-attached prokaryotes that increased their relative abundances toward deeper waters, those acting as seed OTUs. These OTUs belonged to different taxonomic groups in the different stations examined, likely indicating the effect of the initial surface particle colonizers in determining deep ocean microbial communities locally. This implies that sinking particles transport diverse communities, yet some of these taxa [probably dormant or slowly growing (55)] have the potential to grow and dominate deep-ocean communities when the surrounding environmental conditions or the nature of the particles change as they are transported toward deeper waters. Additionally, other taxa can decrease their relative abundance with depth as they find unfavorable conditions when moving from the surface to the deep ocean (51). A similar process has also been observed in other ecosystems, such as the river-to-lake freshwater continuum, where the transport and growth of rare bacteria from terrestrial source environments was shown to strongly determine the structure of the receiving aquatic communities (39). These results concur in highlighting the need to take potential dispersal sources into account to understand observed biogeographic patterns. Focusing on free-living prokaryotes, Wilkins et al. (20) suggested that advection of seawater masses can shape microbial community structure by increasing opportunities for colonization. However, given the limited deep vertical water transport in the open ocean, sinking particles are likely to play a key role in determining and shaping the vertical connectivity of oceanic microbial communities across much of the global ocean by allowing continuous dispersal of viable organisms into the mesopelagic and bathypelagic realms.

Despite a general perception of a homogeneous dark ocean, genomic approaches have unveiled the enormous and dynamic genetic variability of the deep sea microbial communities (reviewed in ref. 3). Indeed, a recent global survey of prokaryotic communities in the bathypelagic realm showed that they differed between oceanic basins and that this biogeographic signal was stronger for the particle-attached members (0.8–20 μm size fraction) than for their free-living counterparts (0.2–0.8 μm size fraction) (36). This

agrees with our observation that at all depths, communities from the largest particles showed much clearer differences between stations than the free-living communities (*SI Appendix*, Table S2). However, we observed that the compositional differences between surface stations of the particle-attached communities were correlated with the compositional differences between deep-sea stations and that the strength of this correlation increased with increasing particle size, whereas no such pattern was observed for surface vs. deep-layer free-living communities (Table 1). Altogether, this indicates that the biogeography of deep-sea communities mirrors that of the overlaying surface communities as a consequence of particle-mediated dispersal and is thus partially determined by that of the attached prokaryotic community originating in the surface. Salazar et al. (36) suggested that submarine mountains that divide the deep ocean into basins might act as ecological barriers for prokaryotic communities, thus favoring their differentiation. However, this explained a limited fraction of the variance in community composition. Our results further suggest that taxonomic differences of the pool of taxa arriving via sinking particles may also be a major mechanism explaining the observed biogeographic patterns of deep-sea prokaryotes.

In our study design based on the comparison of prokaryotic community structure along vertical profiles, we described the variability of community composition along the water column, in a vertical gradient defined by the four depths sampled in each station. We are aware that the described processes are not directly connected along a vertical line, because horizontal transport velocities can be greater than sinking rates. Hence, the sampling points of each depth must be considered as representative samples of a wider area (i.e., they should not be interpreted as points located directly on top of each other but rather as vertical profiles with samples representative of a larger area). Still, similar patterns were observed when analyzing each station separately (*SI Appendix*, Fig. S6) and all stations together (Fig. 2). In addition, the observed similarity in biogeographic patterns between surface and deep particle-attached communities (Table 1), as well as the station-specific signature in the pool of taxa able to grow on sinking particles (*SI Appendix*, Fig. S7), seems to support that there is indeed vertical connectivity occurring within the area represented by each sampled station.

In summary, we show that the global ocean prokaryotic microbiome exhibits a strong vertical connectivity through the entire water column. This connection occurs via particle sinking, which highlights the role of particles as microbial vectors that introduce viable surface taxa into the deep ocean. In addition, our results indicate that all local communities seem to be dominated by OTUs that are already present in surface waters; that there are OTUs from the surface that thrive at depth; and that the biogeography of the bathypelagic realm is influenced, to some extent, by particle colonization events occurring in surface waters. The contributions of both particles and their attached communities to the bathypelagic realm seem therefore crucial. To the existing evidence that sinking particles are an essential source of carbon and nutrients for the development of heterotrophic life in the deep ocean (3, 4), our results add that communities attached to particles provide a source of viable diversity to deeper ocean layers. Given that the bathypelagic realm is constituted mostly by slow sinking or buoyant particles (4), which are resource-rich habitats for microbes (56), and that deep-sea prokaryotes are more adapted to the attached lifestyle than surface ones (57–60), deep-sea microbial activity and life must be concentrated on particles (3, 6, 57). Therefore, this particle-driven dispersal of microbes likely constitutes a fundamental mechanism influencing the structure, assembly, and functioning of deep ocean microbial communities across the global ocean.

Concluding, we describe here the particle-driven vertical dispersion of prokaryotes from the surface to the deep ocean, and our findings suggest a plethora of additional questions that could be explored in the future. Do microbes that sink ever return to the surface ocean, or is there a dispersal mechanism that brings them back to the surface? If the transported microbes depend directly on the processes occurring at the surface, how does variability in particle composition and sinking rates affect the communities in the deep ocean? Is the dispersal of organisms arriving from the surface the main process determining the biogeography of the deep ocean? Here we unveil the importance of particle-driven vertical dispersion, but the relevance of other mechanisms of dispersion in structuring ocean microbial communities at the global scale remains to be assessed, mechanisms that could explain, e.g., how organisms considered endemic of vent systems can colonize geographically and potentially isolated distant hydrothermal habitats (45). The postulated existence of a global seed bank in the ocean (44) requires identifying the various prokaryotic mechanisms responsible for dispersal. Our results provide evidence of one such mechanism occurring at a global scale.

Materials and Methods

Study Area and Sampling. We selected a total of eight stations of those sampled during the Malaspina 2010 Expedition (61) between December 2010 and July 2011. The selected stations were distributed across the global tropical and subtropical ocean (latitudes between 30° N and 40° S): three in the Atlantic Ocean, two in the Indian Ocean, and three in the Pacific Ocean. At each station, four depths were sampled corresponding to the surface (SFC, 3 m), the deep chlorophyll maximum (DCM, 48–150 m), the mesopelagic (MESO, 250–670 m), and the bathypelagic waters (BATHY, 3,105–4,000 m). Surface water was sampled with a Niskin bottle, and water from the other depths was sampled with a rosette of Niskin bottles attached to a conductivity–temperature–depth (CTD) profiler. Vertical profiles of salinity, potential temperature, and dissolved oxygen were recorded continuously with the CTD sensors installed in the rosette sampler. Nutrients (nitrate, phosphate, and silica) were determined using standard procedures as explained in Catalá et al. (62). Bacterial abundance and size were determined by flow cytometry, and bacterial heterotrophic production was estimated using the ^{3}H-leucine incorporation method (63) as described in detail in ref. 64.

Prokaryotic biomass from different size fractions was collected by prefiltering the water through a 200-μm net mesh and sequentially filtering 10 L through 20-, 5.0-, 3.0-, 0.8-, and 0.2-μm pore-size filters, all 47-mm polycarbonate filters (20-μm pore-size filter from GE Water and Process Technologies and the rest of the filters from Millipore), using a peristaltic pump, resulting in five different size fractions (0.2–0.8, 0.8–3.0, 3.0–5.0, 5.0–20, and 20–200 μm). Filter clogging or particle dislodging may affect the taxonomic composition observed in each size fraction (65), but we tried to minimize these issues by filtering at very low speed and pressure and by changing frequently the filters. The filters were flash-frozen in liquid N_2 and stored at −80 °C until DNA extraction. We assume that the 0.2–0.8 μm size fraction will harbor mostly free-living prokaryotic communities that remain suspended in the water column, and the rest of the size fractions will comprise prokaryotes associated to different kinds of particles (e.g., gels, organic and inorganic particles, and living or not living organisms) of variable sizes that will influence their sinking velocities. We define 0.2–0.8 and 0.8–3.0 μm as small size fractions (or small particles, suspended particles), whereas 3.0–5.0, 5.0–20, and 20–200 μm are defined as large size fractions (or large particles, sinking particles).

DNA Extraction, Sequencing, and Sequence Processing. The DNA was extracted with a phenol-chloroform protocol (as described in ref. 66). The hypervariable V4–V5 region of the 16S rRNA gene was PCR amplified with primers 515F-926R (67) and sequenced in an Illumina MiSeq platform using 2 × 250 bp paired-end approach at the Research and Testing Laboratory facility (rtlgenomics.com/). Computing analyses were run at the Marbits bioinformatics platform of the Institut de Ciències del Mar. The amplicons were processed through a protocol (detailed in ref. 68) based on Uparse (69). Briefly, reads were assembled with PEAR (Paired-End reAd mergeR) (70), and those with >100 nucleotides were selected. Quality check, dereplication, OTU clustering (99%), and reference-based chimera filtering (using Silva database v.119) were processed with Usearch (71). Taxonomic assignment of OTUs was generated by BLAST (Basic Local Alignment Search Tool) searches of representative sequences against Silva database v.123. Nonprokaryotic OTUs (eukaryotes, chloroplast, and mitochondria), as well as singletons, were removed. To allow comparisons between samples, the OTU table was randomly subsampled to the number of reads

present in the sample with the lowest amount of reads (which was $n = 5{,}390$), and a total of 13,567 OTUs were obtained.

Data Analysis. Statistical analyses and plots were done in R (www.r-project.org) using the *vegan* (72), *simba* (73), *spaa* (74), *betapart* (75), and *BiodiversityR* (76) packages. The OTU richness of each size fraction and at each depth was calculated using the rarefied OTU table, and the Bray–Curtis metric was used as an estimator of community dissimilarity. To check if the communities were structured stochastically or not, the RC_{Bray} index was calculated. The index consists of the Raup–Crick metric (77) using Bray–Curtis dissimilarities (41). A total of 1,000 randomizations were performed for OTUs with >100 reads in the entire dataset. Communities were clustered using nonmetric multidimensional scaling (nMDS) analyses based on Bray–Curtis distances. Statistical differences among categories such as size fraction, station, and depth were explored with permutational multivariate analyses of variance (PERMANOVA) tests (adonis function, R *vegan* package).

To elucidate the connectivity between communities throughout the water column of a given size fraction, a set of parameters was calculated considering each station separately: Vertical beta diversity (i.e., community differentiation) was calculated using the *trudi* function from R package *simba*. Vertical OTU turnover (i.e., dissimilarity due to species replacement) and nestedness (i.e., dissimilarity due to species loss) were estimated using the *beta.multi* function in the R *betapart* package and were based on the Sorensen index. The vertical niche breadth of each OTU was calculated using the *niche.width* function in the R *spaa* package and applying the Levins (78) index. Niche breadth was defined as the number of different depths where an OTU appeared (i.e., OTUs with niche breadth values of 4 were present across the four depths, whereas OTUs with niche breadth values of 1 were present in only one depth), and an average of all of the niche breadth values of the OTUs of each station was calculated. To test if differences from small to large size fractions were statistically significant in beta diversity, turnover, nestedness, and niche breadth parameters, a Wilcoxon ranked sign test was performed comparing the 0.2–0.8 μm and the 20–200 μm size fractions. Correlations between communities from different size fractions and depths were calculated using Mantel tests (based on Bray–Curtis distances).

To differentiate between the OTUs dominant in (i.e., enriched in, with preference for) smaller or larger size fractions, we defined the PAN-Index. The PAN-Index indicates in which size fraction an OTU is more abundant (and therefore more dominant over the other OTUs) and was calculated using the abundance-weighted mean of each OTU among the five size fractions. This PAN-Index defines the size fraction preference of every OTU in the continuum of sizes (i.e., in which size fraction an OTU is more dominant) and is a modification of the PAN-Index presented in Salazar et al. (52) where only two size fractions (free-living vs. attached) were considered. Values of PAN-Index ranged from 1 to 5, and each number reflects the size preference of a given OTU as follows: 1, preference for the size fraction 0.2–0.8 μm; 2, preference for 0.8–3.0 μm; 3, preference for 3.0–5.0 μm; 4, preference for 5.0–20 μm; and 5, preference for 20–200 μm size fraction. The PAN-Index values presented a bimodal distribution (*SI Appendix*, Fig. S4), and we consequently divided them in two groups: OTUs with PAN-Index $\geq$ 2.7 (i.e., those with preference for large particles) and OTUs with PAN-Index $<$ 2.7 (i.e., those with preference for small particles). To identify the pool of taxa potentially growing in sinking particles, we first selected among the surface OTUs with PAN-Index $\geq$ 2.7 those showing the largest shifts in relative abundance by calculating the Euclidean distance of their relative abundances between all pairs of samples and choosing the OTUs with a mean distance >10, following Ruiz-González et al. (39). We then identified those that increased in relative abundances (dominance) toward deeper layers (i.e., showing higher mean relative abundances in mesopelagic and/or bathypelagic waters than in the surface and/or the DCM). This was done for each station, and these OTUs were named seed OTUs because they might represent taxa seeding deeper communities.

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