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Margot Tragin[1], Adriana Zingone[2], Daniel Vaulot[1*]

[1] Sorbonne Universités, UPMC Univ Paris 06, UMR 7144, CNRS, Station Biologique, Place Georges Teissier, 29680 Roscoff, France

[2] Department of Integrative Marine Ecology, Stazione Zoologica Anton Dohrn, Villa Comunale, Naples, Italy

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[*] Corresponding author : vaulot@sb-roscoff.fr

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## Originality-Significance Statement

Environmental barcoding is now widely used to assess eukaryotic protist communities. The two most common markers correspond to the V4 and V9 regions of the 18S rRNA gene. This paper provides a detailed comparison of the V4 and V9 for the assessment of community composition on a large set of marine samples from the Ocean Sampling Day project using rigorously similar sequencing and analysis pipelines. We demonstrate that, by and large, these two markers provide similar community composition and point out some cases where this may not be true. These data should be very useful to anyone performing metabarcoding of marine protist communities.

## Abstract

We compared the composition of eukaryotic communities using two genetic markers (18S rRNA V4 and V9 regions) at 27 sites sampled during Ocean Sampling Day 2014, with a focus on photosynthetic groups and, more specifically green algae (Chlorophyta). Globally, the V4 and V9 regions of the 18S rRNA gene provided similar images of alpha diversity and ecological patterns. However, V9 provided 20% more OTUs built at 97% identity than V4 and 39% and 56% of the genera were found only in one dataset, respectively V4 and V9. For photosynthetic groups, V4 and V9 performed equally well to describe global communities at different taxonomic levels from the division to the genus and provided similar Chlorophyta distribution patterns. However, at lower taxonomic level, the V9 dataset failed for example to describe the diversity of Dolichomastigales (Chlorophyta, Mamiellophyceae) emphasizing the lack of V9 sequences for this group and the importance of the reference database for metabarcode analysis. We conclude that in order to address specific questions regarding specific groups (e.g. a given genus), it is necessary to choose the marker based not only on the genetic divergence within this group but also on the existence of reference sequences in databases.

## Introduction

Planktonic organisms are distributed throughout all branches of the tree of life (Baldauf, 2008) but share "universal" genes presenting certain degrees of genetic variability which allow them to be used as barcode markers to investigate biological diversity (Chenuil, 2006). The development of High Throughput Sequencing (HTS) allows the acquisition of large metabarcoding datasets (i.e. one marker gene is amplified and sequenced for all organisms), which complement the time-consuming and expertise-demanding morphological inventories to explore the diversity and distribution of protist groups in the ocean. The 18S rRNA gene is commonly used to investigate eukaryotic diversity and community structures (López-García *et al.*, 2001; Moon-van der Staay *et al.*, 2001). The complete 18S rRNA gene (around 1,700 base pairs) from environmental clone libraries can only be sequenced by the Sanger method (Sanger and Coulson, 1975) using a combination of primers. In contrast, HTS provides a very large number of reads but allows only small fragments to be sequenced (van Dijk *et al.*, 2014). Small hypervariable regions of the 18S such as V9 (around 150 bp located near the end of the 18S rRNA gene) or V4 (around 450 bp in the first half of the gene) can be targeted depending on the sequence length allowed by the sequencing technology used. Initially, the Illumina technology only allowed to sequence the V9 region because of its relatively small size (Amaral-Zettler *et al.*, 2009). In recent years longer reads became possible (up to 2*300 bp with current Illumina technology, van Dijk *et al.*, 2014) allowing the sequencing of the V4 region. Both the V4 and V9 regions have been used recently to describe diversity and ecological patterns of protists in several large scale studies (Massana *et al.*, 2014; de Vargas *et al.*, 2015).

The performance of the 18S RNA hypervariable regions as barcodes and the interpretation of results produced remains a matter of debate. Hu *et al.* (2015) showed that the V4 region provides an image of diversity similar to that obtained from the entire 18S rRNA gene. The choice between V4 and V9 depends on the taxonomic levels as well as the specific groups targeted. It is necessary to make detailed comparisons of genetic distances for each targeted region between and within the groups of interest (Dunthorn *et al.*, 2012; Pernice *et al.*, 2013) and to determine whether reference sequences are available for the group of interest in the target region (Tragin *et al.*, 2016). The sequencing platform may also have some impacts: using the 454 technology, Behnke *et al.* (2011) showed that the sequencing error rate was taxon dependent, but V4 error rates were higher than for V9. Analysis of mock communities have highlighted possible biases in molecular methods such as the generation of artificial diversity (Egge *et al.*, 2013). The primers used may also produce a bias against groups whose target fragments are not amplified. For example, some widely used V4 primers miss Haptophyta and Foraminifera, which are important groups of the marine plankton (Massana *et al.*, 2015). Finally bioinformatics steps such as raw sequence filtering based on sequence quality and length, clustering algorithm and threshold to regroup sequences into Operational Taxonomic Units (OTUs) may influence the final results (Majaneva *et al.*, 2015).

Several studies have compared the structure of microbial communities provided by the V4 vs. V9 regions in specific environments such as an anoxic fjord in Norway (Stoeck *et al.*, 2010) or for specific planktonic group such as Radiolaria (Decelle *et al.*, 2014). Some of these studies pointed out that the relative number of V4 and V9 reads may be different depending on the taxonomic levels and groups considered (Stoeck *et al.*, 2010; Giner *et al.*, 2016). Stoeck *et al.* (2010) found that the V9 region recovered more diversity at higher taxonomic levels than the V4 region: the number of unique V4 reads was very low for ciliates and dinoflagellates in comparison to V9, while pelagophytes (Ochrophyta) were not detected at all when using V4. In contrast, both papers (Stoeck *et al.*, 2010; Giner *et al.*, 2016) found that V4 provided more Chlorophyta unique sequences than V9. However, these studies were relying on different technologies for V4 and V9 sequencing. Recently, Piredda *et al.* (2017) used the same sequencing technology to analyze both the V4 and the V9 regions of marine protist communities in different seasons in the Gulf of Naples. They showed that V4 and V9 performed equally well to describe temporal patterns of protist variations and recovered the same number of OTUs (at 95% similarity) with both markers. However, this study was limited to a single sampling site.

The Ocean Sampling Day project has sampled a large number (157 stations) of mostly coastal stations at the summer solstice (June 21) of 2014 with the aim of determining the composition, structure and distribution of prokaryotic and eukaryotic microbial community in marine waters using metabarcode and metagenomic approaches (Kopf *et al.*, 2015). Within this project, the V4 and V9 regions of the 18S rRNA gene from 27 locations were sequenced using the Illumina technology. In the present study, we compare the V4 and V9 metabarcodes using identical sequence processing algorithms. We focus on different levels. First, we analyze the total protist community in terms of richness and diversity. Then we look in detail at the community composition at the Class level for photosynthetic groups. We finally focus on the contribution at each station of Chlorophyta classes and of Mamiellophyceae genera, for which a high quality reference sequence database has been recently constructed (Tragin *et al.*, 2016) and which have been the subject of recent ecological studies in oceanic waters (Monier *et al.*, 2016; Simmons *et al.*, 2016; Clayton *et al.*, 2017).

## Material and Methods

Water samples were collected from 0-2 meter depth at 27 stations in the world ocean (Fig. 1 and Table 1). Metadata (Temperature, Salinity, Nitrates, Phosphates, Silicates and Chlorophyll *a*) are available at https://github.com/MicroB3-IS/osd-analysis/wiki/Guide-to-OSD-2014-data. Samples were filtered on 0.8 µm pore size polycarbonate membranes without prefiltration and flash frozen at -80°C or in liquid nitrogen. DNA was extracted using the Power Water isolation kit (MoBio, Carlsbad, CA, USA) following the manufacturer instructions. The V4 region was amplified using modified universal primer (Piredda *et al.*, 2017): V4_18SNext.For primer (5' CCA GCA SCY GCG GTA ATT CC 3') and V4_18SNext.Rev primer (5' ACT TTC GTT CTT GAT YRA TGA 3'). The V9 region was amplified using modified universal primer (Piredda *et al.*, 2017): V9_18SNext.For (5' TTG TAC ACA CCG CCC GTC GC 3') and V9_18SNexr.Rev (5' CC TTC YGC AGG TTC ACC TAC 3'). The library preparation was based on a modified version of the Illumina Nextera's protocol (Nextera DNA sample preparation guide, Illumina) and sequencing was done on an Illumina Miseq (NE08 Ocean Sampling Day protocols: https://github.com/MicroB3-IS/osd-analysis/wiki/Guide-to-OSD-2014-data#analysis-of-workable-18s-rdna-datasets-sequenced-by-lifewatch-italy). Amplicon PCR and sequencing (V4 region: 2x250 paired end sequencing using MiSeq Reagent kit v3 and V9 region: 2x150 paired end sequencing using MiSeq Reagent kit v2) was done by the Laboratory of Molecular Biodiversity (MoBiLab) of LifeWatch-Italy. R1 and R2 were filtered based on quality and length and assembled by the OSD consortium which provided the so-called "workable" fasta files (https://owncloud.mpi-bremen.de/index.php/s/RDB4Jo0PAayg3qx?path=/2014/silva-ngs/18s/lifewatch/).

All subsequent sequence analyses (Supplementary Data and Fig. S1, Table 2) were done with Mothur version 1.35.1 (Schloss *et al.*, 2009). To compare the two datasets (V4 and V9), twenty-seven OSD stations were selected and subsampled using the lowest number of reads (202,710) at a given station (station 49 for V4, Table 1). Sequences were then filtered by removing sequences shorter than 90 bases for the V9 region and shorter than 170 bp for the V4 region or containing ambiguities (N). Reads were aligned on SILVA release 119 seed alignment (Pruesse *et al.*, 2007) corrected by hand using the Geneious software version 7.1.7 (Kearse *et al.*, 2012). Gaps at the beginning and at the end of the alignment were deleted. Alignments were filtered by removing positions containing only insertions. Chimeras were removed using Uchime version 4.2.40 (Edgar *et al.*, 2011) as implemented in Mothur. The sequences were first pre-clustered and singletons were eliminated. After distance matrix calculation, reads were clustered using the Nearest Neighbor method and OTUs were built at 97% similarity (Supplementary Data). OTUs were assigned using Wang approach (Wang *et al.*, 2007) which is based on the calculation of Bayesian probabilities using kmer (8 bp by default) comparisons between dataset and database sequences. This method is complemented by a bootstrap step to confirm the taxonomical classification: assignation supported at a level lower than 80% were not taken into account.

157 The reference database was a revised version (4.2
158 https://figshare.com/articles/PR2_rRNA_gene_database/3803709/2) of the PR² database (Guillou *et al.*,
159 2013) for which the Chlorophyta sequences had been checked against the latest taxonomy (Tragin *et al.*,
160 2016). The PR² database considers 8 taxonomic levels (from Kingdom to Species). OTUs are considered
161 as assigned when their lowest taxonomic level (Level 8, "Species") differs from “unclassified”. Note
162 that this level may not correspond to a single validly described species but may group several taxa (for
163 example Crustomastigaceae_X_sp., see details in Guillou *et al.*, 2013). Several OTUs can be assigned
164 to the same taxonomy if they, for example, correspond to the same "Species". OTUs assigned to
165 Chlorophyta were BLASTed against GenBank using 97% identity and 0.001 e-value cutoff thresholds
166 (Supplementary Data) and OTUs for which the best hit was not a Chlorophyta were removed from
167 further analysis.

168 Diversity analyses were conducted using the R software version 3.0.2 (http://www.R-
169 project.org/). The Vegan package (https://cran.r-project.org/web/packages/vegan/) was used to compute
170 rarefaction curves and Simpson diversity indexes (*D*, Simpson, 1949) at each station.

171 $$D = 1 - \sum_{i=1}^{S} p_i^{\,2}$$

172 *S* is the number of species in the sample and $p_i$ the proportion of species *i*. *D* is relatively little
173 influenced by sample size and does not require any hypothesis on the species distribution. *D* depends
174 on the number of OTUs recorded as well as the distribution of the sequences within the OTUs. For
175 example, in a sample with two species recorded (*S*=2), *D* will be larger if the two species are equally
176 distributed ($p_1$=$p_2$=0.5) than if one is dominant ($p_1$=0.9 and $p_2$=0.1).

177 Descriptive statistics for V4 versus V9 were computed using the R functions *summary* and *sd*
178 (Table 3). A non-parametric rank Wilcoxon test (Wilcoxon, 1945) was performed to compare both
179 results using the *wilcoxon.test* function from the R package stats. Since the V4 and V9 regions were
180 sequenced from the same DNA sample, the paired option was set as true. This test did not return exact
181 P-values for sample in which null or ex-aequo values occurred.

182 The matrixes of the V4 and V9 relative contribution for photosynthetic groups, Chlorophyta and
183 Mamiellophyceae at each station were compared by the geometry-based procrustean method using the
184 *procrustes* and *protest* functions of Vegan. The distance matrix between stations based on the relative
185 contribution at the Class level for Chlorophyta and genus level for Mamiellophyceae were computed
186 using the Bray-Curtis distance and clustered using the hierarchical clustering “complete” method. Bray-
187 Curtis matrix distance was also computed for the global community (all OTUs considered) and the
188 communities were represented in a 2-dimensional space with the iterative ordination method
189 Nonparametric Multi-Dimensional Scaling (NMDS) plot using the *metaMDS* function of Vegan.

Hierarchical clustering was computed on the same Bray-Curtis distance matrix. The clustering dendrograms were cut with the *rect.hclust* function from the R stats package at a height h=0.9. Resulting groups were traced on the NMDS plot. Available OSD metadata were projected onto the NMDS plots using the *envfit* function from the Vegan with the p.max option set as 0.95.

# Results

## Global eukaryotic community

Twenty-seven stations were selected for which both V4 and V9 metabarcodes were obtained. The two datasets were subsampled in order to process the same number of reads per station. After subsampling, the V4 and V9 datasets were reduced to 62% and 48% of their original size, respectively (Table 2). The number of unique sequences (Table 2) was higher for V4 (around 1,400,000) than for V9 (around 900,000). After filtering based on length and ambiguities, twice more reads were obtained for V4 than for V9 (Table 2). About forty times more chimeras were found for V4 than for V9 (about 7,500 against 170). Following taxonomic assignment, all eukaryotic groups were retained, not just protists.

Rarefaction curves computed for the global datasets as well as for each station (Fig. S2A and S3) reached saturation, suggesting that the sequencing effort was sufficient. Global maximum richness varied between the datasets: 16,383 OTUs (4,311 distinct assignations) were obtained for V9 against 13,169 OTUs (3,412 distinct assignations) for V4. The two datasets yielded similar rank abundance curves (Fig. S2 B) although V9 had larger OTUs as attested by the fact that the curve for V9 was above that for V4. The size of the largest OTU was equivalent (around 180,000 sequences).

The number of OTUs per station varied from 500 to about 3,000 with respective averages of 1,200 and 1,600 for V4 and V9 (Fig. 2A and Table 3). Although a positive correlation was found between the number of OTUs for V4 and V9 per station ($R^2$=0.99, Fig. 2A), the number of OTUs per stations was higher for V9 than for V4 (slope=1.27, Fig. 2A) and this difference was confirmed by a Wilcoxon test (Table 3). The comparison of Simpson's diversity index per station for the two datasets (Fig. 2B and Table 3) showed that V4 and V9 diversity values were similar for large values between 0.9 and 1, irrespective of the OTU richness. For lower values of the Simpson's index (0.6 to 0.9), it was higher for V9 than V4 except at station OSD30 in the Gulf of Finland (Fig. 2B). At the latter station, one specific metazoan OTU (assigned to copepods and corresponding to 105,202 reads) was dominating the V9 reads but this OTU did not dominate the V4 reads. If this copepod OTU is not taken into account (grey star on Fig. 2B), the V4 and V9 datasets have a similar alpha diversity (0.91 and 0.95 respectively). The number of genera (assignations without _X) found in the OSD datasets was equal to 3,669, among which 39% (for V4) and 56% (for V9) were recovered only in one dataset. On average, four OTUs were assigned to the same genus and the maximum number of OTUs per genus reached 128 for V4 and 187 for V9. 98 % of genera found only in one dataset were represented by less than 10 OTUs.

Non-parametric multidimensional scaling analysis (NMDS, Fig. S4A-B) and hierarchical clustering (Fig. S4C-D) were used to visualize the V4 and V9 communities based on OTUs (final stress values were respectively 0.187 and 0.195) using Bray-Curtis dissimilarity. Many stations grouped together in a similar way for both V4 and V9, some according to their geographic location (Fig. S4 and Fig. 1), as in the case of the Mediterranean Sea (OSD14, 22, 49, 76, 77, 99) or of the subtropical Atlantic

coast of the United States (OSD39, 60 and 143). Both V4 and V9 communities were structured by the same combination of environmental parameters with opposite gradients of nitrates, phosphates and chlorophyll on one side vs. silicates, temperature and salinity on the other side (Fig. S4A-B).

## Photosynthetic groups

We next focused on photosynthetic groups for which taxonomic assignation relies on recently validated reference databases (Edvardsen *et al.*, 2016; Tragin *et al.*, 2016). Dinophyceae were excluded from the analysis since about 50% of the species are not photosynthetic (Gómez, 2012). The percent of reads assigned to photosynthetic groups was quite similar between datasets: 28.6% vs. 25.9 % for V4 and V9, respectively. The four major photosynthetic groups were Ochrophyta (mostly diatoms), Chlorophyta (green algae), Haptophyta and Cryptophyta (Fig. 3A). The Rhodophyta, Cercozoa (Chlorarachniophyta) and Discoba (Euglenales) represented less than 1.5% of the photosynthetic groups in the two datasets (Fig. 3A). Procrustean analysis suggested that the relative contribution of photosynthetic groups per station was similar between V4 and V9 ($m^2$=0.17 and r=0.91). The number of OTUs assigned to Ochrophyta was quite similar in the two datasets (1215 and 1250 in V4 and V9, respectively). In contrast, the number of V9 OTUs was almost twice that of V4 for Chlorophyta and Cryptophyta and three times for Haptophyta, but average OTUs size was similar (377, 64, 91 and 573, 100, 241 in V4 versus V9). For these three photosynthetic groups, average pairwise identity between the OTUs reference sequences was higher for V4 than V9 (76 vs. 72% for Chlorophyta, 84 vs. 76% for Cryptophyta and 86 vs. 77% for Haptophyta), indicating that V4 has lower genetic variability for these groups, and therefore is less discriminating.

The relative contribution of photosynthetic groups was very different among the stations which ranged from estuarine to oligotrophic oceanic waters. Ochrophyta contribution was statistically similar for V4 and V9 (Table 3) and varied between 20% (OSD14, 146) and 90% (OSD159, 60) of the photosynthetic metabarcodes (Fig. S5A). Chlorophyta contribution varied between 5% (OSD76, 159) and 70% (OSD14). Chlorophyta contribution was slightly higher in V4, and the difference was confirmed by the Wilcoxon test, except for stations OSD149 and 150 (Fig. S5B). Haptophyta contribution varied across stations from a few percent up to 40% (OSD22, 49, 146) and was larger for V9 than for V4 (Table 3) except for OSD3 (Fig. S5C). Cryptophyta contribution was on average 4% (Table 3) in both datasets and varied between a few percent and 20% (OSD150). It was similar for V4 and V9 (Fig. S5D) except at OSD76, 149 and 150.

Among Ochrophyta, diatoms (Bacillariophyta) largely dominated, followed by Dictyochophyceae and Chrysophyceae-Synurophyceae (Fig. 3B). Diatom relative contribution to photosynthetic metabarcodes per stations was around 50% on average (Table 3) and varied between 15% (OSD30, 146) to 90% (OSD159, 60). Diatom contribution was statistically similar between V4 and V9 (Table 3). Dictyochophyceae relative contribution was below 10% except for five stations (OSD22,

149, 150,152 and 72), where it reached 35% of photosynthetic reads (Fig. S6B). Dictyochophyceae contribution was slightly higher with V4 at these five stations. Chrysophyceae-Synurophyceae relative contribution was below 10% except for OSD76 (25%, Fig. S6C and Table 3) and V4 and V9 contribution were similar except at OSD30, where V9 was higher and OSD49 and 76 where V4 was higher (Fig. S6C). Pelagophyceae relative contribution was below 10% at individual stations but V4 and V9 were similar (Fig. S6D and Table 3). Chlorophyta were dominated by Mamiellophyceae, followed by Trebouxiophyceae, Chlorodendrophyceae and Pyramimonadales (Fig. 3C). Trebouxiophyceae and Chlorodendrophyceae were more represented in V4 while Mamiellophyceae and Pyramimonadales were more represented in V9 (Fig. 3C). Other photosynthetic groups remained similar between the V4 and V9 datasets. Among Haptophyta, Prymnesiophyceae were largely dominating but two environmental clades, HAP3 and HAP4 (Edvardsen *et al.*, 2016), were also recovered (Fig. 3D). For photosynthetic groups, the percentage of genera found either in only one dataset (V4 or V9) or in both was class dependent, but globally 50% of the genera were recovered in both datasets (Fig. S7). Within Ochrophyta, more genera were found using V9 in 5 out of 8 classes, but this was not the case for Bacillariophyta and Xanthophyceae for which more genera were recovered with V4. Raphidophyceae genera were almost all recovered in both V4 and V9 (Fig. S7). More red algae genera (Florideophyceae and Bangiophyceae) were recovered with V9. For Haptophyta, Cryptophyta and Chlorarachniophyceae most genera were found with both markers (Fig. S7).

## Chlorophyta classes

The relative contributions of the 6 major Chlorophyta groups (Mamiellophyceae, Trebouxiophyceae, Chlorodendrophyceae, Pyramimonadales, Ulvophyceae and Pseudoscourfieldiales) in V4 and V9 were similar at most stations (Fig. 4A and Fig. S8) as supported by a procrustean comparison ($m^2$=0.027 and r=0.98) but individual group contributions were not similar except for Mamiellophyceae (Table 3). Mamiellophyceae were dominant at most stations, but the four stations located in the Adriatic Sea (OSD49, 76, 77 and 99) shared a specific pattern with high contributions of Pseudoscourfieldiales and Chlorodendrophyceae in both V4 and V9 datasets (Fig. 4A). Stations OSD30, 54, 55, 141, all located in North Atlantic coastal waters presented differences in Chlorophyta class contribution recovered with V4 and V9 (Fig. 4A and Fig. S9A). For the first three, the Mamiellophyceae contribution in V9 was partially replaced in V4 by classes from "core chlorophytes" such as Chlorodendrophyceae and/or Trebouxiophyceae. At OSD141, prasinophytes clade VII were only recovered with V9, while Chlorophyceae (*Chlamydomonas* sp.) were only recovered with V4 (Fig. S9A). BLAST analysis and alignment of Chlorophyta OTUs (data not shown) revealed that the V9 region of some *Chlamydomonas* is very similar to that of prasinophytes clade VII A5 (Lopes dos Santos *et al.*, 2016). Interestingly, the number of reads recovered in V4 and V9 for these 2 assignations (i.e. *Chlamydomonas* sp. for V4 and prasinophytes clade VII A5 for V9) was similar (51 versus 47 reads, respectively).

In general, Chlorophyta OTUs were well assigned by the Wang approach implemented in the Mothur software (Wang et al., 2007) compared to the results of BLAST (Supplementary data 6 and 7). However, some V9 reads initially assigned as Chlorophyta by the Wang approach hit bacterial sequences and were not considered any further. Some V9 Chlorophyta OTUs shared 100% identity with several different Chlorophyta genera with (mostly in the UTC clade, i.e. Ulvophyceae, Trebouxiophyceae and Chlorophyceae) suggesting that the V9 region might not have the appropriate resolution to investigate UTC clade diversity. A number of genera within the UTC clade were only recovered with one marker in contrast to the Mamiellophyceae and Pyramimonadales for which almost all genera were recovered in both datasets (Fig. S7).

When Chlorophyta communities were clustered using the Bray-Curtis distance, V4 and V9 clustered together for individual stations except for OSD30, 43, 54, 55, 60, 72 and 143, (Fig. 4B). Clustering was strongly influenced by the contribution of Mamiellophyceae, because this class largely dominated in coastal waters and was present at almost all stations. A large group of stations where Mamiellophyceae were dominant formed a first cluster (Fig. 4B), whereas in four other groups of stations either another class was dominant (Trebouxiophyceae, Pseudoscourfieldiales or Chlorodendrophyceae) or none was really dominant (for example OSD141, Fig. 4B).

## Mamiellophyceae genera

Mamiellophyceae that dominated at most OSD stations were further investigated at the genus level. Nine genera of Mamiellophyceae were found in the OSD datasets, seven of which were found in both datasets, one only in V4, assigned to RCC391, and one only in V9, assigned to *Monomastix*. The latter is a freshwater genus and the OTUs assigned to it were badly assigned (BLAST analysis showed 100% identity with sequences of several land plants genera, see Supplementary data), while the RCC391 genus has eight references sequences for V4 against only one for V9. *Micromonas* and *Ostreococcus* were the two dominant genera, except at OSD80 in the Greenland Sea where *Mantoniella* was dominant and in the Adriatic Sea (OSD49, 76, 77 and 99) where Dolichomastigales and *Mamiella* were dominant (Fig. 5A). Procrustean comparison showed that V4 and V9 provided similar Mamiellophyceae genus distribution ($m^2$=0.075 and r=0.96). The relative contributions per station of the four major genera *Micromonas*, *Mamiella*, *Ostreococcus* and *Bathycoccus* (Fig. S10) was overall statistically similar in the two datasets (Table 3), although it could be different for *Mamiella* at specific stations (OSD22, 49, 132, 123). Stations located in the Adriatic Sea (OSD49, 76, 77, 99) showed a different pattern in the heatmap (Fig. S9B) because V9 failed to discriminate the Dolichomastigales clades at the genus level. V9 recorded only *Crustomastix* contribution while V4 found 4 to 6 different clades of Crustomastigaceae and Dolichomastigaceae (Fig. 5A).. Hierarchical clustering based on Bray-Curtis distances always grouped together V4 and V9 (Fig. 5B). Four groups of stations were observed depending on the genus dominant at the station: *Micromonas*, *Ostreococcus*, Dolichomastigales or *Mantoniella* (Fig. 5B).

## Discussion

The OSD LifeWatch dataset, with its uniform sampling protocol, provides a unique opportunity to compare protist communities from a wide range of stations based on the two most widely used 18S rRNA markers, the V4 and V9 regions. In contrast to previous studies (e.g. Giner *et al.*, 2016), sequencing was performed on the same platform (Illumina), the same number of reads was analyzed at all stations for both V4 and V9. Bioinformatics analyses were conducted using exactly the same pipeline with the widespread software Mothur (Schloss *et al.*, 2009). A marked difference between the V4 and V9 datasets was the much larger number of chimeras found in V4. This could be due to the fact that the longer the amplified sequence is, the higher the chance is to have them recombining. Moreover, in contrast to the V9 region, the V4 region is composed of hypervariable regions as well as conserved regions (Monier *et al.*, 2016), which facilitates recombination. Finally bioinformatics programs better detect chimeras on longer amplicons (Edgar *et al.*, 2011).

The choice of an identity threshold to build OTUs affects the number of recovered OTUs and the final taxonomic resolution. An analysis of 2,200 full 18S sequences of protist (Caron *et al.*, 2009) showed that building OTUs at 95% identity provided a number of OTUs close to the expected number of species, but the authors remarked that a 98% identity threshold provides a better taxonomic resolution that allows to investigate interspecific diversity. In the present study, OTUs were built at 97% identity for both the V4 and the V9 regions of the 18S rRNA gene, in agreement with a number of recent studies that used these markers (e.g. Massana *et al.*, 2015; Ferrera *et al.*, 2016; Hu *et al.*, 2016). Clustering regions with different size (V4: 450 bp - V9: 150 bp) at the same identity level should produce more diverse OTUs for V4 than for V9, although regions where nucleotide changes are concentrated do not cover the whole amplicons and can be of different length in V4 and V9. For example in V4, most nucleotide diversity occurs within about 150 bp in the first half of the region (Monier *et al.*, 2016).

The V9 dataset provided 20% more OTUs than the V4. This difference between the number of OTUs for V4 and V9 is the same as the one unveiled in other environmental study such as the Naples times series results (Piredda *et al.*, 2017). Piredda *et al.* (2017) also found 20% more OTUs built at 97% identity for V9 than for V4. This could be linked to the size difference between V4 and V9 as discussed above. Interestingly, these authors showed that the number of OTUs built at 95% identity was similar for V4 and V9, suggesting that at lower identity thresholds, the size difference has a lower impact.

The number of OTUs for the main photosynthetic phyla Ochrophyta, Chlorophyta, Haptophyta and Cryptophyta falls in the range found in European coastal waters using the V4 and 97% identity OTUs (1905, 314, 221 and 77 respectively, Massana *et al.*, 2015) except for the Haptophyta for which three times less OTUs were found in the OSD V4 dataset. The number of OTUs of the main photosynthetic phyla in the OSD V9 dataset were considerably lower than the numbers of Tara Oceans V9 OTUs, 3900, 1420, 713 and 195 respectively (de Vargas *et al.*, 2015). However, the depth of

371 sequencing was much higher than in the OSD dataset (around one to two million reads per sample, i.e.
372 20 to 40 more than for OSD) which increases the occurrence of the rare OTUs that had been filtered out
373 in the OSD dataset because of the relatively low read number.

374 At six stations (OSD30, 80, 123, 141, 143, 152) the same species richness (OTU number) was
375 observed but Simpson index was different between V4 and V9 (Fig. 2 A and B). This means that even
376 if the same number of OTUs was found for V4 and V9, the proportion of each OTU was different. The
377 V9 Simpson index of OSD80 and 123 (0.87 and 0.91 respectively) fall in the range of Simpson index
378 calculated in similar environments: for example in Baffin Bay (0.88, Hamilton *et al.*, 2008) and off the
379 Mediterranean Sea coast (0.92, Ferrera *et al.*, 2016), but the V4 Simpson index was lower (0.68 and
380 0.81 respectively).

381 In the OSD dataset, photosynthetic groups (Dinophyceae excluded) varied widely from between
382 0.8 and 81 and between 1.5 and 65 % at the different stations for V4 and V9, respectively, representing
383 on average 29 and 26% of the sequences recovered, ,. These average numbers are comparable to those
384 observed in other studies. For example, Massana and Pedrós-Alió (2008), synthesizing 35 picoplankton
385 clone libraries of 18S gene from oceanic and coastal waters, found that photosynthetic sequences
386 represented about 30% of eukaryotic sequences. The proportion of the main photosynthetic phyla
387 Ochrophyta, Chlorophyta, Haptophyta and Cryptophyta, roughly 17%, 5-7%, 2-3% and 1.3%,
388 respectively, in the OSD dataset are comparable to those found by Massana and Pedrós-Alió (2008)
389 (15%, 7.7%, 2.4% and 2%, respectively).
390 Mamiellophyceae dominated Chlorophyta in nutrient rich coastal waters, which is consistent
391 with studies in European coastal waters (Massana *et al.*, 2015), in particular in the English Channel (Not
392 *et al.*, 2004), and in the South East Pacific Ocean (Rii *et al.*, 2016). The stations located in the Adriatic
393 Sea (OSD49, 76, 77 and 99) showed a specific pattern with a high contribution of Pseudoscourfieldiales
394 and Chlorodendrophyceae. Several studies using optical microscopy found in the Adriatic Sea a high
395 contribution of phytoflagellates, most of which could not be identified (Revelante and Gilmartin, 1976;
396 Cerino *et al.*, 2012).

397 Within Mamiellophyceae the same genus, most of the time either *Micromonas* or *Ostreococcus*,
398 was dominant in both V4 and V9 datasets. Not *et al.* (2009) found *Micromonas* to be the most prevalent
399 genus in the world ocean coastal waters and at more local scale *Micromonas* dominates coastal
400 picoplankton in the Western English Channel (Not *et al.*, 2004). Rii *et al.* (2016) found that
401 *Ostreococcus* was dominant in the upwelling-influenced coastal waters from Chile. OSD data also
402 unveiled a high genetic diversity of the order Dolichomastigales especially in the Adriatic Sea. Viprey
403 *et al.* (2008) made similar observations in oligotrophic Mediterranean surface waters and Monier *et al.*
404 (2016) in the Tara *Oceans* survey.

Clustering based on taxonomic assignment, either Chlorophyta classes or Mamiellophyceae genera, confirmed that for most stations, the V4 and V9 communities clustered together as observed previously for Illumina vs 454 data obtained on picoplankton (Ferrera et al., 2016). However, for Chlorophyta, V4 and V9 of five stations (OSD30, 43, 54, 55 and 60) did not cluster together (Fig. 4B). OSD43 and 60 were not close in the cluster dendrogram but no clear differences are seen either in the barplot (Fig. 4A) or in the heatmap (Fig. S9A). In contrast, OSD141 V4 and V9 communities clustered together in spite of obvious differences in the barplot (Fig. 4A and B) and in the heatmap (Fig. S9A). At OSD30, 54 and 55, the latter two being spatially close on the Eastern US coast, more Trebouxiophyceae and Chlorodendrophyceae were found with V4 which were replaced by Mamiellophyceae for V9. This could be explained by the fact that the reference sequences of the Trebouxiophyceae and Chlorodendrophyceae found at these stations do not cover the V9 region and that the corresponding V9 OTUs were classified as Mamiellophyceae, because of their similarity to the V9 regions of the latter class.

## Concluding remarks - What is the best choice: V4 or V9?

The first element of choice between these two regions is based on the genetic divergence within and between the groups of interests (Chenuil, 2006). For Chlorophyta, average similarity is in general lower in V9 than V4 (Tragin *et al.*, 2016), which suggests that V9 will be more discriminating than V4 and will be the best choice. This is the case for example for prasinophytes clade VII, an important oceanic group, for which the use of 99% threshold for V9 OTUs allows to discriminate all sub-clades (e.g. A1 and A2) defined to date (Lopes dos Santos *et al.*, 2016), while in V4, several clades collapse together, having identical sequences in that region. The V9 region of some *Chlamydomonas* is very similar to that of prasinophytes clade VII A5, which could lead to misinterpret the distribution of this specific sub-clade when using the V9 region. However this may not be the case for other groups such as Nephroselmidophyceae for which the two markers are equally suitable (Tragin *et al.*, 2016). The second element to take into account is the reference database that contains more representatives of each of the taxa investigated. For example, in the present study, the V9 region of the 18S rRNA gene failed to discriminate clades within Dolichomastigales (Fig. S9B) because there are only four Dolichomastigales V9 reference sequences against 69 for V4 (Tragin *et al.*, 2016). In the same way, obtaining accurate image of communities at stations which host rare or uncultured taxa is more difficult with V9 than V4, because many sequences in public databases are short and do not extend to the end of the 18S rRNA gene. For example, Viprey *et al.* (2008) discovered one novel prasinophyte group (clade VIII) by using Chlorophyta specific primers that only amplified a short (around 910 base pairs) sequence not extending to the V9 region, and therefore this group can only be studied using V4.

Metabarcode analysis methods using assignation rely heavily on carefully curated public database such as $PR^2$ (Guillou *et al.*, 2013) or, even better, on specifically tailored databases that include, besides public sequences, reference sequences for the environment investigated, as for example Arctic specific

441 databases for polar environments (Comeau *et al.*, 2011; Marquardt *et al.*, 2016). Other approaches to
442 analyze metabarcoding datasets do not rely on reference databases. For example, oligotyping relies on
443 nucleotide signatures to cluster sequences and can reveal fine distribution patterns of specific taxonomic
444 groups (Eren *et al.*, 2014; Berry *et al.*, 2017), but to our knowledge it has not been applied to eukaryotes
445 yet. Phylogenetic placement methods such as pplacer (Matsen *et al.*, 2010) allows to investigate
446 phylogenetic diversity without assignation against a reference database. Phylogenetic approach however
447 may be impacted by the lack of reference sequences and have to be complemented by statistical testing
448 of the consistency of phylogenetic signals (Kembel, 2009; Stegen *et al.*, 2012).
449 Despite all these caveats, our analyses demonstrate overall that in most cases V4 and V9 provide
450 similar images of the distribution specific photosynthetic groups such as the Chlorophyta and therefore
451 that global studies using either of these markers are comparable.
452

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## Table legends

Table 1: Location of OSD 2014 stations, number of reads in initial datasets, percentage of reads subsampled and percentage of photosynthetic reads.

Table 2: Evolution of sequence number through the analysis pipeline.

Table 3: General descriptive statistics: maximum, minimum, mean, standard deviation and results of the Wilcoxon test (P value) for V4 versus V9 OTU numbers, Simpson index (data from the Fig. 2) and photosynthetic groups relative contribution (see Fig. S5, S6, S8 and S10). P values in bold are above the 0.05 threshold indicating that V4 and V9 are not significantly different while P values in italics were computed with datasets presenting ex aequo values.

## Figure legends

Fig. 1: Map of the 27 OSD stations sampled 2014 for which both V4 and V9 sequences were available.

Fig. 2: A. Species richness: number of OTUs per stations for V4 versus V9. The grey line corresponds to y=x, and the black line corresponds to the regression y=1.27x+53 ($R^2 = 0.996$). B. Simpson's diversity index per stations for V4 vs. V9. Grey star corresponds to the OSD30 Simpson's index after removal of the metazoan V9 OTU.

Fig. 3: A. Contribution of divisions to photosynthetic metabarcodes (Dinophyceae were excluded) for V4 and V9. B-D. Distribution of reads among classes for the three major photosynthetic divisions for V4 and V9: B. Ochrophyta, C. Chlorophyta D. Haptophyta.

Fig. 4: A. Comparison of Chlorophyta read distribution (assigned at the class level) for 27 OSD stations. B. Comparison of Chlorophyta communities at the class level based hierarchical clustering for V9 and V4. The dissimilarity matrix was computed using Bray Curtis distance. The stations were labelled by marker (V4 or V9). Stations where Mamiellophyceae represent more than 50% of the reads are colored in red (cluster A). Stations in blue are dominated by Pseudoscourfieldiales (cluster C), in brown by Trebouxiophyceae (cluster B) and in purple by Chlorodendrophyceae (cluster D).

Fig. 5: A. Comparison of Mamiellophyceae read distribution (assigned at the genus level) for 23 OSD stations. Stations, where the number of reads assigned to Mamiellophyceae was lower than 100 were removed (OSD14, 22, 37 and 141). B. Comparison of Mamiellophyceae communities at the genus level by hierarchical clustering using V9 and V4. The stations were labelled by marker (V4 or V9) and station name. Stations in blue are dominated by *Micromonas* (cluster D), in red by *Ostreococcus* (cluster A), in green by Dolichomastigales (cluster B) and in grey by *Mantoniella* (cluster C).

## Supplementary Figures

Supplementary Fig. S1: A. Bioinformatics pipeline use to build and assigned OTUs from V4 and V9 datasets. Reference alignment was SILVA seed release 119. The Chlorophyta curated PR² database (Tragin *et al.*, 2016) was used as taxonomic reference. The number of sequences at each step appears in Table 2.

Supplementary Fig. S2: A. Rarefaction curves; B. Rank abundance distribution. x-axis represents OTUs by decreasing number of sequences.

Supplementary Fig. S3: Rarefaction curves per station A. V4; B. V9.

Supplementary Fig. S4: A and B. Non-metric Multi Dimensional Scaling (NMDS) representation of communities based on lowest taxonomic level (OTUs) for V4 (A) and V9 (B). The dissimilarity matrix was computed using Bray Curtis distance. C and D. Hierarchical cluster analysis based on the Bray Curtis matrix for V4 (C) and V9 (D). Stations in panels A and B were grouped together based on clusters from panels C and D using a fixed threshold (0.9).

Supplementary Fig. S5: Correlation between V4 and V9 relative contribution to photosynthetic metabarcodes in major photosynthetic phyla. A. Ochrophyta. B. Chlorophyta. C. Haptophyta. D. Cryptophyta.

Supplementary Fig. S6: Correlation between V4 and V9 relative contribution of the four major Ochrophyta Classes. A. Bacillariophyta. B. Dictyochophyceae., C. Chrysophyceae-Synurophyceae. D. Pelagophyceae.

Supplementary Fig. S7: Percentage of genera from photosynthetic groups found either only in V4 (blue), or only in V9 (red), or in both datasets (grey). Only taxonomically valid genera and only Classes with at least 5 genera were taken into account. Numbers below each group indicate the total number of genera recorded.

Supplementary Fig. S8: Correlation between V4 and V9 relative contribution to photosynthetic metabarcodes for major Chlorophyta classes. A. Mamiellophyceae. B. Trebouxiophyceae. C. Chlorodendrophyceae (OSD14 is not represented on the scatter plot with 65% and 60% for V4 and V9 respectively). D. Pyramimonadales. E. Ulvophyceae. F. Pseudoscourfieldiales.

Supplementary Fig. S9: Heatmap of differences between V9 and V4 (V9-V4) relative contribution: A. Chlorophyta classes B. Mamiellophyceae genera. The colors correspond to the difference from - 50% (-0.5) to + 50 % (0.5).

657 Supplementary Fig. S10: Correlation between V4 and V9 relative contribution to Chlorophyta
658 metabarcodes for major Mamiellophyceae genera. A. *Micromonas*. B. *Mamiella*. C. *Ostreococcu.* D.
659 *Bathycoccus*.

660

## Supplementary data

The data are deposited on Figshare at https://figshare.com/s/ff26853527ced961326a

Supplementary Data 1. Mothur script for sequence analysis

Supplementary Data 2. Fasta file of Chlorophyta OTUs for V4

Supplementary Data 3. Fasta file of Chlorophyta OTUs for V9

Supplementary Data 4. Chlorophyta OTUs for V4 with assignation and read abundance at the different stations (Excel file).

Supplementary Data 5. Chlorophyta OTUs for V9 with assignation and read abundance at the different stations (Excel file).

Supplementary Data 6. Top 10 BLAST hits against Genbank nr database for Chlorophyta V4 OTUs. Red lines correspond to OTUs badly assigned to non-Chlorophyta and green corresponds to OTUs badly assigned to another Chlorophyta representative.

Supplementary Data 7. Top 10 BLAST hits against Genbank nr database for Chlorophyta V9 OTUs. Red lines correspond to OTUs badly assigned to non-Chlorophyta and green lines corresponds to OTUs badly assigned to another Chlorophyta representative.

Fig.S1

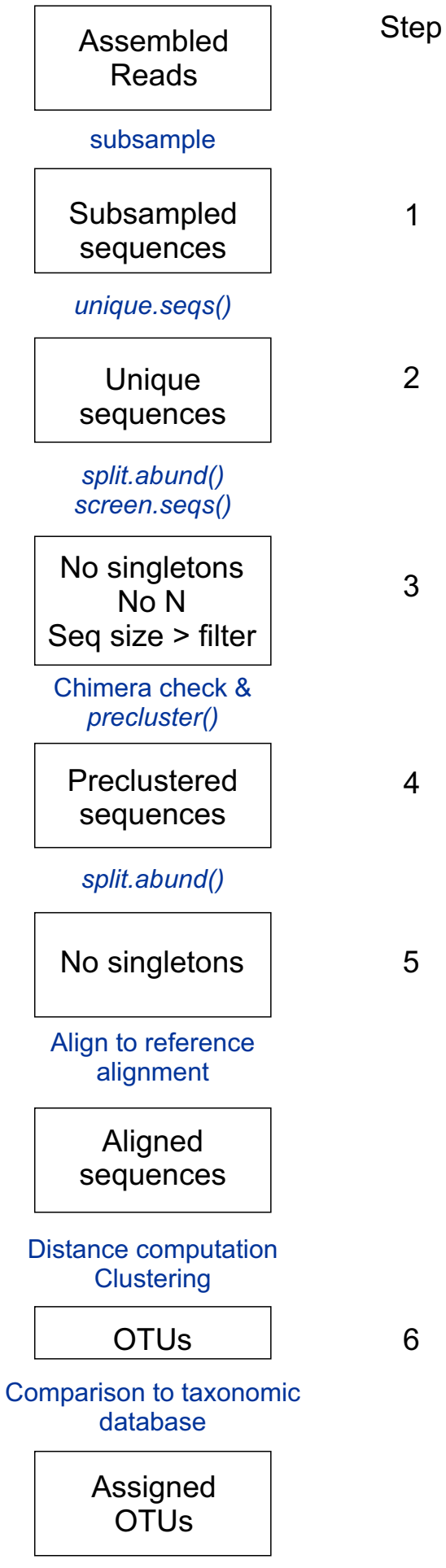
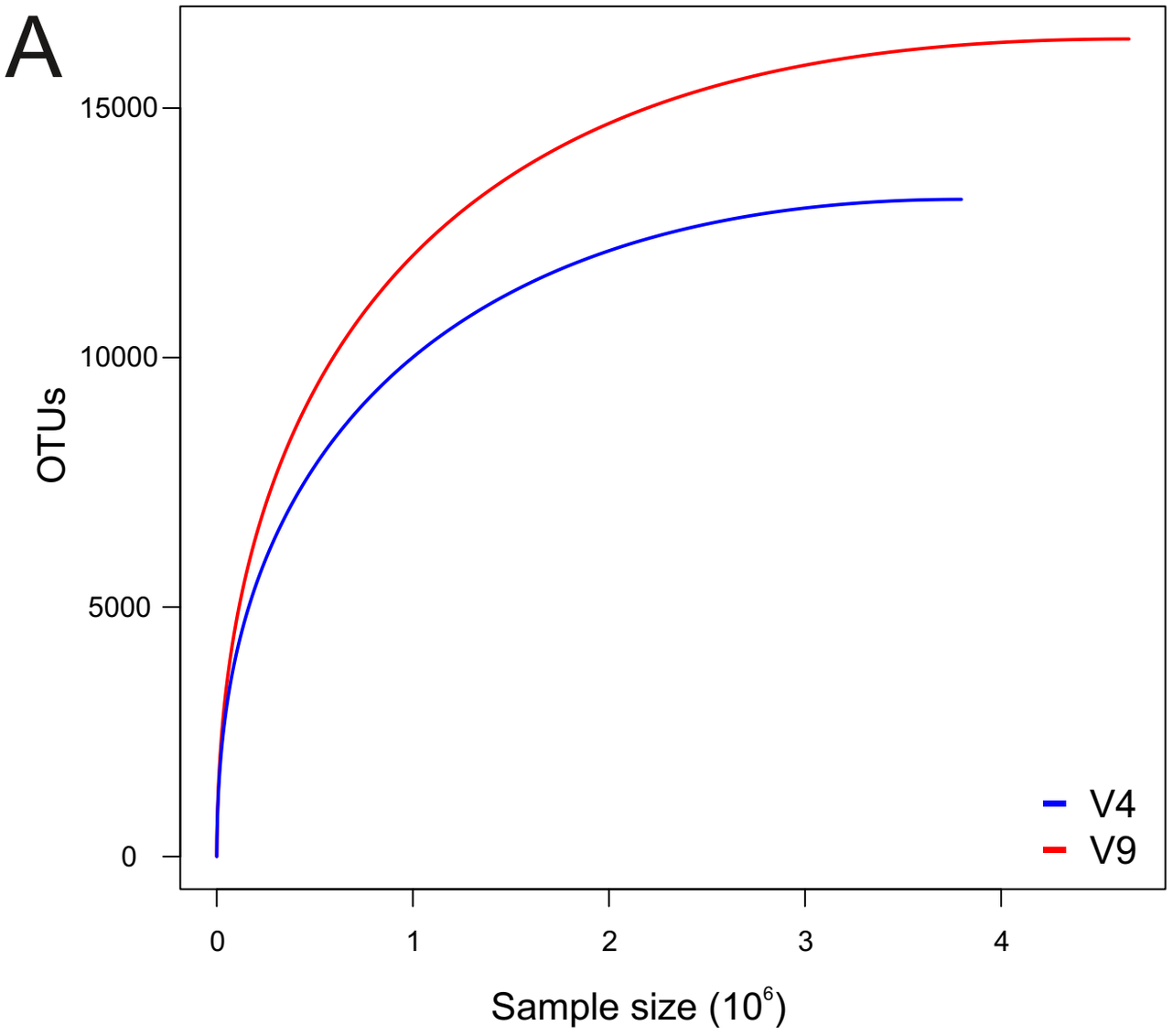

Assembled Reads
Step
subsample
Subsampled sequences
1
unique.seqs()
Unique sequences
2
split.abund()
screen.seqs()
No singletons
No N
Seq size > filter
3
Chimera check &
precluster()
Preclustered sequences
4
split.abund()
No singletons
5
Align to reference alignment
Aligned sequences
Distance computation
Clustering
OTUs
6
Comparison to taxonomic database
Assigned OTUs

Fig.S2


A
15000
10000
5000
0
OTUs
0
1
2
3
4
Sample size ($10^6$)
V4
V9

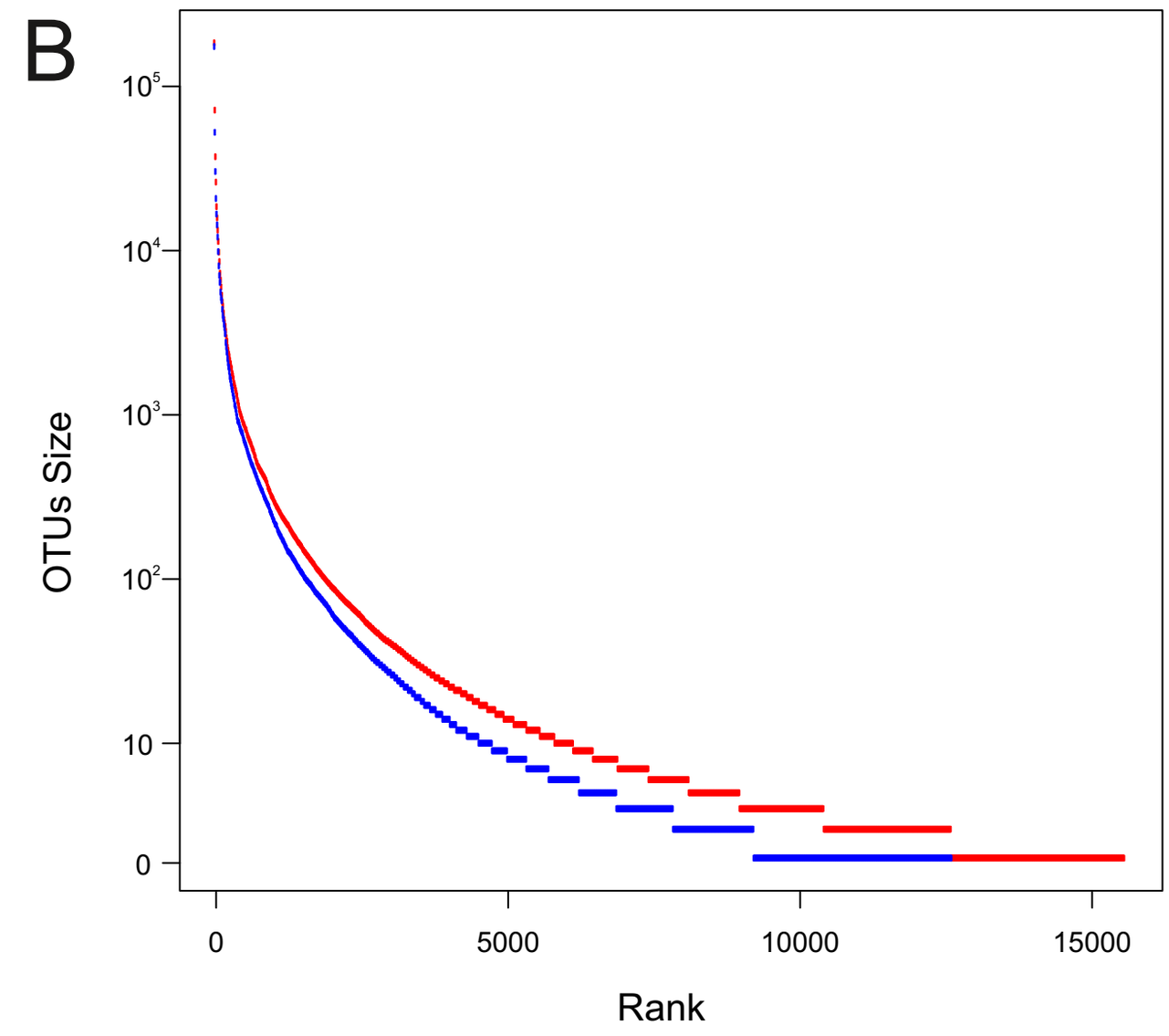

B
$10^5$
$10^4$
$10^3$
$10^2$
10
0
OTUs Size
0
5000
10000
15000
Rank

Fig.S3

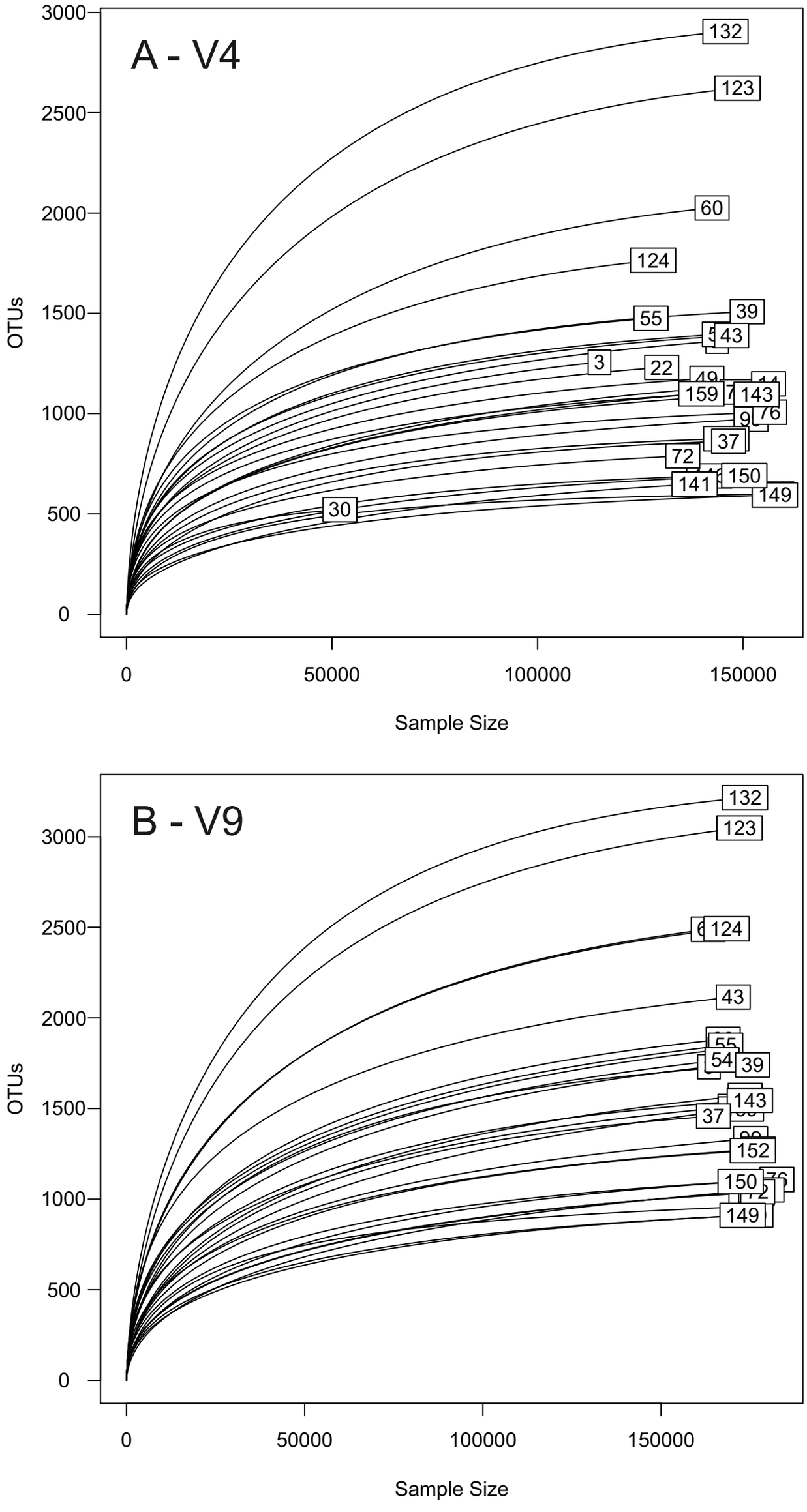
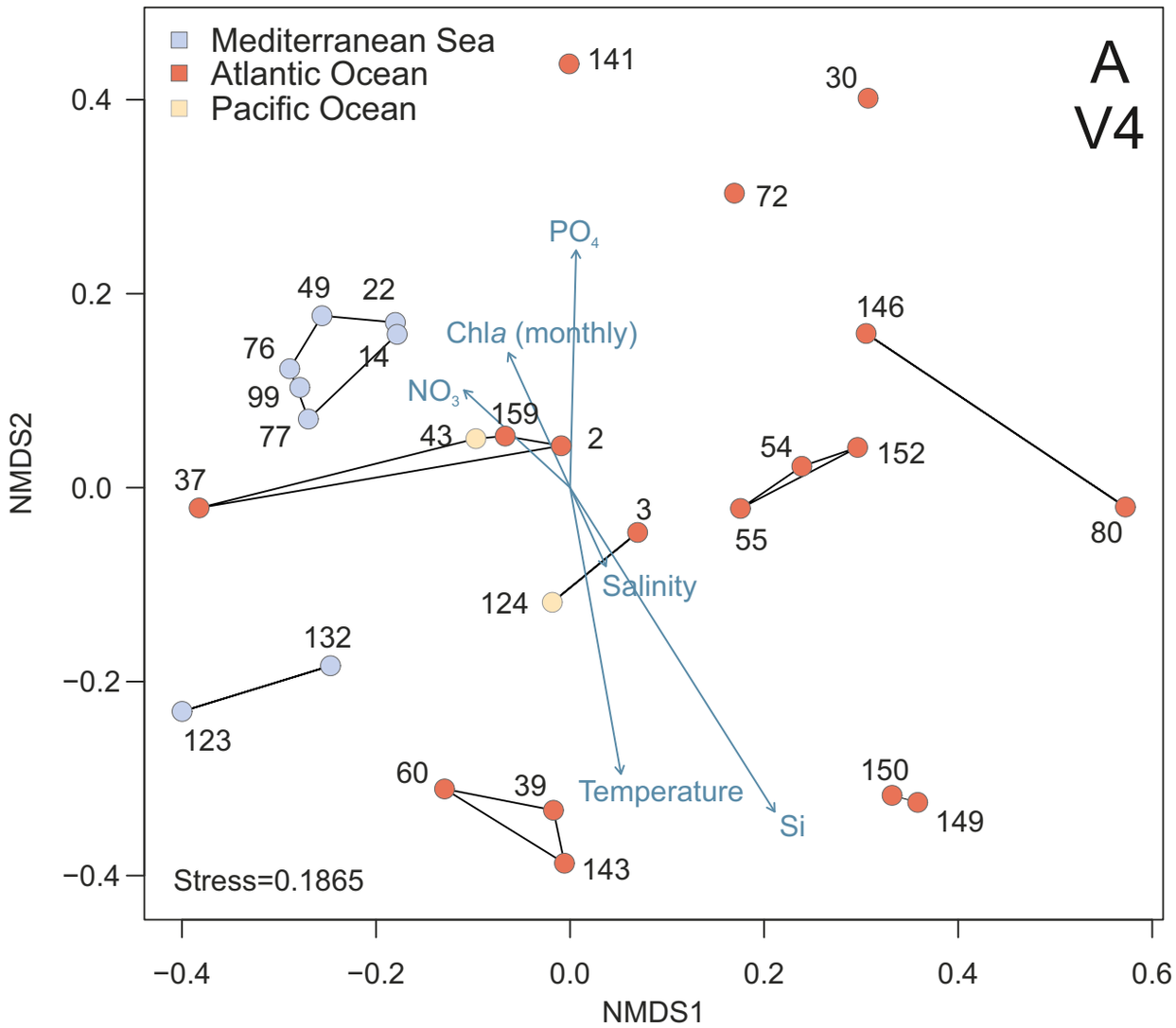
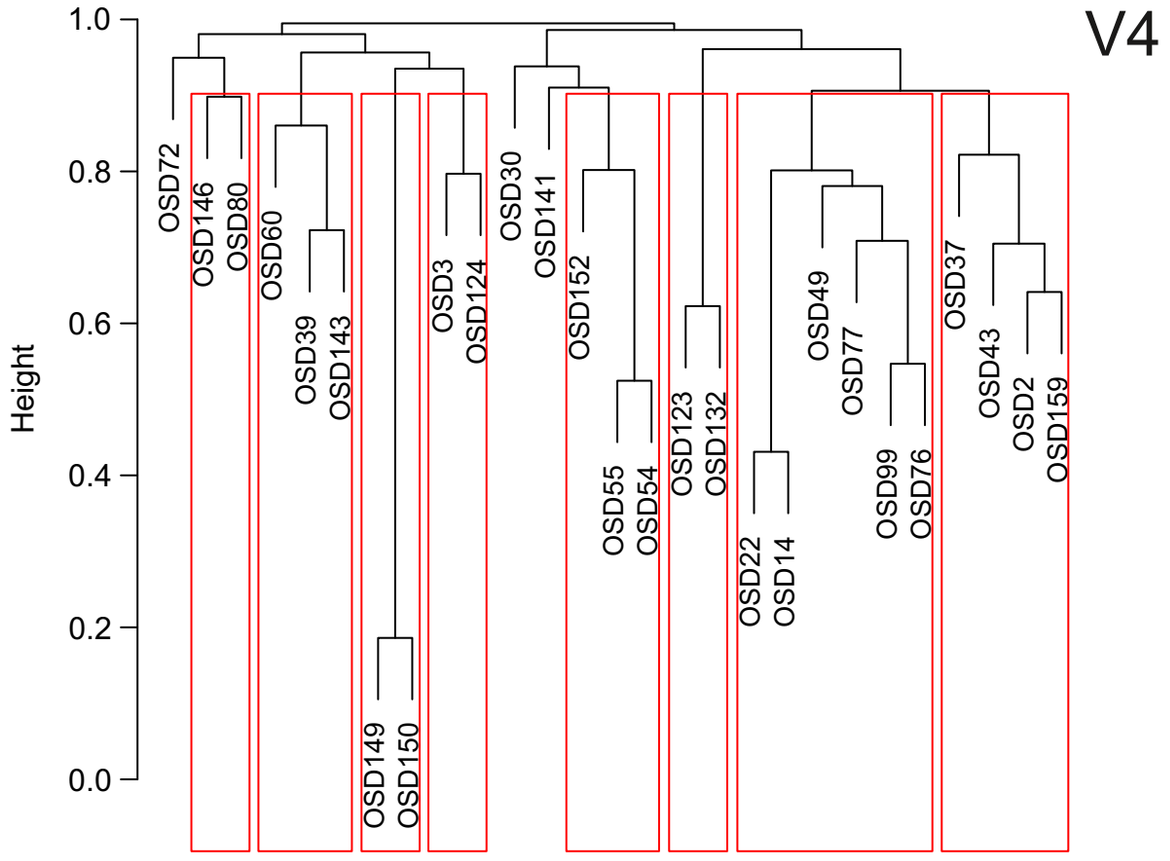
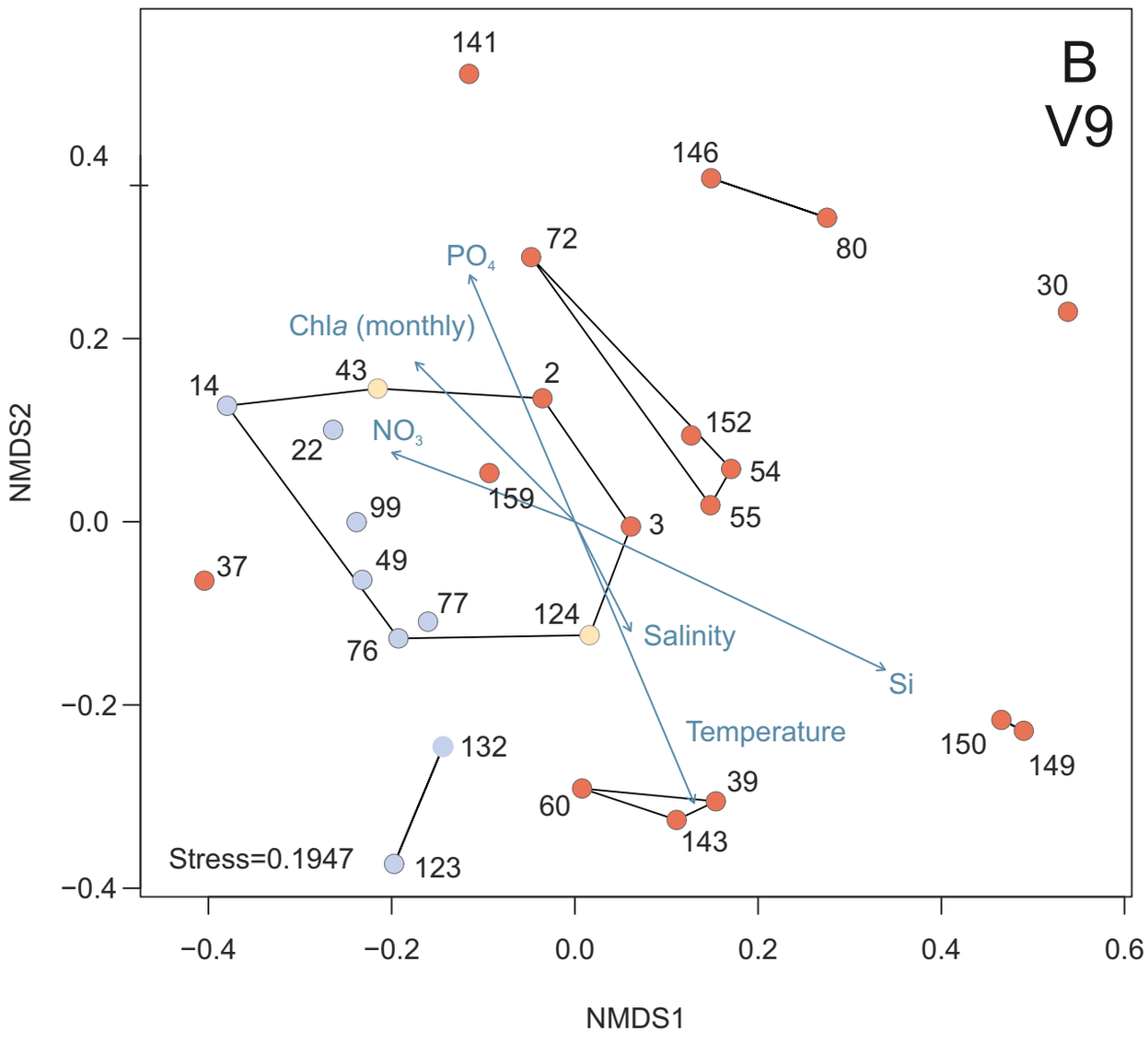

A - V4
OTUs
Sample Size
3000
2500
2000
1500
1000
500
0
0
50000
100000
150000
132
123
60
124
55
39
43
3
22
159
143
37
72
150
141
149
30
B - V9
OTUs
Sample Size
132
123
124
43
54
39
143
37
152
150
149

Fig.S4


Mediterranean Sea
Atlantic Ocean
Pacific Ocean
A
V4
NMDS2
NMDS1
PO4
Chla (monthly)
NO3
Salinity
Temperature
Si
Stress=0.1865
141
30
72
146
80
49
22
76
14
99
77
159
43
2
37
54
152
55
3
124
132
123
60
39
143
150
149


C
V4
Height
OSD72
OSD146
OSD80
OSD60
OSD39
OSD143
OSD3
OSD124
OSD30
OSD141
OSD152
OSD55
OSD54
OSD123
OSD132
OSD22
OSD14
OSD49
OSD77
OSD99
OSD76
OSD37
OSD43
OSD2
OSD159
OSD149
OSD150


B
V9
NMDS2
NMDS1
PO4
Chla (monthly)
NO3
Salinity
Temperature
Si
Stress=0.1947
141
146
80
72
30
14
43
2
22
152
54
55
159
99
3
37
49
77
124
76
132
123
60
39
143
150
149

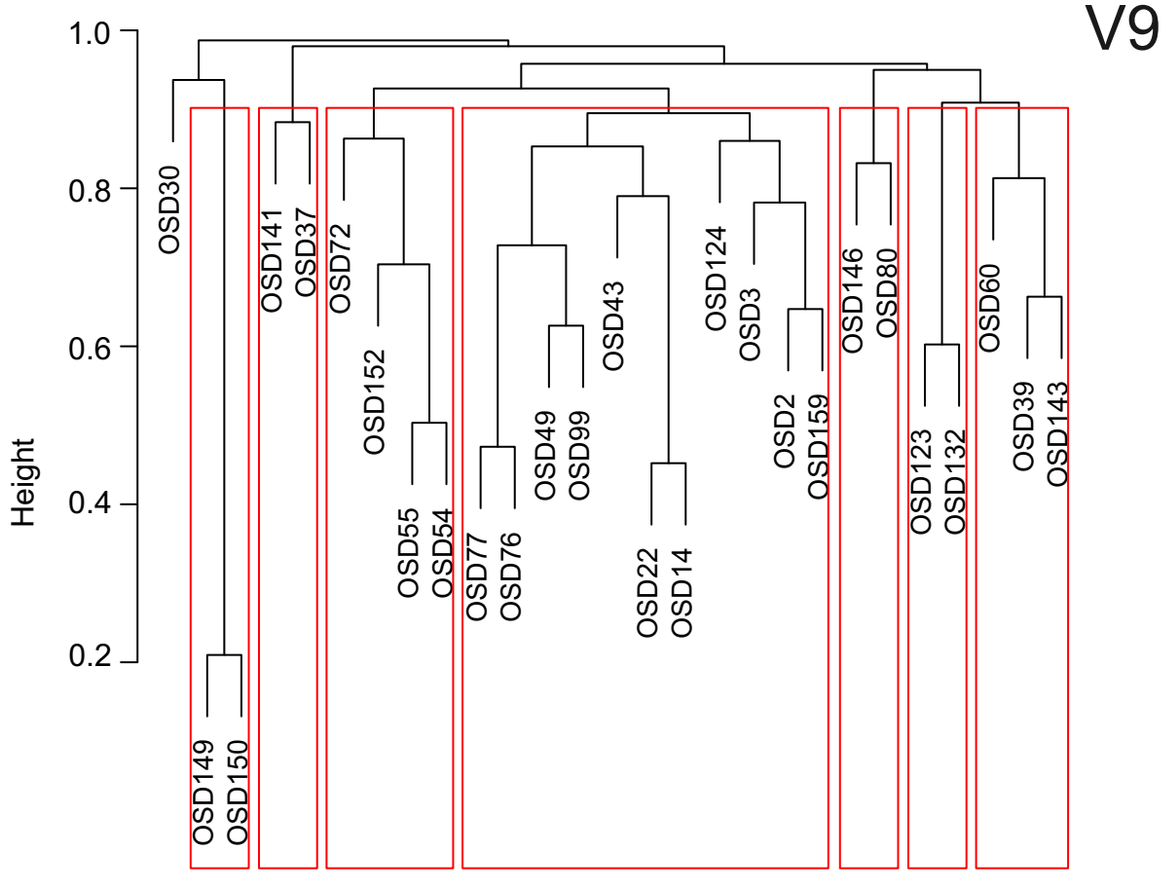

D
V9
Height
OSD30
OSD141
OSD37
OSD72
OSD152
OSD55
OSD54
OSD77
OSD76
OSD49
OSD99
OSD43
OSD22
OSD14
OSD124
OSD3
OSD2
OSD159
OSD146
OSD80
OSD123
OSD132
OSD60
OSD39
OSD143
OSD149
OSD150

Fig.S5

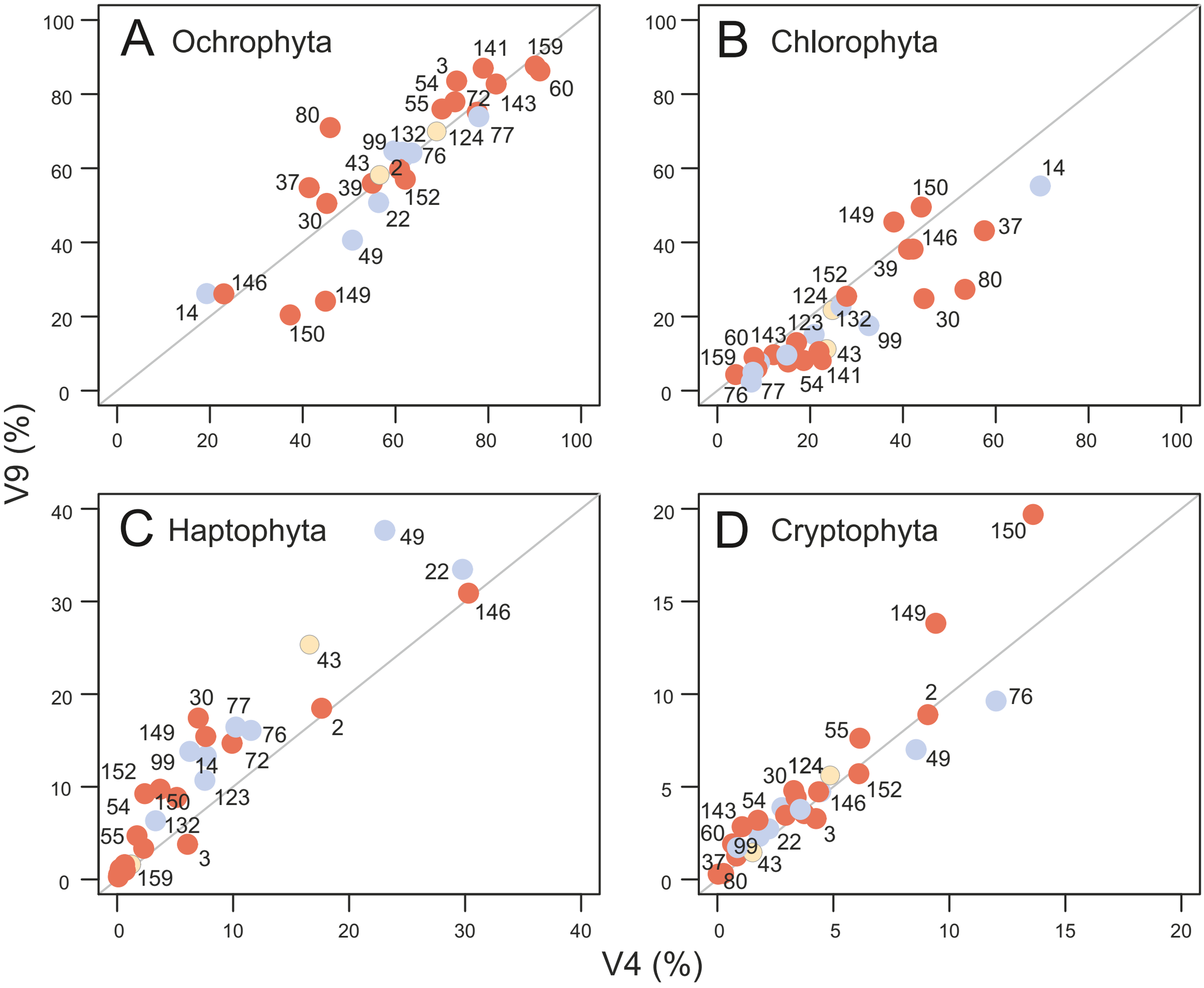

A Ochrophyta
B Chlorophyta
C Haptophyta
D Cryptophyta
V9 (%)
V4 (%)

Fig.S6

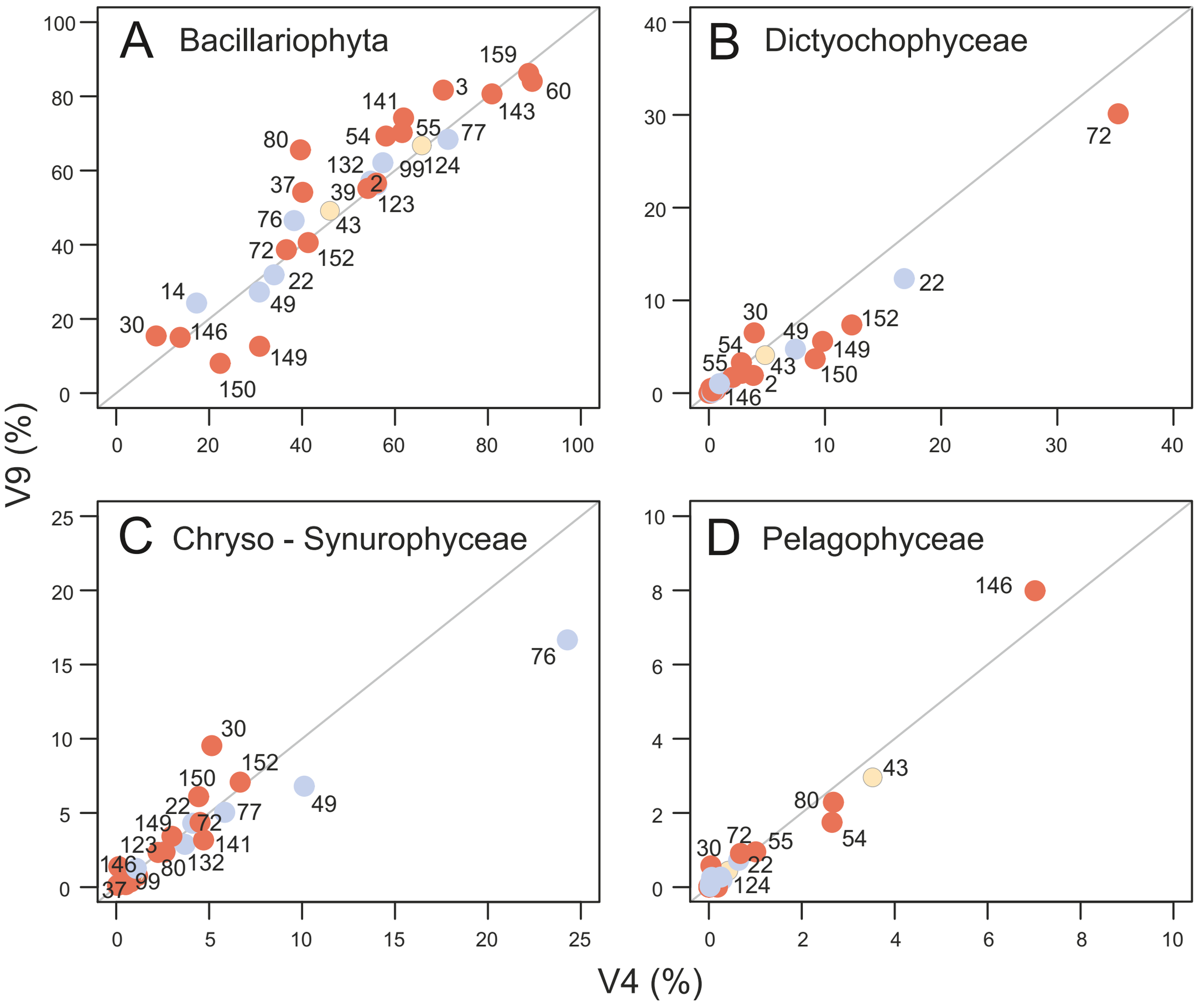

A Bacillariophyta
B Dictyochophyceae
C Chryso - Synurophyceae
D Pelagophyceae
V9 (%)
V4 (%)

Fig.S7

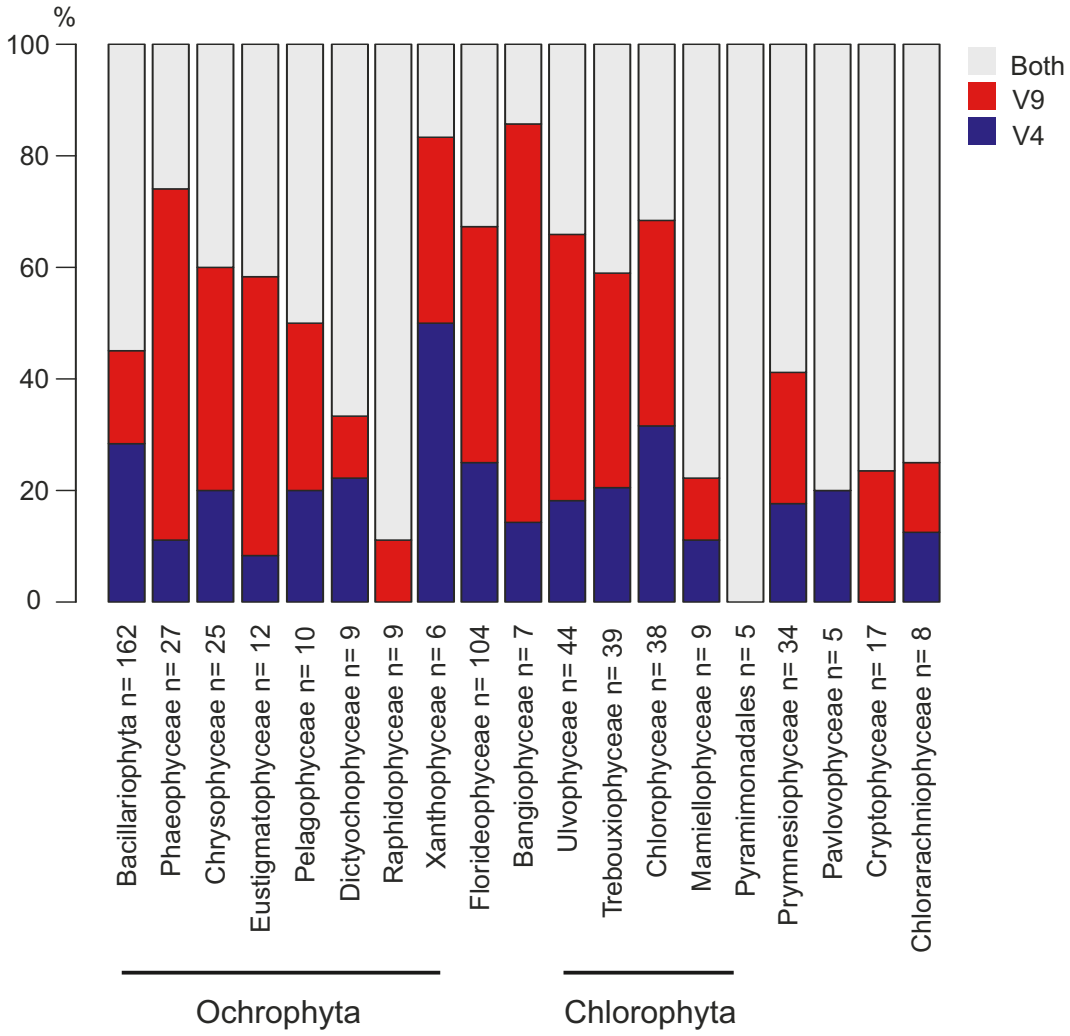

%
100
80
60
40
20
0
Both
V9
V4
Bacillariophyta n= 162
Phaeophyceae n= 27
Chrysophyceae n= 25
Eustigmatophyceae n= 12
Pelagophyceae n= 10
Dictyochophyceae n= 9
Raphidophyceae n= 9
Xanthophyceae n= 6
Florideophyceae n= 104
Bangiophyceae n= 7
Ulvophyceae n= 44
Trebouxiophyceae n= 39
Chlorophyceae n= 38
Mamiellophyceae n= 9
Pyramimonadales n= 5
Prymnesiophyceae n= 34
Pavlovophyceae n= 5
Cryptophyceae n= 17
Chlorarachniophyceae n= 8
Ochrophyta
Chlorophyta

Fig.S8

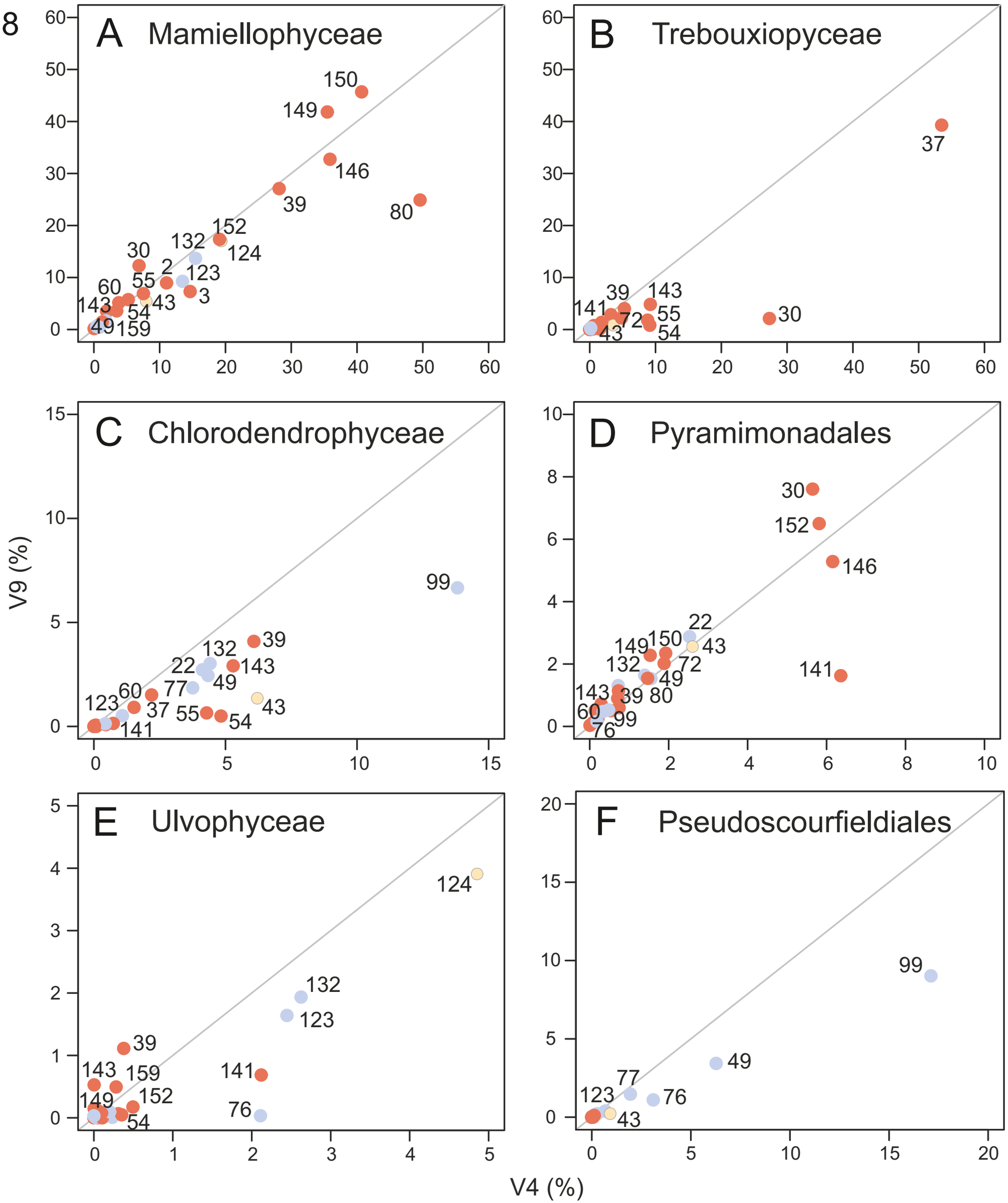

A Mamiellophyceae
B Trebouxiopyceae
C Chlorodendrophyceae
D Pyramimonadales
E Ulvophyceae
F Pseudoscourfieldiales
V9 (%)
V4 (%)

Fig.S9

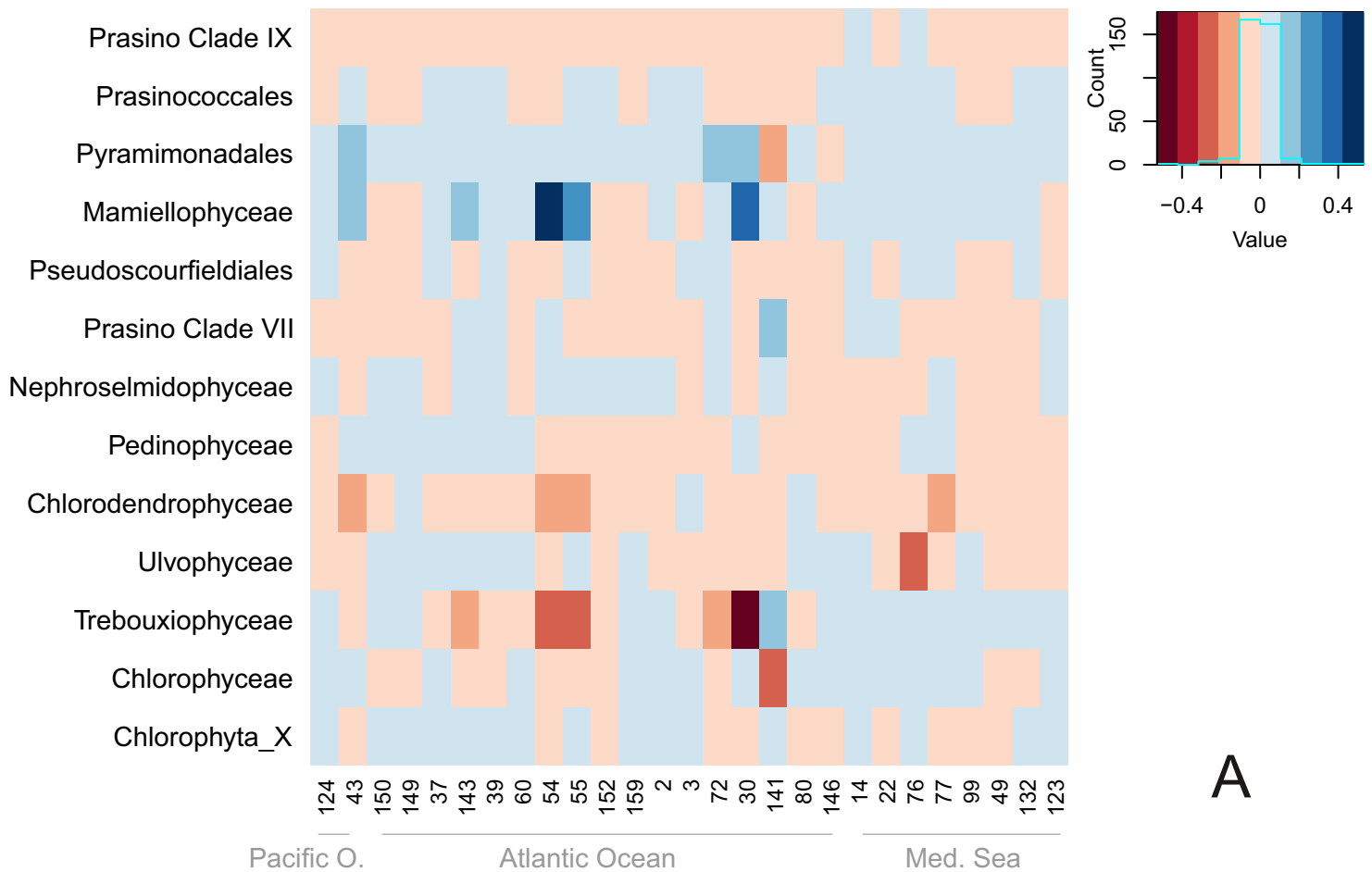

Prasino Clade IX
Prasinococcales
Pyramimonadales
Mamiellophyceae
Pseudoscourfieldiales
Prasino Clade VII
Nephroselmidophyceae
Pedinophyceae
Chlorodendrophyceae
Ulvophyceae
Trebouxiophyceae
Chlorophyceae
Chlorophyta_X
124 43 150 149 37 143 39 60 54 55 152 159 2 3 72 30 141 80 146 14 22 76 77 99 49 132 123
Pacific O.
Atlantic Ocean
Med. Sea
Count
0
50
150
−0.4
0
0.4
Value
A
OSD Station
Bathycoccus
Crustomastix
Dolichomastigaceae−A
Dolichomastigaceae−B
Dolichomastix
Mamiella
Mamiellaceae_X
Mantoniella
Micromonas
Ostreococcus
unclassified
124 43 150 149 143 39 60 54 55 152 159 2 3 72 30 80 146 76 77 99 49 132 123
Pacific O.
Atlantic Ocean
Med. Sea
Count
0
50
150
−0.4
0
0.4
Value
B
OSD Station

Fig.S10

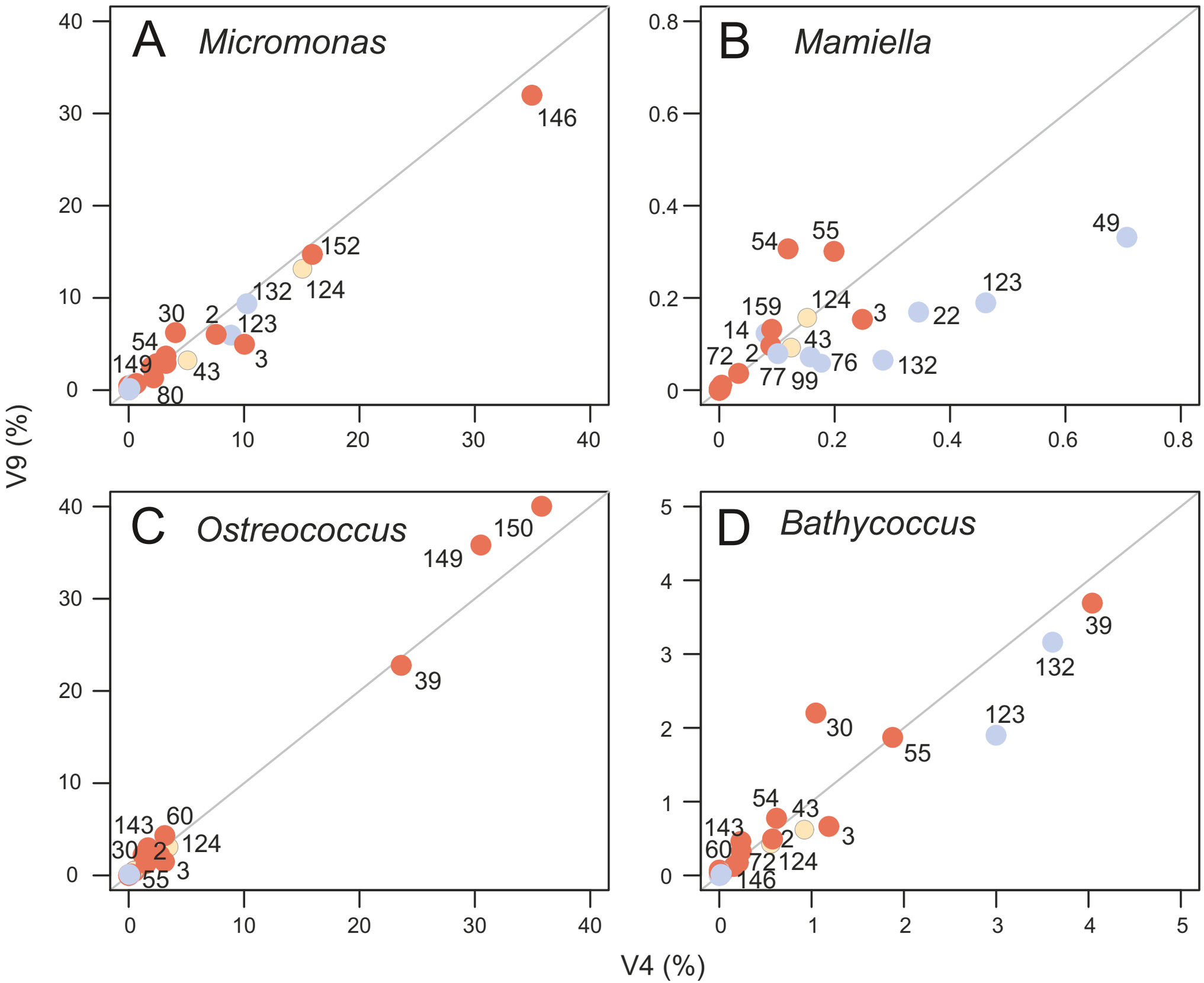


Fig.1

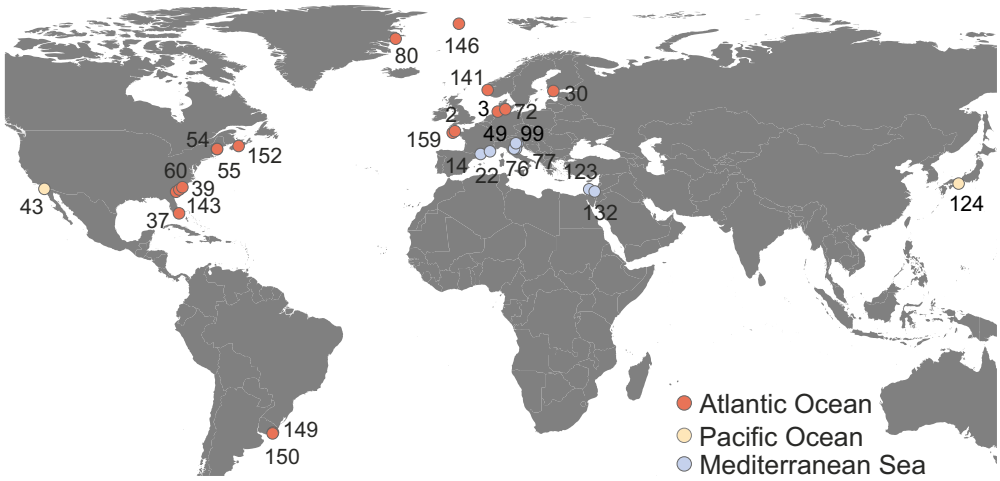

146
80
141
30
3
72
2
159
49
99
14
22
76
77
123
132
54
152
60
55
39
143
43
37
124
149
150
Atlantic Ocean
Pacific Ocean
Mediterranean Sea

Fig.2

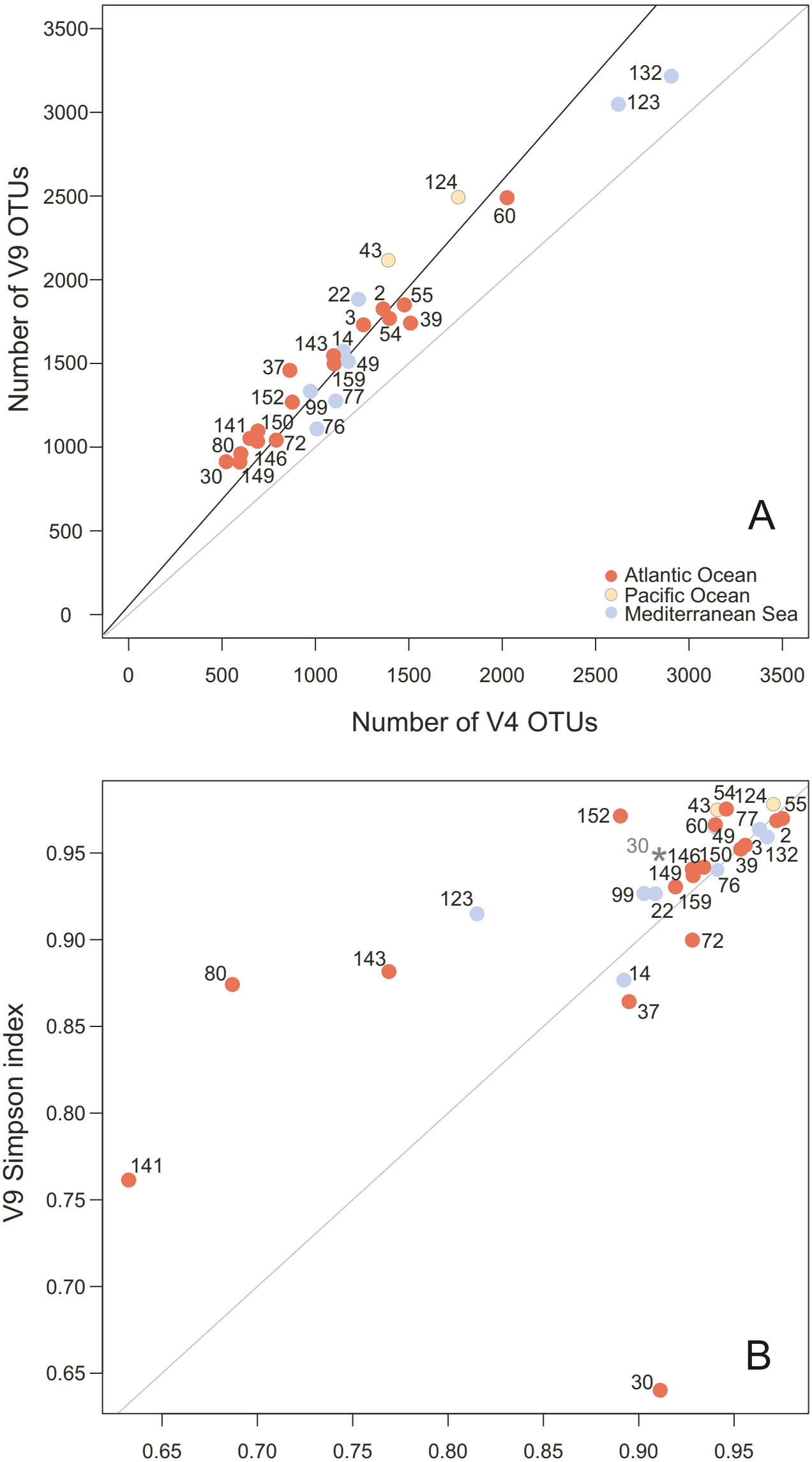

Number of V9 OTUs
3500
3000
2500
2000
1500
1000
500
0
132
123
124
60
43
22
2
55
3
39
54
143
14
37
49
159
152
77
99
141
150
76
80
72
146
30
149
A
Atlantic Ocean
Pacific Ocean
Mediterranean Sea
0
500
1000
1500
2000
2500
3000
3500
Number of V4 OTUs
V9 Simpson index
0.95
0.90
0.85
0.80
0.75
0.70
0.65
54
124
43
55
152
77
60
2
49
3
30
146
150
132
39
149
76
99
159
22
123
72
143
80
14
37
141
B
30
0.65
0.70
0.75
0.80
0.85
0.90
0.95

Fig.3

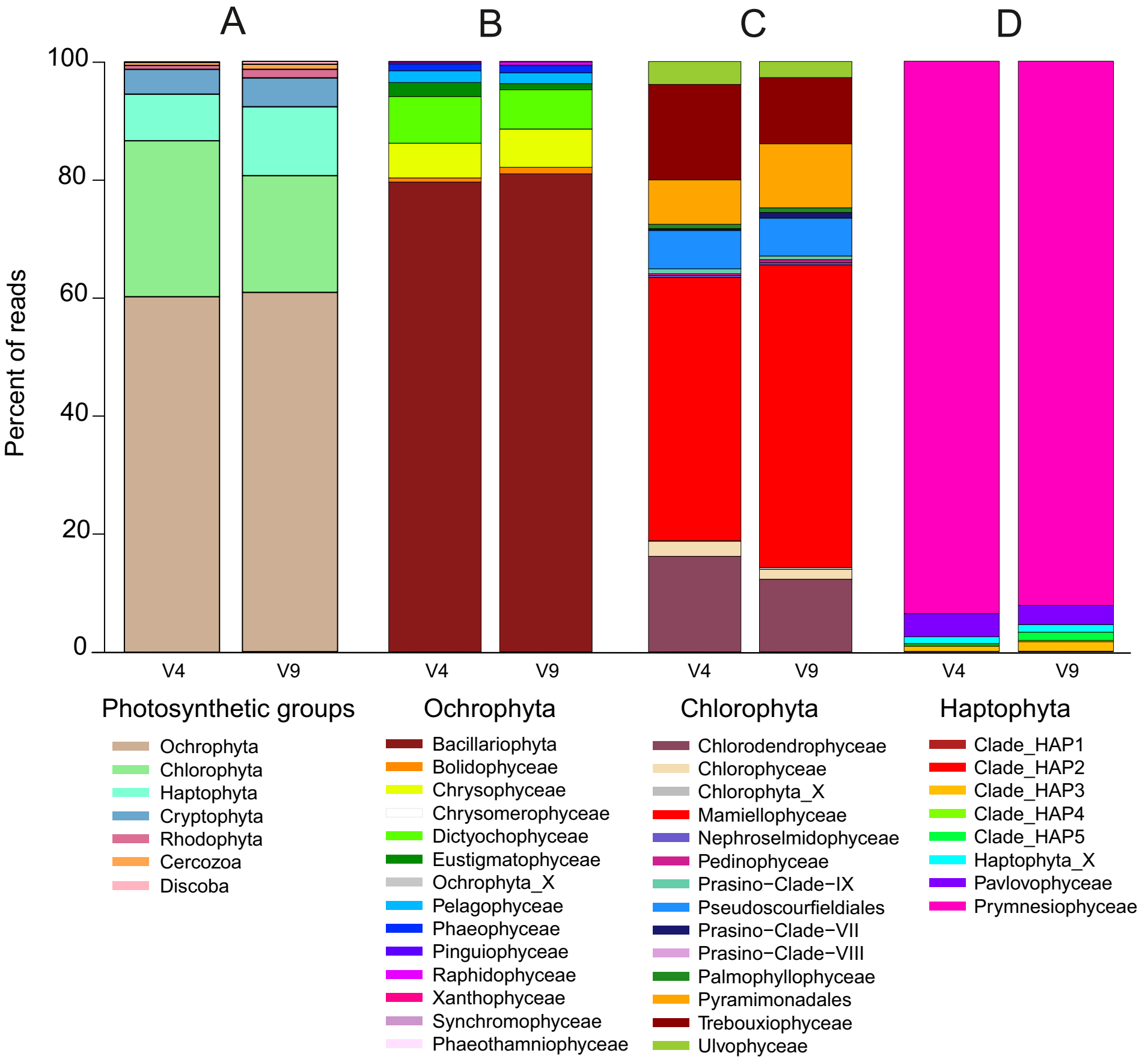
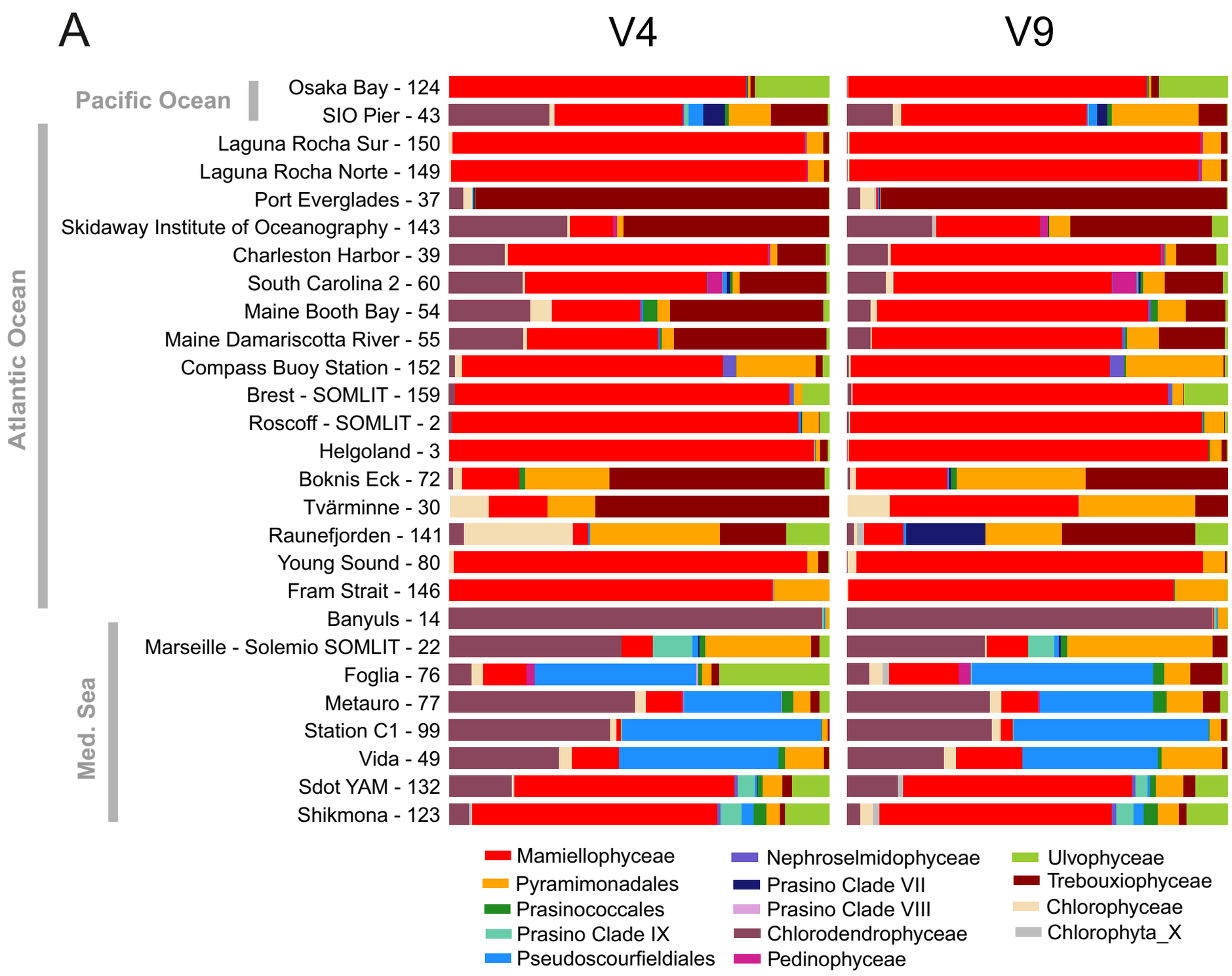
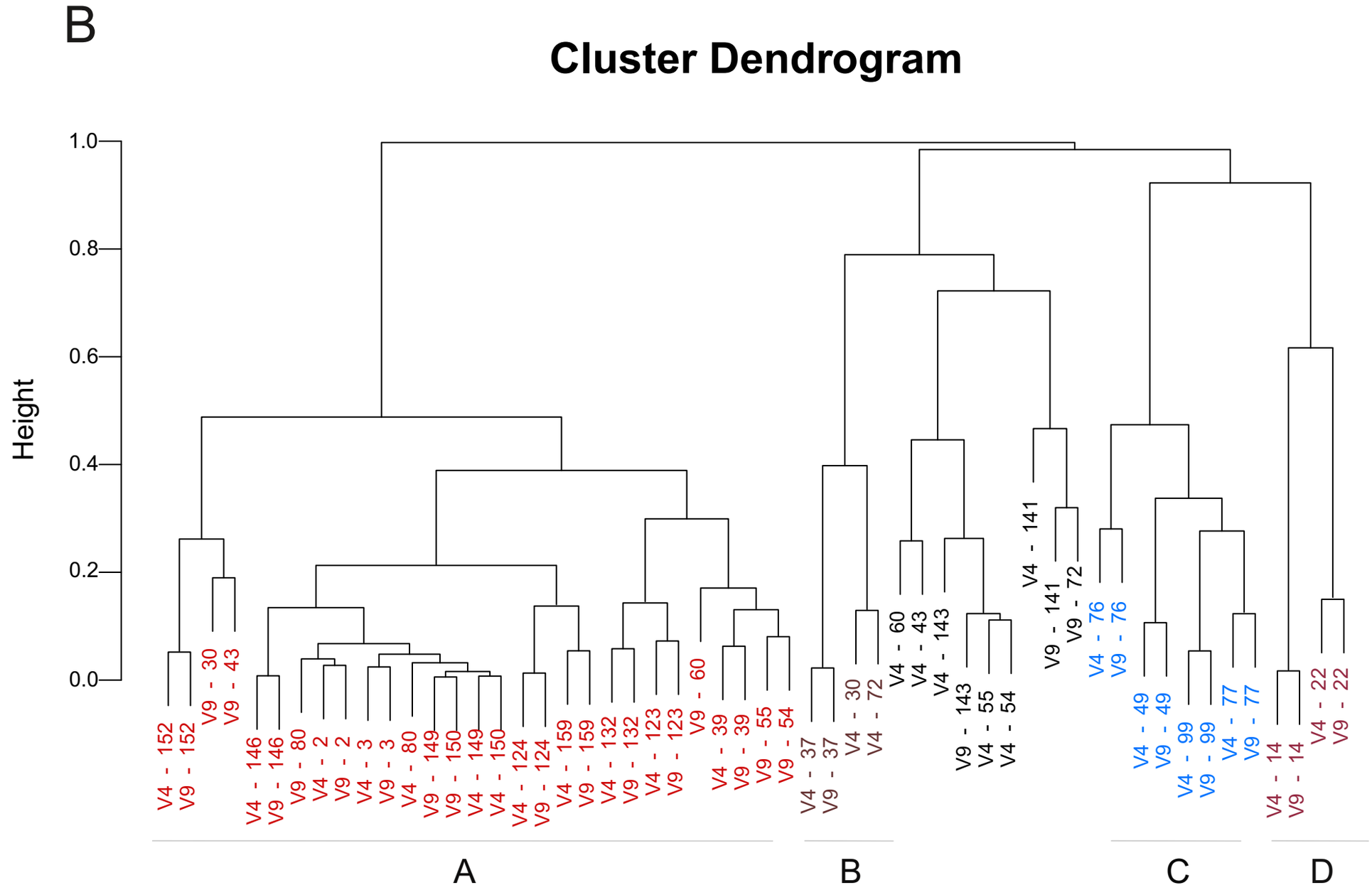

A
B
C
D
100
80
60
40
20
0
Percent of reads
V4
V9
V4
V9
V4
V9
V4
V9
Photosynthetic groups
Ochrophyta
Chlorophyta
Haptophyta
Cryptophyta
Rhodophyta
Cercozoa
Discoba
Ochrophyta
Bacillariophyta
Bolidophyceae
Chrysophyceae
Chrysomerophyceae
Dictyochophyceae
Eustigmatophyceae
Ochrophyta_X
Pelagophyceae
Phaeophyceae
Pinguiophyceae
Raphidophyceae
Xanthophyceae
Synchromophyceae
Phaeothamniophyceae
Chlorophyta
Chlorodendrophyceae
Chlorophyceae
Chlorophyta_X
Mamiellophyceae
Nephroselmidophyceae
Pedinophyceae
Prasino−Clade−IX
Pseudoscourfieldiales
Prasino−Clade−VII
Prasino−Clade−VIII
Palmophyllophyceae
Pyramimonadales
Trebouxiophyceae
Ulvophyceae
Haptophyta
Clade_HAP1
Clade_HAP2
Clade_HAP3
Clade_HAP4
Clade_HAP5
Haptophyta_X
Pavlovophyceae
Prymnesiophyceae

Fig.4

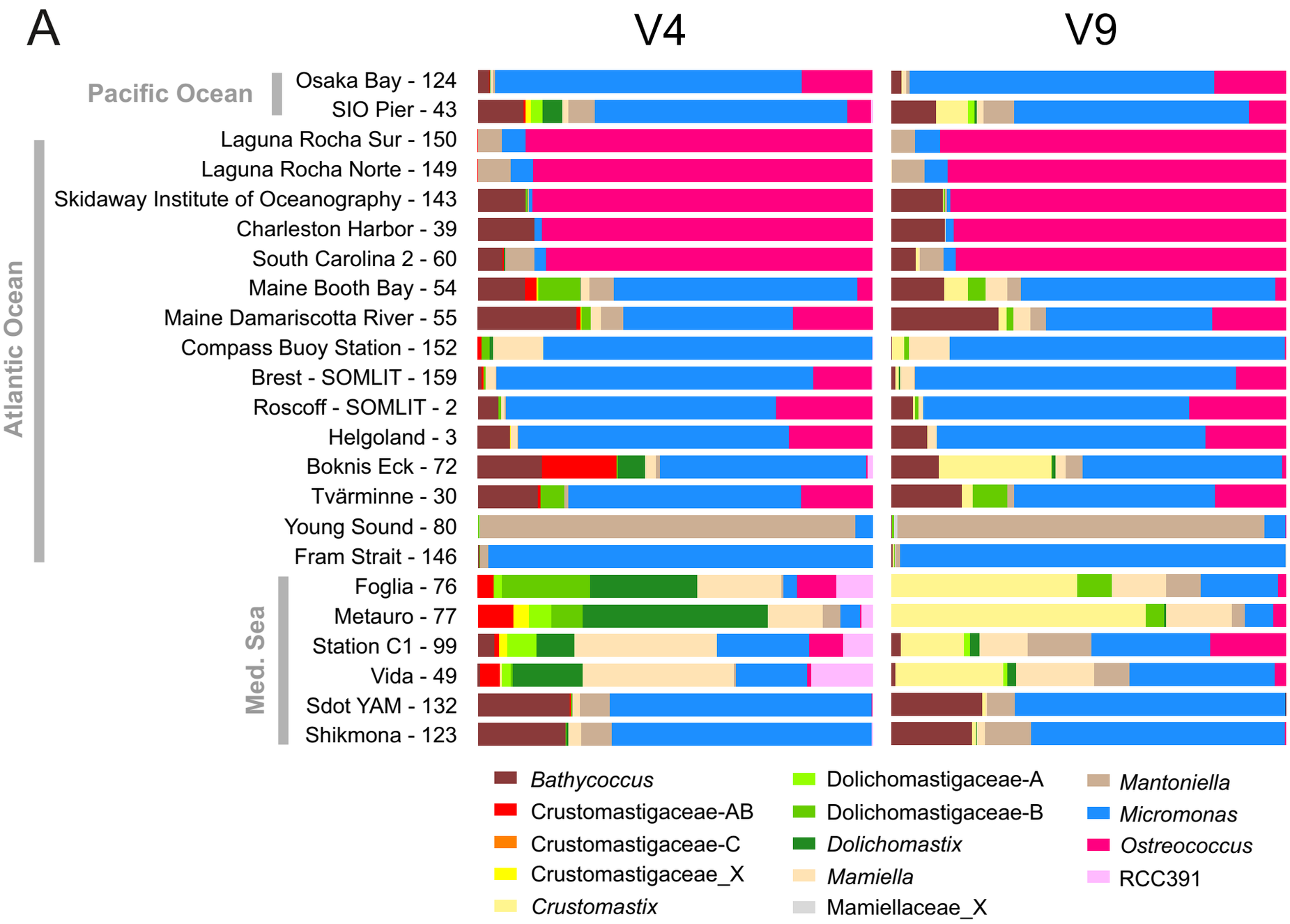

A
V4
V9
Pacific Ocean
Atlantic Ocean
Med. Sea
Osaka Bay - 124
SIO Pier - 43
Laguna Rocha Sur - 150
Laguna Rocha Norte - 149
Port Everglades - 37
Skidaway Institute of Oceanography - 143
Charleston Harbor - 39
South Carolina 2 - 60
Maine Booth Bay - 54
Maine Damariscotta River - 55
Compass Buoy Station - 152
Brest - SOMLIT - 159
Roscoff - SOMLIT - 2
Helgoland - 3
Boknis Eck - 72
Tvärminne - 30
Raunefjorden - 141
Young Sound - 80
Fram Strait - 146
Banyuls - 14
Marseille - Solemio SOMLIT - 22
Foglia - 76
Metauro - 77
Station C1 - 99
Vida - 49
Sdot YAM - 132
Shikmona - 123
Mamiellophyceae
Pyramimonadales
Prasinococcales
Prasino Clade IX
Pseudoscourfieldiales
Nephroselmidophyceae
Prasino Clade VII
Prasino Clade VIII
Chlorodendrophyceae
Pedinophyceae
Ulvophyceae
Trebouxiophyceae
Chlorophyceae
Chlorophyta_X

B


Cluster Dendrogram
Height
1.0
0.8
0.6
0.4
0.2
0.0
V4 - 152
V9 - 152
V9 - 30
V9 - 43
V4 - 146
V9 - 146
V9 - 80
V4 - 2
V9 - 2
V4 - 3
V9 - 3
V4 - 80
V9 - 149
V9 - 150
V4 - 149
V4 - 150
V4 - 124
V9 - 124
V4 - 159
V9 - 159
V4 - 132
V9 - 132
V4 - 123
V9 - 123
V9 - 60
V4 - 39
V9 - 39
V9 - 55
V9 - 54
V4 - 37
V9 - 37
V4 - 30
V4 - 72
V4 - 60
V4 - 43
V4 - 143
V9 - 143
V4 - 55
V4 - 54
V4 - 141
V9 - 141
V9 - 72
V4 - 76
V9 - 76
V4 - 49
V9 - 49
V4 - 99
V9 - 99
V4 - 77
V9 - 77
V4 - 14
V9 - 14
V4 - 22
V9 - 22

A B C D

Fig. 5


A
V4
V9
Pacific Ocean
Atlantic Ocean
Med. Sea
Osaka Bay - 124
SIO Pier - 43
Laguna Rocha Sur - 150
Laguna Rocha Norte - 149
Skidaway Institute of Oceanography - 143
Charleston Harbor - 39
South Carolina 2 - 60
Maine Booth Bay - 54
Maine Damariscotta River - 55
Compass Buoy Station - 152
Brest - SOMLIT - 159
Roscoff - SOMLIT - 2
Helgoland - 3
Boknis Eck - 72
Tvärminne - 30
Young Sound - 80
Fram Strait - 146
Foglia - 76
Metauro - 77
Station C1 - 99
Vida - 49
Sdot YAM - 132
Shikmona - 123
Bathycoccus
Crustomastigaceae-AB
Crustomastigaceae-C
Crustomastigaceae_X
Crustomastix
Dolichomastigaceae-A
Dolichomastigaceae-B
Dolichomastix
Mamiella
Mamiellaceae_X
Mantoniella
Micromonas
Ostreococcus
RCC391

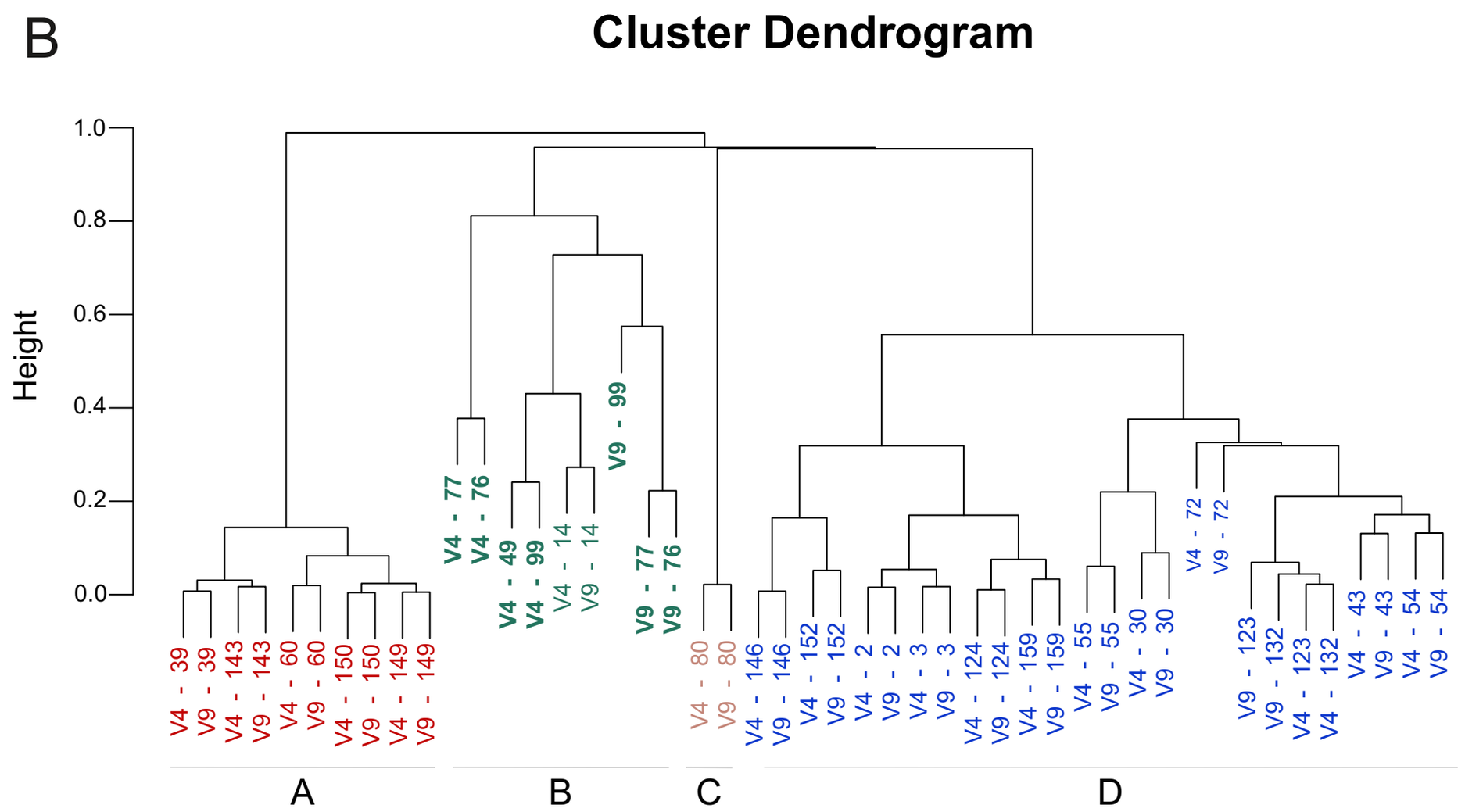

B
Cluster Dendrogram
Height
1.0
0.8
0.6
0.4
0.2
0.0
V4 - 39
V9 - 39
V4 - 143
V9 - 143
V4 - 60
V9 - 60
V4 - 150
V9 - 150
V4 - 149
V9 - 149
V4 - 77
V4 - 76
V4 - 49
V4 - 99
V4 - 14
V9 - 14
V9 - 99
V9 - 77
V9 - 76
V4 - 80
V9 - 80
V4 - 146
V9 - 146
V4 - 152
V9 - 152
V4 - 2
V9 - 2
V4 - 3
V9 - 3
V4 - 124
V9 - 124
V4 - 159
V9 - 159
V4 - 55
V9 - 55
V4 - 30
V9 - 30
V4 - 72
V9 - 72
V9 - 123
V9 - 132
V4 - 123
V4 - 132
V4 - 43
V9 - 43
V4 - 54
V9 - 54
A
B
C
D

TABLE 1

| OSD | Station | Ocean | Region | V4 | | | V9 | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Raw reads | % of reads subsampled | % of photo. reads | Raw reads | % of reads subsampled | % of photo. reads |
| 2 | Roscoff - SOMLIT | North Atlantic Ocean | English Channel | 343 626 | 59.0 | 28.1 | 387 351 | 52.3 | 25.5 |
| 3 | Helgoland | North Atlantic Ocean | North Sea | 315 340 | 64.3 | 27.7 | 257 957 | 78.6 | 34.3 |
| 14 | Banyuls | Mediterranean Sea | Western Basin | 311 053 | 65.2 | 18.2 | 406 871 | 49.8 | 11.2 |
| 22 | Marseille - Solemio SOMLIT | Mediterranean Sea | Western Basin | 302 687 | 67.0 | 7.6 | 353 503 | 57.3 | 7.8 |
| 30 | Tvärminne | North Atlantic Ocean | Gulf of Finland | 296 892 | 68.3 | 8.8 | 346 294 | 58.5 | 4.3 |
| 37 | Port Everglades | North Atlantic Ocean | East coast of USA | 338 053 | 60.0 | 33.6 | 361 524 | 56.1 | 27.7 |
| 39 | Charleston Harbor | North Atlantic Ocean | East coast of USA | 332 841 | 60.9 | 73.1 | 296 868 | 68.3 | 49.0 |
| 43 | SIO Pier | North Pacific Ocean | West coast of USA | 320 295 | 63.3 | 10.9 | 388 996 | 52.1 | 21.0 |
| 49 | Vida | Mediterranean Sea | Adriatic Sea | *202 710* | *100.0* | 14.4 | 302 436 | 67.0 | 14.5 |
| 54 | Maine Booth Bay | North Atlantic Ocean | East coast of USA | 290 311 | 69.8 | 14.5 | 365 441 | 55.5 | 36.9 |
| 55 | Maine Damariscotta River | North Atlantic Ocean | East coast of USA | 237 919 | 85.2 | 30.7 | 276 076 | 73.4 | 43.3 |
| 60 | South Carolina 2 - North Inlet | North Atlantic Ocean | East coast of USA | 268 351 | 75.5 | 50.3 | 353 390 | 57.4 | 33.9 |
| 72 | Boknis Eck | North Atlantic Ocean | Kattegat | 356 529 | 56.9 | 26.5 | 475 461 | 42.6 | 23.3 |
| 76 | Foglia | Mediterranean Sea | Adriatic Sea | 242 825 | 83.5 | 11.5 | 386 655 | 52.4 | 13.2 |
| 77 | Metauro | Mediterranean Sea | Adriatic Sea | 303 448 | 66.8 | 26.5 | 377 917 | 53.6 | 26.4 |
| 80 | Young Sound | North Atlantic Ocean | Greenland Sea | 349 267 | 58.0 | 17.4 | 436 165 | 46.5 | 23.0 |
| 99 | C1 | Mediterranean Sea | Adriatic Sea | 339 739 | 59.7 | 14.2 | 449 242 | 45.1 | 12.7 |
| 123 | Shikmona | Mediterranean Sea | Eastern Basin | 286 203 | 70.8 | 8.9 | 416 420 | 48.7 | 8.3 |
| 124 | Osaka Bay | North Pacific Ocean | Japan Sea | 237 367 | 85.4 | 34.8 | 478 261 | 42.4 | 31.0 |
| 132 | Sdot YAM | Mediterranean Sea | Eastern Basin | 285 592 | 71.0 | 26.4 | 399 001 | 50.8 | 17.7 |
| 141 | Raunefjorden | North Atlantic Ocean | Coast of Norway | 308 267 | 65.8 | 0.8 | 402 413 | 50.4 | 1.6 |
| 143 | Skidaway Institute of Oceanography | North Atlantic Ocean | East coast of USA | 328 039 | 61.8 | 81.4 | 410 937 | 49.3 | 65.3 |
| 146 | Fram Strait | North Atlantic Ocean | Greenland Sea | 369 221 | 54.9 | 44.4 | 447 907 | 45.3 | 41.7 |
| 149 | Laguna Rocha Norte | South Atlantic Ocean | Coast of Uruguay | 324 063 | 62.6 | 52.2 | 323 981 | 62.6 | 44.0 |
| 150 | Laguna Rocha Sur | South Atlantic Ocean | Coast of Uruguay | 338 373 | 59.9 | 50.8 | 367 936 | 55.1 | 44.9 |
| 152 | Compass Buoy Station | North Atlantic Ocean | Baedford Basin | 327 454 | 61.9 | 9.8 | 407 377 | 49.8 | 17.0 |
| 159 | Brest - SOMLIT | North Atlantic Ocean | Celtic Sea | 327 901 | 61.8 | 30.7 | 443 747 | 45.7 | 22.9 |

TABLE 2

| Step | Step description | V4 | V9 |
|---|---|---|---|
| | Total number of sequences initially | 8 844 871 | 11 393 040 |
| 1 | Total number of sequence subsampled | 5 473 170 | 5 473 170 |
| | Total number of sequence subsampled (%) | 61.9 | 48.0 |
| | Total number of sequences per station | 202 710 | 202 710 |
| 2 | Unique sequences | 1 430 038 | 916 411 |
| 3 | Unique sequences after filtering (quality and size) | 203 214 | 103 068 |
| 4 | Unique sequences after chimera check and preclustering | 57 383 | 28 134 |
| 5 | Unique sequences after singleton removal | 53 530 | 26 370 |
| | Total number of sequences finally | 3 796 476 | 4 651 851 |
| 6 | OTUs (97% similarity) | 13 169 | 16 383 |

TABLE 3

| | V4 | | | | V9 | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Parameter | max. | min. | mean | SD | max. | min. | mean | SD | P value |
| OTU number | 2906 | 522 | 1216 | 579 | 3216 | 911 | 1620 | 617.65 | *5.92E-06* |
| Simpson Index | 0.63 | 0.99 | 0.91 | 0.08 | 0.63 | 0.99 | 0.92 | 7.30E-02 | 4.90E-02 |
| | | | | | (% of photosynthetic) | | | | |
| Ochrophyta | 91.1 | 19.3 | 60.3 | 18.1 | 87.6 | 20.4 | 64.1 | 19.8 | **0.4** |
| Chlorophyta | 69.6 | 3.9 | 26.4 | 17.2 | 55.2 | 2.3 | 19.9 | 15.5 | 6.33E-05 |
| Haptophyta | 30.2 | 0.1 | 7.9 | 8.6 | 37.7 | 0.3 | 11.7 | 10.5 | 8.19E-07 |
| Cryptophyta | 13.6 | 0.0 | 4.2 | 3.6 | 19.7 | 0.3 | 4.9 | 4.2 | 8.00E-03 |
| Bacillariophyta | 89.5 | 8.6 | 49.1 | 21.6 | 86.2 | 8.1 | 51.8 | 23.4 | 9.50E-02 |
| Dictyochophyceae | 35.2 | 0.0 | 4.4 | 7.5 | 30.1 | 0.0 | 3.3 | 6.1 | 2.90E-03 |
| Chryso-Synurophyceae | 24.3 | 0.1 | 3.4 | 4.8 | 16.7 | 0.1 | 3.2 | 3.7 | **0.5** |
| Pelagophyceae | 7.0 | 0.0 | 0.7 | 1.6 | 8.0 | 0.0 | 0.7 | 1.6 | ***0.56*** |
| Mamiellophyceae | 49.6 | 0.0 | 12.1 | 14.2 | 45.7 | 0.2 | 10.9 | 12.9 | **0.25** |
| Trebouxiophyceae | 53.5 | 0.0 | 4.9 | 11.2 | 39.3 | 0.0 | 2.3 | 7.5 | 0.023 |
| Chlorodendrophyceae | 68.1 | 0.0 | 4.9 | 13.0 | 53.0 | 0.0 | 3.1 | 10.1 | *2.50E-05* |
| Pyramimonadales | 6.4 | 0.0 | 1.7 | 2.0 | 7.6 | 0.0 | 1.7 | 1.9 | 9.60E-03 |
| Ulvophyceae | 4.9 | 0.0 | 0.6 | 1.2 | 3.9 | 0.0 | 0.4 | 0.9 | 4.60E-02 |
| Pseudoscourfieldiales | 17.1 | 0.0 | 1.2 | 3.5 | 9.0 | 0.0 | 0.6 | 1.8 | *1.00E-03* |
| | | | | | (% of Chlorophyta) | | | | |
| *Micromonas* | 34.9 | 0.0 | 4.8 | 7.6 | 32.0 | 3.00E-02 | 4.3 | 6.8 | **0.5** |
| *Mamiella* | 2.5 | 0.0 | 0.2 | 0.5 | 1.8 | 0.0 | 0.2 | 0.3 | ***0.3*** |
| *Ostreococcus* | 35.8 | 0.0 | 4.0 | 9.6 | 40.0 | 0.0 | 4.4 | 10.6 | ***0.5*** |
| *Bathycoccus* | 4.0 | 0.0 | 0.7 | 1.1 | 3.7 | 0.0 | 0.6 | 1.0 | ***0.6*** |