



**HAL**  
open science

## Functional redundancy of seasonal vitamin B 12 biosynthesis pathways in coastal marine microbial communities

Maxime Beauvais, Philippe Schatt, Lidia Montiel, Ramiro Logares, Pierre E Galand, François-yves Bouget

► **To cite this version:**

Maxime Beauvais, Philippe Schatt, Lidia Montiel, Ramiro Logares, Pierre E Galand, et al.. Functional redundancy of seasonal vitamin B 12 biosynthesis pathways in coastal marine microbial communities. *Environmental Microbiology*, 2023, 25 (12), pp.3753-3770. 10.1111/1462-2920.16545 . hal-04766744

**HAL Id: hal-04766744**

**<https://hal.science/hal-04766744v1>**

Submitted on 5 Nov 2024

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.



L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial - NoDerivatives 4.0 International License

## RESEARCH ARTICLE

# Functional redundancy of seasonal vitamin B<sub>12</sub> biosynthesis pathways in coastal marine microbial communities

Maxime Beauvais<sup>1</sup>  | Philippe Schatt<sup>1</sup> | Lidia Montiel<sup>2</sup> | Ramiro Logares<sup>2</sup> | Pierre E. Galand<sup>3</sup>  | François-Yves Bouget<sup>1</sup>

<sup>1</sup>Sorbonne Université, CNRS, Laboratoire d'Océanographie Microbienne (LOMIC), Observatoire Océanologique de Banyuls, Banyuls sur Mer, France

<sup>2</sup>Department of Marine Biology and Oceanography, Institute of Marine Sciences (ICM-CSIC), Barcelona, Spain

<sup>3</sup>Sorbonne Université, CNRS, Laboratoire d'Écogéochimie des Environnements Benthiques (LECOB), Observatoire Océanologique de Banyuls, Banyuls sur Mer, France

## Correspondence

François-Yves Bouget, Sorbonne Université, CNRS, Laboratoire d'Océanographie Microbienne (LOMIC), Observatoire Océanologique de Banyuls, Banyuls sur Mer, France.  
Email: [francois-yves.bouget@obs-banyuls.fr](mailto:francois-yves.bouget@obs-banyuls.fr)

Pierre E. Galand, Sorbonne Université, CNRS, Laboratoire d'Écogéochimie des Environnements Benthiques (LECOB), Observatoire Océanologique de Banyuls, Banyuls sur Mer, France.  
Email: [pierre.galand@obs-banyuls.fr](mailto:pierre.galand@obs-banyuls.fr)

## Funding information

Agence Nationale de la Recherche (ANR)

## Abstract

Vitamin B<sub>12</sub> (cobalamin) is a major cofactor required by most marine microbes, but only produced by a few prokaryotes in the ocean, which is globally B<sub>12</sub>-depleted. Despite the ecological importance of B<sub>12</sub>, the seasonality of B<sub>12</sub> metabolisms and the organisms involved in its synthesis in the ocean remain poorly known. Here we use metagenomics to assess the monthly dynamics of B<sub>12</sub>-related pathways and the functional diversity of associated microbial communities in the coastal NW Mediterranean Sea over 7 years. We show that genes related to potential B<sub>12</sub> metabolisms were characterized by an annual succession of different organisms carrying distinct production pathways. During the most productive winter months, archaea (*Nitrosopumilus* and *Nitrosopelagicus*) were the main contributors to B<sub>12</sub> synthesis potential through the anaerobic pathway (*cbi* genes). In turn, *Alphaproteobacteria* (HIMB11, UBA8309, *Puniceispirillum*) contributed to B<sub>12</sub> synthesis potential in spring and summer through the aerobic pathway (*cob* genes). Cyanobacteria could produce pseudo-cobalamin from spring to autumn. Finally, we show that during years with environmental perturbations, the organisms usually carrying B<sub>12</sub> synthesis genes were replaced by others having the same gene, thus maintaining the potential for B<sub>12</sub> production. Such ecological insurance could contribute to the long-term functional resilience of marine microbial communities exposed to contrasting inter-annual environmental conditions.

## INTRODUCTION

Marine planktonic communities strongly influence global biogeochemical cycles (Falkowski et al., 2008), and it is now well established that their spatiotemporal dynamics are, in part, driven by the physico-chemical properties of the surrounding environment (Auladell et al., 2022; Fuhrman et al., 2006; Galand et al., 2010; Lambert et al., 2019). However, biotic interactions also shape the structure and the functions of microbial communities (Ahlgren et al., 2019; Chow et al., 2014;

Frischkorn et al., 2018; Han et al., 2022; Joglar et al., 2021; Lima-Mendez et al., 2015; Nemergut et al., 2013; Strom, 2008). Prokaryotic and eukaryotic microorganisms continuously interact by exchanging beneficial metabolites such as nutrients, growth factors, or vitamins (Kouzuma & Watanabe, 2015; Seymour et al., 2017). Among vitamins, vitamin B<sub>12</sub> (cobalamin) appears as a key factor controlling the growth of photosynthetic planktonic communities as demonstrated experimentally (Bertrand et al., 2007; Koch et al., 2011, 2013; Panzeca et al., 2006; Sañudo-Wilhelmy

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial-NoDerivs](https://creativecommons.org/licenses/by-nc-nd/4.0/) License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2023 The Authors. *Environmental Microbiology* published by Applied Microbiology International and John Wiley & Sons Ltd.

et al., 2006). Genomic and culture-based approaches suggest, however, that most eukaryotes are auxotrophs toward B<sub>12</sub> including more than a half of phytoplankton species, compared to about 25% and 8% for thiamin (vitamin B<sub>1</sub>) and biotin (vitamin B<sub>7</sub>), respectively (Croft et al., 2005; Sañudo-Wilhelmy et al., 2014). This high incidence of B<sub>12</sub> auxotrophy is also observed within many marine bacterial lineages, including ubiquitous prokaryotes that are highly abundant in the global ocean, such as members of the SAR11 clade (Gómez-Consarnau et al., 2018; Sañudo-Wilhelmy et al., 2014; Shelton et al., 2019).

Although many microorganisms have an absolute requirement of B<sub>12</sub>-related coenzymes, vitamin B<sub>12</sub> is a scarce micronutrient in the ocean. Several studies have shown that most oceanic and coastal areas of the Pacific Ocean, the Atlantic Ocean, and the Mediterranean Sea are largely B<sub>12</sub>-depleted (picomolar concentrations) and that their bioavailability varies with depth and seasons (Barber-Lluch et al., 2021; Bonnet et al., 2013; Fiala, 1982; Joglar et al., 2021; Koch et al., 2012; Sañudo-Wilhelmy et al., 2012; Suffridge et al., 2018). Auxotrophic organisms thus rely mainly on B<sub>12</sub> de novo biosynthesis, and co-culture experiments have notably shown that some marine bacteria can satisfy the vitamin requirement of auxotrophic organisms via close mutualistic interactions (Cooper et al., 2019; Croft et al., 2005, 2006; Grant et al., 2014; Kazamia et al., 2012). However, B<sub>12</sub> de novo biosynthesis is restricted to certain prokaryotes including some heterotrophic bacteria (essentially *Proteobacteria*), chemoautotrophic archaea, and phototrophic cyanobacteria (Doxey et al., 2015; Gómez-Consarnau et al., 2018; Sañudo-Wilhelmy et al., 2014; Shelton et al., 2019). More precisely, targeted characterizations of B<sub>12</sub> biosynthesis genes in the Ross Sea identified members of *Gammaproteobacteria* (i.e., *Oceanospirillales*) as putative B<sub>12</sub> synthesizers in the Southern Ocean surface waters (Bertrand et al., 2011, 2015). Metagenomics studies also suggest that *Thaumarchaeota* may have an important role in B<sub>12</sub> biosynthesis in the deeper ocean and the Arctic (Doxey et al., 2015; Heal et al., 2017). They were, however, not the main synthesizers during a marine dinoflagellate bloom, during which *Alphaproteobacteria* and *Gammaproteobacteria* (*Oceanospirillaceae*) produced B<sub>12</sub> (Zhou et al., 2020). A metatranscriptomic study carried out in the Atlantic Ocean quarterly, over 1 year, more precisely identified members of *Rhodobacterales* as the main potential B<sub>12</sub> producers (Gómez-Consarnau et al., 2018). Even though several vitamin B<sub>12</sub> producers have been identified punctually at different geographic locations, the extent of their diversity and dynamics over time remains to be explored.

B<sub>12</sub> biosynthesis is a complex metabolic process involving about 20 genes and 30 enzymatic steps (Balabanova et al., 2021; Fang et al., 2017; Martens

et al., 2002; Raux et al., 2000) (Figure 1). Within bacteria, B<sub>12</sub> can be produced through two different ways, called the aerobic (*cob* genes) and anaerobic (*cbi* genes) pathways (Balabanova et al., 2021; Sañudo-Wilhelmy et al., 2014; Shelton et al., 2019), while in archaea, only the anaerobic one is used (Doxey et al., 2015; Fang et al., 2017) (Figure 1A). In contrast to bacteria and archaea, the abundant and globally distributed marine cyanobacteria *Synechococcus* and *Prochlorococcus* rather produce pseudo-cobalamin (named pseudo-B<sub>12</sub>) (Helliwell et al., 2016) (Figure 1B), an analog of cobalamin thought to support lower growth yields in many eukaryotic algae (Guillard, 1968; Heal et al., 2017; Helliwell et al., 2016; Provasoli & Carlucci, 1974). Algal and bacterial pseudo-B<sub>12</sub> remodelers can, however, remodel pseudo-B<sub>12</sub> into vitamin B<sub>12</sub> in an energy-cost-effective way through the *cobC-STU* genes (Heal et al., 2017; Helliwell et al., 2016; Ma et al., 2020) (Figure 1B). The remodelling of pseudo-B<sub>12</sub> requires exogenous DMB (5,6-dimethyl benzimidazole), suggesting that DMB bioavailability in seawater could also impact B<sub>12</sub> cycling in the ocean, and influence the composition of microbial communities (Wienhausen et al., 2022).

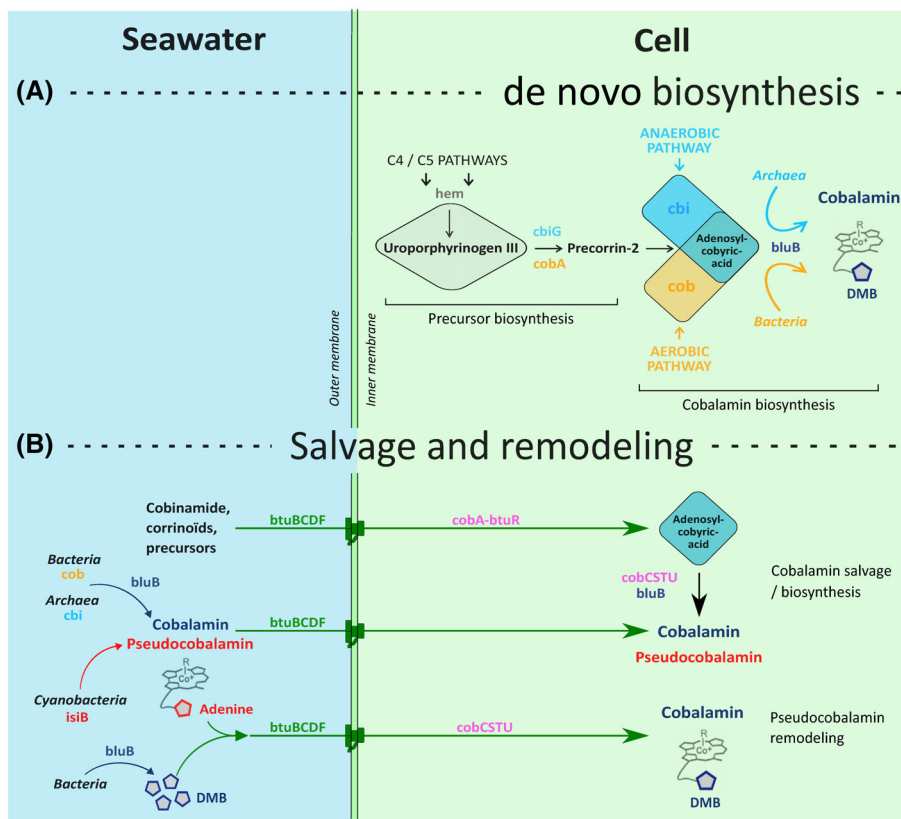
Despite their ecological importance, vitamin B<sub>12</sub> producers and the pathways involved remain poorly studied in marine microbial communities, and their seasonality over several years is not known. The overall aim of this study was to identify the B<sub>12</sub> production pathways present in the coastal NW Mediterranean Sea and test if they show reproducible seasonal patterns. We conducted a 7-year monthly metagenomic time series survey at the SOLA station to monitor the dynamics of genes associated with vitamin B<sub>12</sub> metabolisms (biosynthesis, salvage, and remodelling) and to identify the key taxonomic groups involved in these processes throughout the year. We further verify if environmental perturbations disrupt the vitamin B<sub>12</sub> seasonality.

## EXPERIMENTAL PROCEDURES

### Seawater sampling, environmental parameters monitoring, and DNA extraction and sequencing

Surface seawater (3 m depth) was sampled at the Banyuls Bay microbial observatory (SOLA; 42°31'N, 03°11' E; Northwestern Mediterranean Sea, France) from January 2009 to December 2015. Water sampling, filtration, DNA extraction, and monitoring of environmental parameters have been described elsewhere (Lambert et al., 2019).

DNA samples taken between 2009 and 2011 were shotgun sequenced using Illumina NovaSeq6000 (2 × 150 bp) at the Centre National d'Analisi



**FIGURE 1** Schematic overview of vitamin B<sub>12</sub> de novo biosynthesis, salvage, and remodelling pathways (Balabanova et al., 2021; Fang et al., 2017; Zhou et al., 2021). (A) Vitamin B<sub>12</sub> de novo biosynthesis occurs in the cell (right part; green) and requires the prior generation of the precursor uroporphyrinogen III via the C4 and C5 pathways, which involve the *hem* genes (grey). Depending on the organisms, *cobA* or *cysG* genes' activities catalyse the synthesis of precorrin-2 from uroporphyrinogen III, which can be metabolized in vitamin B<sub>12</sub> in two distinct ways: via the aerobic pathway requiring the *cob* genes present in bacteria, or via the anaerobic pathway relying on the *cbi* genes in archaea (coloured in orange and blue, respectively). Both pathways converge towards adenosylcobyrinic acid, which is finally converted into vitamin B<sub>12</sub>. Archaea and bacteria produce cobalamin, a form of vitamin B<sub>12</sub> that has a dimethylbenzimidazole (DMB) molecule as a lower axial ligand, which is produced through the activity of the gene *bluB* (coloured in dark blue), and then fused to the corring ring to form cobalamin. (B) Molecules structurally close to vitamin B<sub>12</sub> (e.g., cobinamide and other corrinoids) can be directly scavenged from the seawater (left part; blue) through specific transporters such as *btuBCDF* in bacteria (coloured in green). After translocation, these molecules are metabolized in the cell by specific enzymes that ensure the generation of adenosylcobyrinic acid, such as ATP:co(I)rrinoid adenosyltransferases (ACATS) encoded by *cobA-btuR* gene (coloured in pink). Adenosylcobyrinic acid will integrate the biosynthesis pathway and will be transformed into vitamin B<sub>12</sub> by several enzymes encoded by the *cobCSTU* genes (coloured in pink). Cyanobacteria produce pseudo-cobalamin (i.e., pseudo-B<sub>12</sub>) which has an adenine in the lower axial ligand and is not bioactive for most marine microorganisms. The flavodoxin gene *isiB* (coloured in red) is an orthologue of the cobalt reductase *fldA* found in some B<sub>12</sub>-producing bacteria and thus is supposed to be involved in pseudo-B<sub>12</sub> biosynthesis in cyanobacteria (Walworth et al., 2018). Salvaged pseudo-B<sub>12</sub> can be remodelled intracellularly to vitamin B<sub>12</sub> by some microalgae and bacteria that possess *cobCSTU* genes (e.g., *Pavlova* and *Vibrio*) (Helliwell et al., 2016; Ma et al., 2020). In seawater, pseudo-B<sub>12</sub> remodelling requires DMB bioavailability.

Genomica (CNAG), while DNA samples taken between 2012 and 2015 were sequenced using Illumina HiSeq2500 (2 × 100 bp) (Galand et al., 2018). The raw sequences were deposited in the NCBI Sequence Read Archive under accession number PRJEB66489 and PRJEB26919.

### Functional and taxonomic identification of prokaryotic sequences

Metagenomic reads were trimmed with CUTADAPT v1.16 (Martin, 2011), removing adapters and low-quality regions. Clean reads were individually

assembled with MEGAHIT (D. Li et al., 2015). Genes were predicted with Prodigal v2.6.3 and MetaGeneMark v3.38 (Hyatt et al., 2010; Zhu et al., 2010). To remove redundancy, genes were clustered at 95% identity and 90% coverage with Linclust v10 (Steinegger & Söding, 2018). A gene catalogue was generated, and predicted genes were annotated using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (v.2017) with a maximum BLASTP E-value cutoff of 0.1 and a minimum percent coverage of 80%. Gene abundances per sample were estimated by backmapping metagenome reads to the catalogue using BWA (Burrows-Wheeler Alignment tool) (Li & Durbin, 2009). The number of counts per gene was

calculated with HTSeq (Anders et al., 2015) and subsequently normalized within samples by gene length and among samples by a set of 10 single-copy core genes (K06942, K01889, K01887, K01875, K01883, K01869, K01873, K01409, K03106 and K03110) (Salazar et al., 2019). As a result, gene and functional abundance tables were generated. Taxonomic affiliations of the functional genes were analysed with the Genome Taxonomy Database (GTDB) using MMseqs2 and considering the last common ancestor (LCA). KEGGs involved in B<sub>12</sub> biosynthesis were identified by an exhaustive search of key markers in the literature (Balabanova et al., 2021; Fang et al., 2017; Zhou et al., 2021) (Table S1). The catalogue of prokaryotic genes associated with all the B<sub>12</sub>-related KEGGs used in this study is accessible online in the Zenodo repository.

### ***Ostreococcus tauri* cobalamin bioassay**

The Mediterranean strain *Ostreococcus tauri* (RCC745) cultivated in the laboratory was used as a model organism to evaluate the bioavailability of vitamin B<sub>12</sub> in SOLA seawaters collected at different times of the year between 10/09/2020 and 10/09/2021. *O. tauri* is a marine picoeukaryotic phytoplankton that is auxotroph to vitamin B<sub>1</sub> and B<sub>12</sub> and has already been used to develop a vitamin B<sub>1</sub> bioassay (Paerl et al., 2017). Cells from a fresh culture maintained for 6 days in B<sub>12</sub>-free artificial seawater (ASW), with Streptomycin (50 µg.mL<sup>-1</sup>) and Penicillin (100 µg.mL<sup>-1</sup>), were inoculated in a deep well microplate (Nunc) at 5000 cells.mL<sup>-1</sup> in 1.8 mL of SOLA 0.22 µm filtered seawater supplemented with B<sub>12</sub>-free Keller enrichment medium and 1 nM of vitamin B<sub>1</sub> (Sigma-Aldrich). Cyanocobalamin (Sigma-Aldrich) was diluted in MiliQ water at 1 mg.mL<sup>-1</sup> concentrations, aliquoted, and stored at -20°C until use.

First, the B<sub>12</sub> requirement of *O. tauri* was assessed in cultures performed in deep well microplates inoculated at 5000 cells.mL<sup>-1</sup> in 1.8 mL of B<sub>12</sub>-free ASW, complemented with various concentrations of cyanocobalamin ranging between 0 and 100 pM. Above 10 pM of cyanocobalamin, significant cell growth was observed, and the maximal cell concentrations were reached at 25 pM of cyanocobalamin (Figure S2). In the bioassay, 0 to 25 pM of B<sub>12</sub> were added to evaluate B<sub>12</sub> bioavailability in SOLA seawaters at different times of the year.

All cultures were incubated in triplicate for 6 days under constant light conditions (35 µmol.quanta.m<sup>-2</sup>.s<sup>-1</sup>). After 6 days, 66 µL of cultures were fixed with 2 µL of ultrapure glutaraldehyde (Sigma-Aldrich) for 15 min at 4°C. Cell concentrations were determined by flow cytometry using an Accuri C6 flow cytometer (Beckman Coulter).

Of the 31 bioassays performed with seawater collected at SOLA between 10/09/2020 and 21/09/2021, 9 were not conclusive since a high mortality rate was observed even upon B<sub>12</sub> amendment, suggesting that inhibitory compounds or viruses were present in these samples. The failed samples were collected on the following dates: 27/10/2020, 3/11/2020, 24/11/2020, 1/12/2020, 8/12/2020, 15/12/2020, 16/03/21, 30/03/2021 and 06/04/2021.

### **Statistics**

The Lomb Scargle periodogram (LSP) was used to determine if annual rhythmic patterns were present in B<sub>12</sub>-related KEGGs, or genes. Rhythmic means that a gene reoccurs every year at the same time of the year. The LSP (Lomb, 1976; Scargle, 1982) was successfully adapted by biologists to detect periodic signals in unevenly sampled time series (Lambert et al., 2019; Longobardi et al., 2022; Ruf, 1999). The computing of the 'peak normalized power' (PNmax) of LSP was accomplished via the 'lomb' package in the 'R' software (v.4.0.3). KEGGs or genes were considered as rhythmic when they had a PNmax index ≥10 with a significant *p* value (*p* < 0.05) as previously reported (Lambert et al., 2019), and are considered as annual if their period was 365 ± 10 days. To estimate the time of the year of maximal abundance, we determined for each year and each rhythmic pattern the week of the year with the highest number of sequences (Lambert et al., 2019). Then, we selected over the entire time series the week that most often showed the highest number of sequences (named 'phase').

NMDS and PERMANOVA (Permutational multivariate analysis of variance) were based on Bray-Curtis dissimilarity between samples and computed with the 'adonis' (permutations = 999, *p* ≤ 0.001) and the 'metaMDS' functions, respectively implemented in the package vegan (Oksanen et al., 2022). Environmental variables of SOLA time series were fitted onto the ordination space using the 'envfit' function (permutations = 999), and covariance ellipses, that highlight seasonal clustering of samples, were plotted with the 'veganCovEllipse' function. Differences between predefined groups were tested with a two-way crossed analysis of similarities with the 'anosim' function (Anosim, permutations = 999, *p* ≤ 0.001) (Clarke, 1993; Clarke & Warwick, 2001). The correlation between environmental variables (Euclidean distances) and community dissimilarity (Bray-Curtis) was assessed using Mantel tests (*r* ≥ 0.5, *p* ≤ 0.001). All ordinations were plotted using the R package 'ggplot2', and the seasons considered were winter (December to February), spring (March to May), summer (June to August), and autumn (September to November). Despite a log<sub>10</sub> transformation, the abundance data

associated with the KEGG *metE* did not validate the conditions necessary to perform parametric tests (Shapiro–Wilk test, Bartlett test;  $p < 0.05$ ), so non-parametric Kruskal–Wallis tests were performed.

## RESULTS

### Environmental conditions and cobalamin bioavailability in seawater at SOLA

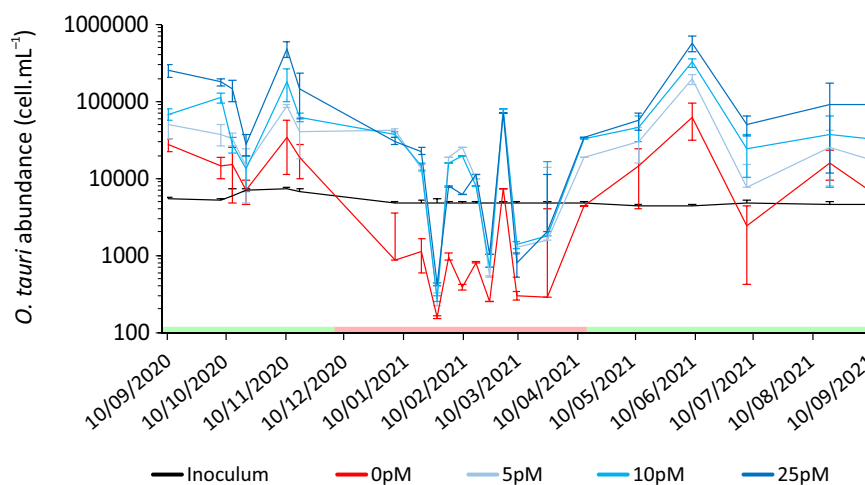
Between 2009 and 2015, surface seawater showed seasonal variations in temperatures that varied from a minimum of 8.5°C in winter to a maximum of 24.3°C in summer. Nutrients ( $\text{NO}_2^-$ ,  $\text{NO}_3^-$  and  $\text{PO}_4^{3-}$ ) and chlorophyll-*a* concentrations were higher in winter and late winter, respectively (Figure S1). Salinity values dropped during short periods in December 2011, March

2013, and January 2014, and corresponded with increases in nitrate and phosphate levels.

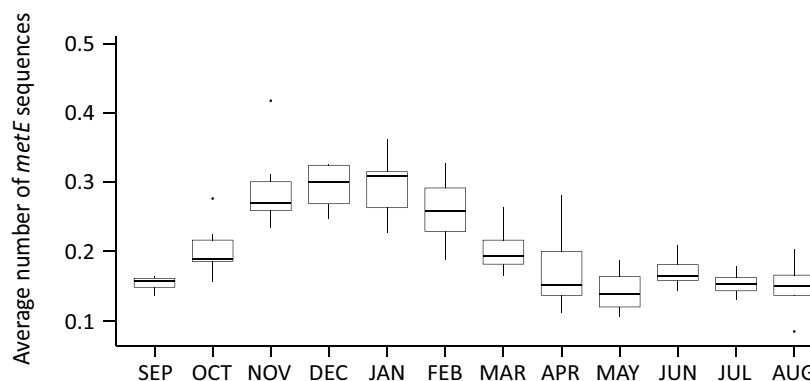
To evaluate the bioavailability of B<sub>12</sub> in seawater at SOLA, we used a B<sub>12</sub> bioassay based on the model organism *Ostreococcus tauri* that was previously used in vitamin B<sub>1</sub> bioassays (Paerl et al., 2017). Assessing the requirement for B<sub>12</sub> of our model species, maximal cell concentrations were reached at 25 pM cyanocobalamin (Figure S2).

B<sub>12</sub>-limited cells incubated in SOLA-filtered seawater collected at different times of the year and enriched with nitrate, phosphate, trace metals, and vitamin B<sub>1</sub>, exhibited different growth patterns (Figure 2A). *O. tauri* grew without B<sub>12</sub> addition in most water samples between April and November. Inversely, no cell growth was observed in the absence of B<sub>12</sub> in most winter water samples. B<sub>12</sub> amendment restored, however, growth or improved cell survival in a dose-dependent

#### (A) *O. tauri* bioassay



#### (B) *metE* annual pattern



**FIGURE 2** (A) Bioassay of *Ostreococcus tauri* RCC745 incubated in SOLA water collected from September 2020 to September 2021 and supplemented with increasing vitamin B<sub>12</sub> concentrations (0–25 pM). The inoculum represents the cell abundance ( $\text{cell.mL}^{-1} \pm \text{SD}$ ,  $n = 3$ ) of the inoculate, and the periods coloured in green indicate the growth of *O. tauri* (i.e., B<sub>12</sub> availability in seawater) while those coloured in red indicate a decline of *O. tauri* cell numbers (i.e., B<sub>12</sub> limitation). (B) Boxplot showing the average relative abundance of *metE* (number of sequences, averaged by month over 7 years between 2009 and 2015) used as an indicator of vitamin B<sub>12</sub> bioavailability in SOLA seawater. *MetE* catalyses the methionine biosynthesis in a vitamin B<sub>12</sub>-independent way.

manner in most winter samples confirming that B<sub>12</sub> was limiting at the time. The annual pattern of the KEGG *metE*, which encodes the cobalamin-independent methionine synthase was used to detect potential B<sub>12</sub> limitations (Figure 2B). *MetE* catalyses methionine biosynthesis in a cobalamin-independent way but is about 50 times less efficient than the cobalamin dependent methionine synthase, encoded by *metH*, which is used when cobalamin is bioavailable (Gonzalez et al., 1992; Helliwell et al., 2011; Matthews et al., 2003). In our 7-year metagenomics time series, *metE* showed significant differences in abundances between seasons (Kruskal–Wallis,  $p < 0.0001$ ; Dunn  $p < 0.01$ ), being more abundant from October to March, than in summer (Figure 2B). Together, Figure 2A, B suggest that B<sub>12</sub> is limiting in SOLA seawater in winter and spring.

## Annual patterns of cobalamin metabolisms over 7 years at SOLA

Over 7 years of monthly sampling at the SOLA station in Banyuls-sur-Mer, we identified 82 KEGGs related to B<sub>12</sub> metabolisms (catabolism, production, salvage, and remodelling) (Figure 1, Table S1) that corresponded to 474,725 genes (Figure 3A). Among them, 30 were significantly rhythmic with a period of  $365 \pm 10$  days as determined by the Lomb Scargle periodogram (LSP) method (PNmax  $\geq 10$ ,  $p$  values  $< 0.05$ ) (Table S1). These rhythmic KEGGs comprised 68.2% of the genes associated with the 82 B<sub>12</sub>-related KEGGs, and 68.6% of the sequences (Figure 3A).

A nMDS analysis based on all 82 B<sub>12</sub>-related KEGGs, fitted to SOLA environmental variables (Figure S1), showed that samples grouped significantly according to seasons (anosim,  $r = 0.393$ ,  $p = 0.001$ ) (Figure 3B). A nMDS focused on the 30 rhythmic KEGGs showed a stronger grouping of the samples according to seasons (anosim,  $r = 0.459$ ,  $p = 0.001$ ) (Figure 3C), while a nMDS based on the non-rhythmic ones showed a weaker seasonal grouping (anosim,  $r = 0.165$ ,  $p = 0.001$ ) (Figure 3D). Environmental parameters had a stronger correlation to rhythmic KEGGs composition (Mantel,  $r = 0.4767$ ,  $p = 0.001$ ) than to non-rhythmic KEGGs composition (Mantel,  $r = 0.2724$ ,  $p = 0.001$ ) (Figure 3C, D).

When considering the genes corresponding to the B<sub>12</sub>-related KEGGs, the nMDS showed that there was also a strong seasonal pattern for all genes (anosim,  $r = 0.568$ ,  $p = 0.001$ ) (Figure 3E), and for the ones related to the rhythmic KEGGs (anosim,  $r = 0.564$ ;  $p = 0.001$ ) (Figure 3F) but also for the genes from the non-rhythmic KEGGs (anosim,  $r = 0.572$ ;  $p = 0.001$ ) (Figure 3G). There was a stronger correlation between samples and environmental variables for the gene-

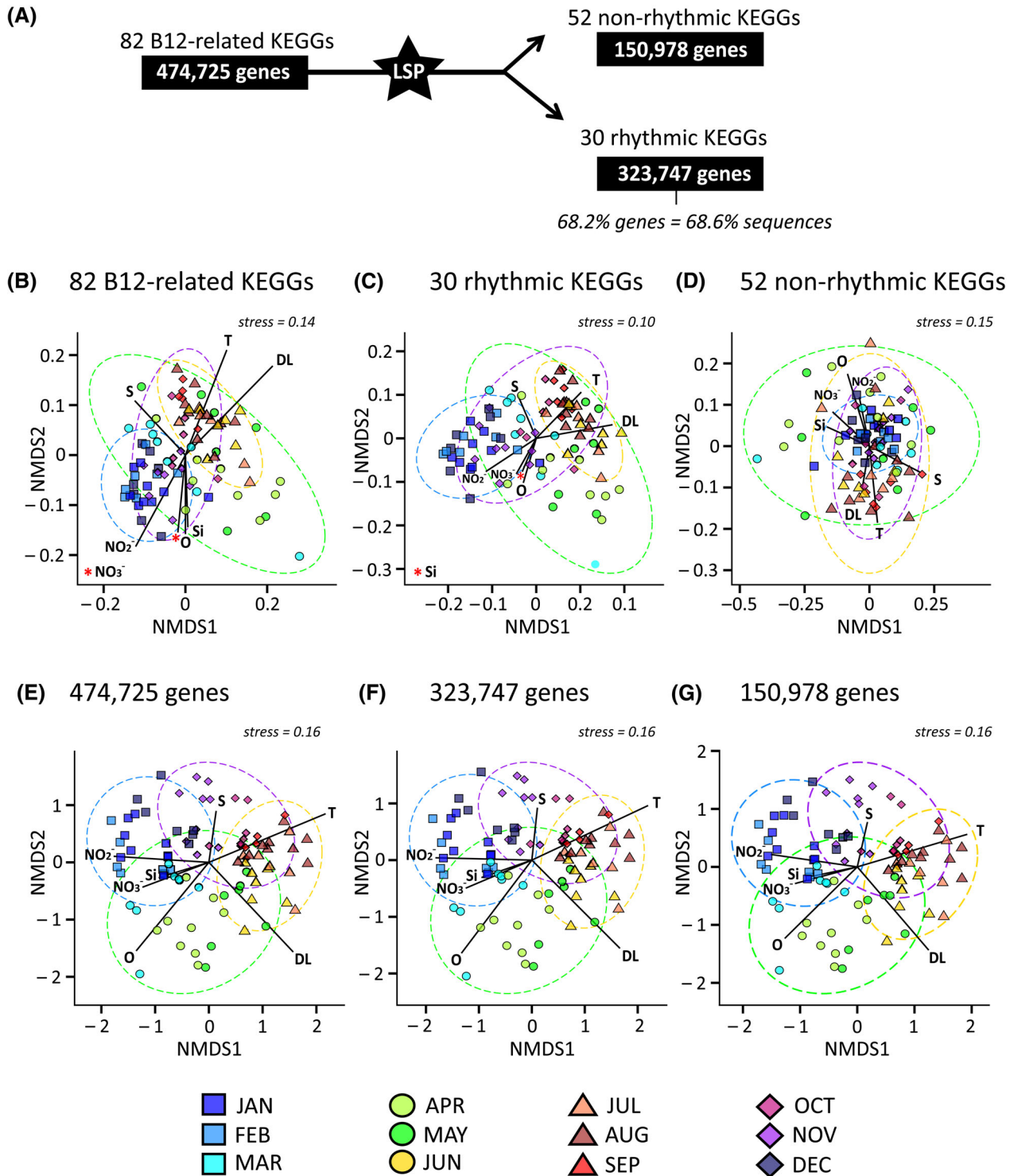
based analysis than for the KEGG-based ones (Figure 3B–G). Environmental parameters were similarly strongly correlated to gene composition for the whole gene dataset (Mantel  $r = 0.5680$ ,  $p = 0.001$ ). Summer samples were correlated to temperature and day length, whereas nutrients, dissolved oxygen concentrations, and salinity rather shaped winter and fall communities (adonis,  $p = 0.001$ ) (Figure 3B–G, Figure S1).

## Seasonality of B<sub>12</sub> and pseudo-B<sub>12</sub> biosynthesis, salvage, and remodelling pathways

Among the 30 rhythmic KEGGs related to vitamin B<sub>12</sub> metabolisms (PNmax  $\geq 10$ ), we selected the 13 KEGGs specifically involved in B<sub>12</sub> or pseudo-B<sub>12</sub> biosynthesis, salvage, and remodelling pathways (Figure 1, Table S1). Overall, we observed that KEGGs related to the anaerobic pathway were present in winter, while the aerobic pathway was predominant in summer, concomitantly with the salvage and the remodelling pathways (Figure 4A). More precisely, KEGGs markers of the anaerobic pathway (*bluB*, *cbiE*, *cbiT* and *cobY*) had peaks of occurrence in February (Figure 4B, C). Rhythmic KEGGs involved in B<sub>12</sub> de novo biosynthesis via the aerobic pathway were more abundant between May and September (Figure 4B), with maximum occurrences varying from May for *cobA*, June for *cobZ*, *cobF*, and *cobG*, and August for *cobW* and *cobK-cbiJ* (Figure 4B, C). Rhythmic KEGGs involved in the salvage pathway (*cobA\_btur*) and in pseudo-B<sub>12</sub> remodelling (*cobC*) peaked in June and August, respectively (Figure 4B, C). The only potential marker of pseudo-B<sub>12</sub> production by cyanobacteria, the KEGG *isiB*, had maximum abundance in December (Figure 4B, C).

## Distinct organisms are involved in seasonal vitamin B<sub>12</sub> and pseudo-B<sub>12</sub> biosynthesis

We next focused on the taxonomic diversity of the genes coding for proteins involved in vitamin B<sub>12</sub> biosynthesis (Figure S3A). A rhythmicity analysis (LSP) performed individually on the 23,045 genes associated with the 13 rhythmic KEGGs related to B<sub>12</sub> and pseudo-B<sub>12</sub> biosynthesis, salvage, and remodelling identified 698 annual rhythmic genes, representing 3% of biosynthesis genes and 29% of the sequences (Figure S3A). These rhythmic genes were annotated taxonomically, and most of them were assigned to the domain of *Bacteria*, accounting for about 80% of the sequences, followed by cyanobacteria and archaea

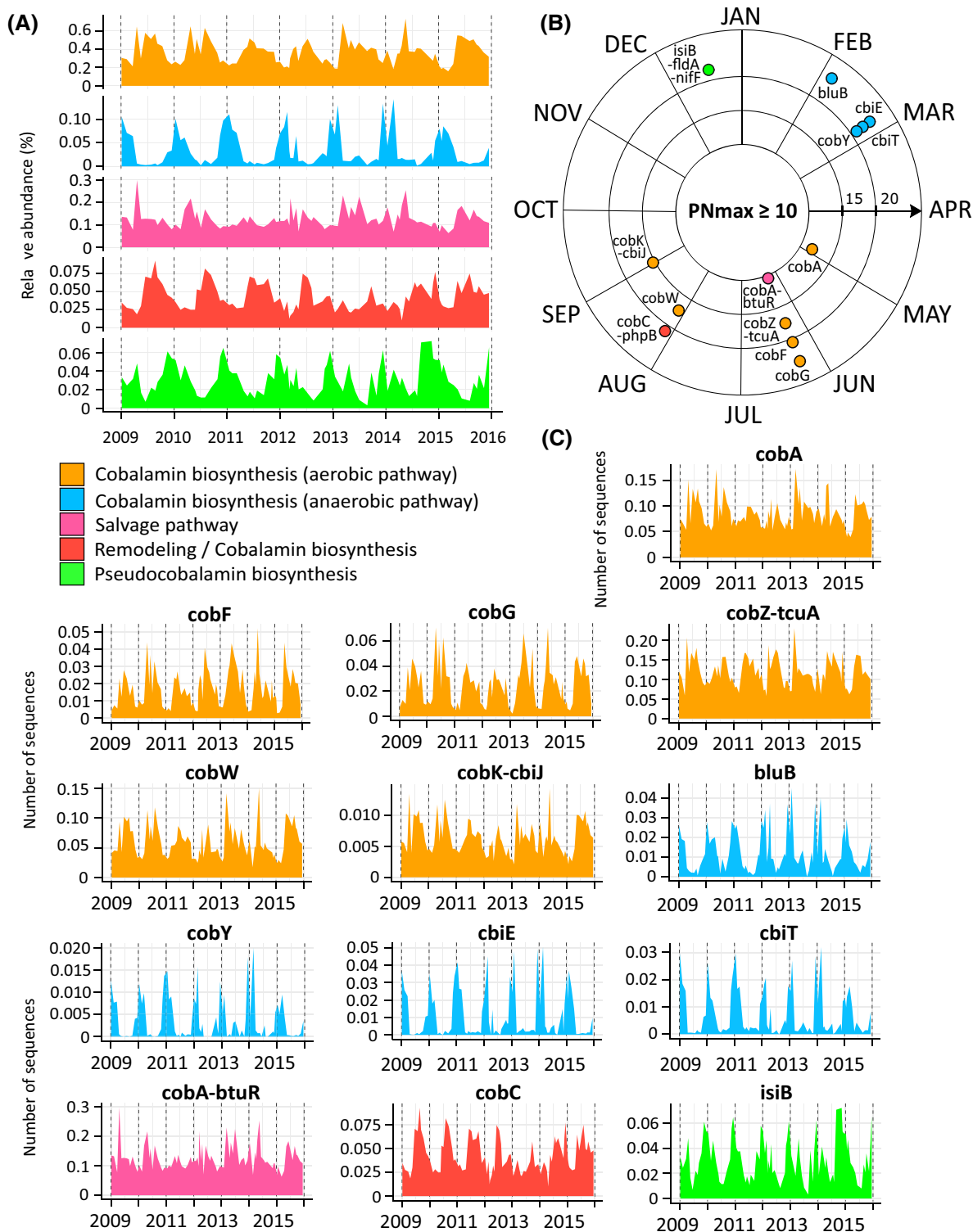


**FIGURE 3** Seasonality of KEGGs related to potential B<sub>12</sub> metabolisms and their associated genes. (A) Schematic representation of the rhythmicity analysis and the number of KEGGs and genes involved. (B) NMDS analysis based on all KEGGs related to vitamin B<sub>12</sub> metabolisms, (C) the 30 rhythmic KEGGs, and (D) the 52 nonrhythmic KEGGs. (E) NDMS analysis based on the genes corresponding to all the KEGGs, (F) the rhythmic ones and (G) the non-rhythmic ones. Covariance ellipses group the samples by seasons: winter (blue), spring (green), summer (red) and autumn (purple). Environmental variables that significantly impact the sample's structure are fitted onto the nMDS. DL, day length; NO<sub>2</sub><sup>-</sup>, nitrite; NO<sub>3</sub><sup>-</sup>, nitrate; O, dissolved oxygen; S, salinity; Si, silicate (Si(OH)<sub>4</sub>); T, temperature.

(Figure S3A). An nMDS analysis of these 13 marker KEGGs and their corresponding 698 rhythmic genes showed marked seasonal patterns (anosim  $r = 0.716$ ,

$\rho = 0.001$ ) and a strong influence of environmental variables, notably at the gene level (Mantel  $r = 0.6879$ ,  $\rho = 0.001$ ) (Figure S3B, C).

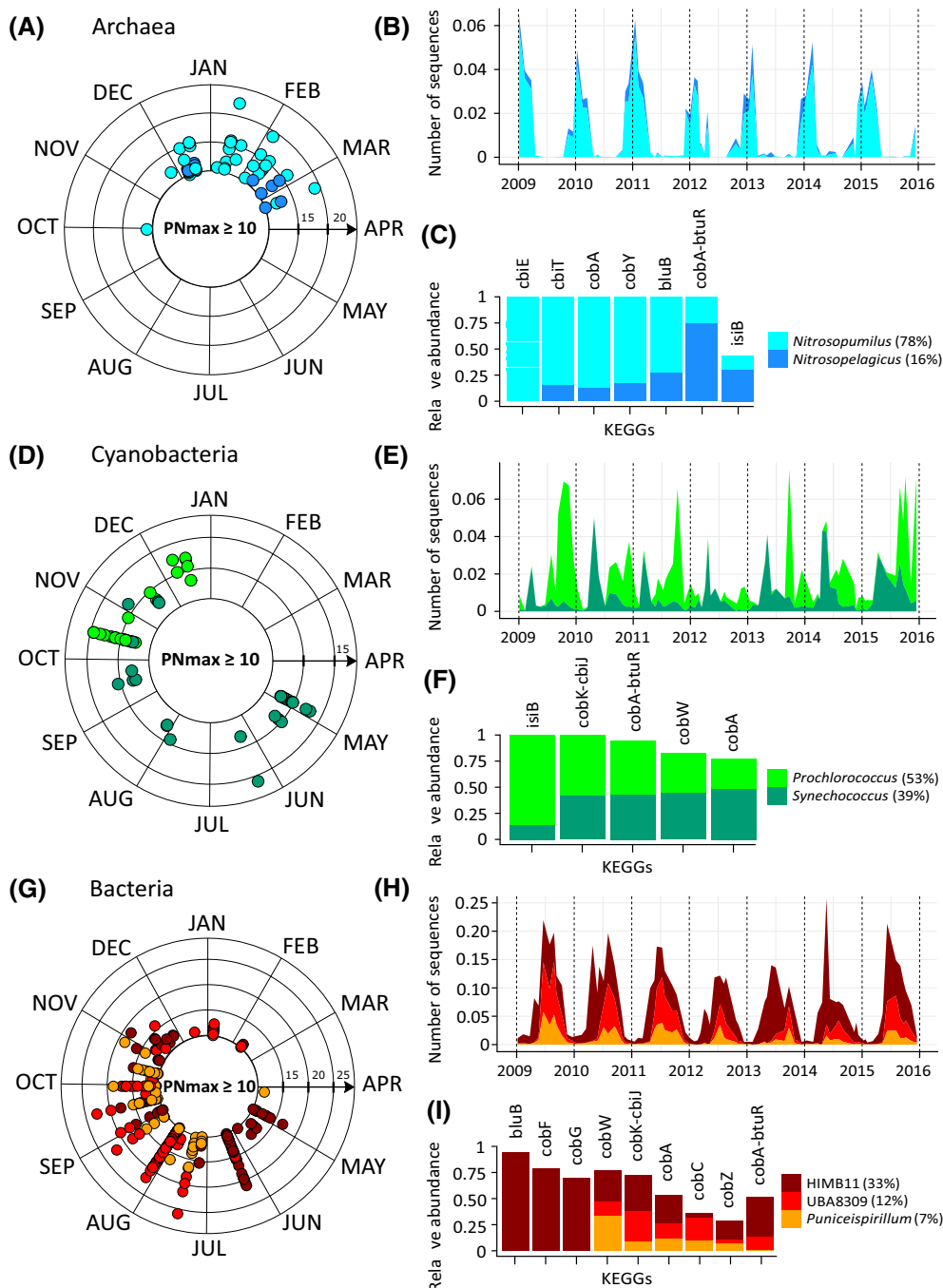




**FIGURE 4** Seasonal patterns of the 13 rhythmic KEGGs related to vitamin B<sub>12</sub> and pseudo-B<sub>12</sub> biosynthesis, salvage, and remodeling pathways at the SOLA station for 7 years. (A) Relative abundance (%) of the KEGGs summed by pathway: aerobic and anaerobic biosynthesis pathways (orange and blue, respectively), salvage pathway (pink), pseudo-B<sub>12</sub> remodeling and cobalamin biosynthesis (red), and pseudo-B<sub>12</sub> biosynthesis (green). (B) The phase of the KEGGs during a year and strength of their rhythmicity (PNmax). The PNmax scale is indicated by a bold arrow on the right branch of the radar plot. (C) Individual abundance patterns of the 13 rhythmic KEGGs over 7 years (number of sequences).

Among archaea, the most abundant genes were affiliated with members of the *Nitrosopumilus* and *Nitrosopelagicus* genera, which belong to the phylum

*Thaumarchaeota* (Figure 5A–C). Both genera were represented by 49 unique genes that accounted for about 94% of the archaeal sequences, which are



**FIGURE 5** Seasonal pattern and metabolic potential of the taxonomical groups involved in vitamin B<sub>12</sub> or pseudo-B<sub>12</sub> production during 7 years at SOLA. For the archaeal (A, B and C), cyanobacterial (D, E and F), and bacterial genes (G, H and I) which are coloured by genus, the phase and the PNmax are indicated on the radar plots (left side). The PNmax scale is indicated by a bold arrow on the right branch of the radar plot. The relative abundance of the genes summed by genus and their dynamics over 7 years is shown on the density plots (top right side), and the proportion of sequences represented by these genes per KEGG is shown on the bar plots (bottom right side). The relative abundance of each genus in their respective dataset is indicated in brackets.

dominated by *Nitrosopumilus* (78% of archaeal sequences) (Figure 5C). *Nitrosopumilus* and *Nitrosopelagicus* had overlapping occurrence patterns in winter, with peaks of abundance observed between December and March (Figure 5A, B). All KEGGs associated with the anaerobic biosynthesis pathway were present in

these archaeal sequences, as well as *bluB*, *cobA-btuR* for the salvage pathway, and *isiB* (Figure 5C). According to our data, rhythmic archaea from the *Nitrosopelagicus* genus do not seem to possess the KEGG *cbiE* (Figure 5C). Archaeal B<sub>12</sub> biosynthesis genes sequences (i.e., *cbiE* and *cbiT* sequences) were

between 3 up to 9 times more abundant than bacterial B<sub>12</sub> biosynthesis genes in winter (i.e., *cobG* and *cobF* sequences) (Figure S4A, B).

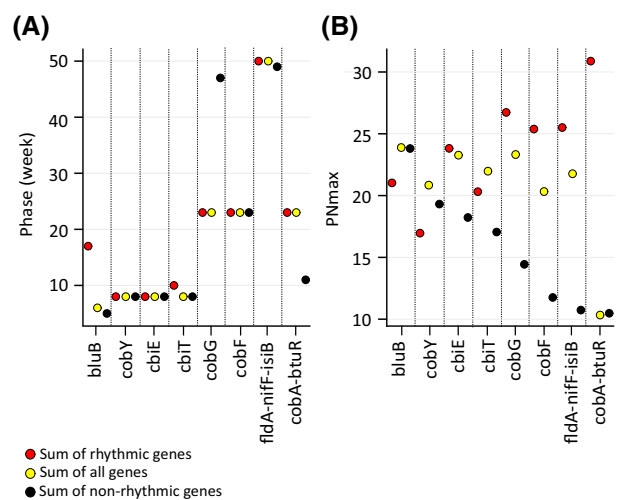
Among *Cyanobacteria*, the most abundant genera detected were *Prochlorococcus* (53%) and *Synechococcus* (39%), represented by 54 unique genes which account for about 92% of cyanobacterial sequences (Figure 5D–F). Genes affiliated with *Prochlorococcus* were present in autumn between October and December, while those affiliated with *Synechococcus* were present between May and November displaying a wider annual distribution (Figure 5D, E). Both genera had the same biosynthesis potential towards pseudo-B<sub>12</sub> and B<sub>12</sub> synthesis or salvage, with five marker KEGGs present in various proportions: *isiB*, *cobK-cbiJ*, *cobW*, *cobA* and *cobA-btuR* (Figure 5F).

For other bacteria, the most abundant genera for B<sub>12</sub> metabolisms were all *Alphaproteobacteria* that belong to the genus HIMB11, which is part of the pelagic *Roseobacter* cluster (PRC), and two genera that belonged to the SAR116 clade, *Puniceispirillum* and UBA8309 (Figure 5G–I). These genera represented 268 unique genes, which accounted for about 51% of bacterial sequences (Figure 5I). Alone, the genus HIMB11 accounted for more than a third of bacterial sequences (Figure 5I). The different species within these bacterial genera had maximal abundances between May and November, with overlapping occurrence patterns during this period (Figure 5G, H). All KEGGs associated with the aerobic biosynthesis pathway were present in these rhythmic bacterial sequences, as well as *bluB* in HIMB11 and *cobA-btuR* for the salvage pathway in HIMB11 and UBA8309 (Figure 5I).

The genera HIMB11 and *Puniceispirillum* were dominated by a single gene belonging to a species that represents more than 80% of the sequences of their respective genera (i.e., HIMB11 *sp001642945* and *Puniceispirillum marinum*) (Figure S5A–D). The five most abundant phylotypes among these dominant species accounted for more than 40% of their associated sequences, with some distinct individual seasonal patterns (Figure S5A–D). The species UBA8309 *sp002457765* accounted for about 54% of the sequences related to the genus UBA8309, but other species were also abundant (Figure S5E, F), and the five most abundant phylotypes accounted for about 60% of the sequences (Figure S5E, F).

## Interannual variability of vitamin B<sub>12</sub> producers

We investigated the interannual variability of vitamin B<sub>12</sub> metabolisms in SOLA by focusing on the 22,347 non-rhythmic genes (PNmax <10) involved in B<sub>12</sub> biosynthesis, which represented 71% of the sequences

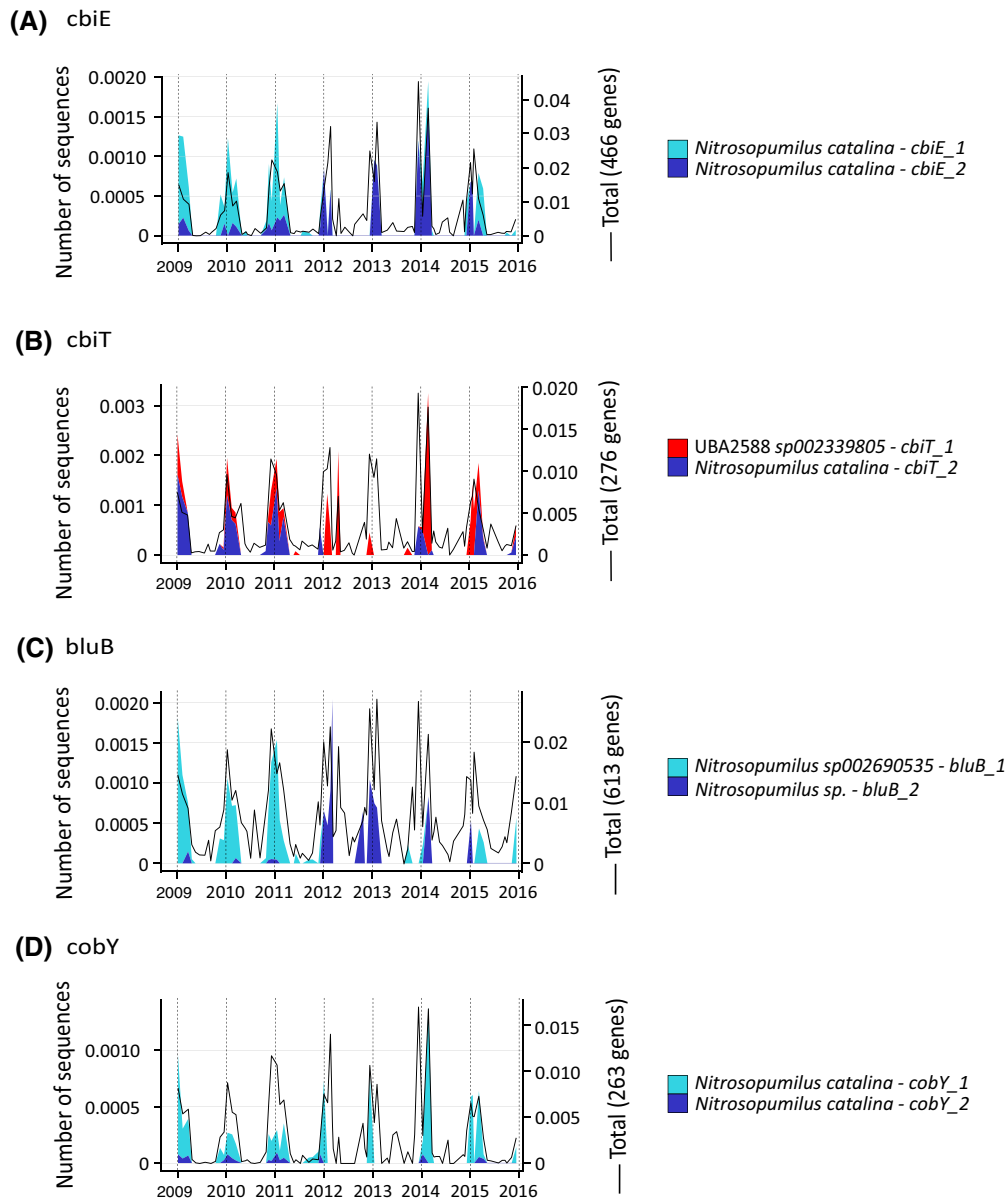


**FIGURE 6** (A) Week of the year during which maximal peak of abundance is observed (i.e., phase) and (B) strength of rhythmicity (i.e., PNmax) for the eight KEGGs calculated after summing all gene abundances, only rhythmic genes abundances, or only non-rhythmic genes abundances. We summed together the abundances of all the genes annotated to the same KEGG, considering either the rhythmic genes only, the non-rhythmic genes only, or all the genes. We then performed a rhythmicity analysis (LSP) on each sum to compare the values of phases and PNmax obtained.

(Figure S3A, Figure S6A). We identified the KEGGs they belonged to and recalculated the KEGG rhythmicity by adding one non-rhythmic gene at the time from the most abundant to the rarest (Figure S6A). We hypothesized that non-rhythmic genes, which together produced an annual rhythmic KEGG (PNmax ≥10), included genes that replaced each other from year to year.

Eight out of our 13 marker KEGGs got an annual rhythm when the abundance of their related non-rhythmic genes was summed (Figure S6B). The phase of five of them (*cobY*, *cbiE*, *cbiT*, *cobF* and *isiB*) was like the one initially determined when summing all the genes, or when considering only the rhythmic genes (Figure 6A). For the other KEGGs (*bluB*, *cobG* and *cobA-btuR*), the phase varied depending on the type of genes summed (Figure 6A). The strength of rhythmicity (PNmax) was usually higher for the KEGGs based on all summed genes or summed rhythmic genes only, than for summed non-rhythmic ones (Figure 6B), excepting for *bluB* and *cobY*, for which lower PNmax values were obtained when summing the rhythmic genes.

We also identified the non-rhythmic genes that were needed to get a rhythmic KEGG (i.e., reaching the PNmax threshold of 10) (Figure 7; Figure S7). Among the KEGGs involved in the anaerobic pathway in winter, the *cbiE* rhythm was recovered when the archaea *N. catalina*—*cbiE*\_2 replaced *N. catalina*—*cbiE*\_1 during winter 2011, 2012 and 2013 (Figure 7A). For *cbiT*, the rhythm was recovered when *N. catalina*—*cbiE*\_2

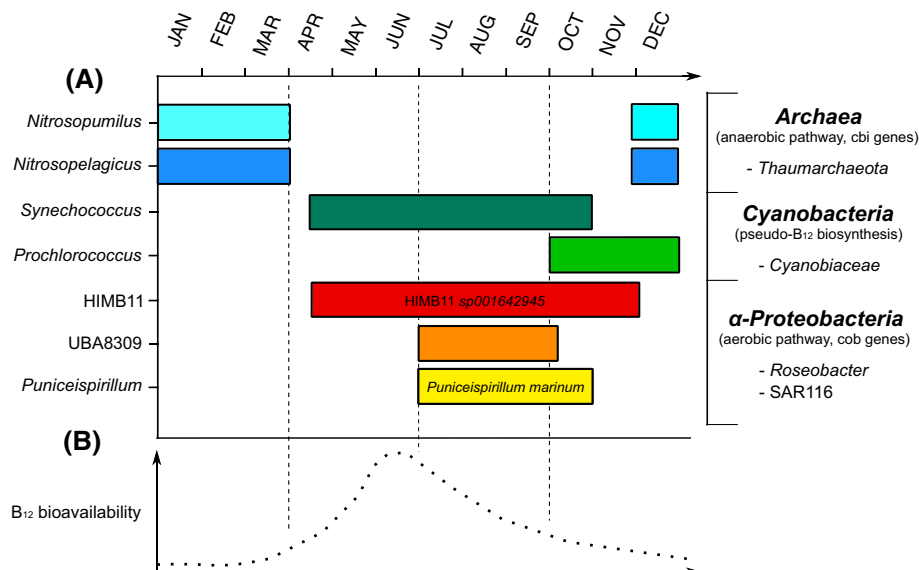


**FIGURE 7** Analysis of non-rhythmic genes associated with four vitamin B<sub>12</sub>-biosynthesis marker KEGGs involved in the anaerobic pathway (A: *cbiE*, B: *cbiT*, C: *bluB* and D: *cobY*). Density plots show the relative abundance (number of sequences) of the first two abundant and non-rhythmic genes (PN<sub>max</sub> <10) sufficient to recover a rhythm at the KEGG level (PN<sub>max</sub> ≥10). The black line shows the sum of all non-rhythmic genes of the respective KEGG.

was replaced by the bacteria UBA2588 *sp002339805*—*cbiT\_1* (Figure 7B). For *bluB* and *cobY*, we also observed a switch between two *Nitrosopumilus* members, belonging to the same or different species for *cobY* and *bluB*, respectively (Figure 7C, D).

For the KEGGs involved in the aerobic biosynthesis pathway (i.e., *cobF* and *cobG*) and in the salvage pathway (i.e., *cobA*-*btuR*) in summer, the rhythmicity threshold was reached after summing more than two nonrhythmic genes (Figure S7). *CobF* and *cobG* recovered a rhythm when adding the three and eight most abundant nonrhythmic genes, which represent 16%

and 8% of the sequences, respectively (Figure S7A, B). For both KEGGs, the genes involved in the recovered rhythm were all affiliated to the genus HIMB11 represented by four species: HIMB11 *sp002684375*; HIMB11 *sp001642945*; HIMB11 *sp003486095*, and HIMB11 *sp000472185* (Figure S7A, B). For *cobA*-*btuR*, the 6 non-rhythmic genes sufficient to get a rhythm at the KEGG level were members of the *Alphaproteobacteria* belonging to *Rhodobacteraceae* (i.e., *Amylibacter sp900197625* and *Planktomarina temperata*) and *Gammaproteobacteria* (i.e., HTCC2207 *sp002469845* and HTCC2207 *sp002334915*) (Figure S7C).



**FIGURE 8** General overview of the seasonality of vitamin B<sub>12</sub> and pseudo-B<sub>12</sub> potential producers and related pathways. (A) Seasonality of potential vitamin B<sub>12</sub> producers identified at the genus level and at the species level for HIMB11 and *Puniceispirillum* (the metabolic pathway used for B<sub>12</sub> biosynthesis is also mentioned). (B) Schematic overview of B<sub>12</sub> bioavailability in SOLA seawater over a year inferred from *Ostreococcus tauri* bioassay and *metE* relative abundance (Figure 3).

## DISCUSSION

Our long-term metagenomic time series revealed that potential vitamin B<sub>12</sub> production in the coastal NW Mediterranean was the result of a seasonal succession of different pathways throughout the year. This succession was characterized by the production of B<sub>12</sub> through the archaeal anaerobic pathway in winter, of pseudo-B<sub>12</sub> in spring and winter by cyanobacteria, and then, from spring to autumn, bacterial B<sub>12</sub> synthesis through the aerobic pathway (Figure 8A).

This 7-year metagenomic study represents the first description of the succession of distinct B<sub>12</sub> metabolic pathways performed by phylogenetically distant organisms over the years.

Archaea appeared to be the main potential B<sub>12</sub> producers in winter. They belonged to the phylum *Thaumarchaeota* (i.e., *Nitrosopumilus* and *Nitrosopelagicus* genera) and could use the anaerobic pathway to produce B<sub>12</sub> through the genes *bluB*, *cbiE*, *cbiT*, and *cobY* (Figure 8A). Marine *Thaumarchaeota*, such as *Nitrosopumilus*, have been shown earlier to possess B<sub>12</sub> synthesis genes and were thus hypothesized to be a source of B<sub>12</sub> in the water column (Doxey et al., 2015; Qin et al., 2017; Santoro et al., 2015) and to play a critical role in the deeper ocean (Heal et al., 2017). *Thaumarchaeota* are, however, known to be much less abundant than bacteria in surface waters, representing only 1%–10% of the prokaryotes (Church et al., 2003; Karner et al., 2001; Massana et al., 2000), so their importance could be limited in the upper water column. Our observations show, nevertheless, that archaeal B<sub>12</sub> production genes were up to 9 times more

abundant than the bacterial ones in winter in the coastal NW-Mediterranean site of SOLA. *Thaumarchaeota*, which are more abundant in winter planktonic communities in temperate seas (Doxey et al., 2015; Galand et al., 2010; Lambert et al., 2019; Pereira et al., 2021), thus certainly play a seasonally important role in B<sub>12</sub> cycling in these areas. Remarkably, *Thaumarchaeota* were present at SOLA when B<sub>12</sub> had the lowest bioavailability, which could indicate that their activity was not sufficient to enrich the water with B<sub>12</sub>. Phytoplankton cells could, however, directly use archaeal-derived B<sub>12</sub> for growth based on functional complementarity towards essential metabolite production and/or regeneration, that is, remineralization of inorganic nutrients and B<sub>12</sub> production versus algal production of carbon- or sulphur-rich organic matter and vitamin B<sub>1</sub> as previously reported for bacteria (Durham et al., 2014; Geng & Belas, 2010; Giovannoni, 2012; Luo & Moran, 2014). The high demand for B<sub>12</sub> by auxotrophic phytoplankton blooms (*Mamiellophyceae* and Dinoflagellates) observed in winter at SOLA could explain why B<sub>12</sub> bioavailability in water is lowest during this period (Figure 8B). Since the isoforms of cobalamin are highly light sensitive, and their lifetime in seawater is very short (Heal et al., 2014; Martens et al., 2002), the balance between B<sub>12</sub> release from prokaryotes and its consumption by other organisms remains hard to estimate (Villegas-Mendoza et al., 2019).

The cyanobacteria *Synechococcus* were the potential producers of pseudo-B<sub>12</sub> from spring to autumn, relayed by *Prochlorococcus* in winter (Figure 8A). *Synechococcus* and *Prochlorococcus* are known to bloom at different times of the year in the coastal NW

Sea (Schauer et al., 2003; Sommaruga et al., 2005) as they are adapted to different ecological niches linked to their sensitivity to sunlight (Sommaruga et al., 2005). Our data suggest that the succession of these known pseudo-B<sub>12</sub> producers (Bonnet et al., 2010; Doxey et al., 2015; Helliwell et al., 2016) provides a continuous input of pseudo-B<sub>12</sub> over several months. Pseudo-B<sub>12</sub> is, however, less bioavailable than B<sub>12</sub> to several microalgae (Heal et al., 2017; Helliwell et al., 2016). If DMB is provided, pseudo-B<sub>12</sub> could nevertheless be scavenged and/or remodelled by specific microorganisms that possess the *cobA-btuR* (i.e., archaea, bacteria, cyanobacteria) and *cobC* genes (i.e., bacteria) (Heal et al., 2017; Helliwell et al., 2016), which are more present in summer. Because bacteria and archaea both can produce DMB (*bluB* gene), cyanobacterial pseudo-B<sub>12</sub> could be remodelled in summer and winter using the DMB produced successively by bacteria and archaea.

Shortly after archaea and partly concomitantly with cyanobacteria, other bacteria potentially produce B<sub>12</sub> via the aerobic pathway (*cobF*, *cobG*, *cobZ\_tcuA*, *cobA*, *cobW* and *cobK-cbiJ* genes) (Figure 8A). Co-occurrences between pseudo-B<sub>12</sub> producing cyanobacteria and other prokaryotic producers (i.e., bacteria and archaea) suggest that pseudo-B<sub>12</sub> and B<sub>12</sub> could coexist in the water column at certain times of the year. Although both have distinct microbial sources and support different enzymatic demands, the coexistence between B<sub>12</sub> and pseudo-B<sub>12</sub> has been observed in the North Pacific subtropical gyre (Heal et al., 2017).

Seasonal bacterial B<sub>12</sub> production from spring to autumn was potentially performed by members of the genera HIMB11 (*Roseobacter* clade) and *Puniceispirillum* (SAR116 clade). HIMB11 had maximal abundance between May and November and could thus be an important player in the B<sub>12</sub> cycle at SOLA over several months. Most members of the marine *Roseobacter* lineage have earlier been described as B<sub>12</sub> producers (Newton et al., 2010), but to date, HIMB11 has been only known for its potential role in carbon and sulphur cycles (Durham et al., 2014, 2015), and its importance in B<sub>12</sub> production has not yet been highlighted. However, HIMB11 belongs to the Pelagic *Roseobacter* Cluster (PRC), whose members have the potential to produce B<sub>12</sub> and other vitamins like B<sub>2</sub> (riboflavin), and B<sub>6</sub> (pyridoxine), but lack genes for the synthesis of vitamins B<sub>1</sub> and B<sub>7</sub> (Billerbeck et al., 2016).

Similarly, a phylogenomic analysis of all available SAR116 genomes showed that SAR116 members also carried the necessary genes for the biosynthesis of vitamins B<sub>2</sub>, B<sub>5</sub> (pantothenate), B<sub>6</sub>, B<sub>9</sub> (folate) and B<sub>12</sub> (Grote et al., 2011; Roda-Garcia et al., 2021). Co-culture approaches have earlier shown potential interactions based on B<sub>12</sub> requirements between *Roseobacter* members and blooming auxotrophic microalgae, including *Prorocentrum* (Wang

et al., 2014), *Thalassiosira* (Durham et al., 2015, 2017) and *Ostreococcus* (Cooper et al., 2019). Interestingly, HIMB11 (*Roseobacter*) and *Puniceispirillum* (SAR116) both possess the DMSP (dimethylsulfoniopropionate) degradation pathway (Varaljay et al., 2012), suggesting that this chemical attractant could be a key metabolite in the regulation of SAR116- and *Roseobacter*-phytoplankton interactions. There might be adaptive associations between *Roseobacter* and phytoplankton strains based on the nature of exudates produced by algal species (Hahnke et al., 2013; Teeling et al., 2012). Genes encoding for the type IV secretion system (T4SS) or involved in motility and chemotaxis have notably been found in B<sub>12</sub>-producing strains of *Roseobacter* (Luo & Moran, 2014; Newton et al., 2010), and they may be important traits for B<sub>12</sub>-based microbial symbiosis between auxotrophic microalgae and B<sub>12</sub> providers (Luo & Moran, 2014; Newton et al., 2010; Wang et al., 2014).

An additional argument for the importance of the association between *Nitrosopumilus*, *Nitrosopelagicus*, HIMB11 and *Puniceispirillum* with phytoplankton lies in recent studies based on co-occurrence networks that showed associations between archaeal or bacterial potential B<sub>12</sub> producers (e.g., *Thaumarchaeota*, *Rhodobacteraceae* and *Puniceispirillaceae*), and auxotrophic microalgae (e.g., *Micromonas*, *Bathycoccus* and *Ostreococcus*) (Joglar et al., 2021; Krabberød et al., 2022; Lambert et al., 2021). A winter interaction hub associated the picoeukaryote *Bathycoccus* with a potential B<sub>12</sub> producer belonging to *Rhodobacteraceae*, in addition to archaea, which also interacts with other B<sub>12</sub> auxotrophs such as dinoflagellates, diatoms, and picoeukaryotes (Krabberød et al., 2022). These findings and ours together suggest an important role of *Puniceispirillaceae* and specific members of *Rhodobacteraceae* in providing vitamin B<sub>12</sub> for phytoplankton blooms. These B<sub>12</sub> producers could be seen as 'core microbes' that would be essential to the seasonal functioning of the marine ecosystem (Krabberød et al., 2022).

We observed that during the 7 years of the study, B<sub>12</sub>-related pathways always re-occurred, but that for some years, the genes involved were carried by different microorganisms. In some cases, the organisms that replaced each other belonged to the same species (*Nitrosopumilus catalina*). These microorganisms could thus be considered as different ecotypes that occupy different ecological niches. In other instances, the change occurred between different species. In both cases, a switch between organisms would indicate changes in environmental niches from time to time. Such changes did occur for 3 years when perturbations changed the environment conditions, and thus possibly the microbial niches at SOLA. Strong rains combined with storms brought lower salinity and higher nutrient loads to the coastal site. We hypothesize that environmental disturbances prevented certain species or

ecotypes from thriving and that they were replaced by other organisms, more or less phylogenetically close, with different or more extensive ecological niches.

The changes between microorganisms allowed the maintenance of metabolic functions at the community level. The gene-level redundancy between phylotypes could thus stimulate the long-term functional resilience of microbial ecosystems exposed to contrasted inter-annual environmental conditions (Awasthi et al., 2014). Similarly, in the human gut microbiome or the soil, functional redundancy between specific taxa maintains stability under disturbances (Li et al., 2021; Moya & Ferrer, 2016). For that process to take place, a large diversity of microorganisms carrying similar genes is needed. If diversity is reduced, the ecological insurance (Awasthi et al., 2014; Yachi & Loreau, 1999) will be lost and the functioning of the ecosystem will be impaired.

## CONCLUSION

Our monthly 7-year metagenomic time series at the SOLA station uncovered a seasonal succession of distinct prokaryotic producers that use different biosynthesis pathways to produce vitamin B<sub>12</sub> de novo. These seasonal microorganisms are essential for the functioning of this coastal marine ecosystem, notably *Roseobacter*, and the SAR116-clade in summer, and *Thaumarchaeota* in winter, when SOLA seawater is B<sub>12</sub>-limited. The fact that some vitamin B<sub>12</sub> producers were replaced by others carrying the same functions during contrasted years indicates the existence of functional redundancy at a gene level in several B<sub>12</sub>-related functions. Environmental perturbations can thus strongly impact biodiversity patterns without perturbing microbial functions. This study contributes to our understanding of the long-term functional resilience of marine microbial communities, especially in coastal ecosystems where they could be exposed to contrasting inter-annual environmental conditions.

## AUTHOR CONTRIBUTIONS

**Maxime Beauvais:** Writing – original draft; writing – review and editing; investigation; funding acquisition; visualization; methodology. **Philippe Schatt:** Resources; methodology. **Lidia Montiel:** Data curation; methodology. **Ramiro Logares:** Data curation; methodology. **Pierre E. Galand:** Supervision; conceptualization; investigation; writing – review and editing; validation; methodology; funding acquisition. **François-Yves Bouget:** Conceptualization; investigation; writing – review and editing; validation; supervision; methodology; funding acquisition.

## ACKNOWLEDGEMENTS

We are grateful to the crew of the ship ‘Nereis II’ for their help in sampling and acquiring the data from the SOLA station. We thank the ‘Service d’Observation’

and the LOMIC team, particularly Eric Maria, and Paul Labatut, for their help in obtaining and processing the SOLA samples. Maxime Beauvais was supported by a PhD fellowship from the doctoral school no. 129 of Sorbonne-University.

## CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

## DATA AVAILABILITY STATEMENT

The catalogue of prokaryotic genes associated with all the B<sub>12</sub>-related KEGGs used in this study is accessible online in the Zenodo repository: 10.5281/zenodo.7409517. The raw sequences were deposited in the NCBI Sequence Read Archive under accession number PRJEB66489: <https://www.ncbi.nlm.nih.gov/sra/PRJEB66489> and PRJEB26919.

## ORCID

Maxime Beauvais  <https://orcid.org/0009-0003-4085-0173>

Pierre E. Galand  <https://orcid.org/0000-0002-2238-3247>

## REFERENCES

- Ahlgren, N.A., Perelman, J.N., Yeh, Y.C. & Fuhrman, J.A. (2019) Multi-year dynamics of fine-scale marine cyanobacterial populations are more strongly explained by phage interactions than abiotic, bottom-up factors. *Environmental Microbiology*, 21(8), 2948–2963. Available from: <https://doi.org/10.1111/1462-2920.14687>
- Anders, S., Pyl, P.T. & Huber, W. (2015) HTSeq-A python framework to work with high-throughput sequencing data. *Bioinformatics*, 31(2), 166–169. Available from: <https://doi.org/10.1093/bioinformatics/btu638>
- Auladell, A., Barberán, A., Logares, R., Garcés, E., Gasol, J.M. & Ferrera, I. (2022) Seasonal niche differentiation among closely related marine bacteria. *ISME Journal*, 16(1), 178–189. Available from: <https://doi.org/10.1038/s41396-021-01053-2>
- Awasthi, A., Singh, M., Soni, S.K., Singh, R. & Kalra, A. (2014) Biodiversity acts as insurance of productivity of bacterial communities under abiotic perturbations. *ISME Journal*, 8(12), 2445–2452. Available from: <https://doi.org/10.1038/ismej.2014.91>
- Balabanova, L., Averianova, L., Marchenok, M., Son, O. & Tekutyeva, L. (2021) Microbial and genetic resources for cobalamin (vitamin b<sub>12</sub>) biosynthesis: from ecosystems to industrial biotechnology. *International Journal of Molecular Sciences*, 22(9), 4522. Available from: <https://doi.org/10.3390/ijms22094522>
- Barber-Lluch, E., Joglar, V., Moreiras, G., Leão, J.M., Gago-Martínez, A., Fernández, E. et al. (2021) Variability of vitamin B<sub>12</sub> concentrations in waters along the northwest Iberian shelf. *Regional Studies in Marine Science*, 42, 101608. Available from: <https://doi.org/10.1016/j.rsma.2020.101608>
- Bertrand, E.M., McCrow, J.P., Moustafa, A., Zheng, H., McQuaid, J.B., Delmont, T.O. et al. (2015) Phytoplankton-bacterial interactions mediate micronutrient colimitation at the coastal Antarctic sea ice edge. *Proceedings of the National Academy of Sciences of the United States of America*, 112(32), 9938–9943. Available from: <https://doi.org/10.1073/pnas.1501615112>
- Bertrand, E.M., Saito, M.A., Jeon, Y.J. & Neilan, B.A. (2011) Vitamin B<sub>12</sub> biosynthesis gene diversity in the Ross Sea: the

- identification of a new group of putative polar B12 biosynthesizers. *Environmental Microbiology*, 13(5), 1285–1298. Available from: <https://doi.org/10.1111/j.1462-2920.2011.02428.x>
- Bertrand, E.M., Saito, M.A., Rose, J.M., Riesselman, C.R., Noble, A.E., Lee, P.A. et al. (2007) Vitamin B12 and iron colimitation of phytoplankton growth in the Ross Sea. *Limnology and Oceanography*, 52(3), 1079–1093.
- Billerbeck, S., Wemheuer, B., Voget, S., Poehlein, A., Giebel, H.A., Brinkhoff, T. et al. (2016) Biogeography and environmental genomics of the Roseobacter-affiliated pelagic CHAB-I-5 lineage. *Nature Microbiology*, 1(7), 1–8. Available from: <https://doi.org/10.1038/nmicrobiol.2016.63>
- Bonnet, S., Tovar-Sánchez, A., Panzeca, C., Duarte, C.M., Ortega-Retuerta, E. & Sañudo-Wilhelmy, S.A. (2013) Geographical gradients of dissolved vitamin B12 in the Mediterranean Sea. *Frontiers in Microbiology*, 4(JUN), 1–10. Available from: <https://doi.org/10.3389/fmicb.2013.00126>
- Bonnet, S., Webb, E.A., Panzeca, C., Karl, D.M., Capone, D.G. & San, S.A. (2010) Vitamin B12 excretion by cultures of the marine cyanobacteria *Crocospaera* and *Synechococcus*. *Limnology and Oceanography*, 55(5), 1959–1964. Available from: <https://doi.org/10.4319/lo.2010.55.4.1959>
- Chow, C.E.T., Kim, D.Y., Sachdeva, R., Caron, D.A. & Fuhrman, J.A. (2014) Top-down controls on bacterial community structure: microbial network analysis of bacteria, T4-like viruses and protists. *ISME Journal*, 8(4), 816–829. Available from: <https://doi.org/10.1038/ismej.2013.199>
- Church, M.J., DeLong, E.F., Ducklow, H.W., Kamer, M.B., Preston, C.M. & Karl, D.M. (2003) Abundance and distribution of planktonic archaea and bacteria in the waters west of the Antarctic peninsula. *Limnology and Oceanography*, 48(5), 1893–1902. Available from: <https://doi.org/10.4319/lo.2003.48.5.1893>
- Clarke, K.R. (1993) Non-parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology*, 18(1), 117–143. Available from: <https://doi.org/10.1111/j.1442-9993.1993.tb00438.x>
- Clarke, K.R. & Warwick, R.M. (2001) A further biodiversity index applicable to species lists: variation in taxonomic distinctness. *Marine Ecology Progress Series*, 216, 265–278. Available from: <https://doi.org/10.3354/meps216265>
- Cooper, M.B., Kazamia, E., Helliwell, K.E., Kudahl, U.J., Sayer, A., Wheeler, G.L. et al. (2019) Cross-exchange of B-vitamins underpins a mutualistic interaction between *Ostreococcus tauri* and *Dinoroseobacter shibae*. *ISME Journal*, 13(2), 334–345. Available from: <https://doi.org/10.1038/s41396-018-0274-y>
- Croft, M.T., Lawrence, A.D., Raux-Deery, E., Warren, M.J. & Smith, A.G. (2005) Algae acquire vitamin B12 through a symbiotic relationship with bacteria. *Nature*, 438(7064), 90–93. Available from: <https://doi.org/10.1038/nature04056>
- Croft, M.T., Warren, M.J. & Smith, A.G. (2006) Algae need their vitamins. *Eukaryotic Cell*, 5(8), 1175–1183. Available from: <https://doi.org/10.1128/EC.00097-06>
- Doxey, A.C., Kurtz, D.A., Lynch, M.D.J., Sauder, L.A. & Neufeld, J.D. (2015) Aquatic metagenomes implicate Thaumarchaeota in global cobalamin production. *ISME Journal*, 9(2), 461–471. Available from: <https://doi.org/10.1038/ismej.2014.142>
- Durham, B.P., Dearth, S.P., Sharma, S., Amin, S.A., Smith, C.B., Campagna, S.R. et al. (2017) Recognition cascade and metabolite transfer in a marine bacteria-phytoplankton model system. *Environmental Microbiology*, 19(9), 3500–3513. Available from: <https://doi.org/10.1111/1462-2920.13834>
- Durham, B.P., Grote, J., Whittaker, K.A., Bender, S.J., Luo, H., Sharon, L. et al. (2014) Alphaproteobacterial strain HIMB11. *Standards in Genomic Sciences*, 9, 632–645. Available from: <https://doi.org/10.4056/sig.1854551>
- Durham, B.P., Sharma, S., Luo, H., Smith, C.B., Amin, S.A., Bender, S.J. et al. (2015) Cryptic carbon and sulfur cycling between surface ocean plankton. *Proceedings of the National Academy of Sciences of the United States of America*, 112(2), 453–457. Available from: <https://doi.org/10.1073/pnas.1413137112>
- Falkowski, P.G., Fenchel, T. & DeLong, E.F. (2008) The microbial engines that drive earth's biogeochemical cycles. *Science*, 320(5879), 1034–1039. Available from: <https://doi.org/10.1126/science.1153213>
- Fang, H., Kang, J. & Zhang, D. (2017) Microbial production of vitamin B12: a review and future perspectives. *Microbial Cell Factories*, 16(1), 1–14. Available from: <https://doi.org/10.1186/s12934-017-0631-y>
- Fiala, M. (1982) Vitamine B12 et phytoplancton au niveau de la thermocline en Méditerranée nord-occidentale. *Oceanologica Acta*, 5(3), 339–347.
- Frischkorn, K.R., Haley, S.T. & Dyhrman, S.T. (2018) Coordinated gene expression between *Trichodesmium* and its microbiome over day-night cycles in the North Pacific subtropical gyre. *ISME Journal*, 12(4), 997–1007. Available from: <https://doi.org/10.1038/s41396-017-0041-5>
- Fuhrman, J.A., Hewson, I., Schwalbach, M.S., Steele, J.A., Brown, M.V. & Naeem, S. (2006) Annually reoccurring bacterial communities are predictable from ocean conditions. *Proceedings of the National Academy of Sciences of the United States of America*, 103(35), 13104–13109. Available from: <https://doi.org/10.1073/pnas.0602399103>
- Galand, P.E., Gutiérrez-Provecho, C., Massana, R., Gasol, J.M. & Casamayor, E.O. (2010) Inter-annual recurrence of archaeal assemblages in the coastal NW Mediterranean Sea (Blanes Bay microbial observatory). *Limnology and Oceanography*, 55(5), 2117–2125. Available from: <https://doi.org/10.4319/lo.2010.55.5.2117>
- Galand, P.E., Pereira, O., Hochart, C., Auguet, J.C. & Debroas, D. (2018) A strong link between marine microbial community composition and function challenges the idea of functional redundancy. *ISME Journal*, 12(10), 2470–2478. Available from: <https://doi.org/10.1038/s41396-018-0158-1>
- Geng, H. & Belas, R. (2010) Molecular mechanisms underlying roseobacter—phytoplankton symbioses. *Current Opinion in Biotechnology*, 21(3), 332–338. Available from: <https://doi.org/10.1016/j.copbio.2010.03.013>
- Giovannoni, S.J. (2012) Vitamins in the sea. *Proceedings of the National Academy of Sciences of the United States of America*, 109(35), 13888–13889. Available from: <https://doi.org/10.1073/pnas.1211722109>
- Gómez-Consarnau, L., Sachdeva, R., Gifford, S.M., Cutter, L.S., Fuhrman, J.A., Sañudo-Wilhelmy, S.A. et al. (2018) Mosaic patterns of B-vitamin synthesis and utilization in a natural marine microbial community. *Environmental Microbiology*, 20(8), 2809–2823. Available from: <https://doi.org/10.1111/1462-2920.14133>
- Gonzalez, J.C., Banerjee, R.V., Huang, S., Sumner, J.S. & Matthews, R.G. (1992) Comparison of cobalamin-independent and cobalamin-dependent methionine synthases from *Escherichia coli*: two solutions to the same chemical problem. *Biochemistry*, 31(26), 6045–6056. Available from: <https://doi.org/10.1021/bi00141a013>
- Grant, M.A.A., Kazamia, E., Cicuta, P. & Smith, A.G. (2014) Direct exchange of vitamin B12 is demonstrated by modelling the growth dynamics of algal-bacterial cocultures. *ISME Journal*, 8(7), 1418–1427. Available from: <https://doi.org/10.1038/ismej.2014.9>
- Grote, J., Bayindirli, C., Bergauer, K., de Moraes, P.C., Chen, H., D'Ambrosio, L. et al. (2011) Draft genome sequence of strain HIMB100, a cultured representative of the SAR116 clade of marine Alphaproteobacteria. *Standards in Genomic Sciences*, 5(3), 269–278. Available from: <https://doi.org/10.4056/sigs.1854551>
- Guillard, R.R.L. (1968) B12 specificity of marine centric diatoms. *Journal of Phycology*, 4(1), 59–64. Available from: <https://doi.org/10.1111/j.1529-8817.1968.tb04677.x>



- Hahnke, S., Brock, N.L., Zell, C., Simon, M., Dickschat, J.S. & Brinkhoff, T. (2013) Physiological diversity of Roseobacter clade bacteria co-occurring during a phytoplankton bloom in the North Sea. *Systematic and Applied Microbiology*, 36(1), 39–48. Available from: <https://doi.org/10.1016/j.syapm.2012.09.004>
- Han, D., Shin, H., Lee, J.H., Kang, C.K. & Kim, D.G. (2022) Phylogenetic diversity and spatiotemporal dynamics of bacterial and microeukaryotic plankton communities in Gwangyang Bay of the Korean peninsula. *Scientific Reports*, 12, 2980. Available from: <https://doi.org/10.1038/s41598-022-06624-7>
- Heal, K.R., Carlson, L.T., Devol, A.H., Armbrust, E.V., Moffett, J.W., Stahl, D.A. et al. (2014) Determination of four forms of vitamin B12 and other B vitamins in seawater by liquid chromatography/tandem mass spectrometry. *Rapid Communications in Mass Spectrometry: RCM*, 28(22), 2398–2404. Available from: <https://doi.org/10.1002/rcm.7040>
- Heal, K.R., Qin, W., Ribalet, F., Bertagnoli, A.D., Coyote-Maestas, W., Hmelo, L.R. et al. (2017) Two distinct pools of B12 analogs reveal community interdependencies in the ocean. *Proceedings of the National Academy of Sciences of the United States of America*, 114(2), 364–369. Available from: <https://doi.org/10.1073/pnas.1608462114>
- Helliwell, K.E., Lawrence, A.D., Holzer, A., Kudahl, U.J., Sasso, S., Kräutler, B. et al. (2016) Cyanobacteria and eukaryotic algae use different chemical variants of vitamin B12. *Current Biology*, 26(8), 999–1008. Available from: <https://doi.org/10.1016/j.cub.2016.02.041>
- Helliwell, K.E., Wheeler, G.L., Leptos, K.C., Goldstein, R.E. & Smith, A.G. (2011) Insights into the evolution of vitamin B12 auxotrophy from sequenced algal genomes. *Molecular Biology and Evolution*, 28(10), 2921–2933. Available from: <https://doi.org/10.1093/molbev/msr124>
- Hyatt, D., Gwo-Liang, C., LoCascio, P.F., Land, M.L., Larimer, F.W. & Hauser, L.J. (2010) Prodigal: prokaryotic gene recognition and translation initiation site identification. *BMC Bioinformatics*, 11, 119. Available from: <https://doi.org/10.1186/1471-2105-11-119>
- Joglar, V., Álvarez-Salgado, X.A., Gago-Martínez, A., Leao, J.M., Pérez-Martínez, C., Pontiller, B. et al. (2021) Cobalamin and microbial plankton dynamics along a coastal to offshore transect in the eastern North Atlantic Ocean. *Environmental Microbiology*, 23(3), 1559–1583. Available from: <https://doi.org/10.1111/1462-2920.15367>
- Karner, M.B., Delong, E.F. & Karl, D.M. (2001) Archaeal dominance in the mesopelagic zone of the Pacific ocean. *Nature*, 409, 507–510 [www.nature.com](http://www.nature.com)
- Kazamia, E., Czesnick, H., van Nguyen, T.T., Croft, M.T., Sherwood, E., Sasso, S. et al. (2012) Mutualistic interactions between vitamin B12-dependent algae and heterotrophic bacteria exhibit regulation. *Environmental Microbiology*, 14(6), 1466–1476. Available from: <https://doi.org/10.1111/j.1462-2920.2012.02733.x>
- Koch, F., Hattenrath-Lehmann, T.K., Goleski, J.A., Sañudo-Wilhelmy, S., Fisher, N.S. & Gobler, C.J. (2012) Vitamin B1 and B12 uptake and cycling by plankton communities in coastal ecosystems. *Frontiers in Microbiology*, 3, 363. Available from: <https://doi.org/10.3389/fmicb.2012.00363>
- Koch, F., Marcoval, M.A., Panzeca, C., Bruland, K.W., Sañudo-Wilhelmy, S.A. & Gobler, C.J. (2011) The effect of vitamin B12 on phytoplankton growth and community structure in the Gulf of Alaska. *Limnology and Oceanography*, 56(3), 1023–1034. Available from: <https://doi.org/10.4319/lo.2011.56.3.1023>
- Koch, F., Sañudo-Wilhelmy, S.A., Fisher, N.S. & Gobler, C.J. (2013) Effect of vitamins B1 and B12 on bloom dynamics of the harmful brown tide alga, *Aureococcus anophagefferens* (Pelagophyceae). *Limnology and Oceanography*, 58(5), 1761–1774. Available from: <https://doi.org/10.4319/lo.2013.58.5.1761>
- Kouzuma, A. & Watanabe, K. (2015) Exploring the potential of algae/bacteria interactions. *Current opinion in biotechnology*, 3, 125–129.
- Krabberød, A.K., Deutschmann, I.M., Bjorbækmo, M.F.M., Balagué, V., Giner, C.R., Ferrera, I. et al. (2022) Long-term patterns of an interconnected core marine microbiota. *Environmental Microbiomes*, 17(1), 22. Available from: <https://doi.org/10.1186/s40793-022-00417-1>
- Lambert, S., Lozano, J.C., Bouget, F.Y. & Galand, P.E. (2021) Seasonal marine microorganisms change neighbours under contrasting environmental conditions. *Environmental Microbiology*, 23(5), 2592–2604. Available from: <https://doi.org/10.1111/1462-2920.15482>
- Lambert, S., Tragin, M., Ghiglione, J.F., Lozano, J.C., Vaulot, D., Bouget, F.Y. et al. (2019) Rhythmicity of coastal marine picoeukaryotes, bacteria and archaea despite irregular environmental perturbations. *The ISME Journal*, 13, 388–401. Available from: <https://doi.org/10.1038/s41396-018-0281-z>
- Li, D., Liu, C.M., Luo, R., Sadakane, K. & Lam, T.W. (2015) MEGAHIT: an ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph. *Bioinformatics*, 31(10), 1674–1676. Available from: <https://doi.org/10.1093/bioinformatics/btv033>
- Li, H. & Durbin, R. (2009) Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics*, 25(14), 1754–1760. Available from: <https://doi.org/10.1093/bioinformatics/btp324>
- Li, Y., Ge, Y., Wang, J., Shen, C., Wang, J. & Liu, Y.J. (2021) Functional redundancy and specific taxa modulate the contribution of prokaryotic diversity and composition to multifunctionality. *Molecular Ecology*, 30(12), 2915–2930. Available from: <https://doi.org/10.1111/mec.15935>
- Lima-Mendez, G., Faust, K., Henry, N., Decelle, J., Colin, S., Carcillo, F. et al. (2015) Determinants of community structure in the global plankton interactome. *Science*, 347(6237), 1–10.
- Lomb, N.R. (1976) Least-squares frequency analysis of unequally spaced data. *Astrophysics and Space Science*, 39(2), 447–462. Available from: <https://doi.org/10.1007/BF00648343>
- Longobardi, L., Dubroca, L., Margiotta, F., Sarno, D. & Zingone, A. (2022) Photoperiod-driven rhythms reveal multi-decadal stability of phytoplankton communities in a highly fluctuating coastal environment. *Scientific Reports*, 12(1), 3908. Available from: <https://doi.org/10.1038/s41598-022-07009-6>
- Luo, H. & Moran, M.A. (2014) Evolutionary ecology of the marine Roseobacter clade. *Microbiology and Molecular Biology Reviews*, 78(4), 573–587. Available from: <https://doi.org/10.1128/mmr.00020-14>
- Ma, A.T., Tyrell, B. & Beld, J. (2020) Specificity of cobamide remodeling, uptake and utilization in *Vibrio cholerae*. *Molecular Microbiology*, 113(1), 89–102. Available from: <https://doi.org/10.1111/mmi.14402>
- Martens, J.H., Barg, H., Warren, M. & Jahn, D. (2002) Microbial production of vitamin B12. *Applied Microbiology and Biotechnology*, 58(3), 275–285. Available from: <https://doi.org/10.1007/s00253-001-0902-7>
- Martin, M. (2011) Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet Journal*, 17, 10–12.
- Massana, R., Delong, E.F. & Carlos, A. (2000) A few cosmopolitan phylotypes dominate planktonic archaeal assemblages in widely different oceanic provinces. *Applied and Environmental Microbiology*, 66(5), 1777–1787. <https://doi.org/10.1128/aem.66.5.1777-1787.2000>
- Matthews, R.G., Smith, A.E., Zhou, Z.S., Taurag, R.E., Bandarian, V., Evans, J.C. et al. (2003) Cobalamin-dependent and cobalamin-independent methionine synthases: are there two solutions to the same chemical problem? *Helvetica Chimica Acta*, 86(12), 3939–3954. Available from: <https://doi.org/10.1002/hlca.200390329>

- Moya, A. & Ferrer, M. (2016) Functional redundancy-induced stability of gut microbiota subjected to disturbance. *Trends in Microbiology*, 24(5), 402–413. Available from: <https://doi.org/10.1016/j.tim.2016.02.002>
- Nemergut, D.R., Schmidt, S.K., Fukami, T., O'Neill, S.P., Bilinski, T.M., Stanish, L.F. et al. (2013) Patterns and processes of microbial community assembly. *Microbiology and Molecular Biology Reviews*, 77(3), 342–356. Available from: <https://doi.org/10.1128/mmr.00051-12>
- Newton, R.J., Griffin, L.E., Bowles, K.M., Meile, C., Gifford, S., Givens, C.E. et al. (2010) Genome characteristics of a generalist marine bacterial lineage. *The ISME Journal*, 4, 784–798. Available from: <https://doi.org/10.1038/ismej.2009.150>
- Oksanen, J., Simpson, G.L., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R. et al. (2022) Package “vegan”: Community Ecology Package (version 2.6-2).
- Paerl, R.W., Bouget, F.Y., Lozano, J.C., Vergé, V., Schatt, P., Allen, E.E. et al. (2017) Use of plankton-derived vitamin B1 precursors, especially thiazole-related precursor, by key marine picoeukaryotic phytoplankton. *ISME Journal*, 11(3), 753–765. Available from: <https://doi.org/10.1038/ismej.2016.145>
- Panzeca, C., Tovar-Sanchez, A., Agustí, S., Reche, I., Duarte, C.M., Taylor, G.T. et al. (2006) B vitamins as regulators of phytoplankton dynamics. *Eos*, 87(52), 4–6. Available from: <https://doi.org/10.1029/2006EO520001>
- Pereira, O., Hochart, C., Galand, P.E. & Boeuf, D. (2021) Seasonality of archaeal proteorhodopsin and associated marine group IIb ecotypes (Ca. Poseidoniales) in the North Western Mediterranean Sea. *The ISME Journal*, 15, 1302–1316. Available from: <https://doi.org/10.1038/s41396-020-00851-4>
- Provasoli, L. & Carlucci, A.F. (1974) *Vitamins and growth regulators*. Botanical Monographs: Algal Physiology and Biochemistry, pp. 741–787.
- Qin, W., Heal, K.R., Ramdasi, R., Kobelt, J.N., Martens-Habben, W., Bertagnoli, A.D. et al. (2017) *Nitrosopumilus maritimus* gen. Nov., sp. nov., *Nitrosopumilus cobalaminigenes* sp. nov., *Nitrosopumilus oxycliniae* sp. nov., and *Nitrosopumilus ureiphilus* sp. nov., four marine ammonia-oxidizing archaea of the phylum thaumarchaeo. *International Journal of Systematic and Evolutionary Microbiology*, 67(12), 5067–5079. Available from: <https://doi.org/10.1099/ijsem.0.002416>
- Raux, E., Schubert, H.L. & Warren, M.J. (2000) Biosynthesis of cobalamin (vitamin B12): a bacterial conundrum. *Cellular and Molecular Life Sciences*, 57(13–14), 1880–1893. Available from: <https://doi.org/10.1007/PL00000670>
- Roda-García, J.J., Haro-Moreno, J.M., Huschet, L.A., Rodríguez-Valera, F. & López-Pérez, M. (2021) Phylogenomics of SAR116 clade reveals two subclades with different evolutionary trajectories and an important role in the ocean sulfur cycle. *MSystems*, 6(5), e0094421. Available from: <https://doi.org/10.1128/msystems.00944-21>
- Ruf, T. (1999) The Lomb-Scargle periodogram in biological rhythm research: analysis of incomplete and unequally spaced time-series. *Biological Rhythm Research*, 30(2), 178–201.
- Salazar, G., Paoli, L., Alberti, A., Huerta-Cepas, J., Ruscheweyh, H.J., Cuenca, M. et al. (2019) Gene expression changes and community turnover differentially shape the Global Ocean Metatranscriptome. *Cell*, 179(5), 1068–1083.e21. Available from: <https://doi.org/10.1016/j.cell.2019.10.014>
- Santoro, A.E., Dupont, C.L., Richter, R.A., Craig, M.T., Carini, P., McIlvin, M.R. et al. (2015) Genomic and proteomic characterization of “Candidatus Nitrosopelagicus brevis”: an ammonia-oxidizing archaeon from the open ocean. *Proceedings of the National Academy of Sciences of the United States of America*, 112(4), 1173–1178. Available from: <https://doi.org/10.1073/pnas.1416223112>
- Sañudo-Wilhelmy, S.A., Cutter, L.S., Durazo, R., Smail, E.A., Gómez-Consarnau, L., Webb, E.A. et al. (2012) Multiple B-vitamin depletion in large areas of the. *Proceedings of the National Academy of Sciences of the United States of America*, 109(35), 14041–14045. Available from: <https://doi.org/10.1073/pnas.1208755109>
- Sañudo-Wilhelmy, S.A., Gobler, C.J., Okbamichael, M. & Taylor, G.T. (2006) Regulation of phytoplankton dynamics by vitamin B12. *Geophysical Research Letters*, 33(4), 10–13. Available from: <https://doi.org/10.1029/2005GL025046>
- Sañudo-Wilhelmy, S.A., Gómez-Consarnau, L., Suffridge, C. & Webb, E.A. (2014) The role of B vitamins in marine biogeochemistry. *Annual Review of Marine Science*, 6, 339–367. Available from: <https://doi.org/10.1146/annurev-marine-120710-100912>
- Scargle, J.D. (1982) Studies in astronomical time series analysis. II. Statistical aspects of spectral analysis of unevenly spaced data. *The Astrophysical Journal*, 263, 835–853.
- Schauer, M., Balagué, V., Pedrós-Alió, C. & Massana, R. (2003) Seasonal changes in the taxonomic composition of bacterioplankton in a coastal oligotrophic system. *Aquatic Microbial Ecology*, 31(2), 163–174. Available from: <https://doi.org/10.3354/ame031163>
- Seymour, J.R., Amin, S.A., Raina, J.B. & Stocker, R. (2017) Zooming in on the phycosphere: the ecological interface for phytoplankton-bacteria relationships. *Nature Microbiology*, 2(7), 17065. Available from: <https://doi.org/10.1038/nmicrobiol.2017.65>
- Shelton, A.N., Seth, E.C., Mok, K.C., Han, A.W., Jackson, S.N., Haft, D.R. et al. (2019) Uneven distribution of cobamide biosynthesis and dependence in bacteria predicted by comparative genomics. *ISME Journal*, 13(3), 789–804. Available from: <https://doi.org/10.1038/s41396-018-0304-9>
- Sommaruga, R., Hofer, J.S., Alonso-Sáez, L. & Gasol, J.M. (2005) Differential sunlight sensitivity of picophytoplankton from surface Mediterranean coastal waters. *Applied and Environmental Microbiology*, 71(4), 2154–2157. Available from: <https://doi.org/10.1128/AEM.71.4.2154-2157.2005>
- Steinegger, M. & Söding, J. (2018) Clustering huge protein sequence sets in linear time. *Nature Communications*, 9(1), 2542. Available from: <https://doi.org/10.1038/s41467-018-04964-5>
- Strom, S.L. (2008) Microbial ecology of ocean biogeochemistry: a community perspective. *Science*, 320(5879), 1043–1045. Available from: <https://doi.org/10.1126/science.1153527>
- Suffridge, C.P., Gómez-Consarnau, L., Monteverde, D.R., Cutter, L., Aristegui, J., Alvarez-Salgado, X.A. et al. (2018) B vitamins and their congeners as potential drivers of microbial community composition in an oligotrophic marine ecosystem. *Journal of Geophysical Research: Biogeosciences*, 123(9), 2890–2907. Available from: <https://doi.org/10.1029/2018JG004554>
- Teeling, H., Fuchs, B.M., Becher, D., Klockow, C., Gardebrecht, A., Bennis, C.M. et al. (2012) Substrate-controlled succession of marine bacterioplankton populations induced by a phytoplankton bloom. *Science*, 336(6081), 608–611. Available from: <https://doi.org/10.1126/science.1218344>
- Varaljay, V.A., Gifford, S.M., Wilson, S.T., Sharma, S., Karl, D.M. & Moran, M.A. (2012) Bacterial Dimethylsulfoniopropionate degradation genes in the oligotrophic North Pacific subtropical gyre. *Applied and Environmental Microbiology*, 78(8), 2775–2782. Available from: <https://doi.org/10.1128/AEM.07559-11>
- Villegas-Mendoza, J., Cajal-Medrano, R. & Maske, H. (2019) B12 production by marine microbial communities and *Dinoroseobacter shibae* continuous cultures under different growth and respiration rates. *Aqua*, 83, 251–262.
- Walworth, N.G., Lee, M.D., Suffridge, C., Qu, P., Fu, F.X., Saito, M.A. et al. (2018) Functional genomics and phylogenetic evidence suggest genus-wide cobalamin production by the globally distributed marine nitrogen fixer *Trichodesmium*. *Frontiers in Microbiology*, 9, 1–12. Available from: <https://doi.org/10.3389/fmicb.2018.00189>

- Wang, H., Tomasch, J., Jarek, M. & Wagner-Döbler, I. (2014) A dual-species co-cultivation system to study the interactions between Roseobacters and dinoflagellates. *Frontiers in Microbiology*, 5(JUN), 1–12. Available from: <https://doi.org/10.3389/fmicb.2014.00311>
- Wienhausen, G., Bittner, M.J. & Paerl, R.W. (2022) Key knowledge gaps to fill at the cell-to-ecosystem level in marine B-vitamin cycling. *Frontiers in Marine Science*, 9(May), 1–9. Available from: <https://doi.org/10.3389/fmars.2022.876726>
- Yachi, S. & Loreau, M. (1999) Biodiversity and ecosystem productivity in a fluctuating environment: the insurance hypothesis. *Proceedings of the National Academy of Sciences of the United States of America*, 96(4), 1463–1468. Available from: <https://doi.org/10.1073/pnas.96.4.1463>
- Zhou, J., Yu, X., Liu, J., Qin, W., He, Z., Stahl, D. et al. (2021) VB12 path for accurate metagenomic profiling of microbially driven cobalamin synthesis pathways. *MSystems*, 6(3), e00497.
- Zhou, J., Zhang, B., Yu, K., Du, X. & Zhu, J. (2020) Functional profiles of phycospheric microorganisms during a marine dinoflagellate bloom. *Water Research*, 173, 115554. Available from: <https://doi.org/10.1016/j.watres.2020.115554>

- Zhu, W., Lomsadze, A. & Borodovsky, M. (2010) Ab initio gene identification in metagenomic sequences. *Nucleic Acids Research*, 38(12), 1–15. Available from: <https://doi.org/10.1093/nar/gkq275>

## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

**How to cite this article:** Beauvais, M., Schatt, P., Montiel, L., Logares, R., Galand, P.E. & Bouget, F.-Y. (2023) Functional redundancy of seasonal vitamin B<sub>12</sub> biosynthesis pathways in coastal marine microbial communities. *Environmental Microbiology*, 25(12), 3753–3770. Available from: <https://doi.org/10.1111/1462-2920.16545>