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1 **Picoeukaryotic photosynthetic potential is functionally redundant**
2 **but taxonomically structured at global scale**

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25

26 **Abstract**

27 Primary production, performed by RUBISCO, and often associated with carbon concentration
28 mechanisms, is of major importance in the oceans. Thanks to growing metagenomic resources
29 (e.g., eukaryotic Metagenome-Assembled-Genomes; MAGs), we provide the first reproducible
30 machine-learning-based framework to derive the potential biogeography of a given function,
31 through the multi-output regression of the standardized number of reads of the associated genes
32 on environmental climatologies. We use it to study the genomic potential of C4-photosynthesis of
33 picoeukaryotes, a diverse and abundant group of marine unicellular photosynthetic organisms. We
34 show that the genomic potential supporting C4-enzymes and RUBISCO exhibit strong functional
35 redundancy and an important affinity towards tropical oligotrophic waters. This redundancy is then
36 structured taxonomically by the dominance of Mamiellophyceae and Prymnesiophyceae in mid and
37 high latitudes. Finally, unlike the genomic potential related to most C4-enzymes, the one of
38 RUBISCO showed a clear pattern affinity for temperate waters.

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40 **Keywords:** carbon concentration mechanisms; metagenomic; biogeography; multivariate
41 boosted tree regressor; picoeukaryotes

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45 INTRODUCTION

46

47 Most of the photosynthetic production on earth relies on the ribulose-1,5-bisphosphate carboxylase
48 oxygenase (RUBISCO; 1). However, because RUBISCO emerged ~2 billion years ago in a period
49 characterized by low oxygen (2), its carboxylase function is surprisingly inefficient relative to its
50 oxygenase function, when considering the contemporary CO₂-to-oxygen ratio (3). To compensate
51 for this metabolic caveat related to RUBISCO-only photosynthesis (i.e., C₃-photosynthesis), carbon
52 fixation pathways evolved ~30 million years ago, when atmospheric CO₂ levels were estimated
53 under 200 ppm. The latter induced selective pressure towards higher carbon fixation efficiency,
54 leading to the development of various Carbon Concentration Mechanisms (CCMs; i.e., biophysical
55 or biochemical) to compensate for the photorespiration affinity of RUBISCO (4). Among
56 biochemical CCMs, C₄-enzymes independently evolved across a large variety of marine and
57 terrestrial lineages (4, 5). The C₄ cycle is performed through 3 acid-decarboxylation types, leading
58 to an increase of the CO₂-to-oxygen ratio at the active site of RUBISCO (6): the MDC-NADP type,
59 the MDC-NAD type, and the PEPCK type. The common enzyme to all C₄ acid decarboxylation
60 types is phosphoenolpyruvate carboxylase (PEPC), fixing CO₂ in the cytosol by producing
61 oxaloacetate. In the MDC-NADP type, oxaloacetate is transferred to the chloroplast and reduced
62 to malate. The latter is then decarboxylated, producing CO₂ and pyruvate, which is converted back
63 to phosphoenolpyruvate. In the MDC-NAD type, oxaloacetate is transferred to the mitochondria
64 and reduced to malate. The decarboxylation reaction transfers CO₂ to the chloroplast by producing
65 pyruvate that is transferred back to the chloroplast to be converted to phosphoenolpyruvate. Finally,
66 the PEPCK type directly converts the mitochondrial oxaloacetate to phosphoenolpyruvate.
67 However, it partially performs the MDH-NAD reduction and MDC-NADP decarboxylation reactions
68 to balance the ATP and NADPH budget, leading to common reactions and enzymes between acid-
69 decarboxylation types (6). In the terrestrial realm, both physiological measurements and stable
70 isotope techniques confirmed the presence of C₃-photosynthesis across a large range of
71 environmental conditions, conversely to C₄-photosynthesis that is adapted to warm, nutrient poor
72 and high irradiance conditions (7, 8). In the marine realm however, only a few studies explored the
73 environmental affinity of C₄-photosynthesis regarding terrestrial-based hypotheses (e.g., 5, 9, 10).
74 The potential for C₄-photosynthesis is highly suspected in key picoeukaryote lineages such as
75 Mamiellophyceae and Prymnesiophyceae. Currently, subcellular evidence for C₄-enzymes include
76 (i) MDC-NADP and PEPC in *Ostreococcus Tauri* (11), (ii) MDC-NADP, PEPC, three different
77 oxoglutarate-to-malate translocator and pyruvate phosphate dikinase (PEPDK) in various
78 *Micromonas* strains (12) and (iii) PEPC in Prymnesiophyceae (*Emiliana Huxleyi*; plastid presence
79 and gene encoding; , 13).

80

81 Marine carbon fixation is largely performed by picoeukaryotes (e.g., 30 to 50 % of global primary
82 production, 14, 15), some of which are suspected to use C₄-photosynthesis (e.g., in picoeukaryotic
83 diatoms; , 5, 9). Picoeukaryotes correspond to the unicellular eukaryotic marine plankton, that are
84 among the most diverse and abundant organisms in the sunlit layer of the world ocean (16–18). In
85 nutrient-poor areas, such as the oligotrophic open ocean, they locally contribute up to 80 % of the
86 phytoplanktonic biomass (19). However, because of their size (i.e., 0.8 to 5 μm), poor
87 representation in culture collections (20) and thus the difficulty for both physiological measurements
88 and stable isotope analysis in natural populations, the genomic potential supporting C₃, and C₄-
89 photosynthesis, its associated biogeography and functioning remains scarcely documented (5, 8,
90 9).

91

92 Recent global expeditions focusing on surface plankton sampling, together with advances in
93 metagenomic sequencing, provided unique data to address the genomic potential and
94 biogeography-related gaps (e.g., 21–24). In this context, metagenomics data are of growing interest
95 to explore the hidden taxonomic and functional diversity potentially related to carbon fixation in
96 picoeukaryotes (e.g., 25, 26). For example, genome-resolved metagenomics (27) based on the
97 *Tara-Oceans* eukaryotic metagenome led to the reconstruction of ~800 Metagenome-Assembled-

98 Genomes (MAGs; 28). The latter are defined as genome-based taxonomic units, functionally and
99 taxonomically annotated, and quantified by their associated genome-wide metagenomic reads.
100 Therefore, MAGs offer the unique opportunity to study the genomic potential supporting carbon
101 fixation and its biogeography, through both a functional and a taxonomic prism.
102

103 Habitat modelling is a popular niche theory-based tool to estimate species biogeography according
104 to the environmental conditions in which they are observed (29). Marine organisms are known for
105 their important sensitivity to their surrounding environmental conditions, influencing growth,
106 reproduction, and metabolic efficiency across all life stages (30). Thus, habitat modelling has been
107 widely used to project the past, present, and future biogeography across various marine organisms,
108 from zooplankton to fishes (e.g., 31). However, omics-based habitat modelling is still an emerging
109 field to explore functional and taxonomic biogeography associated with unicellular planktonic
110 organisms (32–34). Building on the above-mentioned properties associated with MAGs, habitat
111 modelling is transferable to genomic potential, thus exploring the quantitative response of the
112 associated taxonomic and functional gene annotations to environmental conditions.
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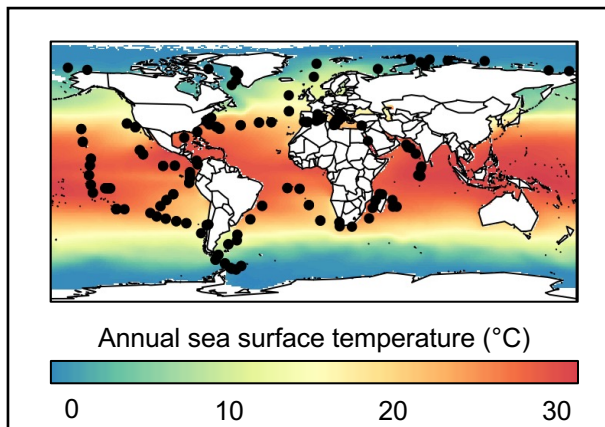
114 Here, complementing recent studies on prokaryote - environment relationships (32), we provide an
115 original, machine learning-based, comprehensive, and reproducible framework to derive the
116 biogeography of the genomic potential related to metabolic functions, from metagenomic-based
117 relative abundances data. Using Multivariate Boosted Tree Regressors (35), we simultaneously
118 project the biogeography of selected genomic functional annotations, while accounting both for
119 their interactions and environmental responses. We applied this framework to metagenome-based
120 Protein Functional Clusters (PFCs; hereafter referred to as “clusters”) linked to RUBISCO and C4-
121 enzymes only, in marine picoeukaryotes. Compared to a more traditional approach (i.e., searching
122 reads in a functional database using sequence similarity), our methodology combining MAGs and
123 PFCs offers several advantages. The quantitative signal resulting from a MAG is (i) standardized
124 by the genome length and (ii) correspond to a taxonomic identity. Combined in PFCs, (iii) it also
125 includes the fraction of signal corresponding to not yet annotated genes. Thus, this approach offers
126 a more robust quantitative framework than traditional approaches, representative of eukaryotic
127 plankton diversity in open oceans (39.1 billion reads recruited, ~97% identity, ~25 Gbp; , 28) and
128 transferable to a variety of functions or enzymes of interest using the already computed PFC
129 network. Finally, habitat modeling provides an interesting tool to estimate the response and co-
130 dominance patterns of C4-enzymes and RUBISCO to environmental conditions representative of
131 the global ocean, conversely to estimates from the samples only, that might be driven by sampling
132 and associated environmental biases.
133

134 RESULTS

136 2.1. C4-CCM enzymes across sampled stations

137
138 From the *Tara Oceans* eukaryotic MAGs, ~1.2 million clusters were built, for which 349 are related
139 to RUBISCO or C4-enzymes (**Fig. S1, Table S1**). This dataset corresponds to 817 unique genes,
140 with a median observed presence across 45 sampled stations per cluster. To avoid considering
141 enzymes related to other metabolic functions, we only selected those related to RUBISCO or C4-
142 enzymes only, corresponding to 240 clusters, distributed across the world Ocean except the Arctic,
143 western Pacific and to a lesser extent Southern Ocean (**Figure 1**). The successive cluster selection
144 criteria (i.e., PFCs exclusive to RUBISCO or C4-enzymes, minimum presence at 10 sampling
145 stations) did not present significant effects on the distribution of clusters across number of reads,
146 number of genes and taxonomic classes (**Fig. S3**). In contrast, we observed a loss of signal for the
147 MDCs (-NAD and -NADP), between functionally exclusive and non-exclusive clusters, highlighting
148 an important fraction of sequence homologs for these enzymes (**Fig. S3**).
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152

153 **Figure 1.** Location of the *Tara Oceans* (TO) sampling stations, represented as black dots. Annual
154 sea surface temperature from World Ocean Atlas (Boyer et al. 2018) are represented in
155 background.

156

157 2.2. Standardized distribution of the genomic potential related to C4-photosynthesis

158

159 Here we present projections for each C4-enzyme and RUBISCO. First, we rescaled the cluster-
160 level projections (i.e., model outputs; **Fig. S1D**) between 0 and 1 (i.e., distribution patterns, **Fig.**
161 **S2**). Then, we aggregated these patterns at the enzyme-level according to their respective
162 functional annotation. We therefore alleviated the propagation of the observed dominance of a
163 given cluster to the aggregated enzyme-level patterns. The resulting enzyme-level projections are
164 referred to as standardized patterns. For each enzyme, it represents a prediction of the genomic
165 potential according to the environmental conditions at each geographical location, and
166 independently of any taxonomic dominance.

167

168 Because most C4-enzymes are involved in several acid-decarboxylation types, we cannot directly
169 infer their corresponding distribution. However, MDC - NAD, MDC - NADP and PEPCK are
170 considered representative of their respective acid-decarboxylation types. We predicted similar
171 standardized patterns (**Figure 2**) for all acid decarboxylation types and RUBISCO. The
172 standardized patterns of all C4-enzymes presented medium to high pairwise Pearson's correlation
173 (0.5 to 0.9), except MDC - NAD and GOT which are weakly correlated (0.3).

174

175 We predicted a high genomic potential (> 0.6) for all standardized patterns in temperate to tropical
176 latitudes, with an associated coefficient of variation below 30 % (**Figure 2A**). We also predicted a
177 high potential (> 0.8) for RUBISCO and PEPDK for temperate to tropical waters only. In contrast,
178 the potential for PEPC, GOT, MDCs and MDHs were high in equatorial latitudes. These patterns
179 suggest a higher affinity of the genomic potential of C4-enzymes for the equatorial ocean, in
180 comparison to RUBISCO. Furthermore, we predicted low-to-moderate potential (between 0 and
181 0.4) in high latitudes (i.e., above polar circles) for all standardized patterns (**Figure 2A**). Predictions
182 in such latitudes also present important calibration and projection-related variability, with
183 coefficients of variations ranging from 30 to 100 % (e.g., for the MDH – NADP and PEPCK).
184 Therefore, our genomic potential predictions remain inconclusive in high latitudes, also subject to
185 lower sampling coverage.

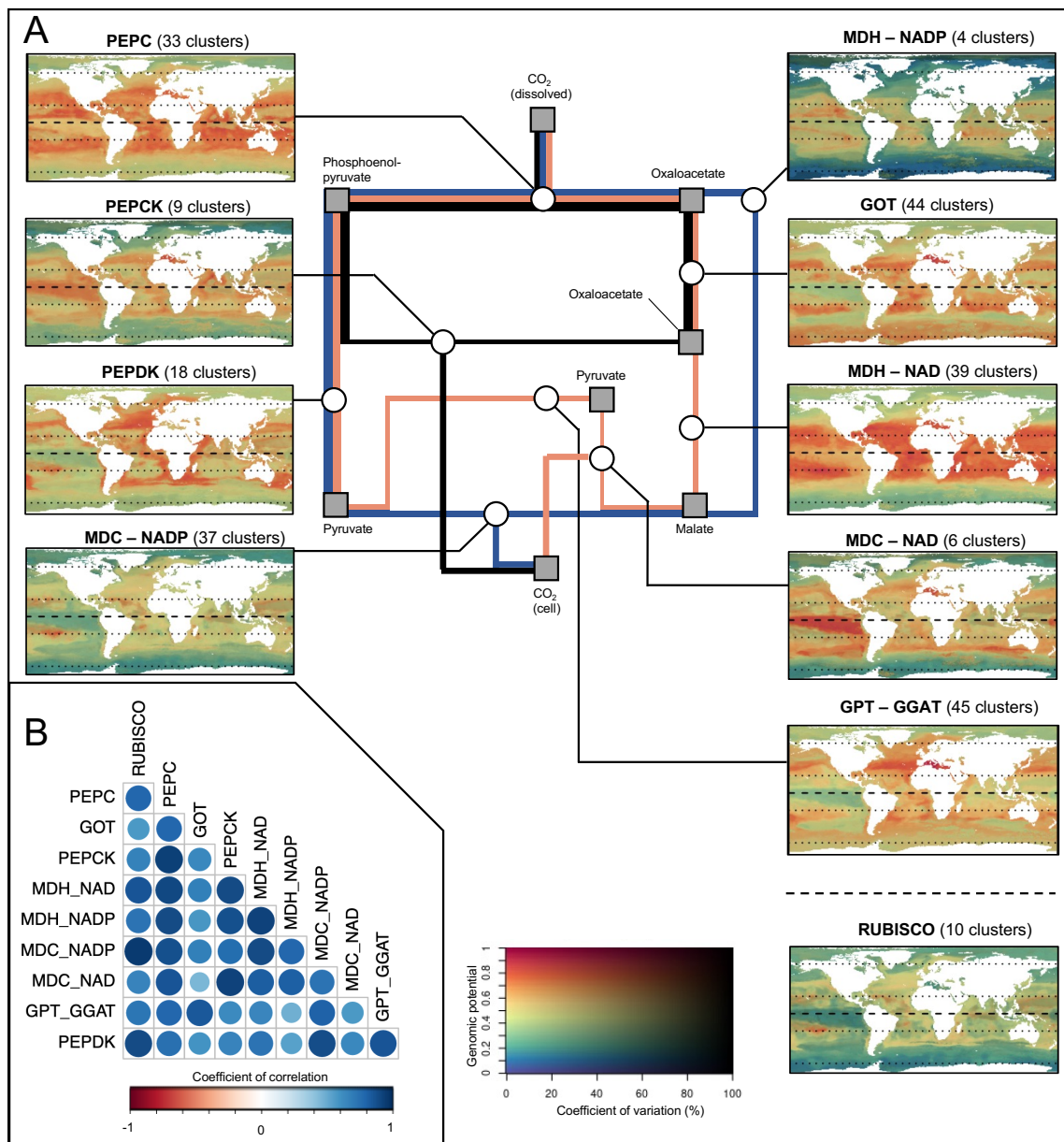
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Figure 2. Standardized patterns corresponding to the relative genomic potential supporting C4-enzymes and RUBISCO. **(A)** Synthetic diagram of the metabolic pathway and corresponding projections. **(B)** Inter-projections Pearson's spatial correlation index. The three main currently described acid-decarboxylation types are represented in blue (MDC-NADP), red (MDC-NAD) and black (PEPCK), respectively. Involved metabolic components and enzymes are indicated on the diagram by squares and circles, respectively. The 2D color scale represents the standardized genomic potential for the target enzyme as the hue value (Y-axis) and the associated coefficient of variation as the saturation (i.e., uncertainty in % of the mean; X-axis). An orange to red hue corresponds to region where environmental conditions yield a high proportion (>0.6) of the target genes in the model. A low saturation level corresponds to an important variance among the underlying cluster-level projections.

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The environmental variables importance in the trained model (**Fig. S4**) highlighted the predominant roles of dissolved oxygen concentration (contributing to 34% of the explained variance) and the yearly variability (i.e., inter-month standard deviation) in Salinity (29%) and, to a lesser extent, of oxygen saturation, chlorophyll a concentration and temperature. Furthermore, we revealed a strong affinity (i.e., maximum potential) of most standardized patterns (**Fig. S5**) for tropical, oligotrophic conditions (e.g., temperature between 15 to 30 °C; phosphate concentration below 0.5 μmol/kg). However, we predicted different responses to the variability in Chlorophyll a concentration and euphotic zone depth across enzymes (**Fig. S5**). Finally, we highlighted no taxonomic dominance across world oceans, according to the taxonomic composition associated to each cluster, suggesting a worldwide functional redundancy in the genomic potential supporting C4-enzymes (**Fig. S7**).

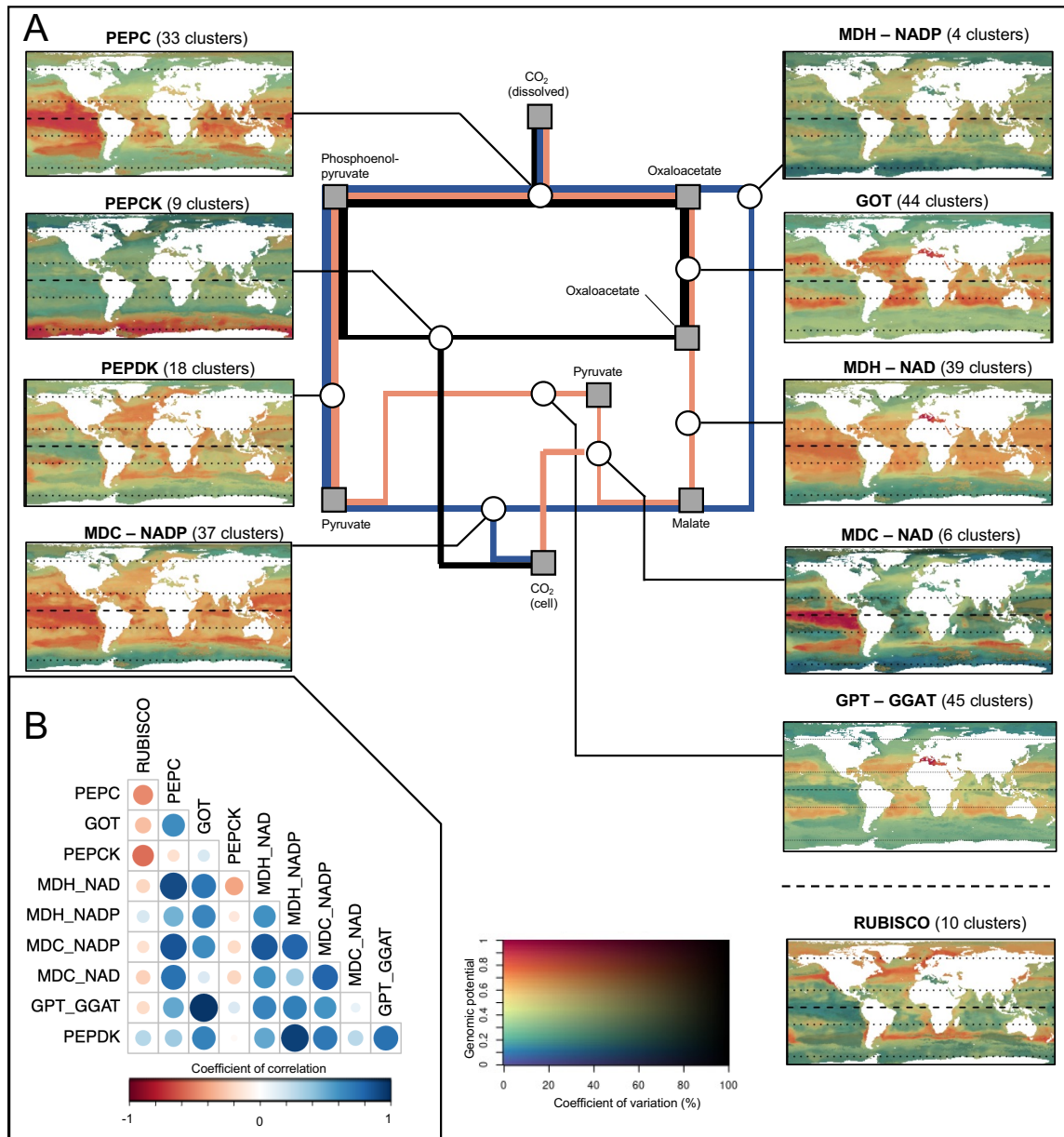
2.3. Weighted distribution of the genomic potential related to C4-photosynthesis

Here we present projections for each C4-enzyme and RUBISCO. First, we rescaled the cluster-level projections (i.e., model outputs; **Fig. S1D**) by their observed metagenomic read abundance (i.e., weighted distribution patterns, **Fig. S2**). Then, we aggregated these patterns at the enzyme-level according to their respective functional annotation. We therefore propagate the observed dominance of a given cluster (i.e., and associated taxa) to the aggregated enzyme-level patterns. The resulting enzyme-level projections are referred to as weighted patterns. For each enzyme, it represents the corresponding genomic potential (i.e., relative to the other considered enzymes), according to the environmental conditions at each geographical location.

We predicted contrasted weighted patterns between RUBISCO and across acid decarboxylation type (**Figure 3A**). Indeed, the weighted pattern of RUBISCO presented maximum potential in temperate areas (**Figure 3B**).

We predicted low-to-moderate potential (< 0.3) and moderate (~ 30 %) uncertainty in high latitudes for the weighted patterns of PEPC, MDCs, MDHs, and transferases (i.e., GOT and GPT – GGAT; **Figure 3A**). These patterns also presented moderate-to-high potential (between 0.5 and 1) in tropical areas, with some discrepancies. We show a Pearson's correlation index above 0.5 between the above-mentioned enzymes, and above 0.7 for GOT and MDHs (**Figure 3B**). The latter presented an important potential in oligotrophic regions (e.g., Pacific gyres), suggesting functional redundancy in the genomic potential from Oxaloacetate to Malate (**Figure 3A**). In contrast, we predicted a high potential (> 0.7) in eutrophic Pacific waters for the weighted patterns of MDCs (Pearson's correlation above 0.7; **Figure 3A**). Overall, we show high confidence in the areas associated to high genomic potential, with coefficient of variations lower than 30 % among all trained algorithms and 100-bootstrap projections. The above-mentioned weighted responses to environmental variables are similar to the ones highlighted in section 3.1., characterized by higher potential in warm, low seasonality, and generally oligotrophic water bodies (**Fig. S4** and **S6**).

Conversely, we predicted moderate to high intensity values in oligotrophic tropical areas, but most importantly in the Southern Ocean (> 0.5; **Figure 3**) for the weighted pattern of PEPC (i.e., a different acid decarboxylation type). The latter was preferentially distributed along water bodies characterized by (i) high seasonality of the Chlorophyll a concentration and the depth of the euphotic zone, (ii) high concentrations of oxygen (presenting the highest explanatory power in the model training; **Fig. S4**) and nutrients (e.g., phosphates and nitrates) and (iii) average temperatures below 8 °C (**Fig. S6**).



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Figure 3. Weighted patterns corresponding to the relative genomic potential supporting C4-enzymes and RUBISCO, re-scaled by the corresponding observed relative metagenomic reads abundance. **(A)** Synthetic diagram of the metabolic pathway and corresponding projections. **(B)** Inter-projections Pearson's spatial correlation index. The three mains currently described acid-decarboxylation types are represented in blue (Malate-NADP), red (Malate-NAD) and black (PEPCK), respectively. Involved metabolic components and enzymes are indicated on the diagram by squares and circles, respectively. The 2D color scale represents the weighted genomic potential for the target enzyme as the hue value (Y-axis) and the associated coefficient of variation as the saturation (i.e., uncertainty in % of the mean; X-axis). An orange to red hue corresponds to region where environmental conditions yield a high proportion (>0.6) of the target genes in the model. A low saturation level corresponds to an important variance among the underlying cluster-level projections.

273
274 Finally, we highlighted that weighted patterns associated with high latitudes (e.g., correlated with
275 the one of PEPCK) were composed at 28 % of Prymnesiophyceae and 50 % of Mamiellophyceae
276 (Shannon index of 1.5), based on the taxonomic composition of each cluster. Mamiellophyceae
277 also composed 40 % of the patterns with a clear temperate affinity (e.g., correlated with the one of
278 RUBISCO; **Fig. S8**). In contrast, we highlight a larger diversity of taxonomic classes, with a
279 Shannon index of 2.1, for patterns associated with equatorial latitudes.

280 281 **DISCUSSION**

282 283 3.1. Genomic potential for C4-CCM in picoeukaryotes

284
285 By selecting clusters (i.e., PFCs) annotated by C4-enzymes or RUBISCO only, we considered a
286 fraction of the available metagenomic information (i.e., ~67 % of the clusters related to C4-enzymes
287 or RUBISCO). In addition, genes related to other metabolic pathways may have responses to
288 environmental variables different from genes related to C4-enzymes, potentially including bias in
289 their corresponding PFC's projection. Therefore, selecting a reduced set of clusters alleviates the
290 risk of metabolic noise in the environmental responses, limited to the effect of C4-enzymes
291 potentially involved in other pathways (e.g., GPT-GGAT transporter).

292
293 Our study focused on planktonic picoeukaryotes, the photosynthetic fraction of which is generally
294 dominated by the Mamiellophyceae, Prasinophyceae, Prymnesiophyceae, Bacillariophyceae, and
295 Dinophyceae lineages in the open ocean (16, 20). The potential for C4-photosynthesis has been
296 suggested for several families, including Bacillariophyceae by combining C4-enzyme inhibition and
297 photosynthetic efficiency monitoring (e.g., PEPDK 36, PEPC and PEPCK, 37). Evidence for genes
298 encoding all C4-enzymes exist in *Micromonas* and *Ostreococcus*, Mamiellophyceae (38, 39). A
299 plastid PEPC enzyme was recently discovered in *Emiliana huxleyi* (38), a Prymnesiophyceae
300 abundant in temperate and polar regions (40). However, to our knowledge, no study provided
301 univocal evidence for C4-CCM usage in situ. Stable isotope measurements would be necessary to
302 fully understand C4-photosynthesis in picoeukaryotes, but they are difficult to apply at species-level
303 in natural, uncultured, plankton communities (e.g., 8, 10). Alternatively, recent literature suggests
304 the need for further studies on deep chlorophyll a maxima and various transporters (e.g.,
305 bicarbonate transporters), some of which are associated with or specific to C4 metabolism, to better
306 understand C4-CCM in natural populations (5, 6).

307
308 Complementing these experimental approaches, we use a data-driven approach to shed more light
309 on the environmental drivers of C4-genes in marine picoeukaryotes. However, MAGs integrate
310 chloroplast and mitochondrial genes corresponding to C4-enzymes but do not distinguish their
311 origin (28), nor provide information on the subcellular location of the corresponding enzymes (9,
312 41). Therefore, the patterns presented here must be interpreted as the potential for the (co-)
313 presence of those pathways in the genome. They should be complemented by culture-based
314 studies, locating enzymes within cells and/or performing carbon isotope discrimination to confirm
315 C4-CCM presence, expression, and its co-existence with C3-photosynthesis in picoeukaryote
316 lineages (8). The present study could be used to locate regions where such mechanisms are most
317 likely to occur.

318 319 3.2. Environment-driven genomic potential

320
321 The modeled distribution patterns revealed that the genomic potential for C4-photosynthesis is
322 more associated with tropical oligotrophic and annually stratified waters. Conversely, the proportion
323 of reads related to RUBISCO (i.e., considered as a representative of all photosynthetic pathways,
324 due to its central role in C3, C4 and CAM photosynthesis) is higher in temperate regions (**Figure**
325 **2A**). The fact that terrestrial C4-plants (4) and the genomic potential for C4-CCM in picoeukaryotes
326 display similar latitudinal distribution, around the tropics, does not imply that the environmental

327 drivers of those distributions are the same. In terrestrial plants, C4-CCMs are considered as an
328 adaptation to drought and are, for example, also associated with a specific leaf structure that
329 reduces their water consumption (4). Drought is of course not an evolutionary driver for marine
330 picoeukaryotes. Alternatively, they present an important surface-to-cytoplasm ratio (i.e., small cells
331 or presence of a vacuole, 42, 43) leading to a high nutrient absorption yield, which is adapted to
332 oligotrophic waters, common in the tropical ocean.

333
334 In addition to environmental conditions, the biogeography of the genomic potential supporting C4-
335 CCM may also relate to irradiance levels, largely controlling ATP generation, necessary to the
336 decarboxylation reaction (42). Indeed, C4-CCM requires additional ATP generation to increase the
337 RUBISCO efficiency in comparison to classical C3-photosynthesis, without impacting the energy
338 available for the latter (42, 44). In contrast, an excess of ATP may lead to photoinhibition, thus
339 lower carbon fixation efficiency (36, 45). Therefore, it has been suggested that C4-photosynthesis
340 is particularly adapted to dissipate excess energy in the cell in high irradiance areas such as tropical
341 oceans (5, 36). Our weighted patterns highlighted differences between PEPCK and MDCs (**Figure**
342 **3**). The latter require 2 extra ATP compared to the C3 carbon fixation to complete the pathway. In
343 a logical way, the PEPCK acid decarboxylation type, which only requires 1 extra ATP and thus is
344 supposed to be more efficient in low irradiance environments (44), showed here the highest
345 genomic potential in polar or sub-polar regions.

346

347 3.3. Functional and ecological implications

348

349 We highlighted functional redundancy among C4-genes in oligotrophic tropical waters (**Fig. S7**).
350 This contrasts with high latitudes, where only a few taxa dominate (**Fig. S8**) (17, 46). More
351 interestingly, we highlighted a biogeographical differentiation between the weighted pattern of
352 RUBISCO – i.e., the baseline photosynthetic enzyme – and those of C4-enzymes. Since 30 million
353 years ago, atmospheric CO₂ concentration has drastically reduced from c.a. 1000 ppm to less than
354 200 ppm 20.000 years ago, resulting in lower dissolved carbon in the oceans (4). This led to a
355 selective pressure towards efficient photosynthetic metabolism, like C4-photosynthesis (7) or, in a
356 lesser extent, RUBISCO of higher carboxylation affinity (e.g., type II in Dinoflagellates, 9). While
357 the evolution of C4-CCM in marine organisms is not yet fully understood, 48 independent evolutions
358 of C4-CCM were identified in the genome of terrestrial plants (e.g., grasses, Caryophyllales, 4),
359 suggesting a higher genomic potential for C4-photosynthesis in taxonomically diverse areas (7).
360 The above-mentioned functional redundancy in the genomic potential for C4-CCM in taxonomically
361 rich tropical waters may relate to a co-evolution between taxonomic diversification and its
362 associated functions (i.e., neutral theory). However, the functional diversity among C4 acid-
363 decarboxylation types may also reflect – or be amplified by – a selection process, as it may present
364 a selective advantage. Moreover, the respective dominance of Mamiellophyceae in temperate
365 latitudes (i.e., correlated with the patterns associated to RUBISCO) and Prymnesiophyceae in polar
366 latitudes, are concordant with the literature (40, 47), thus validating the environmental predictors
367 controlling their biogeography. We identified key environmental predictors shaping the
368 biogeography and (co-)dominance patterns of the genomic potential supporting C4-enzymes and
369 RUBISCO. Such results open new perspectives of exploring the relationship between functional
370 and taxonomic diversity in the oceans, complementing already diverse approaches and data types,
371 and better understand the environmental drivers of key biogeochemical cycles in the current and
372 future climatic context.

373

374 **MATERIAL AND METHODS**

375

376 4.1. Data

377 4.1.1. Genomic data

378

379 We studied the biogeography of the genomic potential related to C4-CCM through the prism of
380 Metagenomic Assembled Genome (MAG, 28) retrieved from the *Tara Oceans* expedition (2009-

2013). Briefly, 280 billion reads from 798 metagenomes, corresponding to the surface and deep chlorophyll maximum layer of 210 stations from the Pacific, Atlantic, Indian, Southern and Arctic Oceans, as well as the Mediterranean and Red Seas (**Figure 1**), encompassing eukaryote-enriched plankton size fractions ranging from 0.8 μm to 2 mm, were used as inputs for 11 metagenomic co-assemblies (6–38 billion reads per co-assembly) using geographically bounded samples. We thus created a culture-independent, non-redundant (average nucleotide identity <98%) genomic database for eukaryotic plankton in the sunlit ocean consisting of 683 MAGs and 30 single-cell genomes (SAGs), all containing more than 10 million nucleotides for a total size of 25.2 Gbp and encoding for 10,207,450 genes. Then, a sequence similarity network was built out using the 683 manually curated MAGs following a similar methodology to the one developed in Faure et al. (32). A pairwise comparison was computed between each protein sequence. The resulting alignment was then filtered, removing self-hits and pairs showing less than 80% of sequence identity and coverage. Resulting Protein Functional Clusters (PFCs, as in 32) were built, hereafter referred to as clusters. The functional annotation performed with eggNOG mapper v2.1.5 was added on the sequences, and the functional homogeneity was checked in each cluster (48, 49). The surface and metagenomic samples correspond to 130 stations.

4.1.2. Environmental data

For each of the 130 selected *Tara Oceans* metagenomic surface samples, we retrieved a set of monthly, global scale, environmental climatologies encompassing the 2005 to 2017 period, at a spatial resolution of $1^\circ \times 1^\circ$ (**Table S2**). The latter corresponds to the available climatology encompassing the sampling period (2009-2013), where we considered temporal environmental variations negligible in comparison to spatial environmental gradients. They correspond to a restricted set of factors characterizing the water body (e.g., oligotrophic, eutrophic) and related to C4-photosynthesis, for which we calculated the yearly average and yearly standard deviation (i.e., proxy of seasonal variations).

4.2. Data selection and pre-processing

4.2.1. Protein functional cluster selection

We first selected a reduced set of clusters, within the 0.8 to 5 μm size fraction and surface samples, for which 100% of the KEGG Orthology (KO, 50) annotated protein members were related to C4-enzymes or RUBISCO (**Fig. S1, Table S1**). To avoid model over-parameterization and because rare clusters were assumed as not influencing the large-scale patterns investigated in this study, we only considered clusters that were present in a minimum of 10 *Tara Oceans* stations.

The corresponding dataset contained 240 clusters distributed across 130 *Tara Oceans* stations. The 240 clusters, functionally annotated with C4-enzymes and RUBISCO, were associated with 234 MAGs. The latter presented an average completeness estimate of 57% (**Table S3**). In comparison, the average completeness estimate across all MAGs from Delmont et al. (28) yield at 37 %. As a supplementary quality check, we estimated a minimum horizontal coverage (i.e., number of bases of a MAG covered with a certain depth) of 68 % for each of the 234 MAGs (**Table S3**). Finally, we show that our MAGs are associated with an average BUSCO completeness (i.e., the percentage of mapped BUSCO genes in each MAG) of 55.7% (**Table S3**). We therefore consider these MAGs of sufficient quality for identifying C4-genes across our samples.

To reduce the number of response variables (clusters; PFCs) to a reasonable amount for multivariate modelling, with respect to the limited number of stations, we performed an Escoufier dimensional reduction (51). The latter iteratively selects the clusters whose pattern across stations minimize the residual variance of the dataset. Here, we selected 50 clusters that represent over 95% of the 240 clusters variance to be included in the multivariate algorithm.

435 4.2.2. Metagenomic data pre-processing

436

437 Genes abundances among samples were determined by mapping raw metagenomic reads against
438 the gene database (28). Briefly, reads were mapped using the bwa tool, and only random best
439 matches with at least 95% of sequence identity over at least 80% of the read length were retained
440 as positive. To alleviate the effect of gene length and sequencing effort variability between samples
441 on the number of reads, we normalized the metagenomic reads by the length of the corresponding
442 gene coding part and the total number of reads per station (i.e., including reads of all non-
443 considered clusters), respectively. Because the total genomic material present at each sampling
444 station is unknown (i.e., non-exhaustive sampling and sequencing effort), the absolute number of
445 reads is not comparable among stations. To compare the abundance between selected clusters at
446 different sampling stations, we transformed the dataset to relative abundance (**Supplementary**
447 **information text** and **Fig. S1**).

448

449 4.3. Multivariate Boosted Regression Tree

450 4.3.1. General principle

451

452 Recently, growing interest for interactions between response variables led to the development of
453 multivariate machine learning algorithms, such as Multivariate Boosted Tree Regressors (MBTR,
454 35). The latter is also particularly adapted to small sample size as the interactions between
455 response variables is considered as supplementary information to calibrate the model. Here, MBTR
456 is used to model the relationship between climatologies and metagenomic relative abundance (i.e.,
457 summed at 1 for each station; **Supplementary information text** and **Fig. S1**). To best reproduce
458 the response of metagenomic reads (i.e., response variable) to the corresponding environmental
459 variables (i.e., explanatory variable), the model sequentially fits decision trees (i.e., boosting
460 rounds) using gradient descent to minimize a specific loss function (see **Supplementary**
461 **information text** for hyperparameter and loss choice). At each boosting round, the algorithm fits a
462 decision tree on the residuals of the previous boosting round and computes a tree loss (i.e., a
463 measure of deviation between observed and predicted response variable values). Decision trees
464 are constructed using the hessian of the loss function (i.e., second order tensor of its partial
465 derivatives) to minimize the loss gradient. Therefore, the information learned by the n^{th} tree is
466 passed to the $n+1^{th}$ tree at a user-defined learning rate (**Supplementary information text** and **Fig.**
467 **S1**). The ensemble of sequentially fitted decision trees are considered in the model until the
468 minimum loss is reached. Finally, one important feature of MBTR is the conservation of the initial
469 correlation structure between the response variables (see methods in 35). The latter is tested by
470 computing a Pearson correlation matrix between response variables before and after model fitting,
471 whose conservation is tested by a Mantel matrix comparison test (**Supplementary Information**
472 **text**).

473

474 4.3.2. Model training and evaluation

475

476 To avoid over-fitting, the explanatory and response datasets were split between training set and
477 test set using a n -fold cross-validation procedure. For each model, n algorithms were trained on
478 different $n-1$ folds, while the remaining fold was used for testing only (i.e., computing the loss at
479 each boosting round). To minimize the effect of spatial and temporal autocorrelation in our data
480 (i.e., leading to over-optimistic model evaluation, 52), the n -folds were defined according to the
481 *Tara Oceans* station number. Because the cruise followed a continuous trajectory in time and along
482 the sampled stations, the resulting folds are spatially and temporally distant (i.e., spatial and
483 temporal block splitting, as recommended in 52). The resulting n -algorithms predictions were
484 aggregated in an average response and its corresponding coefficient of variation (CV). The ability
485 of the final model to reproduce the observed clusters relative abundance across environmental
486 conditions has been measured by the R^2 criteria and the root mean square error (RMSE, between
487 0 and 1 according to the distribution pattern scale).

488

489 *4.3.3. Spatial projections*

490

491 To better estimate projection uncertainty, our spatial projections were constructed using a bootstrap
492 procedure. For each 100-bootstrap round, we first re-sampled the original dataset (i.e., train and
493 test response dataset and corresponding explanatory variable values) with replacement. Then, we
494 re-fitted an MBTR algorithm on the re-sampled data by using the hyperparameters corresponding
495 to the validated model, including the number of boosting rounds corresponding to the minimum loss
496 across all n -algorithms. Finally, the re-fitted MBTR algorithm was used to predict the relative
497 abundance of clusters worldwide, using the corresponding climatologies values at each
498 geographical cell.

499

500 4.4. From model projections to final outputs

501

502 We only modelled the 50 clusters representing 95% of the dataset variability. Therefore, we
503 indirectly reconstructed the projections of the 190 others by identifying their most representative
504 Escoufier-selected cluster. To this extent, we performed a correspondence analysis based on the
505 observed relative abundance of all clusters. By using the dimensions of the correspondence
506 analysis space corresponding to a minimum of 80% variance explained, we calculated the
507 Euclidean distance between each non-selected cluster, and its nearest neighbor selected by the
508 Escoufier criteria. Because the 50 Escoufier selected clusters represented over 95% of the dataset
509 variability, we considered that a cluster and its nearest neighbor in the correspondence analysis
510 space share the same relative abundance pattern. In addition, we calculated the scale of each non-
511 selected cluster with respect to their nearest Escoufier-selected neighbors using the sum of their
512 observed relative abundance across all stations (**Fig. S2**). We then reconstructed the spatial
513 projections of the 190 clusters not considered in MBTR according to their projected nearest
514 Escoufier-selected neighbor. The resulting 240 cluster-level projections of the genomic potential
515 were then aggregated at the enzyme level according to their functional annotation (see Result
516 section, **Fig. S2**).

517

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Fundings:

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Author Contributions:

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Competing Interests:

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Data availability

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Code availability

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All R and Python codes, the corresponding pipeline, libraries, and associated technical documentation are available in the Blue-Cloud catalogue at: <https://data.d4science.net/qa7Z>

Instructions on how to build the Sequence Similarity Network and associated Protein Functional Clusters are available at: <https://data.d4science.net/BN9t> . Instructions and credentials on how to access the genomic database used in this study (PostgreSQL) are available in the technical documentation at <https://data.d4science.net/qa7Z> .

The ensemble of enzyme-level projections are available upon registration at [\[https://data.d4science.net/Zraq\]](https://data.d4science.net/Zraq). Additional data that support the findings of this study are available from the corresponding author upon reasonable request.

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