ECOLOGY

Global biogeography of the smallest plankton across ocean depths

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Tiny ocean plankton (picoplankton) are fundamental for the functioning of the biosphere, but the ecological mechanisms shaping their biogeography were partially understood. Comprehending whether these microorganisms are structured by niche versus neutral processes is relevant in the context of global change. We investigate the ecological processes (selection, dispersal, and drift) structuring global-ocean picoplanktonic communities inhabiting the epipelagic (0 to 200 meters), mesopelagic (200 to 1000 meters), and bathypelagic (1000 to 4000 meters) zones. We found that selection decreased, while dispersal limitation increased with depth, possibly due to differences in habitat heterogeneity and dispersal barriers such as water masses and bottom topography. Picoplankton β-diversity positively correlated with environmental heterogeneity and water mass variability, but this relationship tended to be weaker for eukaryotes than for prokaryotes. Community patterns were more pronounced in the Mediterranean Sea, probably because of its cross-basin environmental heterogeneity and deep-water isolation. We conclude that different combinations of ecological mechanisms shape the biogeography of the ocean microbiome across depths.

INTRODUCTION

The smallest eukaryotes and prokaryotes (picoplankton, 0.2 to 3 μm) play essential roles in the global ocean: from trophic interactions (*1*) to biogeochemical cycles (*2*, *3*). They account for 57% (~3.8 gigatons of carbon) of the ocean's biomass (*4*) and are the main contributors to the taxonomic and functional diversity of the ocean (*5*–*7*). Therefore, understanding the mechanisms determining their global biogeography is fundamental to predicting how they will respond to environmental changes. Picoplankton abundance, diversity, and composition are relatively well described across ocean depths (*8*, *9*): Prokaryotes' diversity increases with depth (*5*, *10*), while picoeukaryotes' diversity sharply decreases (*11*). These depth-related patterns are strongly shaped by gradients in sunlight, temperature, oxygen, and nutrients (*5*, *10*) as well as by physical barriers such as water masses, currents, and fronts (*12*–*14*). However, the ecological processes underpinning picoplankton biogeography are only partially understood (*15*, *16*), especially when considering different ocean depth zones and geographic scales. The deep ocean is the largest ecosystem on our planet and harbors a massive microbial genetic diversity (*17*) responsible for essential global ecosystem services. Therefore, understanding the ecological processes that shape the microbiota in the vast and understudied deep ocean is particularly important.

The biogeography of organisms is the result of four high-level ecological processes that act in different proportions: selection, dispersal, ecological drift, and evolutionary diversification (*18*). Selection is a deterministic force emerging from combinations of biotic and abiotic variables that lead to differences in the fitness of individuals of a species and, as a consequence, to changes in community structure. Environmental selection is also mentioned in the literature as niche partitioning/differentiation or environmental filtering (*19*–*21*). Selection can either restrict (homogeneous selection) or promote (heterogeneous selection) the divergence of communities (*22*). Dispersal is the movement of organisms across space and their establishment in new locations, affecting local community assembly by adding individuals from the regional species pool. Dispersal is a stochastic process for small plankton as they passively drift with currents (*22*). Microbial dispersal rates may be high (homogenizing dispersal), moderate, or low (dispersal limitation) (*22*), depending on organisms and population sizes, geographic scale, and the presence of physical barriers (*16*, *23*, *24*). Dispersal limitation occurs when species are not present in suitable habitats because colonizers cannot reach them (*25*). Thus, the relative importance of dispersal limitation usually increases with geographic scales (*26*) or barriers (*23*). Ecological drift (hereafter drift) refers to random changes in community structure due to stochastic demographic events (i.e., birth, death, immigration, and emigration) in a local community (*18*). Drift is a stochastic process that tends to be most important for the local extinction of low-abundant microbial taxa with small populations (*27*), especially under a low dispersal scenario (*24*). Last, diversification (also referred to as "speciation") is the emergence of new species by evolution (*18*), which occurs more frequently for microbes than for larger organisms due to their short

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generation times, high mutation rates, and horizontal gene transfer (*22*, *27*). Yet, diversification is expected to have a relatively small impact on the turnover of communities connected via dispersal (*28*), as is the case for ocean picoplankton (*23*). Diversification, as measured by the evolution of the ribosomal RNA (rRNA) gene sequence, will not be further considered here, given that its impact on measured ecological processes is likely minor considering the relatively low evolutionary rates of this marker (*29*).

A recent study—using datasets from the global cruises *Malaspina* and TARA oceans—found that the relative importance of these processes differs between the components of the surface ocean picoplankton community: While prokaryotes are shaped by a balanced combination of dispersal, selection, and drift, picoeukaryotes are mainly driven by dispersal limitation (*15*). However, we do not fully understand whether these processes change across ocean depth zones. These zones display notable differences in environmental and physical features that may influence selection, dispersal, and drift. First, environmental heterogeneity—potentially exerting heterogeneous selection on microbial communities (*15*, *30*)—is higher in the upper ocean due to stronger horizontal environmental gradients (*31*) than in the deep ocean (*32*). Second, aerial dispersal (*33*) and fast oceanic currents likely increase microbial dispersal at the surface ocean (*34*, *35*). In turn, the presence of sharp geographical barriers (e.g., water masses and bottom topography) may limit microbial dispersal in the low-turbulent deep ocean (*16*, *36*). Third, smaller population sizes in the deep ocean (*8*) may lead to reduced dispersal and increased drift (*24*), as compared to the surface ocean (*15*, *35*). Recently, using a subset of the *Malaspina* cruise dataset, it has been shown that picoplankton community assembly differed between a water layer in the surface ocean (3 m) and a counterpart in the deep ocean (~4000 m), with dispersal limitation being relatively more important in the deep layer than in the surface counterpart (*16*).

In addition, we do not know whether these processes would be different in an ocean basin presenting strong environmental gradients and clear geographic barriers. In this regard, the Mediterranean Sea—the largest semienclosed sea on Earth—is an ideal model to test ecological hypotheses at a smaller scale (*37*). The Mediterranean Sea is connected to the Atlantic Ocean through the Strait of Gibraltar, but the connection is limited (*38*). Consequently, the Mediterranean Sea has developed unique oceanographic features compared to the open ocean, such as higher temperature and salinity in deep waters as well as a west-to-east gradient of decreasing nutrient concentration and increasing salinity in surface waters (*10*, *39*). In addition, the Mediterranean Sea deep (>1000 m) waters are physically divided by the Sicily Strait (500 m deep) into western and eastern basins. These features are expected to influence the processes shaping picoplankton communities and, ultimately, be reflected in their biogeography (*10*).

Previous work found that different ecological processes shape prokaryotic and picoeukaryotic communities in the surface ocean (*15*), while differences in picoplankton biogeography were found when comparing specific water layers in the surface (3 m) versus deep ocean (4000 m) (*16*). Despite these advances, we lacked a broad understanding of the ecological processes driving picoplankton community assembly and biogeography across all depth zones of the global ocean, taking into account environmental heterogeneity, potential dispersal barriers, and geography. Here, we address this knowledge gap. We determined the relative importance of the ecological processes structuring picoplanktonic communities inhabiting three ocean depth zones at the global and basin scales: the epipelagic (0 to 200 m), mesopelagic (200 to 1000 m), and bathypelagic (1000 to 4000 m) zones. We also aimed to understand to what extent water masses, deep-sea topography, and environmental heterogeneity are potentially limiting dispersal or exerting selection on the picoplanktonic communities. To do so, we used 16*S* and 18*S* rRNA gene amplicon sequence variants (ASVs) from both prokaryotes and picoeukaryotes collected during global and regional expeditions covering the tropical and subtropical global ocean as well as the Mediterranean Sea. We hypothesize that the role of heterogeneous selection will decrease with depth due to a potential decrease in habitat heterogeneity, while homogeneous selection is expected to be higher in the bathypelagic compared to the meso- and epipelagic. In turn, the relative importance of dispersal limitation is expected to increase with depth, given the decrease in current speed in deep waters, the presence of geographical barriers (e.g., fronts and deep sea topography), and the absence of aerial dispersal. We also hypothesize that these patterns should be more pronounced in the Mediterranean Sea due to its strong environmental gradients and constrained connectivity in deep waters.

RESULTS

Different ecological processes shape the smallest members of the ocean microbiome across depth zones

We analyzed picoplankton community composition in 451 samples across three ocean depth zones: epipelagic (0 to 200 m—including the deep chlorophyll maxima, DCM), mesopelagic (200 to 1000 m), and bathypelagic (1000 to 4000 m) using metabarcoding of the 16*S* and 18*S* rRNA genes (Fig. 1A and fig. S1A; see Materials and Methods for details on standard protocols). These zones display contrasting environmental features across the water column (Fig. 1B and fig. S1B), reflected in a depth-structured picoplankton community composition (Fig. 1C). Our data also make evident an inverted diversity pattern between the two main components of the picoplankton community: While prokaryotic diversity (richness, Shannon index, and phylogenetic diversity) increased with depth, picoeukaryotic diversity decreased toward the deep ocean (Fig. 1D and fig. S2). Although the Mediterranean Sea displayed higher temperature and salinity as well as lower nutrients than the oceanic basins, particularly in the meso- and bathypelagic (Fig. 1B), the diversity patterns were similar in both ocean datasets, pointing to general patterns. The different environmental features, however, were reflected in differences in picoplankton community composition (Bray-Curtis dissimilarity) between the Mediterranean Sea and the rest of the oceanic basins (Fig. 1C). The Mediterranean Sea was evaluated separately from the open ocean in downstream analyses to test whether the observed large-scale patterns were reflected at the regional scale of a smaller basin with strong environmental gradients and clear geographic barriers.

We found differences in the β-diversity metrics [β-Nearest Taxon Index (βNTI), RC_{Bray}, and β-diversity partitioning, figs. S3 and S4] and, ultimately, in the balance between ecological processes shaping picoplankton communities across depth zones of the ocean (Fig. 2A). Selection explained a similar percentage of the turnover of picoeukaryotes as compared to prokaryotes in the epipelagic (~37 versus ~36%), mesopelagic (~32 versus ~31%), and bathypelagic (~32 versus ~26%) of the open ocean (Fig. 2A). Heterogeneous

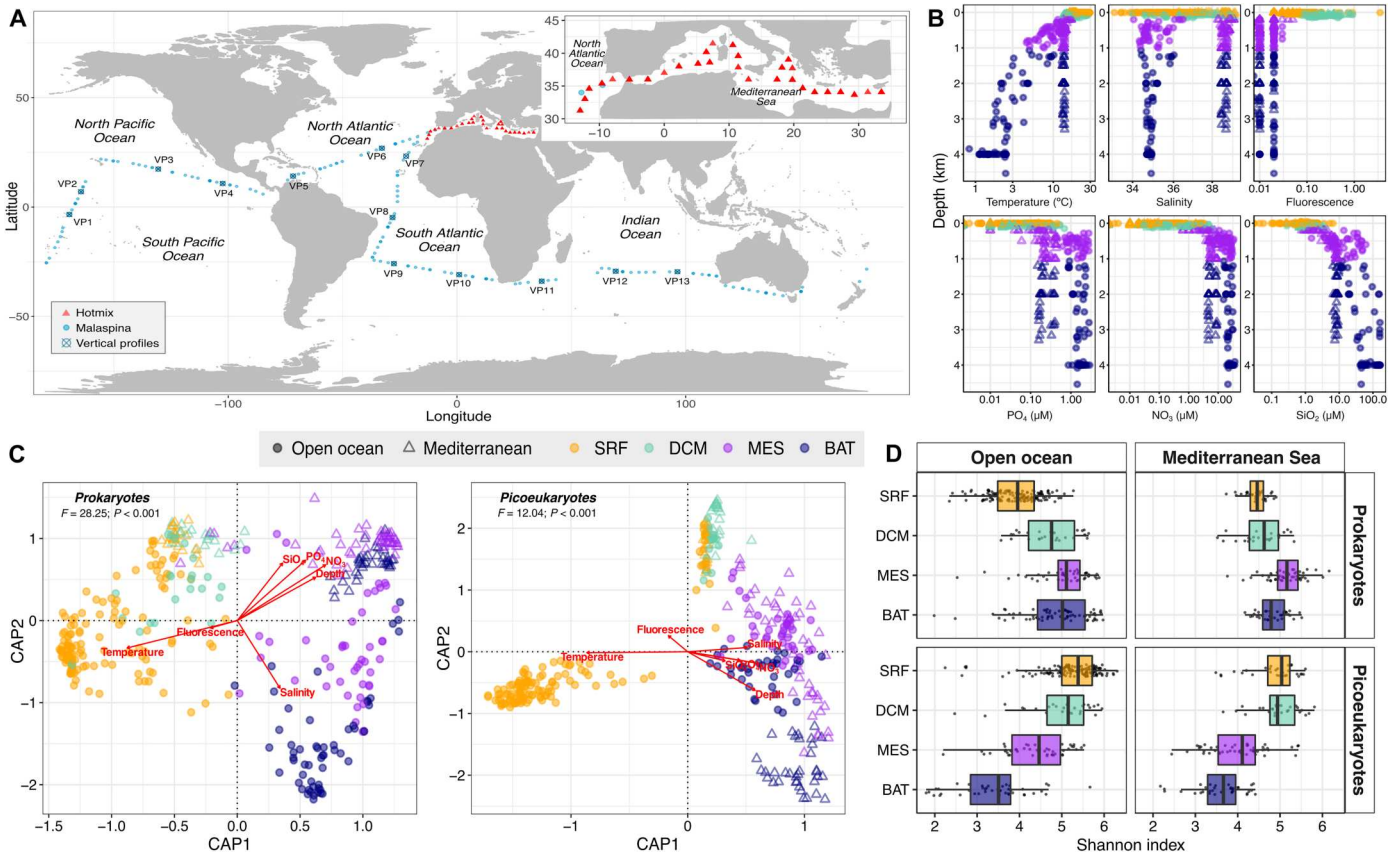

Fig. 1. The analyzed dataset covers environmentally and biologically contrasting depth zones of the ocean. (**A**) Geographic distribution of the stations (N = 149) from which seawater samples and environmental data were collected at different depth zones (see fig. S1 for sample vertical distributions) in the two cruises used in this study: *Malaspina-2010* (circumglobal expedition) and *HotMix* (trans-Mediterranean expedition). Stations for which the whole vertical profile was sampled in *Malaspina* are represented by crossed squares (13 stations). Samples were separated into "open ocean" (*Malaspina-2010* + *Hotmix*'s North Atlantic samples) and "Mediterranean Sea" (see reasoning in Materials and Methods). (**B**) Vertical profiles of the measured environmental parameters: temperature, salinity, and fluorescence (chlorophyll a proxy) decrease with depth, while nutrient concentration (NO_3, PO_4, and SiO_2) increases with depth. Note that higher temperature and salinity values and lower nutrient concentrations were observed in the Mediterranean Sea, especially in the meso- and bathypelagic (see also fig. S1B). (**C**) Distance-based redundancy analyses (based on Bray-Curtis dissimilarities) performed on picoplankton community composition of both prokaryotic (left) and picoeukaryotic (right) samples based on 16*S* rRNA and 18*S* rRNA genes, respectively. Both communities were structured by depth zones and segregated between the tropical and subtropical open ocean and the Mediterranean Sea. (**D**) Picoplankton diversity expressed as Shannon index by depth zones (SRF, surface; DCM, deep chlorophyll maxima; MES, mesopelagic; BAT, bathypelagic). See also fig. S2 for picoplankton phylogenetic diversity, gamma diversity, ASVs richness, and Pielou's evenness index variation by depth zones and correlations with environmental variables.

selection, that is, selection promoting the differentiation of communities, tended to increase with depth for both domains. For prokaryotes, it increased from ~10% in the epipelagic to ~19 and ~13% in the meso- and bathypelagic, respectively (Fig. 2A). Similarly, for picoeukaryotes, it increased from ~13% in the epipelagic to ~27 and ~31% in the meso- and bathypelagic (Fig. 2A). Accordingly, the relative importance of homogeneous selection, that is, selection acting against community differentiation, decreased from ~26% in the epi- to ~13% in the bathypelagic for prokaryotes. Similarly, the relative importance of homogeneous selection for picoeukaryotes drastically decreased from ~26% in the epipelagic to ~0.7% in the bathypelagic (Fig. 2A).

The importance of selection in shaping picoeukaryotic communities was slightly different in the Mediterranean Sea compared to the tropical and subtropical open ocean. The relative importance of selection for prokaryotic community assembly was consistently higher than its counterpart for the picoeukaryotic community. This was observed in the epipelagic (~54% prokaryotes versus ~44% picoeukaryotes), mesopelagic (~39% versus ~25%), and bathypelagic (~32 versus ~6%), respectively (Fig. 2A). The proportion of heterogeneous selection for prokaryotes dramatically dropped from 37% in the epipelagic to ~5% in deep waters, while the role of homogeneous selection increased from the epipelagic (~18%) to the mesopelagic (~34%) and bathypelagic (~28%) (Fig. 2A). For picoeukaryotes, both heterogeneous and homogeneous selection decreased from the epipelagic (33 and 10%) to the bathypelagic (6 and 0.2%, respectively) (Fig. 2A).

Dispersal limitation was a more important driver of picoeukaryotic than prokaryotic community assembly in the deep zones, especially in the open ocean's mesopelagic (~60 versus ~29%). We found that, for picoeukaryotes, the proportion of dispersal limitation increased from ~31% in the epipelagic to ~60% in the mesopelagic and ~38% in the bathypelagic (Fig. 2A). In the Mediterranean Sea, the relative importance of dispersal limitation was much higher

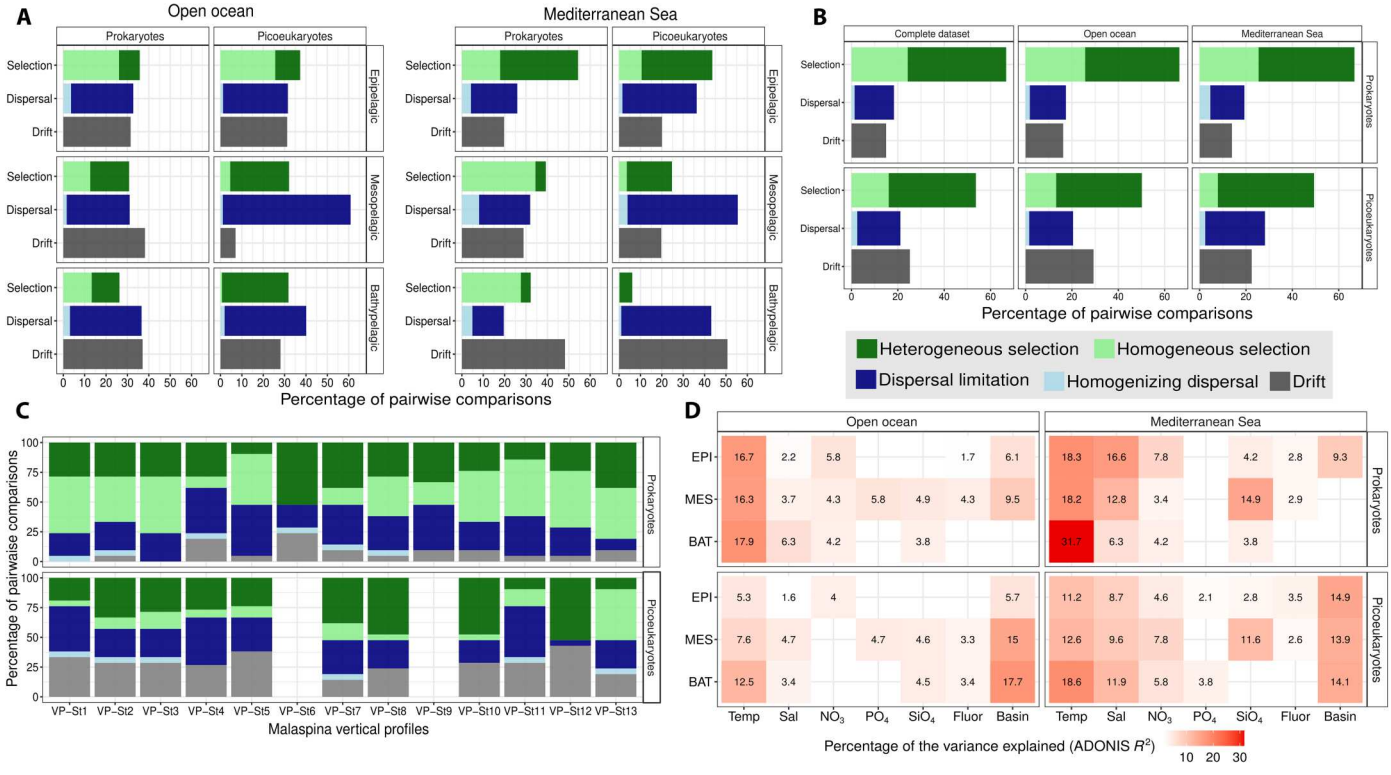

Fig. 2. Picoplankton community assembly processes and environmental drivers across ocean depth zones. Relative importance of the ecological processes structuring picoplankton communities in (**A**) different depth zones of the global ocean: epipelagic ($N = 240$), mesopelagic ($N = 97$), and bathypelagic ($N = 86$); (**B**) pooling all samples together of the complete dataset as well as separated by the open ocean, and the Mediterranean Sea; and (**C**) integrating all depths (from 3 to 4000 m, i.e., seven different depths) in each of the 13 vertical-profile (VP) stations (labeled as in Fig. 1A). Note that the results using standardized and evenly distributed sample sets were nearly identical (fig. S5). The epipelagic results separated by surface and deep chlorophyll maximum are shown in fig. S7. (**D**) Percentage of variance (Adonis R^2) in picoeukaryotic and prokaryotic community composition (Bray-Curtis dissimilarities) explained by each of the measured environmental variables and ocean basin. Blank spaces depict nonsignificant results ($P > 0.05$). EPI, epipelagic; MES, mesopelagic; BAT, bathypelagic; Temp, temperature; Sal, salinity; Fluor, fluorescence; Basin, ocean basin.

for picoeukaryotic than for prokaryotic community assembly in the epipelagic (~35 versus ~22%), mesopelagic (~52 versus ~24%), and bathypelagic (~42 versus ~15%). Conversely, homogenizing dispersal had a minimal role in the structuring of the microbiota in all depth zones of the open ocean (<2% for picoeukaryotes and <4% for prokaryotes) and the Mediterranean Sea (<5% for picoeukaryotes and <8% for prokaryotes) (Fig. 2A).

Drift explained a higher fraction of community turnover for prokaryotes than picoeukaryotes in the mesopelagic (~38 versus ~7%) and bathypelagic (~37 versus ~28%) of the open ocean (Fig. 2A). This pattern was partially observed in the Mediterranean Sea, with drift explaining a higher proportion of community turnover for prokaryotes (~29%) and picoeukaryotes (~20%) in the mesopelagic (Fig. 2A). In the open ocean, the percentage of community turnover explained by drift increased with depth for prokaryotes, but it decreased for picoeukaryotes (Fig. 2A). In contrast, drift sharply increased with depth for both prokaryotes and picoeukaryotes in the Mediterranean Sea (Fig. 2A). When estimated using a standardized dataset including 39 samples in each depth zone and evenly distributed samples (figs. S5A and S6), the different ecological processes explained fairly similar percentages of variability, and the values were strongly correlated [correlation coefficient (r) ~ 0.9, $P < 0.001$] to those found with the complete dataset (fig. S6).

When selection was estimated by pooling all depths together, it was by far the most relevant ecological process shaping both prokaryotic (~67%) and picoeukaryotic (~54%) communities (Fig. 2B). This aligns with the strong vertical physicochemical gradients in the ocean. Dispersal limitation also tended to play a relatively more important role in shaping picoeukaryotic than prokaryotic communities when estimated across all depth zones (Fig. 2B). Because of the vertical connectivity between the surface and the deep ocean (*66*), we also estimated the relative importance of ecological processes after integrating all depths (from 3 to 4000 m) in each of the 13 vertical profile stations included in our dataset (Fig. 1A). Compared to the previous analyses, this approach can inform on the relative importance of ecological processes acting across depths at single points in the ocean. We found that selection was the most important factor vertically shaping picoplankton communities in the analyzed stations, explaining ~52 to 81% of the prokaryotic community turnover (Fig. 2C) and ~24 to 52% of the picoeukaryotic community turnover (Fig. 2C). The relative importance of vertical dispersal limitation ranged from 10 to 43% in prokaryotes and from 5 to 43% in picoeukaryotes (Fig. 2C). Drift was greater in picoeukaryotes (~15 to 43%) than in prokaryotes (~5 to 24%) across depths (Fig. 2C).

Environmental heterogeneity across depth zones dictates the relative importance of selection

We evaluated the abiotic drivers of selection across ocean depth zones. Water temperature was the most important environmental driver of prokaryotic community composition in the open ocean (~16 to 18% Adonis) and the Mediterranean Sea (~18 to 32%) (Fig. 2D). In turn, temperature explained a moderate or low percentage of the variance of the picoeukaryotic community composition, being the highest values in the bathypelagic (~12% in the open ocean and ~18% in the Mediterranean Sea) (Fig. 2D). In the Mediterranean Sea, the percentages of the variance in community composition explained by temperature in prokaryotes and picoeukaryotes increased from the surface (~18 and ~11%, respectively) to the deep zones (~32 and ~19%, respectively). Salinity, another potentially important driver of selection, explained a moderate fraction of prokaryotic (up to 16%) and picoeukaryotic (up to 12%) community variance in the Mediterranean Sea but not in the open ocean (Fig. 2D). Geography (ocean basin) could affect the structure of picoplankton communities if it is linked with differential dispersal or selection regimes. Geography (ocean basin) explained most of the variation of the picoeukaryotic community composition in the open ocean and the Mediterranean Sea (Fig. 2D). The percentage of community variance explained by geography in picoeukaryotes increased markedly from the surface to the meso- and bathypelagic in the open ocean (Fig. 2D). In turn, geography explained a limited fraction of community variance in prokaryotes.

Environmental heterogeneity (measured as the average pairwise dissimilarity between samples in terms of their temperature, salinity, fluorescence, PO_4^{3-}, NO_3^-, and SiO_2) was significantly higher in the epi- than in the meso- and bathypelagic of the open ocean and the Mediterranean Sea (fig. S8). We found that the picoplankton communities' dissimilarity increased with environmental distance in all depth zones (Fig. 3). This positive relationship was always stronger in the epipelagic than in the bathypelagic (Fig. 3). Prokaryotes displayed a stronger coupling with environmental distances than picoeukaryotes in all depth zones of both the open ocean and the Mediterranean Sea (Fig. 3). This coupling was stronger in the Mediterranean Sea than in the open ocean across all depth zones (Fig. 3). When estimated considering all depth zones together, the correlation between community dissimilarity and environmental distances was stronger for prokaryotes than for picoeukaryotes in both the open ocean ($r = 0.62$ versus $r = 0.46$, $P < 0.001$) and the Mediterranean Sea ($r = 0.69$ versus $r = 0.35$, $P < 0.001$) (fig. S9). The metric used to estimate selection (βNTI) was positively correlated, in prokaryotic and picoeukaryotic communities, with environmental distances in both the open ocean ($r = 0.55$ and $r = 0.50$, $P < 0.001$) and the Mediterranean Sea ($r = 0.55$ and $r = 0.50$, $P < 0.001$) (fig. S9).

Water masses and deep sea topography modulate picoplankton assembly

Water masses, determined for the meso- and bathypelagic, were vertically structured and segregated by basins in the open ocean and the Mediterranean Sea (fig. S11). We found that prokaryotic community composition (Bray-Curtis dissimilarities) displayed a modest or moderate positive correlation with differences in water mass composition (Euclidean distances) in the bathypelagic of the open ocean ($r = 0.2$ and $r = 0.4$, $P < 0.001$). Similarly, differences in prokaryotic community composition were moderately correlated with water mass composition in the meso- and bathypelagic of the Mediterranean Sea ($r = 0.46$ and $r = 0.33$, $P < 0.001$) (Fig. 4). For picoeukaryotes, this moderate coupling between differences in community composition and water masses was only observed in the meso- and bathypelagic of the Mediterranean Sea ($r = 0.49$ and $r = 0.29$, $P < 0.001$) (Fig. 4). In general, in the Mediterranean Sea, the link between picoplankton community composition and water masses was stronger in the meso- than in the bathypelagic (Fig. 4). For the open ocean, picoplankton community composition

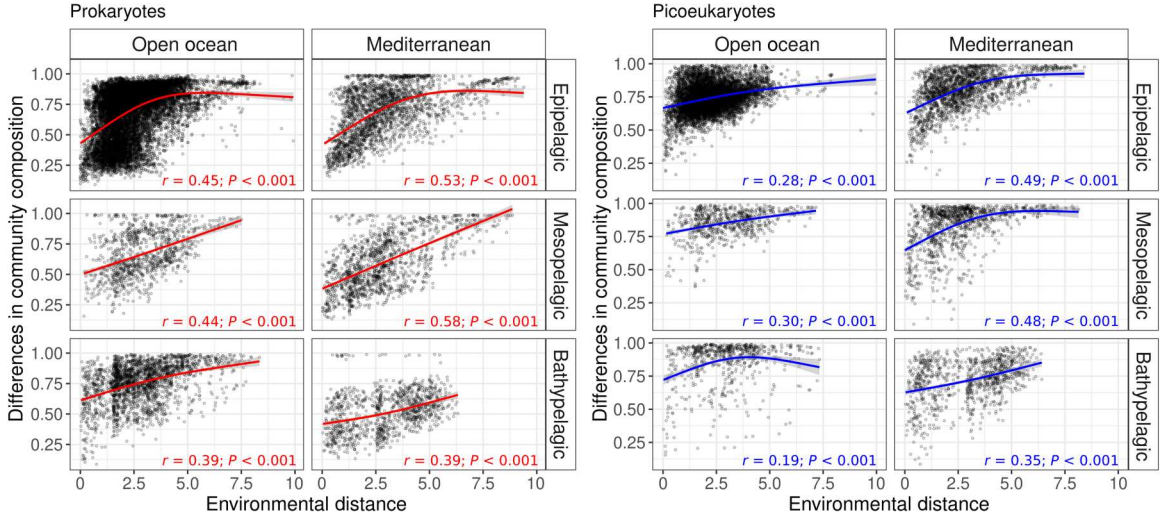

Fig. 3. Picoplankton community composition was positively correlated to environmental heterogeneity. Bray-Curtis dissimilarities for all pairwise community comparisons as a function of environmental distance for both prokaryotes and picoeukaryotes in the epi-, meso-, and bathypelagic of the open ocean and Mediterranean Sea. The solid curves illustrate the nonlinear regressions. Spearman's rank correlation coefficients are depicted on the panels. Outliers with high environmental distances (>10) corresponding to pairwise comparisons with epipelagic samples from the Costa Rica Dome upwelling system were removed from the open ocean plot (see fig. S10).

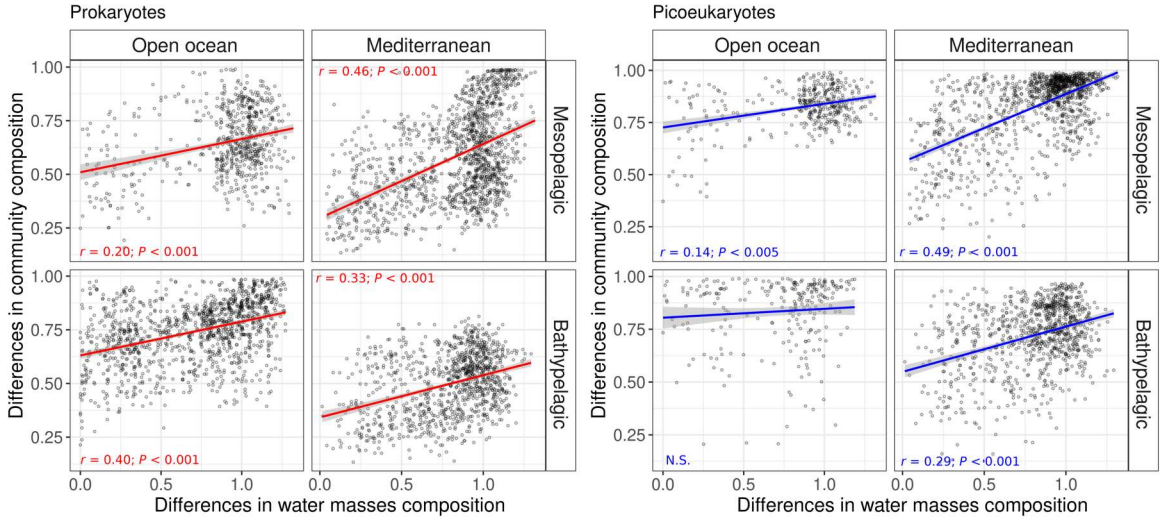

Fig. 4. Relationships between picoplankton community differentiation and differences in water mass composition. Bray-Curtis dissimilarities between pairwise picoplankton community comparisons as a function of water mass composition dissimilarity (based on Euclidean distances) for both prokaryotes and picoeukaryotes in the meso- and bathypelagic of the open ocean and Mediterranean Sea. The solid curves illustrate the nonlinear regressions. Spearman's rank correlation coefficients are depicted on the panel. N.S., nonsignificant.

and water masses were positively linked within most individual vertical-profile stations, with variable slopes in each station (fig. S12).

In the open ocean, changes in prokaryotic and picoeukaryotic community composition (β-diversity) displayed positive correlations with geographic distances (distance-decay) in four depth zones (Fig. 5A). These correlations were normally weaker for prokaryotes than for picoeukaryotes. Prokaryotes displayed positive correlations with distances up to ~2000 km in the surface and 1000 km in the deep ocean, while picoeukaryotes showed positive correlations up to ~3000 km in the surface and ~4000 km in the deep ocean (Fig. 5A). For picoeukaryotes, these positive correlations were stronger in the bathypelagic (Mantel $r = 0.5$, $P < 0.05$) than in the surface (Mantel $r = 0.3$, $P < 0.05$) (Fig. 5A). Picoeukaryotes also displayed negative correlations with increasing distances up to ~20,000 km across the deep zones (Fig. 5A). Picoeukaryotes had a higher variation in the spatial autocorrelations than prokaryotes in the deep ocean, especially in the bathypelagic. When evaluating these spatial autocorrelations at a regional scale, as in the Mediterranean Sea, we found that prokaryotes and picoeukaryotes did not display such contrasting correlation scores as in the open ocean

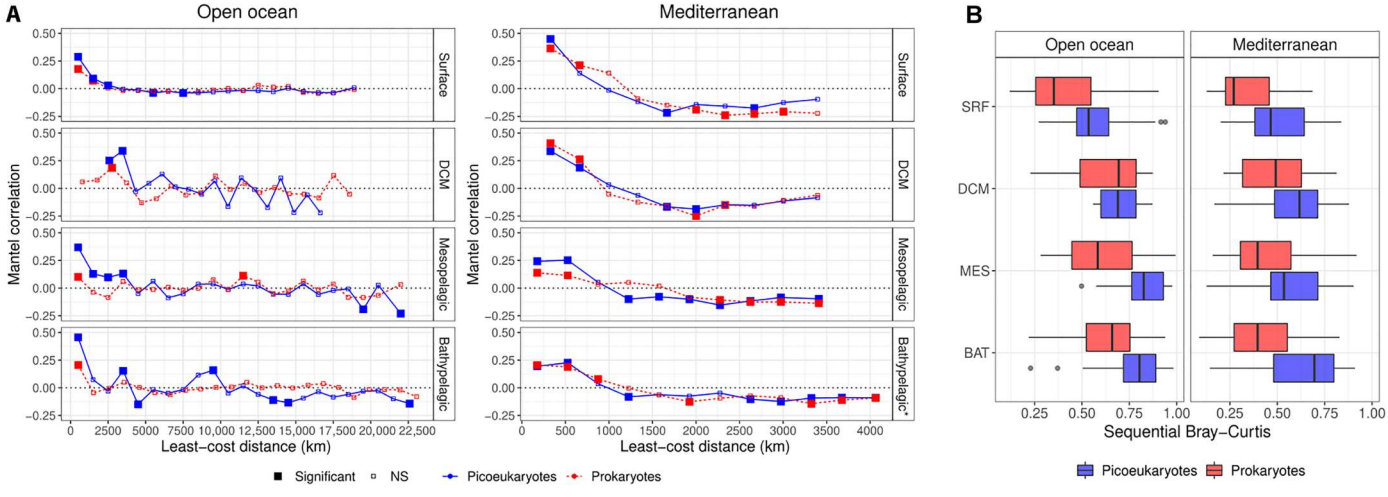

Fig. 5. Distance-decay and sequential spatial differentiation in picoplankton communities across ocean depth zones. (**A**) Mantel correlograms between β-diversity and least-cost geographic distances featuring distance classes of 1000 km for the open ocean and 350 km for the Mediterranean Sea. Filled squares depict significant correlations ($P < 0.05$). NS, nonsignificant correlations. (**B**) Sequential Bray-Curtis dissimilarities for prokaryotes and picoeukaryotes in all depth zones [means were significantly different between domains (Wilcoxon test, $P < 0.05$) in all depth zones, except in the DCM]. The averages were also significantly different [analysis of variance (ANOVA), Tukey post hoc test; $P < 0.001$] between the surface (SRF) and the deep zones [mesopelagic (MES) and bathypelagic (BAT)] for picoeukaryotes, but not for prokaryotes. See fig. S13 for maps showing the sequential change in community composition across space in the surface and bathypelagic ocean. The epipelagic was here separated into surface and DCM because we aimed at evaluating only the horizontal geographic distance in each depth.

(Fig. 5A). These two domains had similar patterns of positive correlations in the first 350 to 850 km of the Mediterranean Sea (Fig. 5A). Picoeukaryotes had higher mean sequential changes in communities (β-diversity) than prokaryotes in all depth zones (Fig. 5B). Overall, sequential community change tended to increase with depth in picoeukaryotes, with significant differences between the surface and the meso- and bathypelagic in picoeukaryotes, but not in prokaryotes (Fig. 5B).

Microbial abundances and activity may regulate dispersal limitation and ecological drift

Microbial abundances and activity may also be potential regulators of dispersal limitation and drift. Here, microbial abundances—as measured by flow cytometry—sharply decreased with depth in both the open ocean and the Mediterranean Sea (Fig. 6A). Similarly, prokaryotic activity—as measured by leucine incorporation rates—drastically decreased from surface to deep ocean waters (Fig. 6B), with statistically significant differences between epipelagic (SRF and DCM) and deep zones (MES and BAT).

DISCUSSION

Selection decreases while dispersal limitation and drift increase with ocean depth

Our results supported our main hypothesis, indicating that a different combination of ecological processes shapes picoplankton biogeography across ocean depth zones at global and regional scales (Fig. 7). Selection was the most important process shaping picoplankton communities in the epipelagic ocean (see also Supplementary Text), likely as a response to a higher overall environmental heterogeneity when compared to the deep ocean. In particular, microalgal blooms (*31*, *40*), the magnitude of the DCM (*5*, *41*), ocean fronts and eddies (*13*, *14*, *42*), and differences in physicochemical variables (Fig. 1B) together increase environmental heterogeneity in the upper ocean (Fig. 7). The higher relative importance of heterogeneous selection in the epipelagic of the Mediterranean Sea than in the open ocean is probably linked to strong environmental gradients. The main surface gradients in the Mediterranean Sea are a north-south increase in temperature (*43*), a west-east increase in salinity (*43*), and a west-east decrease in nutrient concentrations (*10*). This result contradicts a previous hypothesis indicating that homogeneous selection should be the most important process structuring microbial communities in all ocean basins (*44*). Instead, the relative importance of ecological processes shaping picoplankton communities will change depending on environmental heterogeneity, ocean circulation, and geographic scale (*45*, *46*). In our study, the overall role of selection decreased, for both prokaryotes and microbial eukaryotes, when transiting from the epipelagic into the deep waters, where there is lower environmental heterogeneity in comparison to the epipelagic (fig. S8). Moreover, the coupling between picoplankton community differentiation and environmental distances was stronger in the epipelagic than in the deep ocean, further indicating that the relative importance of heterogeneous selection rises with increasing environmental variability. Selection was also the most important process shaping picoplankton communities when these processes were estimated considering all samples in our dataset, thus capturing environmental differences from surface to deep waters. This represents further evidence that heterogeneous selection is enhanced as environmental heterogeneity increases. These findings are coherent with ecological theory and other studies that show that high environmental heterogeneity leads to higher heterogeneous selection (*18*) in terrestrial (*28*, *47*) and aquatic ecosystems (*30*, *48*, *49*).

Conversely, dispersal limitation and drift were overall higher in the deep than in the surface ocean. This suggests that factors such as microbial abundances (i.e., small population sizes) (*24*) and physical barriers (water masses and deep-sea bathymetry) (*16*) play an important role in the structuring of deep ocean picoplankton communities (Fig. 7). Dispersal limitation increased with depth probably because of decreasing turbulence (stable water masses and slow currents) (*32*) and the presence of straits and seamounts (*50*) that may work as geographical barriers for microbial dispersal in the deep ocean (Fig. 7). Other studies have shown that physical barriers can limit microbial dispersal in soils (*48*), sediments (*28*), ponds (*49*), and, possibly, in the ocean (*23*).

Water masses affect the distribution of prokaryotic communities

Water masses may affect microbial communities in at least two ways: (i) as a selective force—since they have different temperatures and salinity (*51*) as well as organic matter composition (*52*–*54*)—or (ii) as a barrier to dispersal due to differences in water density (*12*).

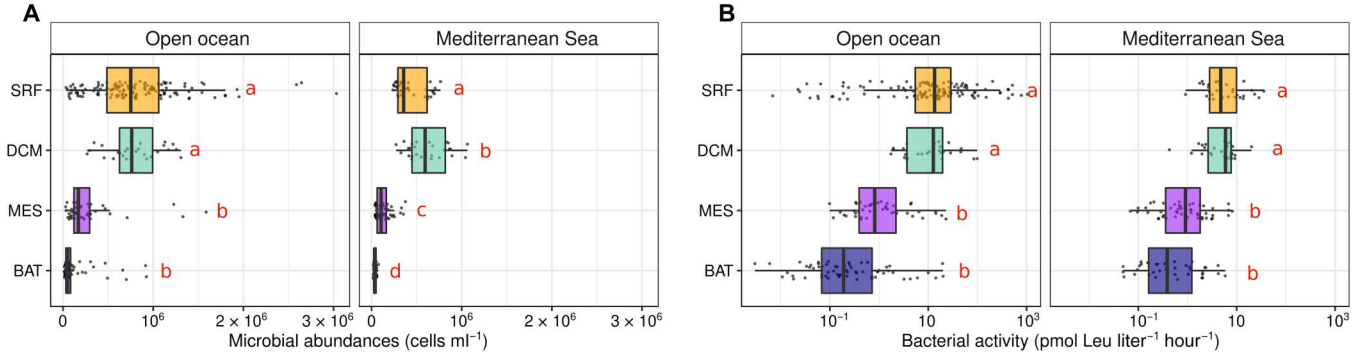

Fig. 6. Microbial abundances and bacterial activity sharply decrease in deep waters. (**A**) Microbial abundances (prokaryotes + picoeukaryotes) as measured by flow cytometry. (**B**) Bacterial activity as measured by leucine incorporation rates in each depth zone of the open ocean and the Mediterranean Sea. Different red letters represent significantly different means (ANOVA, Tukey post hoc test, $P < 0.05$) between depth zones. SRF, surface; DCM, deep chlorophyll maxima; MES, mesopelagic; BAT, bathypelagic.

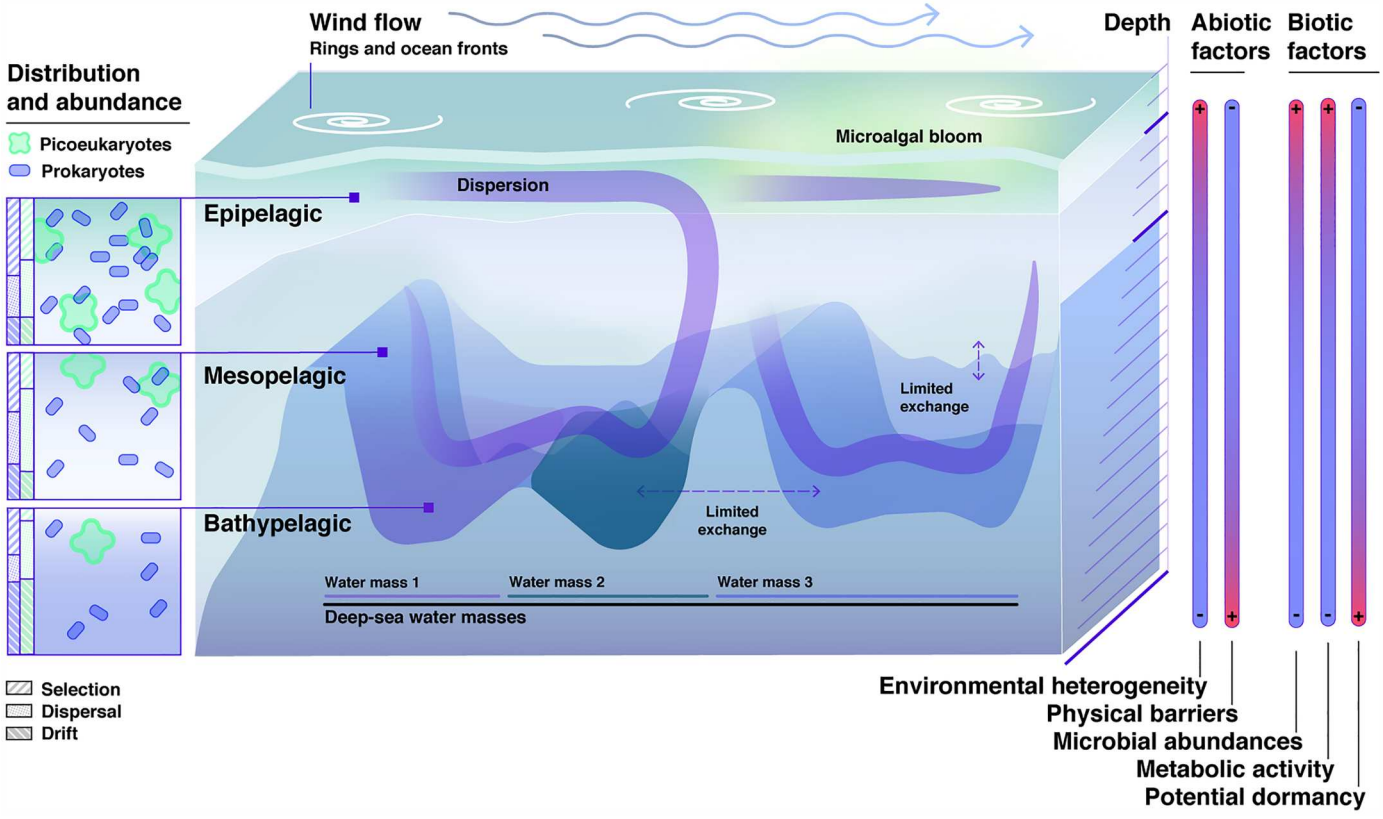

Fig. 7. Conceptual model synthesizing the ecological processes assembling picoplankton communities across ocean depth zones. We used the main findings of this study and the knowledge available in the literature to construct this hypothetical model. Vertical variation of biotic and abiotic factors, as well as geography (e.g., bathymetry), would affect the ecological processes that generate community distribution patterns. According to this model, dispersal limitation would increase with depth: Dispersal limitation would be weaker in the epipelagic than in the meso- and bathypelagic due to faster currents and, potentially, aerial dispersal in surface waters, compared to more isolated deeper zones. Other mechanisms in deep waters, such as (i) barriers to dispersal (e.g., water mass boundaries and deep sea topography) or (ii) limited random dispersal due to low cell abundances, could also explain this pattern. Selection would be the most important process structuring picoplankton communities in the epipelagic and would display decreasing importance with depth due to higher habitat heterogeneity—driven by microalgal blooms, the magnitude of the DCM, and mesoscale processes (e.g., ocean rings and fronts)—in upper than in bottom waters. The relative importance of drift would increase toward the deep, likely because of decreasing microbial abundances with depth. The importance of dispersal limitation would always be higher in picoeukaryotes than in prokaryotes, given the smaller population sizes of picoeukaryotes and their limited capability to generate dormant stages to sustain long-range dispersal compared to prokaryotes. Thus, a different balance of ecological processes would assemble these domains, even when they share the same ocean zones.

We found significant positive correlations between prokaryotic community structure and water mass compositions in the open ocean, which aligns with previous studies that found bacterial communities associated with specific water masses (*12*, *14*, *52*). This relationship is likely linked to different types of organic matter in each water mass (*53*, *55*), which likely select for different prokaryotes (*52*, *54*). In turn, picoeukaryotes were poorly correlated with differences in water mass in the open ocean, implying that some of them could swim across boundaries or that they are weakly linked to the composition of organic matter in each water mass. Instead, the high dispersal limitation of picoeukaryotes would be mainly regulated by their smaller populations (*8*) as well as by their limited capability to enter into dormancy when compared to prokaryotes (*56*).

In the Mediterranean Sea, the coupling between community structure and water mass composition was significant for both prokaryotes and picoeukaryotes in the meso- and bathypelagic, which agrees with previous reports (*57*). This is likely linked to the strong horizontal cross-basin separation imposed by the straits of Sicily and Gibraltar (*10*). Our results also indicate that differences in both prokaryotic and picoeukaryotic communities are coupled with differences in water mass composition in vertical profiles (fig. S12). The strength (R^2) and the slope of the regressions between differences in picoplankton community composition and differences in water mass composition varied among vertical profile stations (fig. S12). This indicates that local-scale events (e.g., upwellings and dense water propagation) may also regulate the impact of water masses on microbial communities in the vertical dimension (*58*, *59*). Accordingly, a recent study has shown that picoeukaryotic communities are strongly shaped by vertically structured water masses in the Weddell Sea (*60*).

Weaker picoplankton biogeography in the surface than in the deep ocean

Distance-decay analyses revealed that the autocorrelation in community and geographic distances was stronger in the deep sea than at the surface. This agrees with our sequential analysis results (Fig. 5B), suggesting more marked changes across space in the deep ocean, particularly in the picoeukaryotic community. In

agreement, a recent study found larger eukaryotic community dissimilarity between pairs of sites in the deep than in the surface global ocean (*61*). Such changes in community composition with increasing geographic distance (that is, distance decay) can be generated by selection and/or dispersal limitation (*62*, *63*). For picoeukaryotes, the fact that changes in community composition were better explained by geography (ocean basin) than by environmental variation (Fig. 2D) supports that the distance-decay pattern in the deep sea is predominantly related to dispersal limitation (*16*, *61*). In turn, prokaryotic community structure was predominantly explained by environmental variables rather than by geography (Fig. 2D), which indicates that, in this domain, distance decay is mostly driven by selection. It is important to notice that as many prokaryotes may be dormant (*64*), the distance decay could have been stronger if we had analyzed the active prokaryotic community (using RNA) instead of the total community (using DNA), as previously shown (*64*).

The decreasing microbial population sizes from surface to deep waters could increase the role of dispersal limitation and drift in the deep ocean. Rare species with small populations are less likely to disperse (*65*) and more likely to randomly become locally extinct than species with large populations (*24*). We found that depth-related patterns in ecological processes were more pronounced in the Mediterranean Sea than in the open ocean. This is partially explained by the Mediterranean Sea's characteristics: a semienclosed basin with unique oceanographic features such as limited circulation, sharp geographic barriers, and strong environmental gradients (*10*, *39*).

Differences between picoplankton domains govern the balance of assembly processes in the different depth zones

A different balance of ecological processes shapes prokaryotic and picoeukaryotic communities in several ecosystems (*35*, *48*, *49*, *66*), including the surface ocean (*15*, *44*). We found that such differences between domains persist in the deep ocean. Dispersal limitation was always higher for picoeukaryotes than for prokaryotes, which agrees with previous studies using similar approaches conducted in Antarctic lakes (*48*) and in basin-scale oceanic regions (*45*). This contrast between domains in terms of dispersal is partially due to organismal and population size differences (*35*, *65*, *67*). Unicellular eukaryotes are, on average, three times larger than prokaryotes and, therefore, are expected to be more limited by dispersal (*35*, *67*). Picoeukaryotes have populations in the open ocean that are about three orders of magnitude smaller (~10^3 cells ml^{-1}) than prokaryotes (~10^6 cells ml^{-1}), which decreases their likelihood of dispersing (*65*).

Homogeneous selection was, in general, higher in prokaryotes than in picoeukaryotes, which is in line with previous findings in the Pacific Ocean (*44*). This supports the idea that environmental heterogeneity can act differently on prokaryotes and picoeukaryotes. This may be due to different adaptations in prokaryotes and picoeukaryotes to the same environmental heterogeneity (*56*). For instance, a given environmental heterogeneity could select for a few generalist species that have wide niches or many specialist species with narrow niches. Moreover, the relatively higher homogeneous selection in prokaryotes than in picoeukaryotes suggests that dormancy could play an important role in modulating prokaryotic assembly in the deep ocean. Dormancy is common in prokaryotes to overcome harsh environmental conditions (*68*) and can affect metacommunity structure by dampening distance-decay relationships and maintaining local diversity (*64*). Many prokaryotes reach the deep ocean from the surface through vertical dispersal (*69*) or disperse as endospores from sediments (*70*). DNA-based community composition data, such as ours, include nonactive bacterial cells (*71*), likely in a dormancy state, to survive the conditions of the deep ocean (*70*). Therefore, a higher proportion of dormant bacteria can create an apparent "homogenization" of prokaryotic communities in deep zones. Evidence exists that bacteria decrease their activity toward the deep ocean (Fig. 6) (*8*, *72*). So far, there is very little evidence pointing to dormancy in picoeukaryotes (*56*), which could partially explain the negligible role of homogeneous selection in the assembly of this domain in the deep ocean.

Drift was similar for prokaryotes and picoeukaryotes in the epipelagic, but it was higher for prokaryotes compared to picoeukaryotes in the deep ocean. However, drift was greater in picoeukaryotes than in prokaryotes both in the depth-integrated analysis (Fig. 2B) and across vertical profiles (Fig. 2C). This contradiction is most likely related to the methodology, as results derived from the used method are dependent on the samples considered in the calculation. Thus, drift, and other ecological processes, should be interpreted in the context of the dataset used in each calculation, and one should be careful when interpreting results from different datasets.

Microbial domains may respond differently to environmental changes across ocean depths

The ocean is experiencing changes in important variables such as temperature, pH, salinity, and nutrient concentrations (*73*), which are likely affecting all domains of life, their community structure, and interactions (*74*). Climate change is also affecting ocean currents (*75*), which may affect plankton dispersal rates (*76*). Water masses have also been modified by anthropogenic change in temperature and salinity, even in the deep ocean (*77*, *78*), which may affect picoplankton communities (*14*, *52*) by changing selective pressure and dispersal rates. Our results suggest that the prokaryotic and eukaryotic components of the ocean's smallest plankton are likely to respond differently to global change as a result of the different balance of ecological processes structuring their communities. Prokaryotes seem to be more sensitive to selective forces than picoeukaryotes (*15*), so that changes in environmental drivers (e.g., temperature and organic matter composition) may have a higher potential to affect prokaryotic community composition at a global scale (*15*, *54*) than changes in dispersal drivers (e.g., currents and fronts). In turn, picoeukaryotic community composition at a global scale may be more affected by changes in factors regulating horizontal and vertical dispersal—such as current circulation (*34*) and thermal stratification (*79*)—than by environmental drivers.

Our work suggests that the microbial communities inhabiting the deep ocean are likely to respond differently to environmental changes than those living in the surface ocean. This is particularly relevant in the context of increasing multiple stressors caused by climate change (e.g., warming, acidification, and deoxygenation) and human exploitation activities (i.e., mining, oil and gas extraction, and waste disposal) in the deep ocean (*80*). While upper ocean picoplankton communities would be relatively more sensitive to changes in environmental selective forces (e.g., temperature and nutrient concentration), deep ocean picoplankton communities may be more affected by the removal or creation of dispersal pathways,

such as currents and stratification patterns (*75*, *76*), as well as micro- and nanoplastic pollution (*80*, *81*).

Developing a conceptual framework for the global biogeography of picoplankton across ocean depths

Historically, many studies have focused on the effects of selection—also referred to in the literature as niche modeling or environmental filtering—on marine microbial communities (*19*–*21*). Other studies aimed to model how dispersal influences microbial biogeography in the global surface ocean (*35*, *82*–*84*). More recently, there have been important efforts bringing together environmental selection and dispersal in the study of the ocean microbiome (*16*, *34*, *76*). Nevertheless, besides selection and dispersal, picoplankton community assembly is also ruled by drift (*15*, *30*). A key piece remained missing in integrating these processes into a unified framework that accounts for the organismal, environmental, and physical differences across depth zones. By combining empirical evidence, we propose a novel conceptual framework that expands the current understanding of plankton community assembly in environmentally distinct ocean depth zones (Fig. 7). It synthesizes how environmental heterogeneity, water mass structure, deep-sea topography, microbial abundance, and activity mediate the action of ecological processes assembling communities of the smallest ocean plankton (Fig. 7). In summary, it indicates that there is an increasing relative importance of dispersal limitation with depth, which agrees with our initial hypothesis that dispersal limitation would be weaker in the epipelagic than in the meso- and bathypelagic due to faster currents and, potentially, aerial dispersal in surface waters. Other mechanisms taking place in deep waters, such as (i) barriers to dispersal (e.g., fronts, currents, and deep sea topography) or (ii) limited random dispersal due to low cell abundances, could also explain this pattern. Selection was the most important process structuring picoplankton communities in the epipelagic and displayed decreasing importance with depth. This aligns with the hypothesis that the relative importance of selection decreases with depth due to higher habitat heterogeneity in surface than bottom waters. The relative role of drift increased toward deep layers, likely as a result of decreasing microbial abundances with depth. The importance of dispersal limitation was always higher in picoeukaryotes than in prokaryotes, which suggests that different ecological processes are acting on the assembly of these domains even when they share the same ocean zones.

This framework may be used to delineate hypothesis-driven studies to predict how plankton assemblages will respond across depths to multiple stressors in a changing ocean (*85*). For instance, on the basis of this framework, we can expect that the balance between determinism (selection) and stochasticity (dispersal limitation or drift) would decrease with plankton size. Large particles are also expected to be more limited by dispersal than small particles. Thus, we can also foresee that particle-attached prokaryotes—which are particularly relevant in the deep ocean (*86*)—should be more limited by dispersal than free-living prokaryotes. In general, our framework suggests that the importance of dispersal limitation relative to that of selection should increase not only with organism and particle sizes, as expected by the size-dispersal hypothesis (*35*), but also with ocean depth, due to more dispersal barriers and less environmental heterogeneity in the deep ocean compared to the surface. In other words, this dispersal-selection balance, regulated by organism size, should be more pronounced in the deep than in the upper ocean.

MATERIALS AND METHODS

Dataset, sampling, and analytical methods

We compiled a dataset (Fig. 1) composed of 451 samples from surface (3 m depth) to deep waters (up to 4800 m), covering three depth zones of the ocean: epipelagic (0 to 200 m, including DCM), mesopelagic (200 to 1000 m), and bathypelagic (1000 to 4000 m). This dataset combines samples obtained during two oceanographic expeditions with similar sampling strategies: (i) the *Malaspina-2010* circumglobal expedition (*40*) with 263 samples from 120 stations distributed along the tropical and subtropical portions (latitudes between 35°N and 40°S) of the Pacific, Atlantic, and Indian oceans (Fig. 1), and (ii) the *HotMix* trans-Mediterranean cruise (*10*, *55*) with 188 samples from 29 stations distributed along the Mediterranean Sea (from −5°W to 33°E) and the adjacent northeast Atlantic Ocean (Fig. 1A). This dataset, therefore, allows the comparison of the tropical and subtropical ocean (samples hereafter called "open ocean") to a semienclosed basin such as the Mediterranean Sea (see detailed reasoning in Supplementary Text). In addition, the *Malaspina-2010* dataset contains 13 stations where the whole vertical profile was sampled (vertical-profile stations in Fig. 1). A detailed vertical distribution of the samples is available in the Supplementary Materials (fig. S1). Because of differences in sampling sizes between depth zones, we also generated subsets with a standardized number of samples (n = 39) that were evenly distributed across space (figs. S5 and S6).

This dataset includes contextual data of six standardized environmental parameters (temperature, salinity, fluorescence, PO_4^{3-}, NO_3^-, and SiO_2) as well as prokaryote and picoeukaryote abundances determined by flow cytometry and bacterial activity measurements (see details in Supplementary Text). To obtain picoplankton biomass, seawater samples were sequentially filtered through a set of different pore-size polycarbonate filters using a peristaltic pump (see details in Supplementary Text). Here, only the free-living "picoplankton" size fraction (0.2 to 3 μm) was used in downstream analyses.

Nucleic acid extraction, sequencing, and bioinformatics

DNA extraction was conducted with a standard phenol-chloroform protocol (*87*), a Nucleospin RNA kit (Macherey-Nagel) procedure, or a PowerWater Sterivex DNA Isolation Kit (MO BIO Laboratories) (see details in Supplementary Text). DNA extracts were used for both the 16*S* [V4-V5 region (*88*)] and 18*S* [V4 region (*89*)] rRNA gene amplification and sequencing (see details in Supplementary Text). Raw Illumina miSeq reads (2 × 250 or 2 × 300) were processed using DADA2 (*90*) to determine ASVs. For the 16*S* rRNA gene, forward reads were trimmed at 220 base pairs (bp) and reverse reads were trimmed at 200 bp, while for the 18*S* rRNA gene, we trimmed the forward reads at 240 bp and the reverse reads at 180 bp. Then, for the 16*S*, the maximum number of expected errors (maxEEs) was set to two for the forward reads and four for the reverse reads, while for the 18*S*, the maxEE was set to seven and eight for the forward and reverse reads, respectively. Last, DADA2 was used to estimate error rates for both the 16*S* and 18*S* rRNA genes to delineate the ASVs (see details in Supplementary Text). Prokaryotic ASVs were assigned taxonomy using the naïve

Bayesian classifier method (*91*) alongside the SILVA v.132 database (*92*) as implemented in DADA2, while Eukaryotic ASVs were BLASTed (*93*) against the Protist Ribosomal Reference database [PR^2, version 4.11.1; (*94*)]. Eukaryotes, chloroplasts, and mitochondria were removed from the 16*S* ASVs table, while Streptophyta, Metazoa, and nucleomorphs were removed from the 18*S* ASVs table. Both the 16*S* and 18*S* ASVs tables were rarefied to 20,000 reads per sample with the function rrarefy from the Vegan R package. To be consistent with our previous study (*15*), for the calculation of ecological processes and associated analyses, ASVs with total abundances <100 reads across all samples were removed to avoid biases (see details in Supplementary Text). Phylogenetic trees were built for both the 16*S* and 18*S* rRNA gene datasets using the full ASV sequences (see details in Supplementary Text).

Environmental heterogeneity, water mass characterization, and least-cost distance calculations

We calculated the average pairwise dissimilarity (EnvHt) as an index of environmental heterogeneity based on the main standardized environmental variables: temperature, salinity, fluorescence, PO_4^{3-}, NO_3^-, and SiO_2. We first computed a Euclidean distance matrix for each depth zone using the vegan R package and then determined the dissimilarity among samples by dividing the Euclidean distance matrix (*Euc*) by the maximum Euclidean distance (Euc_{max}) of a given depth zone as described in (*30*) and summarized here: $EnvHt = (Euc/Euc_{max}) + 0.001$ (see details in Supplementary Text). The percentage of different water types contributing to the water mass composition of each sample (from 200 m to the bottom) was calculated using an optimum multiparameter water mass analysis (see details in Supplementary Text) (*95*). Least-cost geographical distances—considering continents and deep-sea bathymetry as geographic barriers—were calculated using the "lc.dist()" function of the marmap R package (see details Supplementary Text) (*96*).

Quantification of the ecological processes

The action of ecological processes (selection, dispersal, and drift) was quantified using a null model approach (*28*) that has been successfully applied to microbial ecology studies in diverse aquatic environments (*30*, *48*, *49*, *97*). This analysis consists of two main sequential steps: (i) inference of selection from ASV phylogenetic turnover and (ii) subsequent inference of dispersal and drift from ASV compositional turnover (*28*). Since phylogenetic signal (*98*) is an assumption of this method (*28*), we first tested whether closely related taxa (based on the 16*S* and 18*S* rRNA-gene phylogeny) were more similar in terms of habitat preferences than distantly related taxa. Mantel correlograms between ASV's niche and phylogenetic distances were used to test the phylogenetic signal using the environmental variables that explained the highest fraction of community variance in each depth zone. We detected a phylogenetic signal within short phylogenetic distances, which aligns with the literature (*15*, *28*, *30*).

Afterward, we determined the phylogenetic turnover using the abundance-weighted β-mean nearest taxon distance (βMNTD) metric (*28*), which computes the mean phylogenetic distances between each ASV and its closest relative in each pair of communities (pairwise comparisons). Then, we run null models with 999 randomizations to simulate random phylogenetic turnover ($\beta MNTD_{null}$), in other words, phylogenetic turnover without the influence of selection (*28*). Last, the βNTI was calculated from the differences between the observed βMNTD and the mean $\beta MNTD_{null}$ values. βNTI values lower than −2 or higher than +2 denote that observed βMNTD deviates by more than 2 SDs from the null model expectation. Overall, $|\beta NTI| > 2$ indicates that taxa are phylogenetically more related or less related than expected by chance, pointing to an influence of selection on community assembly (*28*). Specifically, βNTI values higher than +2 indicate the action of heterogeneous selection, while βNTI values lower than −2 point to homogeneous selection (*28*).

The β-diversity of communities not governed by selection ($|\beta NTI| \leq 2$) was evaluated in a second step, which consisted of computing ASV taxonomic turnover to calculate the influence of either dispersal or ecological drift on community structure. To do so, we calculated the Raup-Crick metric (*99*) based on the Bray-Curtis dissimilarities (RC_{bray}) (*28*). RC_{bray} compares the measured β-diversity against the β-diversity obtained from null models (999 randomizations), representing a random community assembly (or ecological drift). The RC_{bray} metric varies from −1 to 1. A value of 0 represents no difference in the observed dissimilarity from the null expectation. The threshold of $|RC_{bray}| > 0.95$ (two-tailed test, $\alpha = 0.05$) indicates that a pair of communities is significantly different from the null expectation (*28*). Absolute RC_{bray} values smaller than $|RC_{bray}| \leq 0.95$ indicate a community assembled by ecological drift alone (i.e., by chance). On the other hand, RC_{bray} values >+0.95 or < −0.95 indicate that community assembly is structured by dispersal limitation or homogenizing dispersal, respectively (*99*). To further investigate the community assembly patterns in each depth zone, we used the "betapart" R package (*100*) to calculate the partitioning of β-diversity (Jaccard, Sorensen, and Bray-Curtis) into turnover or nestedness (*101*).

The relative importance of ecological processes was calculated for each depth zone. In addition, we calculated these processes by integrating all depths of both datasets (i.e., *Malaspina* and *Hotmix* cruises; fig. S5). Since there are processes taking place along the water column (vertically) that may affect the biogeography that we observe horizontally in each depth zone, we also estimated the ecological processes integrating all depths (from 3 to 4000 m) in each of the 13 vertical profile stations (Fig. 1A; see also fig. S1 for sample vertical distribution).

Although this statistical approach has been proven useful to estimate the relative contribution of ecological processes to microbial community assembly, it has some limitations (*15*, *22*). For instance, the βNTI and Raup-Crick metrics are used to estimate the processes at the whole-community level, which may not be adequate since different taxa may be under different ecological processes (*102*). Furthermore, the estimated ecological processes might vary according to the chosen molecular marker, clustering method, and spatial scale (*15*). Detailed technical limitations of this approach have been discussed in (*22*) in an overall context and in (*15*) in the context of the ocean microbiota.

General analysis

Distance-based redundancy analyses were performed on community composition (based on Bray-Curtis dissimilarities) of both prokaryotic (16*S* rRNA gene) and picoeukaryotic (18*S* rRNA gene) samples using the "capscale()" function of the vegan R package (*103*). Analyses of dissimilarities were conducted using the "adonis2()" function of the vegan R package to investigate the

percentage of variance in community composition explained by environmental or geographic variables (*104*). We used ocean basins as a categorical explanatory variable in the open ocean and the Mediterranean Sea (see details in Supplementary Text). Spearman correlations were computed between β-diversity (Bray-Curtis and βNTI) and environmental Euclidean distances matrices using the "cor.test()" function of the stats R package. Spearman correlations were also carried out to test the association between community (Bray-Curtis dissimilarity) and water masses composition (Euclidean distances) in the meso- and bathypelagic. Mantel correlograms were carried out with the "mantel.correlog()" function in Vegan to test for the decrease in picoplankton community similarity (β-diversity) with increasing geographic distances (distance decay) (see details in Supplementary Text). Statistical differences between depth zones in sequential Bray-Curtis horizontal analyses were tested using analysis of variance (ANOVA) followed by a Tukey post hoc test. Statistical analyses were conducted in the R statistical environment (*105*), and all plots were generated using ggplot2.

Supplementary Materials

This PDF file includes:

Supplementary Text
Figs. S1 to S13
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