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Long term seasonality of microbial vitamin B1 and B12 metabolisms and their potential interplay in the Northwestern Mediterranean Sea

Maxime Beauvais

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École doctorale des Sciences de l'environnement d'Ile-de-France (ED129)

Laboratoire d'Océanographie Microbienne (UMR 7621)

Observatoire Océanologique de Banyuls-sur-Mer

**Long term seasonality of microbial vitamin B₁ and B₁₂
metabolisms and their potential interplay in the
Northwestern Mediterranean Sea**

Par Maxime BEAUVAIS

Thèse de doctorat en Écologie Microbienne

Dirigée par **François-Yves BOUGET** et **Pierre GALAND**

Soutenue publiquement le 15 décembre 2023 à Banyuls-sur-Mer

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List of publications

- Beauvais. M., Schatt. P., Montiel. L., Logares. R., Galand. PE., and Bouget. FY. (2023). *Functional redundancy of seasonal vitamin B₁₂ biosynthesis pathways in coastal marine microbial communities*. *Environmental Microbiology* (in press)
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Contribution to scientific conferences



18th International Symposium on Microbial Ecology (ISME)

(14-19 August 2022 Lausanne, Switzerland)

Seasonality and functional redundancy of vitamin B₁₂ biosynthesis pathway in a Northwestern Mediterranean coastal site over 7 years

(Poster presentation)



ASLO Aquatic Sciences Meeting (4–9 June 2023 · Palma de Mallorca, Spain)

Seasonality, functional redundancy, and interplay of vitamin B₁ and B₁₂ metabolisms in a Northwestern Mediterranean coastal site over 7 years

(Oral presentation)

I. CHAPTER I: Introduction

1. Role, dynamics, and functions of marine microbial communities

A. Microbial communities are key players of ocean biogeochemical cycles

Marine microbial communities are characterized by a tremendous diversity of organisms that exhibit an outstanding variety of functions (Carradec et al., 2018; de Vargas et al., 2015; Salazar et al., 2019; Sunagawa et al., 2020). Marine phytoplankton and cyanobacteria ensure about a half of the worldwide primary production (Field et al., 1998) while about a half of the organic carbon fixed by marine autotrophs is directly consumed by heterotrophic bacteria (Ducklow & Kirchman, 2000). It is now established that these organisms are the main drivers of ocean biogeochemical cycles, significantly influencing critical element fluxes (C, H, O, N, P, S) through their metabolic activities (Falkowski et al., 2008) (**Fig. I.1**). Photosynthetic organisms (*i.e.*, phytoplankton and cyanobacteria) capture energy from sunlight to convert inorganic carbon dioxide (CO₂) into complex organic molecules *via* the photosynthesis (Field et al., 1998) (**Fig. I.1**). Phytoplanktonic cells such as diatoms, dinoflagellates, *Prymnesiophyceae* and *Chlorophyceae*, can proliferate significantly in a transient but sudden manner in localized to massively scaled events, named blooms (Buchan et al., 2014; Shutler et al., 2013). These blooms strongly stimulate biogeochemical activities by contributing directly to CO₂ and inorganic nutrients assimilation, dissolved organic matter (DOM, < 0.45µm) and particulate organic matter (POM, > 0.45µm) release, but also indirectly by stimulating nutrient recycling by prokaryotes. Phytoplanktonic blooms play a key role in carbon export and long-term sequestration through the microbial carbon pump (Buchan et al., 2005; Jiao et al., 2010) (**Fig. I.1**). The pool of organic and inorganic substrates produced by phytoplankton is thus at the basis of nutrient cycling and recycling in the ocean in the microbial loop (Azam & Malfatti, 2007; Buchan et al., 2014; Falkowski et al., 2008) (**Fig. I.1**), influencing community structure and functions over short (*i.e.*, bloom events) (Hahnke et al., 2013; Teeling et al., 2012, 2016;

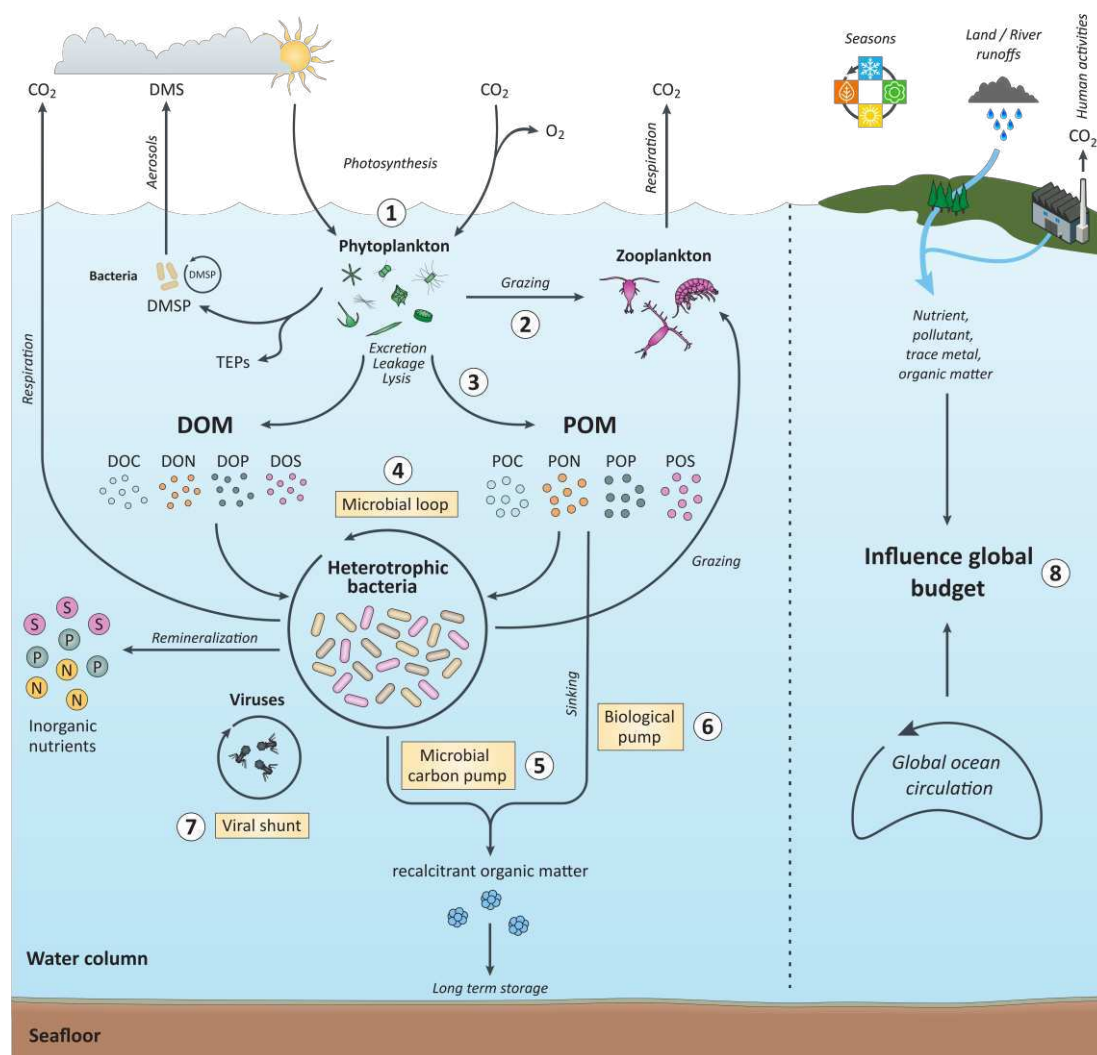


Figure I.1: Overview of the key processes involved in ocean biogeochemical cycles, in which marine microbial communities play a central role. (1) Photosynthetic phytoplankton communities capture energy from sunlight and uptake dissolved carbon dioxide (CO₂) from the seawater to produce organic molecules such as carbohydrates, and dioxygen (O₂) as a side-product (photosynthesis). (2) Grazers, such as zooplankton, regulate phytoplankton communities *via* predation (grazing) and release CO₂ via respiration. (3) Phytoplankton release a considerable amount of dissolved (DOM, left side) and particulate (POM, right side) organic matter, which is enriched in carbon (DOC, POC), nitrogen (DON, PON), phosphate (DOP, POP) and sulfur (DOS, POS). This pool of organic matter may result from cell leakage, viral lysis, or excretion. Secondary metabolites such as dimethylsulfoniopropionate (DMSP) and transparent exopolymer particles (TEPs), could also be secreted by phytoplankton. DMSP could be produced and metabolized by some bacteria and algae into the aerosol dimethylsulfide (DMS), which influence sulfur cycle and together with TEPs, the climate. TEPs are large, sticky, and acidic polysaccharides released by senescent cells and involved in particle aggregation. (4) Heterotrophic bacteria remineralize and recycle organic matter into inorganic forms, which are bioassimilable for phytoplankton, and releases CO₂ *via* respiration (microbial loop). A fraction of the carbon stored in heterotrophic bacteria is transferred up the food web *via* grazing. (5) Another part of this carbon pool, which resists microbial degradation, sinks, and is sequestered for thousands of years as recalcitrant organic matter on the seafloor (microbial carbon pump). (6) The biological pump refers to the export of phytoplankton-derived organic matter from the surface to the depths *via* sinking. (7) The viral shunt describes the contribution of virus-mediated cell lysis (phytoplankton and bacteria) to the release of DOM and POM. (8) The global budget of these processes is largely influenced by environmental parameters and physicochemical properties of seawater, which are driven by seasons, land runoffs (sources of natural and anthropogenic inputs) and oceanic currents. Adapted from (Buchan et al., 2014).

Francis et al., 2021; Huang et al., 2018; Matsumoto et al., 2021) and long timescales (Chen et al., 2021). Phytoplankton also produce a great diversity of secondary metabolites such as dimethylsulfoniopropionate (DMSP), a sulphur-rich compound that can be converted into dimethylsulphide (DMS) aerosol by major bacterial (*i.e.*, *Roseobacter*, *SAR11*, *SAR116*) (Reisch et al., 2008, 2011; Roda-Garcia et al., 2021; Todd et al., 2009) and phytoplankton groups (coccolithophores and dinoflagellates) (Alcolombri et al., 2015) (**Fig. I.1**). DMS emissions from surface seawaters are notably one of the major sources of sulphur in the atmosphere (Hopkins et al., 2023). Phytoplankton-derived organic matter could be released into the seawater *via* several mechanisms, including export from living cells (either passive or active) (Landa et al., 2017, 2019), viral lysis (Lønborg et al., 2013), protist grazing (Sherr & Sherr, 2016) or by leakage from senescent cells (Thornton, 2014) (**Fig. I.1**). Viral lysis and grazing are important top-down controls of phytoplankton and bacterial communities in ocean, which redirect carbon and nutrients to higher trophic levels (Buchan et al., 2014; Cram et al., 2016) (**Fig. I.1**).

The quality, amount, and fate of phytoplanktonic-derived organic matter in the oceans are thus primarily mediated by marine heterotrophic bacteria activities' which remineralize and recycle these organic compounds (**Fig. I.1**). In ocean, heterotrophic bacteria have adopted several trophic strategies to cope with organic matter bioavailability, from fast responding copiotrophs which rapidly exploit increasing carbon and nutrient loads (*i.e.*, *Roseobacter*, *Flavobacteria* and *Alteromonadales*) (Buchan et al., 2014; Ivars-Martinez et al., 2008; Tada et al., 2011) to slow growing oligotrophs thriving on lower organic carbon concentrations (*i.e.*, *Pelagibacterales* such as *SAR11*) (Carini et al., 2013). Marine bacteria have a short generation time in ocean surface (from a few hours to days) (Kirchman, 2016), and therefore respond rapidly to the physicochemical gradients of metabolites in the seawater and to environmental conditions changes. In summary, marine microbial communities exhibit a wide range of trophic strategies mirrored by different lifestyles which underly a multitude of population dynamics.

B. The composition of marine microbial assemblages is dynamic over time

Marine microbial communities respond to a coupling of environmental (*i.e.*, *abiotic*) and biological (*i.e.*, *biotic*) forcings that shape their composition, functions, and dynamics (Fuhrman et al., 2015; Lima-Mendez et al., 2015; Louca et al., 2016; Seymour et al., 2017). Temperature, photoperiod, salinity, and nutrient concentrations are the main environmental drivers of marine microbial community structure, functions, and dynamics (Carradec et al., 2018; Fuhrman et al., 2015; Gilbert et al., 2012; Lambert et al., 2019; Louca et al., 2016; Salazar et al., 2019; Sunagawa et al., 2015), but their respective contributions vary from one region to another (Bunse & Pinhassi, 2017).

i. Microbial community successions in temperate regions

From Sverdrup’s “Critical Depth Hypothesis” to Behrenfeld’s “Dilution Recoupling Hypothesis”, it is now thought that phytoplanktonic bloom initiation is governed by the balance between biomass production (growth) and biomass loss from physical (sinking) and biological (grazing) processes (Behrenfeld, 2010; Sverdrup, 1953). This balance is strongly influenced by light and by the internal movements of water masses, such as water mixing events, which could reduce the predation pressure in sunlit waters by dilution (Fischer et al., 2014).

In temperate regions, the predictable and recurrent patterns of phytoplankton dynamics is a key feature to link prokaryotic population composition to phytoplankton succession (Bunse & Pinhassi, 2017). Most phytoplankton blooms occur during the yearly transitions from winter to spring, following water mixing events that brings nutrients to the surface. In addition to higher nutrient levels, an increased irradiance, photoperiod, sea surface temperature, and a reduced grazing pressure, trigger these phytoplankton blooms which are often dominated by diatoms and dinoflagellates (Bunse & Pinhassi, 2017). Seasonal variation in environmental conditions and phytoplankton composition strongly impact the microbial community structure and dynamic (Bunse & Pinhassi, 2017). Prominent shifts in bacterioplankton composition were

reported in the North Sea and in the Baltic Sea in response to phytoplanktonic blooms, during which copiotrophic bacteria (*i.e.*, *Alteromonadales*, *Roseobacter*, *Flavobacteria*), actively degrade phytoplankton-derived organic matter (Buchan et al., 2014; Francis et al., 2021; Hahnke et al., 2013; Lindh et al., 2015; Teeling et al., 2012, 2016). Increased abundances of *Verrucomicrobia* were also reported during phytoplanktonic blooms in the North Sea (Teeling et al., 2016), and during cyanobacterial bloom in the Atlantic Ocean (Alonso-Sáez et al., 2015) and in the Baltic Sea (Lindh et al., 2015). Moreover, higher temperature during warmer seasons increased the metabolic potentialities associated with phytoplankton-derived DOM utilization by marine heterotrophic bacteria, underlying potential adaptive associations between bacteria and phytoplankton based on the chemical nature of algal exudates (Morán et al., 2023).

The spring bloom leads to rapid nutrient depletion in seawater during more stratified summer periods, and smaller-sized phytoplankton and cyanobacteria, which are adapted to oligotrophic conditions and higher temperatures, take over (Bunse & Pinhassi, 2017). *Synechococcus*, SAR11, *Roseobacter*, and SAR86 are often dominating summer stratified waters in temperate seas (Alderkamp et al., 2006; Alonso-Sáez et al., 2015; Bunse & Pinhassi, 2017; Lindh et al., 2015). During the autumn to winter transition, decreasing temperature and light combined with nutrient renewal promote diatom and dinoflagellate blooms, which are notably associated with increased abundances of *Actinobacteria*, *Flavobacteria*, *SAR11*, and *Planctomycetes* in the Baltic Sea (Lindh et al., 2015). Members of *Roseobacter* and *Gammaproteobacteria* also showed recurrent maximal abundances during winter nutrient-rich conditions in the Atlantic Ocean (Alonso-Sáez et al., 2015). An elevated diversity within bacterioplankton communities in winter mixed waters has been reported in the English Channel (Gilbert et al., 2012) and in the coastal Atlantic Ocean (García et al., 2015) and appears to be a general feature in mid- to high-latitude environments (Ladau et al., 2013).

ii. Microbial community successions in polar regions

In polar regions, the strong seasonal variability observed in environmental conditions (*i.e.*, long ice cover and darkness periods in winter, and low temperature combined with extended irradiance in summer) results in pronounced shifts in marine microbial community (Bunse & Pinhassi, 2017; Grzymalski et al., 2012; Iversen & Seuthe, 2011; Williams et al., 2012). As observed in temperate regions, increasing phylogenetic and functional diversity are reported in winter, which are correlated with temperature and brine salinity (Ghiglione & Murray, 2012; Torstensson et al., 2015; Williams et al., 2012). Chemolithoautotrophic processes also increase in importance during winter, and ammonia oxidizing *Thaumarchaeota* can represent up to 16% of cells (Alonso-Sáez et al., 2008; Church et al., 2003), with notably *Nitrosopumilus maritimus* which contributed to 30% of polar metaproteomes (Williams et al., 2012). In late spring, runoffs (from land and snowmelt) and increasing irradiance trigger phytoplanktonic blooms of unicellular microalgae which lead to prominent shifts in bacterioplankton composition (Boetius et al., 2015; Torstensson et al., 2015). *Roseobacter*, *Alteromonadales* and *Flavobacteria* displayed strong seasonal variations in polar regions, reflecting potential differences in DOM utilization by these bacteria (Alonso-Sáez et al., 2008; Ghiglione & Murray, 2012; Williams et al., 2012). In polar metaproteomes, proteins related to *Alteromonadales* and *Flavobacteria* showed higher abundances in summer and were associated with non-labile polymers degradation, while *Roseobacter* and SAR11 were stable and abundant contributors of the community, and primarily expressed proteins for labile DOM utilization (Williams et al., 2012).

All these studies covering a wide range of oceanic biotopes that differ in terms of temperature, salinity, and photoperiod, improved our understanding of marine microbial community structuring in response to the contrasting environmental conditions of the global ocean. However, they do not consider the inter-annual variations and the fine-scale dynamic of marine microbial communities, which are hard to capture by such discrete sampling schemes.

iii. Marine microbial community composition is strongly predictable

Long-term time series studies recently offer unique opportunities to better understand the seasonal variability of marine microbial community structure over long timescales. Time-series studies have been conducted over a decade at several geographic locations such as the Hawaiian Ocean Time series (HOT) in the North Pacific, the San Pedro Ocean Time-series (SPOT) off the Californian coast, and the microbial observatories of Blanes Bay (BBMO) and Banyuls Bay (SOLA), coastal stations 100 km apart in the northwestern (NW) Mediterranean Sea.

In the northern Pacific gyre (HOT station), bacterioplankton dynamics showed inter-annually recurring patterns in surface layers, and environmental drivers such as wind speed and light intensity, influence inorganic nutrient concentrations and phytoplankton composition, which ultimately control bacterial community composition (Bryant et al., 2016; Eiler et al., 2011). Seasonal and recurrent patterns of diversity were also reported at SPOT station, with annually reoccurring bacteria (Chow et al., 2013; Cram et al., 2015; Fuhrman et al., 2006) and archaea communities (Beman et al., 2011; Cram et al., 2015; Parada & Fuhrman, 2017) which could reliably be predicted from seasonal environmental parameters (light and temperature). Key taxa of the microbial food web exhibited pronounced seasonal patterns at SPOT both in surface (e.g., *Prochlorococcus*, *SAR11*, *SAR86*) and deep-water layers (*Gammaproteobacteria*, *Deltaproteobacteria*, *Flavobacteria*) (Chow et al., 2013; Cram et al., 2015). The dynamics of marine archaea over 5 years at SPOT revealed distinct marine group I (MGI, *Thaumarchaeota*) and II (MGII, *Euryarchaeota*) assemblages along the water column, which were seasonal at all depths (0 – 890 m) and could realize potential dynamic associations with bacteria (Parada & Fuhrman, 2017). Various assemblages of MGI (ammonia oxidizers) and *Nitrospina* (mostly nitrite oxidizers) observed along the water column suggest that these taxa could complement each other in the nitrification process at different depths (Parada & Fuhrman, 2017).

A strong inter-annual predictability of microbial community composition was reported in the NW Mediterranean Sea for bacteria (Alonso-Sáez et al., 2007; Auladell et al., 2019, 2022; Krabberød et al., 2022; Lambert et al., 2019; Salter et al., 2015), archaea (Galand et al., 2010; Hugoni et al., 2013; Lambert et al., 2021; Pereira et al., 2021) and eukaryotes (Giner et al., 2019; Krabberød et al., 2022; Lambert et al., 2019). These studies improved our understanding on these recurrent seasonal patterns, as well as the potential interplay between marine microbes (Arandia-Gorostidi et al., 2022a; Auladell et al., 2019; Deutschmann et al., 2023; Krabberød et al., 2022) or their responses to irregular environmental changes (Lambert et al., 2021).

The long-term (7 years) taxonomic study of SOLA planktonic communities showed a strong seasonality of microbial groups belonging to all kingdoms, including phytoplankton, bacteria, and archaea, which were mainly driven by day length and temperature (Lambert et al., 2019) (**Fig. I.2**). The bacterial fraction of SOLA planktonic community is much more diverse and dynamic than the others, with alternating abundance patterns in the rhythmic sequences (~30 to 40%) of four main groups: *Alphaproteobacteria* (~20% all year round), *Gammaproteobacteria* from January to March (~10%), cyanobacteria from March to September (~10 to 20%) and *Flavobacteria* from April to June (~10%) (Lambert et al., 2019) (**Fig. I.2A, B**). The analysis of SAR11-clades dynamics in the open Mediterranean Sea showed a shift in bacterial composition at the ecotype-level following deep water mixing events in winter, which resets SAR11 communities to a high diversity state compared to the low diversity exhibited by ecotypes which dominate in summer (Salter et al., 2015). These observations further emphasize the impact of seasonal vertical mixing periods on community diversity and dynamics in many oceanic regions, impacting the most dominant bacteria in ocean surface waters (Bolaños et al., 2022; Carlson et al., 2009). Within cyanobacteria, *Synechococcus* and *Prochlorococcus* are very abundant in the Mediterranean Sea and are known to share distinct ecological niches as they bloom at different times of the year (*i.e.*, from spring to autumn and

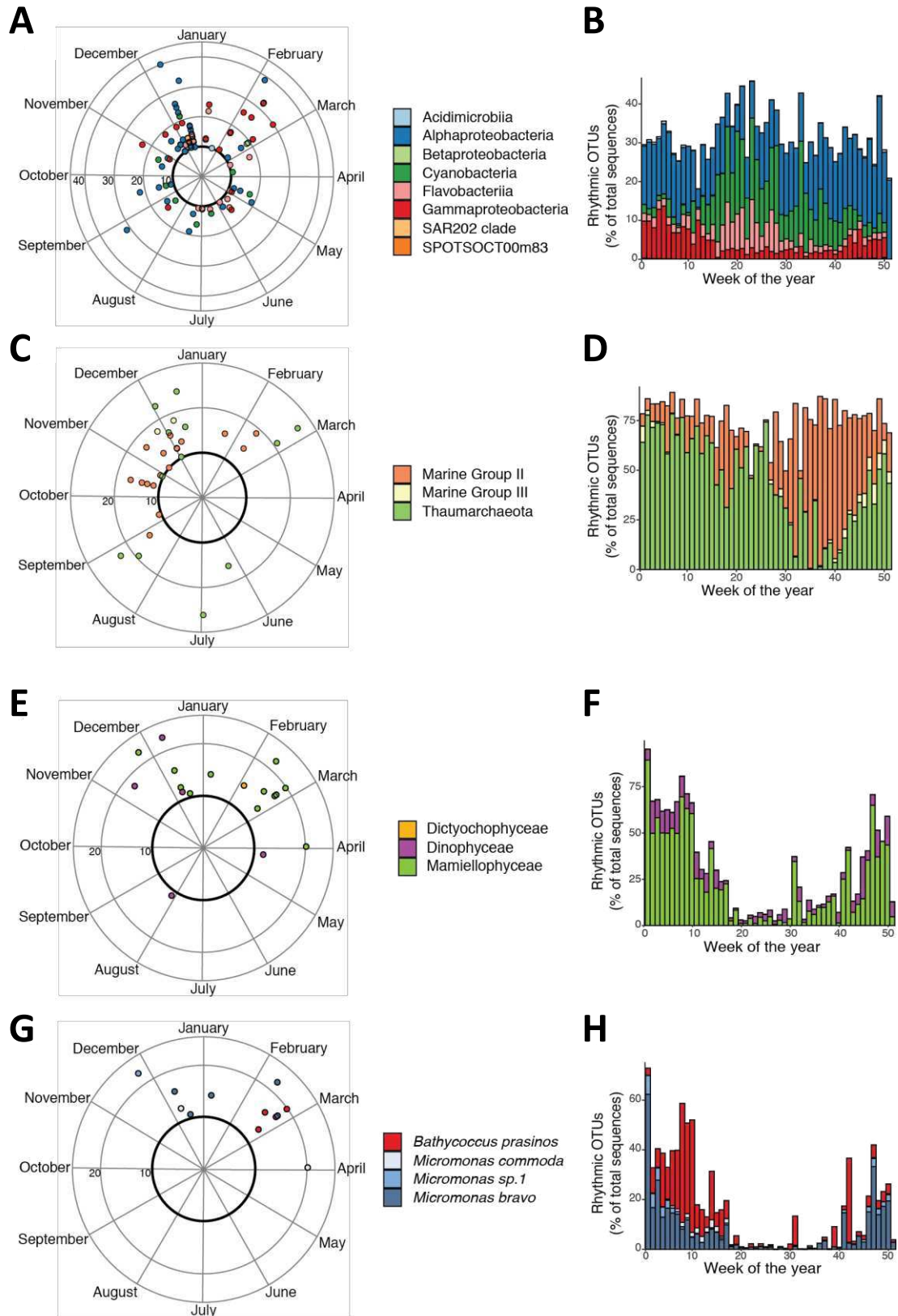


Figure I.2: Overview of the seasonality observed within SOLA planktonic communities from a 7-year metabarcoding study. The polar plot represents the rhythmic ASVs belonging to bacteria (A), archaea (C), eukaryotic phytoplankton (E) and *Mamiellophyceae* (G). Their respective bar plots show the proportion of sequences belonging to rhythmic ASVs averaged per week of the year for bacteria (B), archaea (D), eukaryotic phytoplankton (F) and *Mamiellophyceae* (H). Adapted from (Lambert et al., 2019).

from autumn to winter, respectively) (Schauer et al., 2003; Sommaruga et al., 2005). Within archaea, rhythmic sequences accounted for more than a half of total sequences throughout the year (~80% in autumn/spring) and showed an alternation between *Thaumarchaeota* from November to June and MGII from July to October (Lambert et al., 2019) (**Fig. I.2C, D**). Long term survey of archaeal communities at SOLA showed that distinct MGII subclades had different seasonal dynamics (Galand et al., 2010; Hugoni et al., 2013). MGIIa were abundant during summer in nutrient-limiting conditions, while MGIIb thrive in winter under nutrient-rich conditions (Galand et al., 2010; Hugoni et al., 2013). Along with temperature and nutrient availability, the photo-inhibition of archaeal ammonia oxidation could explain their repartition in the ocean (Lu et al., 2020; Merbt et al., 2017; Peng et al., 2013). Phytoplankton represented few rhythmic sequences in summer but over 50% of total sequences from autumn to winter at SOLA station and were dominated by Dinoflagellates and *Mamiellophyceae* (**Fig. I.2E, F**). A recurrent succession between *Micromonas bravo* from November to January and *Bathycoccus prasinos* from January to March is notably observed (Lambert et al., 2019) (**Fig. I.2G, H**).

It is striking to note that the strong predictable pattern of microbial communities appears to be maintained over 7 years despite irregular environmental perturbations at SOLA station (Lambert et al., 2019). Nevertheless, distinct co-occurrence patterns were revealed in a high frequency timeseries covering 3 years at SOLA station (Lambert et al., 2021). By carrying out weekly, and bi-weekly sampling schemes, this study shows that the networks linking microbial community members changed year to year in response to contrasted environmental conditions (Lambert et al., 2021). Impacts on microbial communities were also observed at the individual scale, with less interconnected taxa changing their first neighbors under perturbations (Lambert et al., 2021). Thus, changes in community composition could be detected by carrying a higher sampling frequency or by looking at a finer taxonomic level (Lambert et al., 2021).

C. Seasonal dynamics of microbial functions inferred from omics data

The advent of metagenomic approaches has recently expanded the scope for the taxonomic and functional characterization of marine microbial communities at local (Bryant et al., 2016; Francis et al., 2021; Galand et al., 2018; Grzymiski et al., 2012; Huang et al., 2018; Matsumoto et al., 2021; Pan et al., 2020; Pereira et al., 2021; Teeling et al., 2016; S. Wang et al., 2020; Zhang et al., 2023; Zhou et al., 2020) and global scale (Louca et al., 2016; Rusch et al., 2007; Sunagawa et al., 2015, 2020; Yooseph et al., 2007; Yutin et al., 2007).

i. Functional characterization of phycospheric communities

Even though often incomplete (*i.e.*, not covering the full year at regular sampling interval), several metagenomic studies have been conducted to characterize the functions of prokaryotic communities associated with phytoplankton blooms (Francis et al., 2021; Huang et al., 2018; Matsumoto et al., 2021; Pan et al., 2020; Teeling et al., 2016; Zhou et al., 2020).

In the North Sea, *Flavobacteria*, *Gammaproteobacteria*, and *Alphaproteobacteria* exhibited strong recurrence patterns during 4 annual spring blooms surveyed from 2009 to 2012, and successively degrade algal-derived organic matter using distinct carbohydrate-active enzymes and phosphate acquisition strategies (Francis et al., 2021; Teeling et al., 2016). Bacterioplankton also displayed distinct organic matter utilization strategies in response to phytoplankton succession in the Kerguelen Islands over 4 months (Zhang et al., 2023) in the Sendai Bay (Japan) over a year (Matsumoto et al., 2021). Early phytoplankton bloom activity increased the abundance of genes involved in amino acid metabolisms, while genes involved in signal transduction and cellular communication were prevalent in mid to post-bloom phases, suggesting the prevalence of particle-attached lifestyles (Matsumoto et al., 2021). Functional genes dynamics are consistent with the dynamic observed in the chemical nature of algal-derived exudates during blooms, with low molecular weighted molecules at early stages (amino acids) and high molecular weight molecules in late stages (polysaccharides) (Buchan et al.,

2014; Zhou et al., 2020). These observations suggests that distinct degradations processes are carried seasonally by different organisms in response to the chemical nature of algal exsudates (Francis et al., 2021; Matsumoto et al., 2021; Teeling et al., 2012; Zhou et al., 2020). A weekly metagenomic assessment of genes related to quorum sensing during a dinoflagellate bloom suggest the importance of cellular communication processes for the regulation of ecological and chemical relationships between prokaryotes and bloom-forming algae (Huang et al., 2018).

ii. Anoxygenic Aerobic Photosynthesis (AAP) and Proteorhodopsins (PR)

Targeted analysis of the AAP marker gene *pufM* at the Blanes station (monthly sampling) revealed seasonal (Ferrera et al., 2014) and interannual (Auladell et al., 2019) variability of marine AAP bacterial assemblages with a succession of distinct phylotypes along the year. Among the 8 major bacterial APP phylogroups which dominate GOS sequences (Yutin et al., 2007), the phylogroup K (*Gammaproteobacteria*) was the greatest contributor to community structure over all seasons in the Mediterranean Sea, with phylogroups E (*Rhodobacter*-like) and G (*Roseobacter*-like) being prevalent in spring (Auladell et al., 2019). AAP bacteria and AAP related genes are more abundant during spring/summer (from April to October) than during autumn/winter (from November to March), both in the Mediterranean Sea (Auladell et al., 2019; Ferrera et al., 2014; Galand et al., 2018) and in the Atlantic Ocean (Alonso-Sáez et al., 2020).

A focus on archaeal PR associated with MGIIb group (*Candidatus Thalassarchaeaceae*) at SOLA station revealed a seasonal succession of different spectral tunings of the archaeal PR, with green-tuned rhodopsins in autumn followed by blue in the middle of the winter, and green again in spring (Pereira et al., 2021). Among archaea, different subclades of MGIIb possessed distinct lifestyles and physiologies linked to their respective ecological niches (Pereira et al., 2021). Some of these ecotypes (*i.e.*, O3 and WHARN) had recurrent seasonal patterns of abundance associated to a reproducible seasonal succession in winter. About bacterial PR, they were higher than *pufM* transcripts in the Atlantic Ocean and showed seasonal trends with

maximal abundances in summer during two consecutive years (Alonso-Sáez et al., 2020). PR-related genes were mainly expressed by *Pelagibacteraceae*, but distinct organisms achieved PR-mediated phototrophy during spring 2012 compared to spring 2011, such as *Gammaproteobacteria* (including *Thioglobus*), *Bacteroidetes* and *Euryarchaeota* (Alonso-Sáez et al., 2020). This year was contrasted considering low bacterial abundances reported *in-situ*, mirrored by important changes in community composition (Alonso-Sáez et al., 2015; Morán et al., 2015). Similarly, PR-related gene transcripts were mostly dominated by SAR11 at SPOT station, excepting during a spring algal bloom during which *Gammaproteobacteria* dominated PR-related transcripts (Sieradzki et al., 2018). These observations suggest that PR-mediated phototrophy is widespread and is mostly ensured by *Alphaproteobacteria* (*i.e.*, SAR11), but could be handled by *Gammaproteobacteria* members during contrasted abiotic or biotic conditions, both in temperate (Atlantic Ocean) and subtropical oceanic regions (Pacific Ocean).

iii. Biogeochemically relevant functions

In the Atlantic Ocean, biogeochemically relevant functions such as *nifH* (nitrogen fixation), *amoA* (ammonia oxidation), *soxB* (sulfur oxidation) and *coxL* (CO oxidation) have shown seasonal expressions patterns. *NifH* and *amoA* occurred successively in summer and autumn as they are carried by distinct organisms (Alonso-Sáez et al., 2020). The diazotrophic cyanobacterium *Candidatus Atelocyanobacterium Thalassa*, formerly UCYN-A (Thompson et al., 2012), accounted for 96% of *nifH*-related transcripts, while *amoA* genes were represented by ammonia oxidizing *Thaumarchaeota* (Alonso-Sáez et al., 2020). *SoxB* and *coxL* expressions were dominated by *Rhodobacteraceae* and showed distinct expression profiles from *amoA*, with higher abundances reported in summer (Alonso-Sáez et al., 2020). These observations suggest an alternation between different trophic strategies towards N, C and S requirements over time, suggesting a temporal partitioning of these energy conservation strategies. In the coastal NW Mediterranean Sea, relevant markers involved in ocean biogeochemical cycles also

showed seasonal abundance patterns, such as the genes *puf*, *cox* and *ure* (urea oxidation) during spring, and *amo* genes during autumn and winter (Auladell et al., 2023).

iv. Vitamin B₁-related metabolisms in the Baltic Sea

To date, only one study combining metagenomic and short-term time series approaches has been conducted weekly over a year in the Baltic Sea (Paerl, Sundh, et al., 2018). This study mainly explores the presence of key markers genes related to vitamin B₁ production and salvage in marine metagenomes. Most metagenomes from the Baltic Sea, North Sea and Eastern Pacific Ocean lacked the gene *thiC* involved in HMP production (B₁ precursor), suggesting that HMP auxotrophs dominate wild bacterioplankton communities in these areas (*i.e.*, *Pelagibacterales*, *Methylophilales* in the Baltic Sea) (Paerl, Sundh, et al., 2018). Over 60% of metagenomes lacked at least one B₁ biosynthesis core gene (*thiC*, *thiG* or *thiE*), consistently with observations made from Tara Oceans metagenomes (Paerl, Sundh, et al., 2018). Other important types of auxotrophs were also highlighted in the Baltic Sea, including auxotrophs to HMP and cHET precursors (*i.e.*, *Rhodobacterales*) and exclusive B₁ salvagers (*i.e.*, *Flavobacteriales*) (Paerl, Sundh, et al., 2018). Temporal changes in the abundances of genes related to exogenous B₁ and precursors utilization suggest that their bioavailability could be strong drivers of marine microbial communities' structure and dynamic (Paerl, Sundh, et al., 2018), as suggested for B₁₂ and DMB (Suffridge et al., 2018; Wienhausen, Dlugosch, et al., 2022).

D. Biotic forcings also shape microbial community structure and functions

While the abiotic determinism of community assembly (*i.e.*, prediction of community structure and dynamic from environmental conditions) is well described in the literature, the contribution of biotic forcings remains poorly explored. Moreover, abiotic factors remain incomplete predictors of microbial community structure while biotic interactions could be strong drivers of community assembly (Gralka et al., 2020; Lima-Mendez et al., 2015; Seymour et al., 2017).

i. Top-down controls: viral lysis and grazing

In the context of the TARA Ocean project, a global analysis of 313 plankton samples collected from 68 stations across the photic zone of 8 oceanic provinces revealed the importance of biotic interactions (Lima-Mendez et al., 2015). This study further emphasizes the role of grazing, viral lysis, and parasitism as major top-down controls of phytoplankton and bacterial communities.

Interannual dynamics of microbial communities at SPOT station provided interesting insights into the relationships between bacteria, archaea, protists, and viruses (Ahlgren et al., 2019; Beman et al., 2011; Chow et al., 2014; Cram et al., 2016; Dart et al., 2023; Steele et al., 2011). Potential antagonistic relationships such as viral lysis (mediated by T4-like phage), predation (protist grazers) and competition were highlighted over 3 years (Chow et al., 2014; Yeh & Fuhrman, 2022). Opposite vertical diversity patterns between prokaryotes and protists were reported, with a higher prokaryotic and protistan diversity at mid-depth and at the surface, respectively (Yeh & Fuhrman, 2022). Grazing and viral lysis are selective processes that have different implications for ecosystems, as grazers are rather influenced by the size than the taxonomy of their prey. However, T4-like phage could have broad interspecies host range. The fine-scale dynamic of *Prochlorococcus* and *Synechococcus* over 5 years showed recurrent and specific patterns of interactions between T4-like phage and ecotypes from both cyanobacterial genera (Ahlgren et al., 2019; Dart et al., 2023). At a fine taxonomic level, phage community structure has a stronger influence on cyanobacterial communities' composition than environmental factors (Ahlgren et al., 2019; Dart et al., 2023). These results support the Kill the Winner (KTW) model which hypothesized that seasonally forced abiotic factors are primary drivers of broad-level community structure, while sequential booms and busts of interacting viruses and hosts rather drive fine scale, intra-ecotype dynamics (Thingstad, 2000). At SPOT station, viruses followed their host dynamics rather than controlling their abundances, suggesting potential positive associations until the trend reverses parasitism (Chow et al., 2014).

ii. Biotic interactions: phytoplankton-bacteria interplay

Among the most studied biotic interactions, those between phytoplankton and bacteria are of particular interest in microbial ecology, as they exert a strong influence on fundamental ecosystem-scale processes such as nutrient supply and regeneration, primary production, vitamin production and biogeochemical cycles (Buchan et al., 2014; Seymour et al., 2017). In the ocean, a tremendous diversity of prokaryotes and phytoplankton co-occur and continuously interacts by exchanging beneficial (growth factors, antibiotics, vitamins) or deleterious compounds (proteases, anti-quorum sensing/biofilm compounds) (Kouzuma & Watanabe, 2015; Seymour et al., 2017) (**Fig. I.3**). Phytoplankton and bacteria synergistically influence their respective physiologies and metabolisms, and their interactions can range from the mutual exchange of resources required for growth (syntrophy) to the competition for limiting inorganic nutrients in the ocean (Seymour et al., 2017) (**Fig. I.3**). Although phytoplankton–bacteria interactions have been extensively associated with the recycling of algal detritus by copiotrophic degraders (Buchan et al., 2014), further studies has revealed a greater complexity in their chemical exchange within the proximate environment of the phycosphere, which are at the basis of syntrophic relationships for key metabolites (Seymour et al., 2017) (**Fig. I.3B**).

For example, the diatom *Pseudo-Nitzschia multiseriis* supplies *Sulfitobacter* sp. (*Rhodobacteraceae*) with organosulfur molecules (DMSP, taurine) in exchange for bacterially-derived ammonium (Amin et al., 2015) (**Fig. I.3B.a**). In turn, the bacteria converted the algal-derived amino-acid tryptophan into the grow hormone indole-3-acetic acid (IAA), promoting diatom cell division (Amin et al., 2015). Interestingly, production of IAA by *Rhodobacteraceae* is widespread in the ocean (Amin et al., 2015; Seymour et al., 2017) and microalgae strongly respond to IAA (Kozlova et al., 2017; Labeeuw et al., 2016; Lau et al., 2009), which indicate that these bacteria could be involved in numerous ecological interactions with phytoplankton in ocean. Another example of mutualistic chemical exchange involves *Ruegeria pomeroyi*

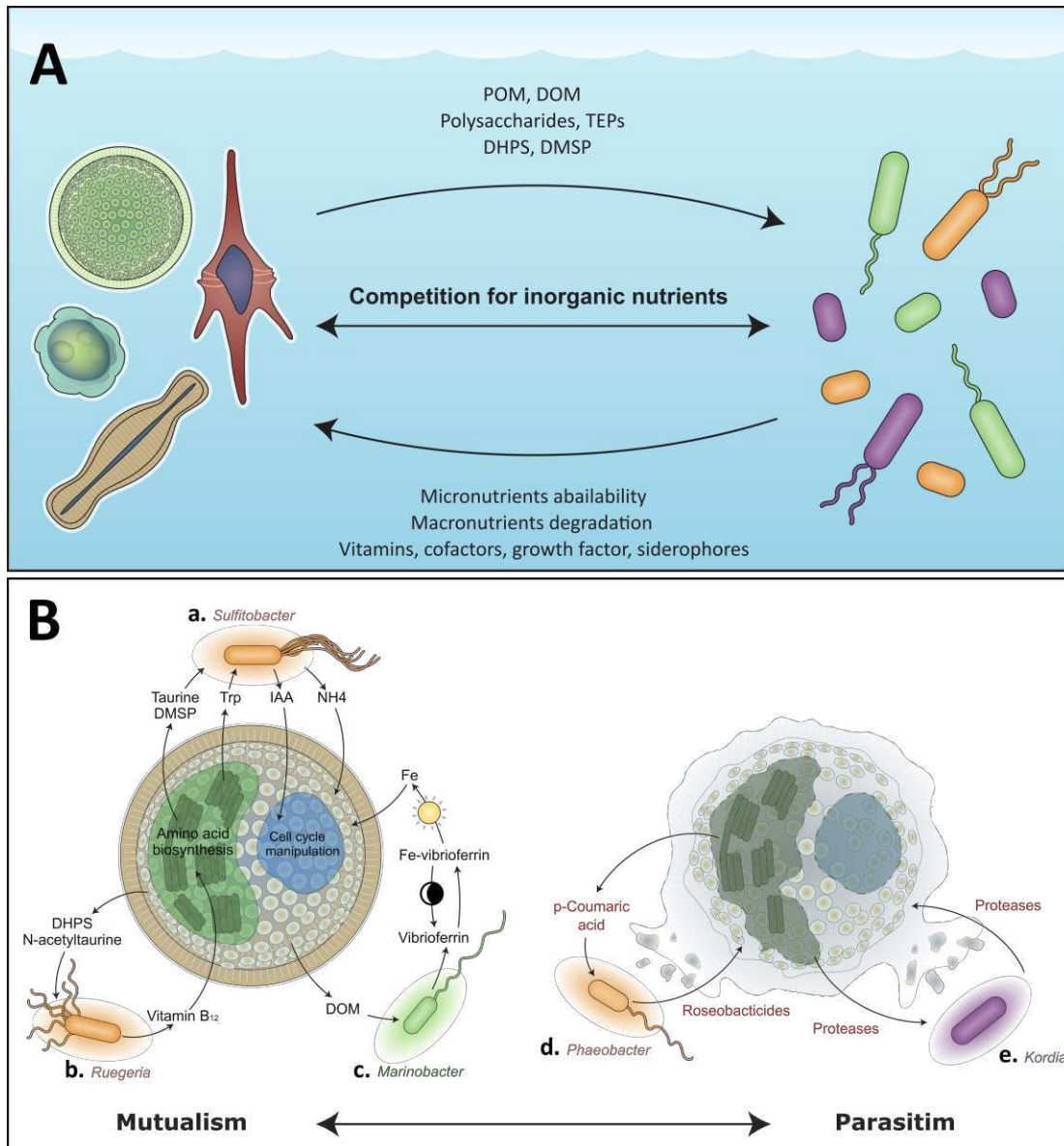


Figure 1.3: Phytoplankton–bacteria interactions in the ocean. **(A)** Phytoplankton cells release a great amount of dissolved (DOM) and particulate (POM) organic matter (*i.e.*, polysaccharides, DMSP) which are used by marine prokaryotes in exchange of vitamins or growth factor. **(B)** Examples of mutualistic (left) and parasitic (right) phytoplankton–bacteria interactions. **(a)** *Sulfitobacter* enhances the growth of the diatom *Pseudo-Nitzschia multiseriis* through mutual exchanges involving tryptophan (Trp), ammonium, and growth-promoting hormone (indole-3-acetic acid, IAA) from bacteria, and carbon or sulfur-rich exudates from the phytoplankton. **(b)** *Ruegeria pomeroyi* provide vitamin B₁₂ to the diatom *Thalassiosira pseudonana* in exchange for several carbon and sulfur sources, including N-acetyltaurine and 2,3-dihydroxypropane-1-sulfonate (DHPS). **(c)** *Marinobacter* secretes the siderophore vibrioferin to acquire iron in the dark. The iron–vibrioferin complex is highly photolabile and degrades during the day, releasing bioavailable iron that is taken up by phytoplankton in exchange for DOM. **(d)** *Phaeobacter gallaeciensis* senses secretion of p-coumaric acid from the coccolithophore *Emiliania huxleyi* during senescence, which activates the production and release of the algicidal roseobactinoids, which lyse *E. huxleyi* and release DOM. **(e)** *Kordia algicida* produces extracellular proteases that lyse diatom cells in order to acquire DOM, while the diatom also produce algal proteases in defense. The phytoplankton cell illustrated could represent multiple species. Bacteria are colored according to their phylogeny with *Rhodobacteraceae* in orange (*Sulfitobacter*, *Ruegeria*, *Phaeobacter*), *Alteromonadaceae* in green (*Marinobacter*) and *Flavobacteriaceae* in purple (*Kordia*). Adapted from (Seymour et al., 2017).

(*Roseobacter*-clade) and the diatom *Thalassiosira pseudonana*. *R. pomeroyi* provide the essential vitamin B₁₂ in exchange of diatom-derived molecules, including carbohydrates and the organosulfur molecule DHPS (Durham et al., 2015, 2017) (**Fig. I.3B.b**). Other co-culture experiments involving *Roseobacter* member (*Dinoroseobacter shibae*) and *Ostreococcus tauri* (*Mamiellophyceae*) showed that bacteria could produce vitamin B₁₂ in exchange of vitamins B₃ and B₇ produced by the microalgae (Cooper et al., 2019). Genes encoding for the type IV secretion system (T4SS) or involved in motility and chemotaxis have notably been found in B₁₂ producing strains of *Roseobacter*, and they may be important traits for B₁₂-based microbial symbiosis between auxotrophic microalgae and B₁₂ providers (Luo & Moran, 2014; Zoccarato et al., 2022). Finally, the green algae *Lobomonas rostrata* uses the vitamin B₁₂ produced by *Mesorhizobium loti*, which in turn uses algal-derived organic matter (Grant et al., 2014; Kazamia et al., 2012). Other important chemical interactions involve the excretion of small organic molecules that binds iron with high affinity (siderophores) (Vraspir & Butler, 2009). *Marinobacter* species feed on phytoplankton-derived DOM and produce vibrioferrin, which forms a highly photolabile iron complex that provides iron to the bacteria in the absence of light, and to the phytoplankton after photolysis during the day (Amin et al., 2012) (**Fig. I.3B.c**).

Competitive or antagonistic relationships between phytoplankton and bacteria have also been largely considered, often involving competition for inorganic nutrients or the algicidal activities of bacteria and related defense mechanisms of phytoplankton (Amin et al., 2012; Seymour et al., 2017). For example, *Kordia algicida* (*Bacteroidetes*) infects diatoms such as *Chaetoceros didymus*, and causes cell lysis using extracellular proteases, while in response to this attack *C. didymus* secretes algal proteases to kill *K. algicida* (Paul & Pohnert, 2011, 2013) (**Fig. I.3B.e**). Phytoplankton–bacteria interactions can also change dynamically according to the physiological state of the partners. *Phaeobacter gallaeciensis* (*Rhodobacteraceae*) establishes a mutualistic relationship with healthy cells of the coccolithophore *Emiliania*

huxleyi in exchange for organic carbon by producing the growth-promoting hormone phenylacetic acid and the antibiotic tropodithietic acid, which may kill algicidal bacteria (**Fig. I.3B.d**). However, the bacterium shifts its mutualistic lifestyle to become an opportunistic pathogen when *E. huxleyi* cells become senescent and released the algal by-product p-coumaric acid. Responding to this chemical signal, *P. gallaeciensis* releases roseobacticides A and B which are algicidal molecules that lyse *E. huxleyi* (Seyedsayamdost et al., 2011) (**Fig. I.3B.d**). Such ambivalent interactions depending on host physiological status have also been documented between the symbiotic *D. shibae* and the toxic dinoflagellate *Prorocentrum minimum* (H. Wang et al., 2014). Co-cultures dynamic of *D. shibae* and *P. minimum* can be divided into two distinct phases: a first mutualistic where bacteria provide essential vitamin B₁ and B₁₂ to the dinoflagellate, which in return provides essential carbon sources and vitamin B₃ to the symbiotic bacteria, and a second pathogenic phase where bacteria kill the starved dinoflagellate cells (Wagner-Döbler et al., 2010; H. Wang et al., 2014). These "Jekyll and Hyde" strategies might be widespread within *Roseobacter*-clade and enable them to maximize their access to algal-derived organic matter, first by achieving a positive association with healthy phytoplankton cells, then by killing senescent cells (Seyedsayamdost et al., 2011; Wagner-Döbler et al., 2010; H. Wang et al., 2014).

In conclusion, microbial seasonal patterns are the result of a coupling between different biotic and abiotic factors, whose respective contributions vary from region to region and season to season due to the specific biotic and abiotic features of each region and their evolution over short and long timescales. Seasonally driven environmental factors govern the physicochemical conditions of the environment and seawater (light, temperature), which have a significant influence on the composition and functions of microbial communities over time (season) and space (latitude, longitude, distance from the coast). On a more localized scale and over shorter periods of time, the effect of large-scale factors is modulated by biological processes involving

beneficial (syntrophism) or deleterious (viral lysis, grazing, parasitism) biotic interactions, which strongly influence community turnover. Over the long term (*i.e.*, seasonal, and inter-annual scales), long term time series studies have shown the strong predictability in the structure and dynamics of various microbial assemblages. Beyond the rhythm of the seasons, certain irregular, and brutal environmental disturbances (*i.e.*, storms, floods) challenge the resilience of marine ecosystems, whose composition and functions could be strongly impacted. However, most studies to date have focused on changes in the taxonomic structure of marine microbial communities and do not address functional changes related to environmental perturbations.

E. How do microbial diversity and functions impact ecosystem functioning?

More than 50 years ago, Hutchinson formulated the plankton paradox which asked how a tremendous diversity of phytoplanktonic species using the same resources could be maintained in a restricted number of ecological niches? (Hutchinson, 1961). This paradox can be transposed to marine microbes, within which a great diversity of organisms is supported by a limited range of available resources. Several ecological interpretations suggest that this great diversity results from the presence of different taxa able to perform the same set of metabolic processes in the ecosystem, introducing the concept of functional redundancy (Allison & Martiny, 2008; Louca et al., 2018; Yin et al., 2000) (**Fig. I.4**).

i. Functional redundancy and ecological insurance

The functional redundancy is defined as the coexistence of multiple distinct (or similar) organisms able to perform the same set of biochemical functions within the microbial community, and thus could be seen as a measure of diversity into functional groups (Louca et al., 2018) (**Fig. I.4**). Functional redundancy is an important component of the insurance mechanism, which theoretically maintains the ecosystem stability by preventing the loss of ecological functions consequently to species loss, which could be induced by environmental or anthropogenic disturbances (Awasthi et al., 2014; Yachi & Loreau, 1999) (**Fig. I.4**).

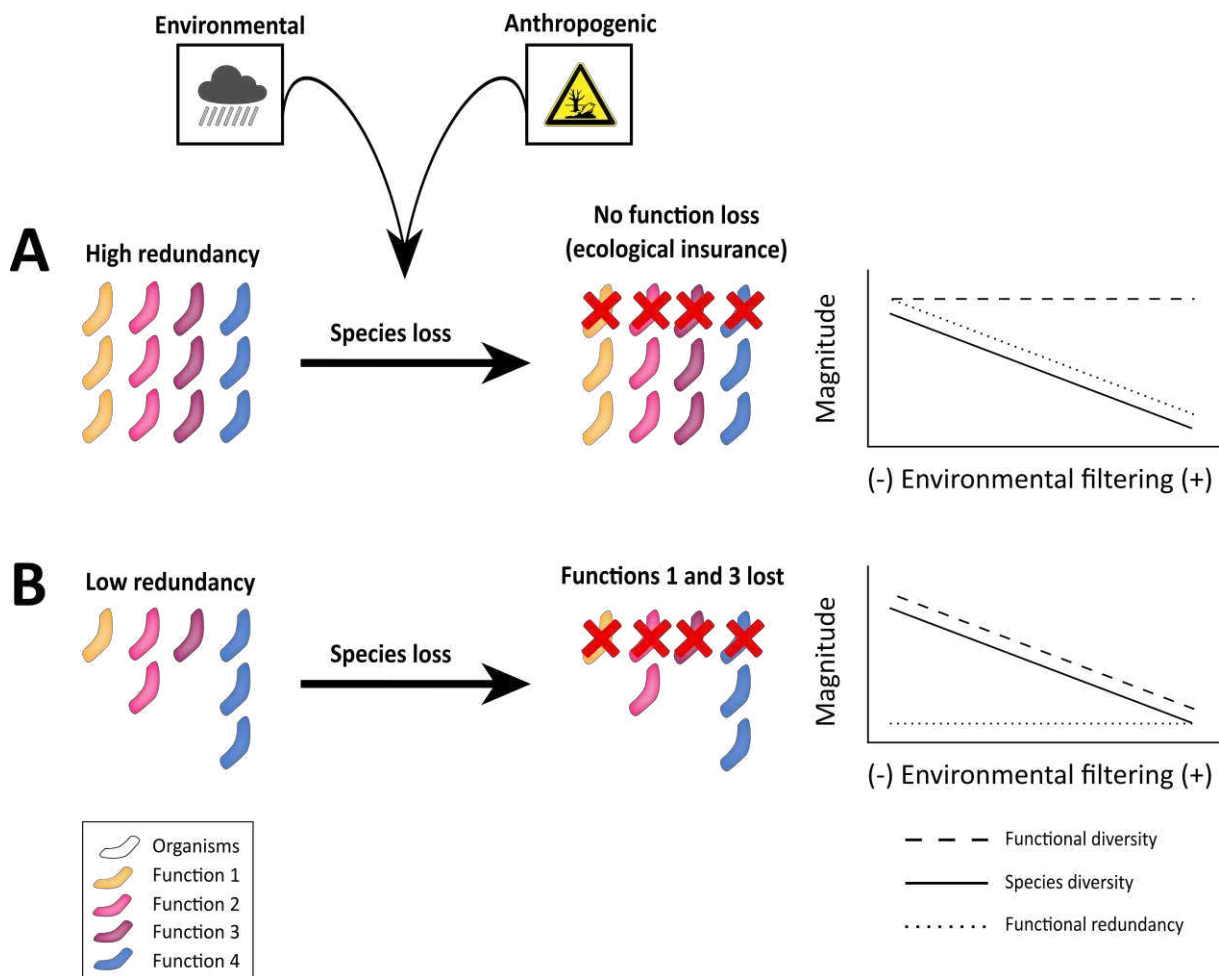


Figure I.4: Conceptual diagram showing how functional redundancy can contribute to the ecological insurance of microbial functions under disturbances. **(A)** Under environmental or anthropic perturbations, a high level of partial functional redundancy within a microbial community (*i.e.*, diverse taxa within a same function) increases the probability of losing functionally redundant rather than functionally unique organisms. In this case, species loss leads to a reduction in the overall (*i.e.*, strict) functional redundancy, but not in the overall functional diversity (*i.e.*, ecological insurance). **(B)** Conversely, a low level of functional redundancy does not prevent the loss of functions associated with the loss of species following perturbations, which impaired the ecosystem functioning and compromise its stability. Adapted from (Stotz et al., 2021).

Two different levels of functional redundancy could be distinguished: the strict (redundancy of all functions at the organism level) and the partial functional redundancy (redundancy between different organisms for a given function) (Galand et al., 2018) (**Fig. I.4**). A low level of strict redundancy was reported in microbial communities from soil (Fierer et al., 2013) but specific taxa which are partially redundant also maintains ecosystem stability under

disturbances (Li et al., 2021). In contrary, a high level of strict redundancy was reported in plant-associated microbial communities (Louca et al., 2017) and in the Human microbiome (Moya & Ferrer, 2016; Tian et al., 2020). Indeed, a convergence of metabolic functions do not necessarily mirror a phylogenetic convergence between taxa (Louca et al., 2018). This concept is however challenged in marine ecosystems, where contrasting amounts of strict functional redundancy were reported (Galand et al., 2018; Louca et al., 2017; Sunagawa et al., 2015). A high taxonomic richness and variability within functional groups indicate a high level of strict functional redundancy in the global ocean microbiome, where community structure and functions were shaped by markedly different processes (Louca et al., 2016; Sunagawa et al., 2015). However, a shift in the community composition altered the overall functional attributes of seasonal communities from the NW Mediterranean Sea, leading to debates on strict functional redundancy in time series studies (Galand et al., 2018). In addition, assessment of partial redundancy levels in key microbial functions is even more lacking in marine ecosystems.

Contradictorily, some studies suggest that "core species" carrying key functions might not be easily replaced although they are fundamental for ecosystem (Krabberød et al., 2022; Shade & Handelsman, 2012). A small and dynamic core microbiota were reported in the NW Mediterranean Sea, associated with other abundant non-core taxa, which are functionally redundant (Krabberød et al., 2022). Together, these observations are thus balanced between functionally redundant organisms from a global point of view, and these core species carrying essential but not widespread functions which are keystone of ecosystem functioning.

ii. Genome streamlining and the Black Queen Hypothesis

Marine microbial communities are dynamic systems of co-occurring species structured as a complex network of interactions (Ahlgren et al., 2019; Arandia-Gorostidi et al., 2022a; Aylward et al., 2015; Dart et al., 2023; Deutschmann et al., 2023; Faust & Raes, 2012; Krabberød et al., 2022; Lambert et al., 2021).

Microbial connectedness usually derives from metabolic dependencies toward relevant metabolites (*i.e.*, public goods), which are crucial for marine microbes. However, the more bioavailable a metabolite is in the environment, the lower the selection pressure exerted to maintain these biosynthesis genes. Consequently, gene deletion is favored over gene insertion, leading to an overall reduction in genome size and metabolic costs (*i.e.*, genome streamlining) (Giovannoni et al., 2005, 2014) (**Fig. I.5A**). Known streamlined genomes included ubiquitous taxa such as SAR11 and *Prochlorococcus* and are typically characterized by: (1) small genomes with highly conserved core genes and few pseudogenes; (2) low ratios of intergenic spacer DNA to coding DNA; (3) low GC content and low numbers of paralogs (Giovannoni et al., 2014) (**Fig. I.5A**).

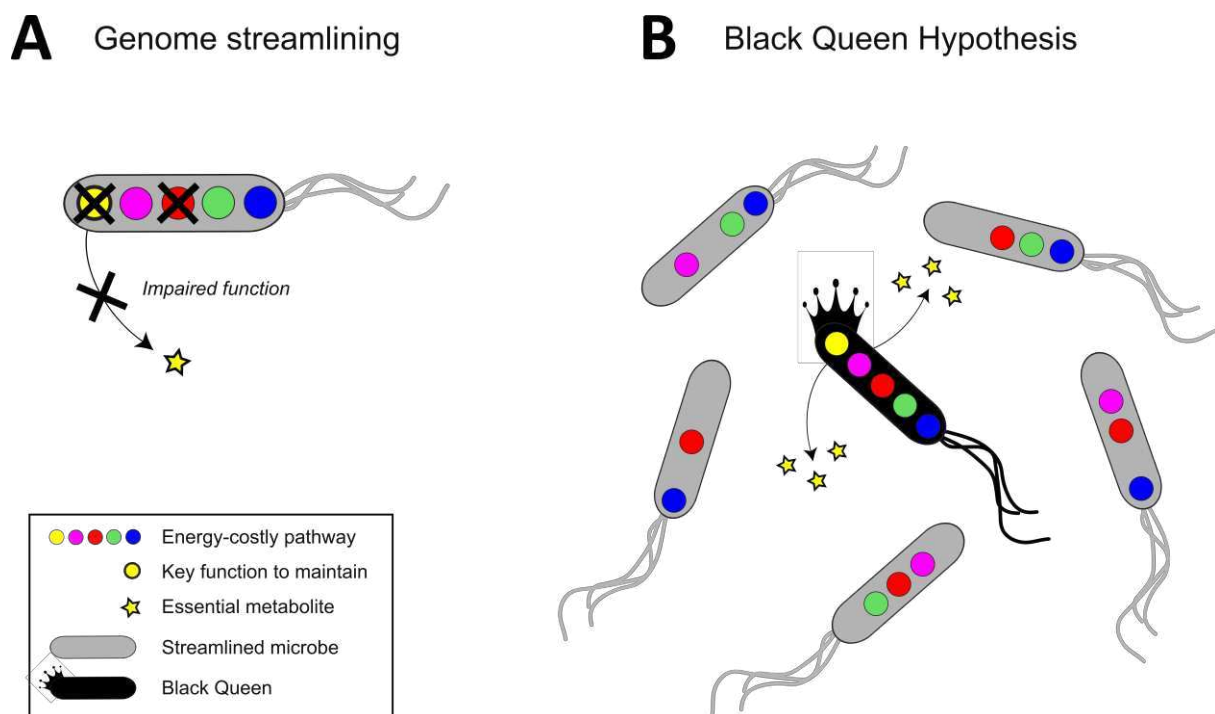


Figure I.5: Schematic representation of the theoretical concepts of genome streamlining and Black Queen Hypothesis. **(A)** The concept of genome streamlining illustrates the loss of at least one key gene involved in a metabolic pathway, whose maintenance is costly from an energetic point of view. Gene loss leads to impaired function but reduced metabolic costs and makes the streamlined microbe dependent on an exogenous source of the metabolite that it can no longer produce. **(B)** The Black Queen Hypothesis describes the maintenance of these leaky functions by the Black Queen organism, which maintains the balance of the leaky metabolite for the whole streamlined community, sustaining ecosystem stability. Adapted from (Gerrit Mathias Wienhausen, 2018; Morris et al., 2012).

Morris later proposes the Black Queen Hypothesis (BQH), which describes the mechanism which allows microbes to lose essential genes by natural selection rather than drift, providing a selective advantage assuming that the gene's function is not vital and ensured by other organisms (Morris, 2015; Morris et al., 2012) (**Fig. I.5B**). The Black Queen refers the queen of spades in the card game “Hearts”, where the aim is to avoid holding this card last. In this case, the queen of spades represents an essential but metabolically costly function to maintain at the community level, and the gene reduction forcing applied to this function can only proceed until the production of public goods is sufficient to support the ecosystem's demand. The Black Queen organisms would thus be forced to keep this essential genetic trait to sustain ecosystem stability and provide the leaky metabolite to the microbial community (**Fig. I.5B**). The level of metabolite privatization (*i.e.*, leakiness) is a key factor shaping the microbial metabolic interdependencies (Yamagishi et al., 2021). A low metabolite leakiness favors mutualistic interdependencies between organisms, whereas an easily accessible metabolite rather leads to one-way dependencies due to low privatization (D’Souza et al., 2018). Together, the genome streamlining and the BQH theories thus suggest that, through these gene losses, metabolic interdependencies would tend to increase in conjunction with an increasing production of public goods by Black Queens for streamlined microbes (Estrela et al., 2016).

Genome streamlining and the BQH are quite recent theories in marine microbial ecology but have provided interesting insight to decipher metabolic interdependencies between marine microbes and understand how they manage to thrive in a diluted environment where success is determined by resource competition. Although genome streamlining is widespread in marine, metagenomes, improving fitness by outsourcing metabolic functions is equivalent to becoming dependent on neighboring partners, which could offset the benefit. In competition experiments conducted with *E. coli* in the presence of amino acids, auxotrophic mutants acquired a significant advantage over their evolutionary ancestor, suggesting that their emergence was

selectively favored when amino acids were available (D'Souza & Kost, 2016). However, further studies suggest that both the genetic background and the environmental conditions determine the adaptive value of a gene loss, and that the fitness gain is inversely proportional to the number of streamlined functions (D'Souza et al., 2015). Moreover, streamlined organisms must also conserve salvage strategies to meet their needs for the leaky metabolite (Cordero et al., 2012).

Disentangling microbial interdependencies within complex natural communities remain a challenge since these theories lacks to elucidate the whole complexity of the microbial world. To disentangle the contribution of key metabolite exchanges in the structure and dynamic of marine planktonic communities, their functional characterization is of particular interest to assess the involvement of specific organisms in the production of essential but scarce micronutrients such as B vitamins. Our understanding on the possible interconnections between marine organisms has strongly increased over time using ecological networks (Ahlgren et al., 2019; Arandia-Gorostidi et al., 2022a; Aylward et al., 2015; Dart et al., 2023; Deutschmann et al., 2023; Faust & Raes, 2012; Krabberød et al., 2022; Lambert et al., 2021). To date, only one study has inferred potential B₁ interdependencies from metagenomics-informed networks within soil communities grown in bioreactor, which were further validated by co-culture experiments (Hessler et al., 2023). However, the complexity of natural communities' interactions in ocean may requests the coupling of various tools for in depth studies, notably modeling approaches (Boza et al., 2023; Grant et al., 2014; Yamagishi et al., 2021; Zelezniak et al., 2015). The way in which auxotrophy structure complex natural communities within the microbial “social network” is a key question in marine microbial ecology that remains difficult but necessary to answer (Zengler & Zaramela, 2018).

2. B-vitamins are essential but scarce coenzymes in the ocean

All domain of life requires coenzymes, which are non-protein organic molecules that bind specifically with their respective enzymes as kinetic regulators to catalyze essential biochemical reactions (*i.e.*, enzymatic cofactor) (Monteverde et al., 2017). It is estimated that about 32% of characterized enzymes (n = 5554) require a cofactor to operate, within which 46% require a coenzyme-like cofactor, such as B-vitamins, and 54% require a trace-metal cofactor (Monteverde et al., 2017). Trace metals (*i.e.*, Fe, Mn, Ni, Zn, Mo, Co, Cu) are the most studied cofactors in the ocean, and the "metallome" has been proposed as the third keystone in the "trinity of life", linking the genome and proteome to the mineral chemical environment (Fraústo da Silva & Williams, 2004). Their distribution in the global ocean was explored during the GEOTRACES project which revealed striking patterns for key trace metals, such as Fe, Co, Zn (Aparicio-González et al., 2012). However, coenzymes and notably B-vitamins have received much less attention in marine microbial ecology, although they match the features of inorganic metal cofactors by catalyzing a wide range of biochemical reactions essential to life (**Fig. I.6**).

A. B-vitamins are key enzymatic cofactors for all living marine organisms

B-vitamins are characterized by eight unique water-soluble and light-sensitive molecules with distinct chemical structures ranging from simple to complex organic heterocycles, which include vitamin B₁ (thiamin), B₂ (riboflavin), B₃ (niacin), B₅ (pantothenate), B₆ (pyridoxine), B₇ (biotin), B₉ (folate) and B₁₂ (cobalamin) (Monteverde et al., 2017) (**Fig. I.6A**). Although their structural differences, many B-vitamin biosynthesis pathways are co-dependent as they require other B-vitamins as catabolized reactants (*i.e.*, vitamin cannibalism) (Ealick & Begley, 2007) (**Fig. I.6B**). These vitamin co-requirements in biosynthesis pathways were suggested to be an important and necessary criterion for evolution through symbiosis (King, 1980). For example, several vitamin co-requirements are present both in B₁ and B₁₂ production pathways (**Fig. I.6B**).

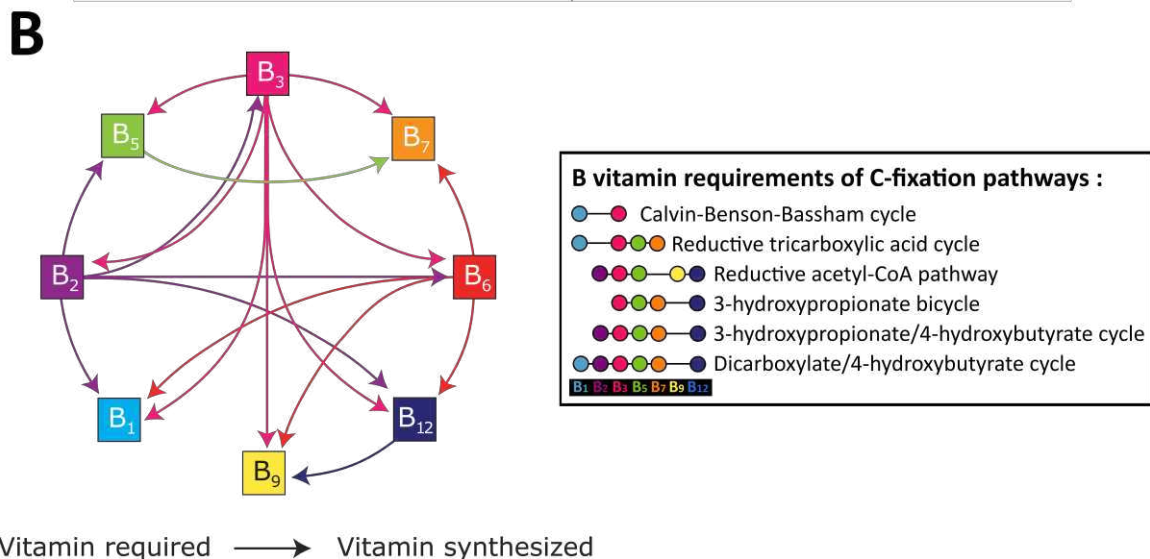
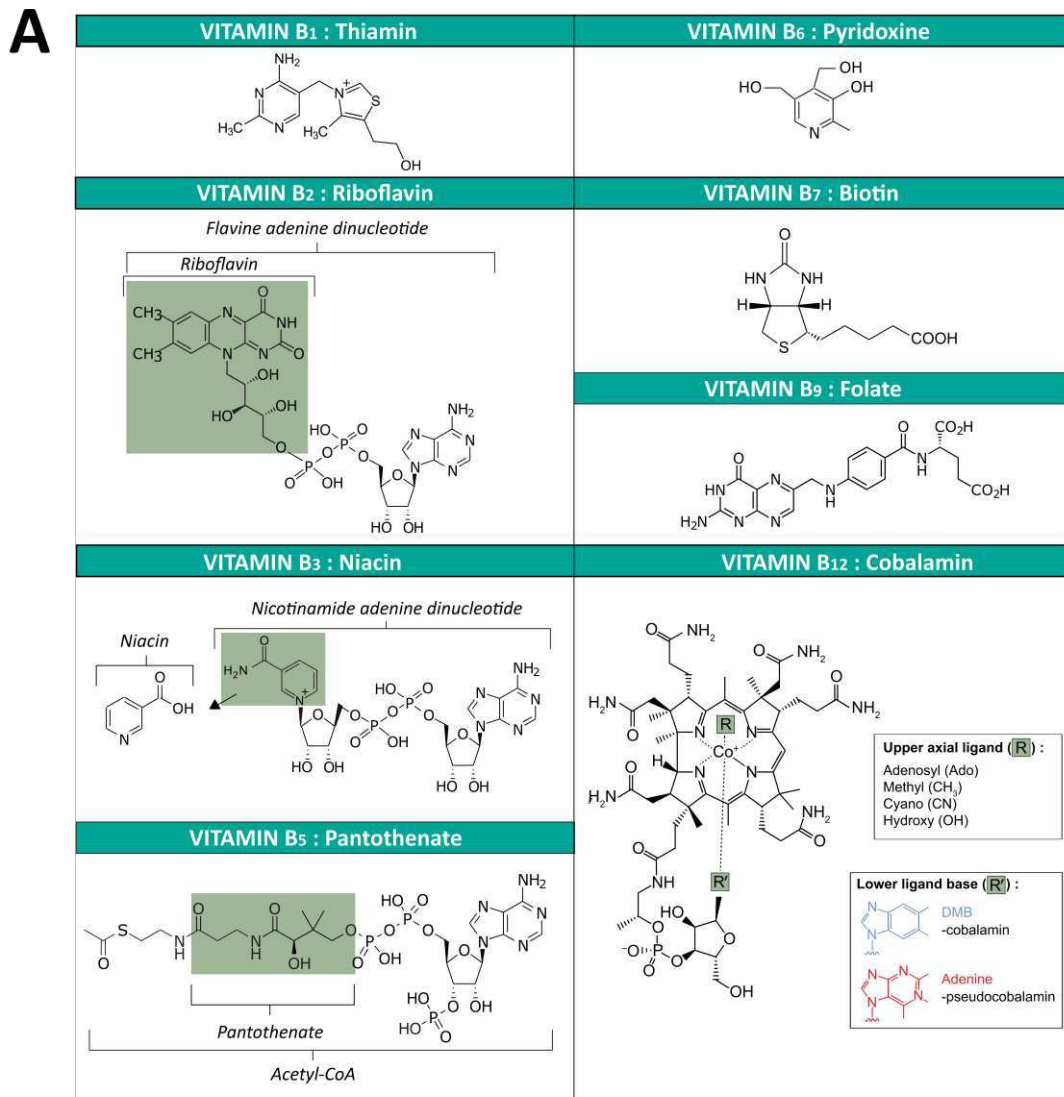


Figure I.6: B-vitamin structures, biosynthesis co-requirement and roles in carbon fixation pathways. (A) B₁ (thiamin), B₂ (riboflavin), B₃ (niacin), B₅ (pantothenate), B₆ (pyridoxine), B₇ (biotin), B₉ (folate) and B₁₂ (cobalamin) chemical structures. (B) B-vitamin biosynthesis co-requirements (left panel) and their involvements in the six known carbon-fixation pathway (right panel). Adapted from (Monteverde et al., 2017).

The flavin derived from vitamin B₂ is catabolized to form the lower ligand DMB (5,6-dimethylbenzimidazole) of vitamin B₁₂ (Taga et al., 2007). The NAD (Nicotinamide Adenine Dinucleotide) derived from vitamin B₃, and the vitamin B₆, are catabolized for the synthesis of the 4-amino-5-hydroxymethyl-2-methylpyrimidine (HMP) and the 5-(2-hydroxyethyl)-4-methyl-1,3-thiazole-2-carboxylic acid (cHET), respectively, which are the two precursors of vitamin B₁ (Chatterjee et al., 2007; Jurgenson et al., 2009). Interestingly, all B-vitamins production pathways require vitamin B₃, while vitamin B₁ and B₁₂ are not involved in almost any of other B-vitamin production pathways and require a great deal of vitamin co-requirements (*i.e.*, vitamin B₂, B₃ and B₆) (Monteverde et al., 2017) (**Fig. I.6B**). Together, these data suggest that certain B-vitamins, such as B₃, could be rather synthesized with the aim of producing other essential B-vitamins which are harder to produce *de-novo*, such as vitamin B₁ and B₁₂.

Each of the six known carbon-fixation pathways require B-vitamins, and some of them require at least two B-vitamins (*i.e.*, CBB cycle require B₁ and B₃) while most require a complex combination of B-vitamins (Monteverde et al., 2017) (**Fig. I.6B**). In addition to carbon fixation, these strong vitamin dependencies affect a wide range of key functions involved in biogeochemical cycles such as anoxygenic and oxygenic photosynthesis and sulfate reduction, which include multiple B-vitamins dependent steps (Monteverde et al., 2017). However, a few vitamin-dependent enzymes constitute the bulk of B-vitamin requirements reported among marine microbes (Sañudo-Wilhelmy et al., 2014). B₁- and B₁₂-dependant enzymes are among the most widespread B-vitamin-requiring enzymes within microbial lineages (*i.e.*, *Cyanobacteria*, *Alpha-* and *Gammaproteobacteria*, *Bacteroidetes*) (Sañudo-Wilhelmy et al., 2014). Taken together, these observations suggest that B-vitamins, and notably vitamin B₁ and B₁₂, have a central role in the bulk enzymatic activity of marine microbial communities and could significantly influence community composition and functions, the fate of organic matter in the marine food web, and ultimately, global ocean biogeochemical cycles and climate.

i. Vitamin B₁ (thiamin) biosynthesis and salvage pathways

Vitamin B₁ is a water-soluble sulfur containing vitamin that is used to form thiamine diphosphate (TDP), which is the cofactor of several core enzymes involved in the Krebs cycle (*i.e.*, pyruvate dehydrogenase, α -ketoglutarate dehydrogenase), and in the pentose phosphate pathway (acetolactate synthase, transketolase) (Sañudo-Wilhelmy et al., 2014).

Vitamin B₁ biosynthesis requires two precursors which are synthesized by distinct chemical routes: HMP which is produced *via* the HMP synthase encoded by the gene *thiC*, and cHET which is produced *via* the thiazole synthase encoded by the gene *thiG* (bacteria, algae), or *thi4-thi1* in (archaea) (Helliwell, 2017; Maupin-Furlow, 2018; Paerl, Sundh, et al., 2018) (**Fig. I.7**). After phosphorylation steps, precursors are condensed in thiamine monophosphate (TMP) by *thiE* in bacteria or *thiN* and *thiDN* in archaea. TMP is then phosphorylated by the TMP kinase encoded by *thiL* to finally produce the active form of vitamin B₁, thiamine diphosphate (TDP) (**Fig. I.7**). Thiamine and related precursors can also be salvaged from the natural environment *via* specific transporters, such as those encoded by *thiXYZ* genes for HMP or by *thiBPQ* genes for B₁ (**Fig. I.7**). These precursors are phosphorylated in the cell by specific enzymes encoded by the gene *thiM* for cHET or by the gene *thiD* for HMP, to be then condensed and phosphorylated in TDP *via* the common route of the *de-novo* synthesis pathway (**Fig. I.7**). Thiamin salvagers rather use a specific diphosphorylase encoded by the gene *thiN-TPK1* to produce TDP. These “cheaters” can thus scavenge vitamin and/or their related precursors from the seawater with obvious energy benefits over synthesizing it *de-novo* (Gutowska et al., 2017; Jurgenson et al., 2009; Paerl, Bertrand, et al., 2018) (**Fig. I.7**). Several types of B₁ auxotrophs that partially lack the biosynthetic pathway but could salvage exogenous precursors are known, namely HMP auxotroph (lack *thiC*) and dual HMP/cHET auxotroph (lack *thiC* and *thiG* but have *thiM*), while other could only salvage exogenous vitamin B₁ (B₁-auxotrophs, lack *thiC* and *thiG* but have *thiN-TPK1*) (Paerl, Sundh, et al., 2018).

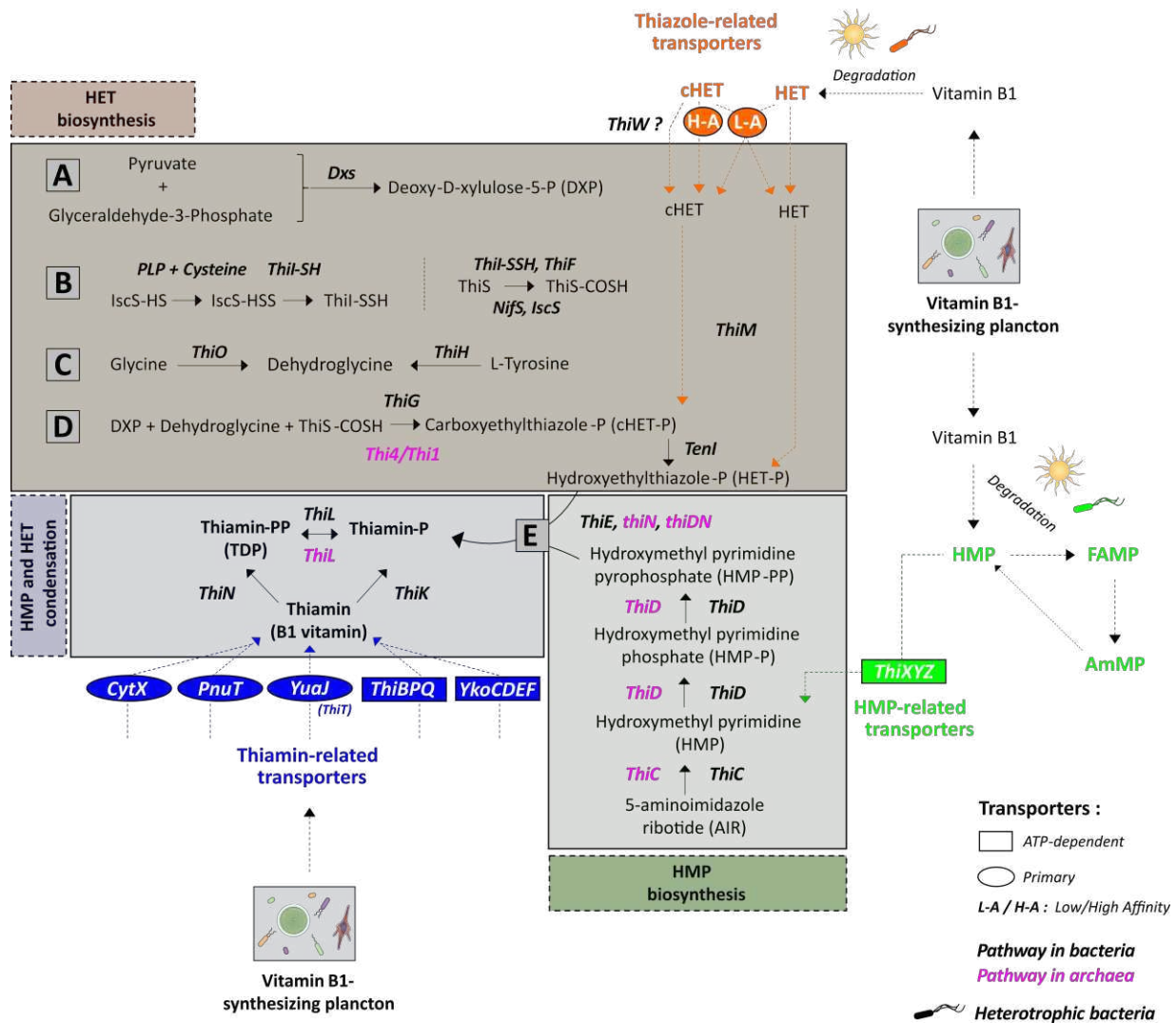


Figure I.7: Schematic overview of vitamin B₁ *de-novo* biosynthesis pathway, and precursor and vitamin scavenging pathways. The *de-novo* biosynthesis of vitamin B₁ requires the condensation of two precursors which are previously synthesized by two distinct pathways: 4-amino-5-hydroxymethyl-2-methylpyrimidine (HMP) produced *via* the HMP synthase encoded by the *thiC* gene (green box) and 5-(2-hydroxyethyl)-4-methyl-1,3-thiazole-2-carboxylic acid (cHET) produced *via* thiazole synthase encoded by the *thiG* gene in bacteria (black), or *thi4-thi1* in archaea (pink) (orange box). The upstream genes involved in the production of (A) Deoxy-D-xylulose-5-phosphate (DXP), (B) dehydroglycine and (C) the thiocarboxylated *ThiS* (sulfur donor) condensed in the *ThiG* mediated synthesis of cHET, are also indicated. The two precursors are then condensed into thiamine monophosphate (TMP, blue box) *via* *thiE* in bacteria or *thiN* in archaea (E), which is then phosphorylated by the TMP kinase encoded by *thiL* to finally produce the active form of the vitamin B₁, thiamine diphosphate (TDP). Thiamine and associated precursors can also be recovered from the natural environment *via* specific transporters (ATP dependent, boxed; or primary; circled), such as those encoded by the *thiXYZ* genes for HMP (light green) or by the *thiBPQ* genes for B₁ (in blue). *ThiW* was predicted to be involved in thiazole uptake based on its colocalization with *thiM* genes, encoding the thiazole salvage enzyme hydroxyethyl thiazole kinase which phosphorylates the salvaged precursor. Phosphorylated form of both precursors is condensed into TMP which is then phosphorylated into TDP by the same pathway. Thiamine scavengers use a specific diphosphorylase encoded by the *thiN-TPK1* gene to produce TDP or take over the normal pathway *via* the thiamine kinase *thiK*. Precursors and vitamins can come mainly from neighboring planktonic producers, and from biotic (heterotrophic bacteria) and abiotic (light) degradation of B₁ in seawater. Pyrimidine degradation products such as N-formyl-4-amino-5-aminomethyl-2-methylpyrimidine (FAMP) or 4-amino-5-aminomethyl-2-methylpyrimidine (AmMP) could also be used for HMP generation by auxotrophs. Adapted from (Helliwell, 2017; Maupin-Furrow, 2018; Paerl et al., 2023).

Some marine plankton have been shown to salvage HMP or HMP degradation products more efficiently than the active vitamin B₁, such as the 4-amino-5-aminomethyl-2-methylpyrimidine (AmMP) which is produced from the N-formyl-4-amino-5-aminomethyl-2-methylpyrimidine (FAMP) via *YlmB*, *TenA_E* or *TenA_C* genes (Gutowska et al., 2017; Paerl et al., 2023) (**Fig. I.7**). Experimental data and global survey among TARA Ocean metagenomes also showed that eukaryotic phytoplankton including HAB species and picoeukaryotes mostly use FAMP while bacterioplankton more likely use FAMP (Paerl et al., 2023). Additionally, picoeukaryotes (*i.e.*, *Ostreococcus lucimarinus*) were found to salvage and use cHET together with HMP precursor to produce B₁ (Paerl, Bertrand, et al., 2018). Strikingly, *Emiliana Huxleyi* can grow using either B₁ or HMP while lacking cHET synthesis genes, suggesting an alternative and yet uncharacterized B₁ synthesis pathway (McRose et al., 2014).

ii. Vitamin B₁₂ (cobalamin) biosynthesis and salvage pathways

Cobalamins includes closely related organometallic corrinoids consisting of a tetrapyrrole corrin ring surrounding a central cobalt ion, which is linked to an upper (β) and a lower (α) axial ligand (Helliwell et al., 2016) (**Fig. I.6**). The nature of these ligand defines the chemical nature and the affinity of the molecule with its enzyme, which ensures the specificity of the reaction catalyzed. Methyl- and adenosylcobalamin are the most important active forms of B₁₂, as they are the specific cofactor of the B₁₂-dependent methionine synthase (encoded by *metH*) and the B₁₂-dependant methylmalonyl-CoA mutase (MCM) (**Fig. I.6**). These enzymes are involved in methionine synthesis in the cytosol and odd-chain fatty-acid metabolism in the mitochondrion, respectively, and both require chaperone proteins (Helliwell et al., 2011; Sañudo-Wilhelmy et al., 2014). The B₁₂-dependent methionine synthase *metH* is the most ubiquitous B₁₂-requiring enzymes within the microbial realm, and more than 80% of representative genomes from abundant marine microbes (*i.e.*, *Cyanobacteria*, *Alphaproteobacteria*, *Gammaproteobacteria*, *Bacteroidetes*) possess this gene. When B₁₂ is available in seawater, most organisms could use

the B₁₂-dependent methionine synthesis pathway mediated by *metH*, but a few harbor the B₁₂-independent pathway, mediated by *metE*, which have a 50 times less efficient turnover rate in *E. coli* (Gonzalez et al., 1992; Matthews et al., 2003). Despite a high metabolic cost, the presence of a vitamin B₁₂-independent homologous enzyme could provide a selective advantage under vitamin B₁₂-limited conditions to auxotrophic organisms, *metE* being actively expressed under B₁₂ scarcity by the diatom *Grammonema islandica* (Cohen et al., 2017).

Vitamin B₁₂ is produced through a complex and energy-costly metabolic process involving about twenty genes and thirty enzymatic steps (Balabanova et al., 2021; Fang et al., 2017; Martens et al., 2002; Warren et al., 2002) (**Fig. I.8**). The tetrapyrrole precursor biosynthesis (*i.e.*, uroporphyrinogen III) is the first step of the pathway and is common to the heme, chlorophyll, and coenzyme F430 biosynthesis pathways (**Fig. I.8**). The precursor is then metabolized through two distinct but convergent routes leading to the corrin ring formation, namely the aerobic (*cob* genes) and the anaerobic (*cbi* genes) pathways, which are used by bacteria and archaea, respectively (Balabanova et al., 2021; Doxey et al., 2015; Fang et al., 2017) (**Fig. I.8**). These two distinct ways differ by the genes involved (*i.e.*, *cob* for aerobic and *cbi* for anaerobic), the timing of cobalt insertion (*i.e.*, late for aerobic and early for anaerobic), and by the molecular oxygen requirement of the precorrin-3B synthase encoded by the gene *cobG*, which catalyzed the third step of the aerobic pathway (Balabanova et al., 2021; Doxey et al., 2015; Fang et al., 2017) (**Fig. I.8**). Both aerobic and anaerobic pathways converge toward the same molecule (*i.e.*, cob(I)yrinic acid, *c*-diamide), which is further processed into adenosylcobyrinic acid during the nucleotide loop assembly (**Fig. I.8**). The latter portion of the pathway involves the synthesis and attachment of the aminopropanol linker and the lower α -axial ligand (Balabanova et al., 2021; Doxey et al., 2015; Fang et al., 2017) (**Fig. I.8**).

In contrast to bacterial and archaeal cobalamins, which have DMB as specific lower α -axial ligand, cyanobacteria (*e.g.*, *Synechococcus*, *Prochlorococcus*) rather produce pseudo-B₁₂

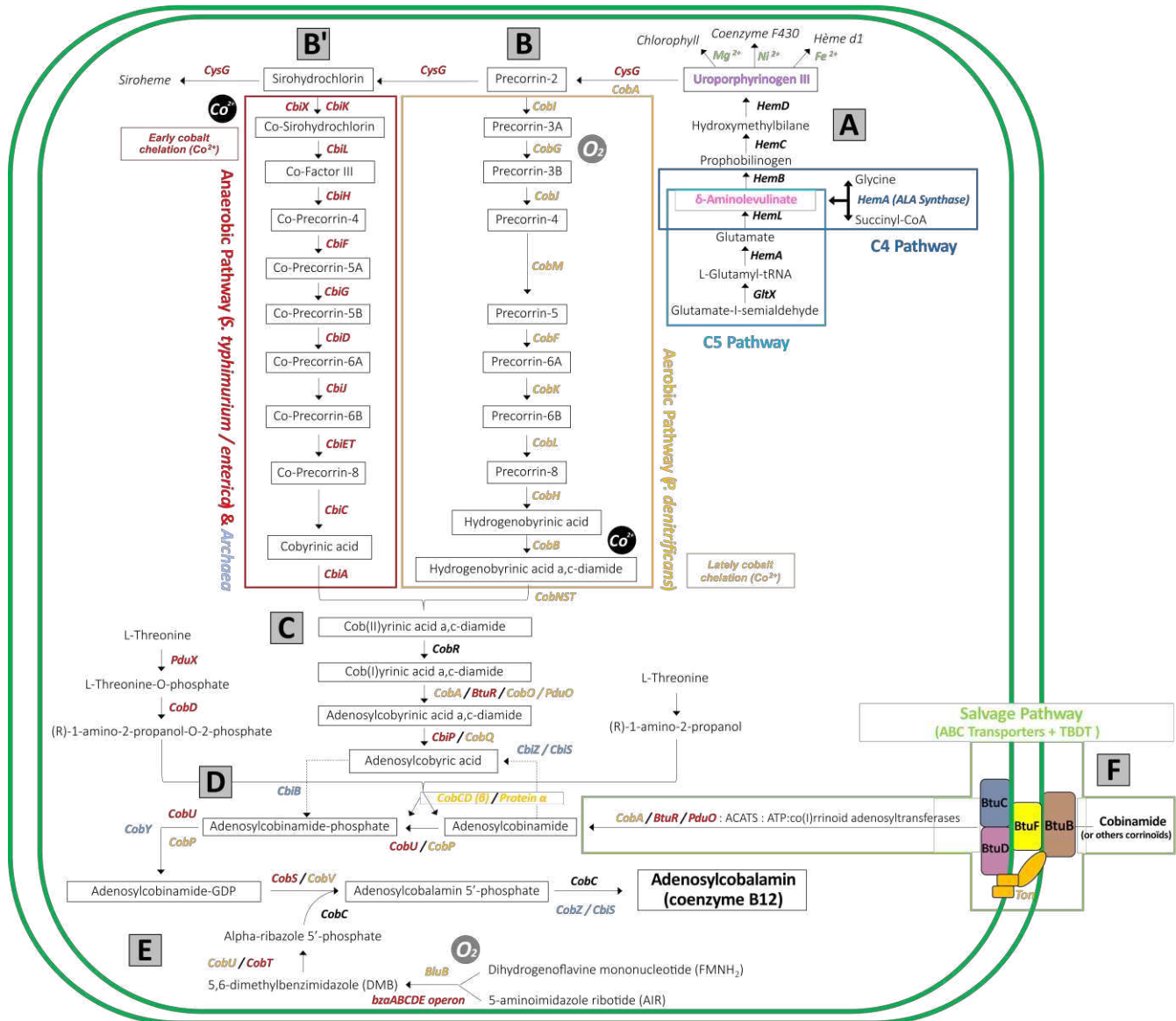


Figure I.8: Schematic overview of *de-novo* biosynthesis and salvage pathways of vitamin B₁₂. We took the random example of adenosylcobalamin biosynthesis in bacteria and archaea to simplify, knowing that a diversity of B₁₂ form exists (Fig. I.6). (A) Vitamin B₁₂ *de-novo* biosynthesis requires the prior generation of the precursor uroporphyrinogen III *via* the C4 and C5 pathways (in blue), which involve the *hem* genes, and which is a common precursor to heme, chlorophyll, and coenzyme F430 biosynthesis pathways. Depending on the organism, the activities of the *cobA* or *cysG* genes catalyze the synthesis of precorrin-2 from this precursor, which can then be metabolized into vitamin B₁₂ in two distinct ways: by (B) the aerobic biosynthesis pathway requiring the *cob* genes present in bacteria (yellow box), or (B') by the anaerobic biosynthesis pathway requiring the *cbi* genes present in archaea and certain bacteria (red box). (C) Both pathways converge on the same compound, adenosylcobyric acid, which is ultimately converted to vitamin B₁₂ by distinct pathways depending on the organisms considered (D). (E) Archaea and bacteria produce cobalamin, a form of vitamin B₁₂ that has a dimethylbenzimidazole (DMB) molecule as a lower axial ligand. DMB is produced through the activity of the gene *bluB* (colored in dark blue) or the *bza* operon (*bzaABCDE*), and then fused to the coring ring to form cobalamin. (F) Molecules structurally close to vitamin B₁₂ (e.g., cobinamide and other corrinoids) can be directly extracted from seawater (green box) *via* specific transporters such as *btuBCDF* in bacteria (colored in green). After translocation, these molecules are metabolized in the cell by specific enzymes which ensure the generation of adenosylcobyric acid, such as ATP:co(I)rrinoid adenosyltransferases (ACATS) encoded by the *cobA-btuR* gene. Adapted from (Balabanova et al., 2021; Fang et al., 2017).

(*i.e.*, pseudo-cobalamin), which have an adenine as lower α -axial ligand and which coexists with B₁₂ in the water column (Heal et al., 2017a; Helliwell et al., 2016). Pseudo-B₁₂ is an active cofactor for the cyanobacterial methionine synthase (Tanioka et al., 2010) but poorly supports the growth of eukaryotic phytoplankton in culture (Heal et al., 2017a; Helliwell et al., 2016). While cyanobacterial exudates are strong chemoattractants (Seymour et al., 2010), they meet their own cofactor requirement and not supply an active cofactor to other competitors. Bacteria, and archaea can also use cost-efficient salvage pathways to scavenge exogenous B₁₂ or corrinoids from the seawater (Rodionov et al., 2009; Woodson et al., 2005; Zayas et al., 2006). Other studies notably demonstrated the ability of several phytoplankton (*e.g.*, *Pavlova*) and bacteria (*i.e.*, *Vibrio*) to remodel pseudo-B₁₂ into B₁₂ when DMB is (Grossman, 2016; Helliwell et al., 2016; Ma et al., 2020). Together, these observations indicate that B₁ and B₁₂ cycles in ocean are complex and driven by diverse organisms which use a wide range of metabolic strategies to meet their vitamin requirements, including auxotrophs that scavenge and remodel exogenous vitamin or precursors, and prototrophs that can produce them *de-novo*.

B. Vitamin B₁ and B₁₂ are scarce and showed patchy distribution in ocean

Although many marine microbes have an absolute requirement of B₁ and B₁₂, numerous studies have shown that most oceanic and coastal regions of the global ocean are largely B₁ and/or B₁₂-depleted (picomolar concentrations), including large areas of the Pacific Ocean (Heal et al., 2017a; Sañudo-Wilhelmy et al., 2012, 2014), of the Atlantic Ocean (Barber-Lluch et al., 2021; Carini et al., 2014; Joglar, Pontiller, et al., 2021; Koch et al., 2012, 2013; Suffridge et al., 2020) and of the Mediterranean Sea (Bonnet et al., 2013; Fiala, 1982; Suffridge et al., 2018).

i. The Pacific and the Atlantic Oceans are globally B₁ and B₁₂-depleted

In the Pacific Ocean, B₁ and B₁₂ are below the detection limit in large areas (hundreds of kilometers), including at SPOT and HOT stations, with overall B₁₂ concentrations below 5 pM (Sañudo-Wilhelmy et al., 2012, 2014). Patchy B₁ and B₁₂ distributions patterns were also

reported regionally in several stations of the California margin, with highest concentrations of B₁ and B₁₂ detected from coastal shallow waters to epipelagic zone (0 – 200 m), and the lowest in the open ocean (Sañudo-Wilhelmy et al., 2012, 2014).

In the Atlantic Ocean, regional studies from the northwestern Iberian Peninsula also reported very low concentrations of vitamin B₁₂ throughout the year (< 3 pM), with higher concentrations detected in shallower depths (40 – 80 m), especially during winter (Barber-Lluch et al., 2021; Joglar, Álvarez-Salgado, et al., 2021). In coastal waters of Sapelo Island, low vitamin B₁ and B₁₂ concentrations were detected (< 4.5 pM), and B₁ concentrations followed diel oscillations with higher concentrations during the day (Gómez-Consarnau et al., 2018). Vertical profiles (0 – 300 m) of vitamin B₁ and related precursors concentrations in the Sargasso Sea (Carini et al., 2014) and along a large latitudinal transect in the North Atlantic Ocean (Suffridge et al., 2020) have shown that vitamin B₁ precursors (*i.e.*, HMP, HET, cHET, AmMP) could be more abundant than vitamin B₁ in the water column, suggesting that precursors salvage is a workable strategy in B₁-depleted areas, as demonstrated experimentally (Gutowska et al., 2017; Paerl, Bertrand, et al., 2018; Paerl et al., 2023). Overall, vitamin B₁ and related precursors displayed similar depth distribution patterns, with low concentrations near the surface and higher concentrations at the bottom of the photic zone (100 m) (Suffridge et al., 2020).

ii. Large areas of the Mediterranean Sea are devoid of vitamins B₁ and B₁₂

Although very low vitamin B₁₂ concentrations were detected along a longitudinal transect in the Mediterranean Sea (< 6 pM), higher B₁₂ concentrations were reported in the Western Mediterranean basin (Bonnet et al., 2013). However, a larger scale study spanning the whole Mediterranean-Atlantic transect highlighted patchy distribution patterns for vitamin B₁ and B₁₂, with low concentrations in large areas of the Mediterranean Sea (Suffridge et al., 2018) (**Fig. I.9**). Vitamin B₁ concentrations could sometimes reach maximum values close to 500 pM in restricted areas of the transect and was on average 33 times more abundant than vitamin B₁₂,

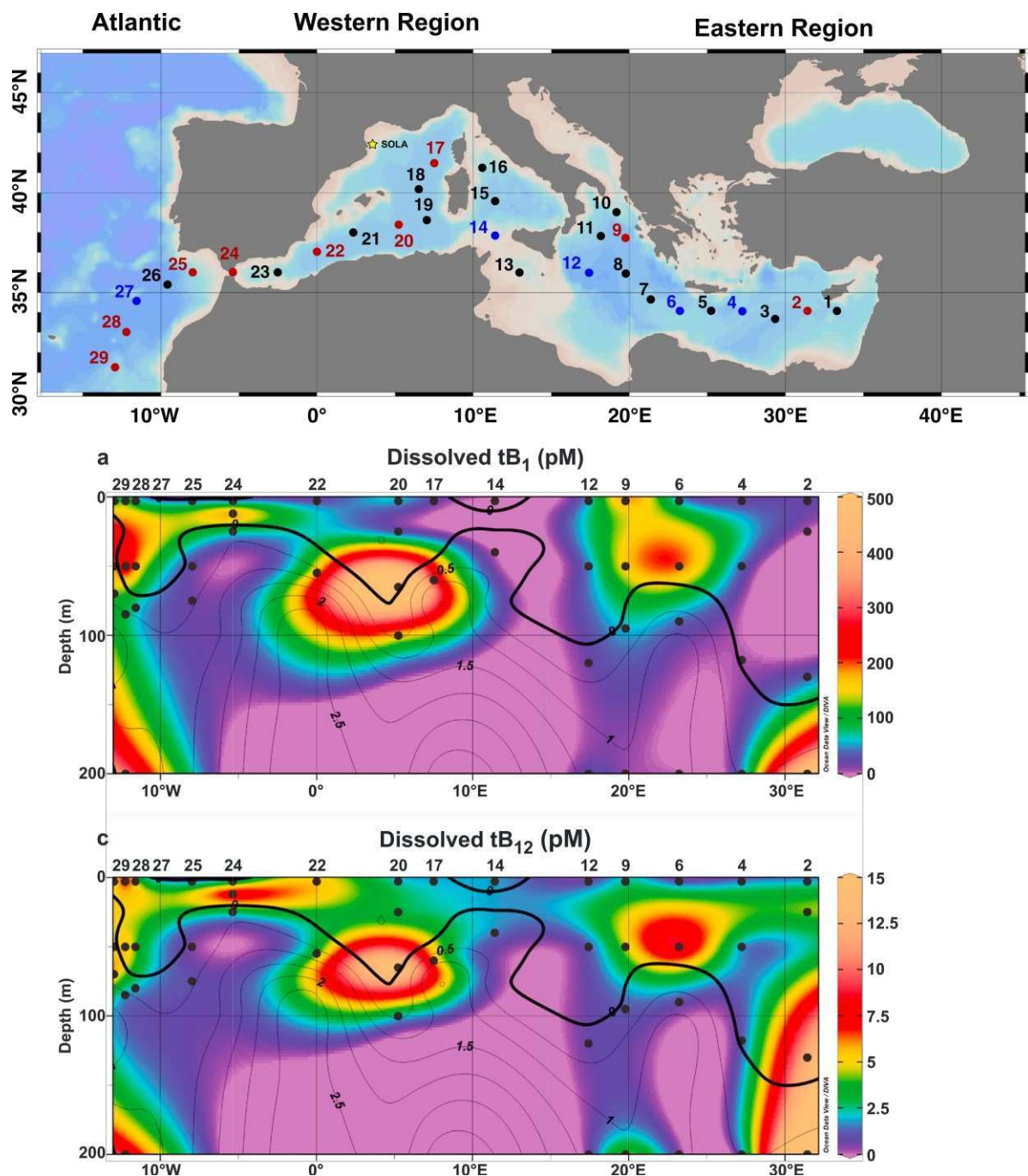


Figure I.9: Distribution of vitamins B₁ and B₁₂ in the water column along a Mediterranean-Atlantic Ocean transect. The oceanographic campaign took place in spring 2014 and covers 29 stations from station 1 near Cyprus to station 29 in the Eastern Atlantic Ocean. The stations labelled in blue have their respective concentrations of dissolved vitamin B₁ (top panel) and B₁₂ (bottom panel) indicated above, while the stations labelled in red have paired dissolved and particulate B-vitamin measurements. Distributions of total dissolved tB₁ and tB₁₂ (in picomolar) are plotted as a function of depth and longitude. Stations are labeled on the upper x axis of each plot. tB₁ is the sum of B₁, thiamin monophosphate, and thiamin pyrophosphate, while tB₁₂ is the sum of cyano-cobalamin, methyl-cobalamin, hydroxy-cobalamin, and deoxyadenosyl-cobalamin. The SOLA station is indicated by a yellow star on the map. Adapted from (Suffridge et al., 2018).

which barely exceeded 15 pM in the same sites (Suffridge et al., 2018) (**Fig. I.9**). In this study, higher vitamin B₁ and B₁₂ concentrations were reported at the deep chlorophyll maximum (DCM, 0 – 100 m) (**Fig. I.9**) and were correlated positively with phytoplankton and negatively with bacterial abundances (Suffridge et al., 2018). Both dissolved and particulate pools of vitamins and precursors appear tightly interconnected by dynamic exchanges, as surface and deep-water layers (Suffridge et al., 2018). Authors have also stressed the importance of measuring all forms of vitamin B₁, B₁₂ and their related precursors to properly assess vitamin bioavailability in seawater, as precursors could be more abundant than vitamins in different oceanic regions (Suffridge et al., 2018, 2020). Moreover, these studies identify vitamin B₁, B₁₂ and their congeners as potential drivers of microbial community composition, notably in the Mediterranean Sea (Suffridge et al., 2018, 2020).

To sum up, vitamin B₁ and B₁₂ concentrations are often very low or even below the detection limit of actual analytical methods, which are however quite sensitive (0.18 pM for vitamin B₁₂ and 0.81 pM for vitamin B₁) (Sañudo-Wilhelmy et al., 2012, 2014). The general trend observed in the distribution of vitamins along the water column (*i.e.*, depletion at the surface versus maximum near the bottom of the euphotic zone and low concentrations at depth) and along the coastal to offshore transect (*i.e.*, higher concentration in coastal areas than in open ocean) suggest a global pattern of vitamin distribution in the ocean. Moreover, it is necessary to consider diverse chemical forms of B-vitamins and their associated precursors to properly assess vitamin bioavailability *in-situ*, since precursors are abundant in the water column and scavenging strategies are reliable in ocean. Taken together, these observations suggest that vitamin B₁, B₁₂ and their related precursors are strong structuring factors of marine planktonic communities, as they are scarce and unevenly distributed along the water column, while being essential to key microbial metabolisms in ocean.

3. Vitamin B₁ and B₁₂ auxotrophy are widespread in marine ecosystems

A. Most eukaryotes rely on exogenous sources of B₁, B₁₂, or precursors

Decades of culture-based experiments and subsequent genomic advances have demonstrated that vitamin B₁₂ and in a lesser extent, vitamin B₁, are required by many marine organisms. Laboratory growth culture experiments showed that about 50% of cultivated phytoplankton species require vitamin B₁₂, while about 25% require vitamin B₁ (n = 332 species) (Croft et al., 2005, 2006; Sañudo-Wilhelmy et al., 2014). In culture, *Haptophyceae*, *Chlorophyta* and diatoms notably rely on exogenous B₁ and B₁₂ (Gutowska et al., 2017; McRose et al., 2014; Paerl, Bertrand, et al., 2018; Paerl et al., 2017). Whole-genome sequencing efforts have further confirmed that B₁ and B₁₂ auxotrophy are widespread within *Haptophyceae* (*Emiliana*, *Pavlova*) and *Chlorophyta* (*Chlorella*, *Micromonas*, *Ostreococcus*), while diatoms (*Thalassiosira*, *Phaeodactylum*) and Pelagophytes (*Aureococcus*) could only produce B₁ (Helliwell, 2017; Sañudo-Wilhelmy et al., 2014).

Vitamin B₁ and B₁₂ have also a significant role in regulating the dynamics of most HAB species (Koch et al., 2013; Tang et al., 2010). Among 27 HAB species surveyed, 74% and 96% were dependent to exogenous vitamin B₁ and B₁₂, respectively, including toxic dinoflagellates (*Alexandrium*, *Gymnodinium*, *Karenia*, *Prorocentrum*) and diatoms (*Pseudo-Nitzschia*) (Tang et al., 2010). HAB species thus depends on their prokaryotic neighbors' that can produce B-vitamins, which imply proximity interactions (Croft et al., 2005), scavenging due to B₁₂-containing cell lysis (Droop, 2007) or direct B₁₂ excretion (Bonnet et al., 2010). Dual co-culture experiments notably showed potential interactions between B₁₂-producing bacteria and auxotrophic phytoplankton, including *Chlorophyceae* (*Ostreococcus*, *Lobomonas*) (Cooper et al., 2019; Grant et al., 2014), dinoflagellates (*Prorocentrum*) (H. Wang et al., 2014), diatoms (*Thalassiosira*) (Durham et al., 2015, 2017). These observations suggest that such mutualistic associations between bacteria and auxotrophic phytoplankton could be widespread in the ocean.

B. Abundant and ubiquitous marine microbes are vitamin auxotrophs

This high incidence of B₁ and B₁₂ auxotrophy is also observed within many marine bacterial lineages. Important vitamin auxotrophs including ubiquitous prokaryotes that are abundant in the global ocean, such as members of the SAR11 clade (*Alphaproteobacteria*), SAR86 clade (*Gammaproteobacteria*) and *Flavobacteria* (*Bacteroidetes*) (Carini et al., 2014; Gómez-Consarnau et al., 2018; Rusch et al., 2007; Sañudo-Wilhelmy et al., 2014; Shelton et al., 2019)

i. Proteobacteria

SAR11 is the most abundant bacterioplankton in the ocean (> 60% in GOS sequences) and specifically requires exogenous HMP to complete B₁ synthesis (*i.e.*, *Candidatus Pelagibacter ubique*) (Carini et al., 2014; Rusch et al., 2007). Among 11 SAR11 genomes, all were putative B₁ and B₁₂ auxotrophs but strikingly, none appear to require B₁₂, owing to the absence of B₁₂-requiring enzymes in these organisms (Sañudo-Wilhelmy et al., 2014). Similar observations based on metatranscriptomic data in the North Atlantic Ocean showed that *Pelagibacterales* members (*i.e.*, SAR11) are HMP auxotrophs and participate actively to B₁ uptake, but neither synthesize B₁₂ nor have B₁₂ dependency (Gómez-Consarnau et al., 2018; Joglar, Pontiller, et al., 2021). A half of *Rhodobacterales* genomes (25/50) lacked *thiC* and were thus also unable to produce HMP, while 72% (36/50) were able to produce cHET (Sañudo-Wilhelmy et al., 2014). Other *Alphaproteobacteria* (*i.e.*, *Sphingomonadales*, *Caulobacterales*, *Parvularculales*) could produce B₁ while all of them exhibit strong reliance on exogenous B₁₂ (Sañudo-Wilhelmy et al., 2014). Within *Gammaproteobacteria*, members of the SAR86 clade lack B₁ and B₁₂ biosynthesis pathways (Dupont et al., 2011), as previously observed in SAR11. Although it includes diverse organisms, most *Gammaproteobacteria* (*i.e.*, *Alteromonadales*, *Pseudomonadales*, *Thiotrichales*, *Vibrionales*) lack the B₁₂ biosynthesis pathway but almost all could produce B₁ (Sañudo-Wilhelmy et al., 2014). However, 46% and 37% of *Oceanospirillales* (6/13 genomes) and *Chromatiales* (3/8 genomes) could produce vitamin B₁₂.

ii. Bacteroidetes

The absence of B₁₂ biosynthesis pathways was reported among all 40 *Bacteroidetes* genomes surveyed, within which 24% of *Flavobacteria* (6/25 genomes), 60% of *Cytophagia* (6/10 genomes) and 60% of *Sphingobacteria* (3/5 genomes), have the genes require to B₁ biosynthesis (Sañudo-Wilhelmy et al., 2014). A metatranscriptomic analysis conducted in the Northwestern Iberian margin identify *Flavobacteriales* as major contributors of vitamin B₁ and B₁₂ uptake, further supporting their reliance on exogenous vitamins (Joglar, Pontiller, et al., 2021). Although B₁₂-auxotrophy is widespread among *Bacteroidetes* genomes (including *Flavobacteriales*) (Sañudo-Wilhelmy et al., 2014), their low contribution to B₁₂ uptake in the Atlantic Ocean suggest a greater dependency for B₁, while their low contributions to B₁ and B₁₂ synthesis further confirm their inability to produce them (Gómez-Consarnau et al., 2018).

4. Bacteria, cyanobacteria, and archaea produce B-vitamins in ocean

Vitamin B₁ and B₁₂ biosynthesis pathways are restricted to a few prokaryotic groups including heterotrophic bacteria (mostly *Proteobacteria*), chemoautotrophic archaea (*Thaumarchaeota*), and photo- / diazotrophic cyanobacteria (Gómez-Consarnau et al., 2018; Heal et al., 2017a; Sañudo-Wilhelmy et al., 2014; Shelton et al., 2019). A recent study on the functional capacity of marine bacteria to produce B-vitamins identify *Cyanobacteria*, *Gammaproteobacteria*, and *Actinobacteria* as important sources of B₁ and B₁₂ in oceans, while *Alphaproteobacteria* rather produce B₁₂ and require B₁ (Zoccarato et al., 2022). This study also showed that an exclusive reliance on exogenous vitamins is a risky strategy in the pelagic ocean, which further underly the mosaic pattern of metabolic complementarities for vitamins (Gómez-Consarnau et al., 2018; Romine et al., 2017; Zoccarato et al., 2022). *Alpha-* and *Gammaproteobacteria*, displayed more interaction traits than other microbial lineages, including B-vitamin synthesis, and are thus predicted to preferentially interact with bacteria and phytoplankton (Zoccarato et al., 2022).

A. Proteobacteria

Decades of whole genome sequencing efforts has demonstrated that 76% and 37% of genome from abundant and representative marine bacteria (*i.e.*, *Cyanobacteria*, *Alphaproteobacteria*, *Gammaproteobacteria*, *Bacteroidetes*) had the *de-novo* pathways required for vitamin B₁ and B₁₂, respectively (n = 413 genomes) (Sañudo-Wilhelmy et al., 2014).

Within *Alphaproteobacteria*, the *Rhodobacterales* order represented important potential B₁₂ producers, as 86% of their representative genomes (43/50) have the genes required for B₁₂ biosynthesis (Sañudo-Wilhelmy et al., 2014). *Rhodobacterales* also contributed actively to B₁₂ biosynthesis in the Atlantic Ocean (Gómez-Consarnau et al., 2018), and were identified as important B₁₂ synthesizers in marine surface waters (Doxey et al., 2015a; Heal et al., 2017a). Although they were also identified as potential B₁ producers in the North Atlantic Ocean (Suffridge et al., 2020), they accounted for only 7% of B₁ synthesis gene expression while representing a twofold high percentage of B₁-dependent gene transcripts (Gómez-Consarnau et al., 2018). Other *Alphaproteobacteria*, such as *Roseobacter* and SAR116, were identified as important B-vitamin producers in oceans. A phylogenomic analysis of 185 SAR116 genomes, including UBA1172, *Puniceispirillaceae*, and AAA536-G10, suggest that these organisms are an important source of B-vitamins, as they have the necessary genes for B₂, B₅, B₆, B₉ and B₁₂ synthesis (Grote et al., 2011; Roda-Garcia et al., 2021b). Among SAR116, *Puniceispirillum marinum* were identified as important B₁₂ producer in the Atlantic Ocean, which however require exogenous HMP (Gómez-Consarnau et al., 2018). An analysis of 52 *Roseobacter* genomes shows evidence for a functional vitamin B₁₂ synthesis pathway in all of them (Luo & Moran, 2014). In another study, all *Roseobacter* strains genomes (n = 32) could potentially produce B₁₂ while 22 strains had the potential to produce both B₁ and B₁₂ (*i.e.*, *Roseobacter*, *Phaeobacter*, *Ruegeria Sillicibacter*, *Sulfitobacter*, *Oceanicola*, *Roseovarius*, *Octadecabacter*, *Dinoroseobacter*) (Newton et al., 2010), further confirming their role in B-vitamins production.

Although B₁₂ auxotrophy is common within *Gammaproteobacteria* genomes, 43% of *Oceanospirillaceae* representative genomes (6/13) could produce B₁₂ (Sañudo-Wilhelmy et al., 2014). A targeted analysis of B₁₂-synthesis genes in the Ross Sea notably identified *Oceanospirillaceae* as putative B₁₂ producers in the Southern Ocean surface waters (Bertrand et al., 2015; Bertrand, Saito, Jeon, et al., 2011). Moreover, *Alteromonadales* and *Oceanospirillales* were one of the major contributors to the transcription of vitamin B₁ and B₁₂ synthesis genes in the Atlantic Ocean (NW Iberian Peninsula) (Joglar, Pontiller, et al., 2021).

B. Thaumarchaeota

Metagenomics surveys suggests that *Thaumarchaeota* are important B₁₂ producers in the English Channel (Doxey et al., 2015a) and in the Arctic (Heal et al., 2017). *Thaumarchaeota*, are also more abundant during winter in temperate regions such as the Mediterranean Sea (Galand et al., 2010; Hugoni et al., 2013; Lambert et al., 2019; Pereira et al., 2021) and in polar regions (Williams et al., 2012), and therefore could play a seasonally important role in B₁₂ production in these areas. *Nitrosopumilus ubiquus* have been shown to possess B₁₂ synthesis genes and were thus hypothesized to be a major source of B₁₂ in the water column (Doxey et al., 2015b; Qin et al., 2017; Santoro et al., 2015) and in the deeper ocean (Doxey et al., 2015, Heal et al., 2017). Furthermore, B₁₂-related genes from *Thaumarchaeota* dominated polar marine metagenomes, while those from bacteria dominated at the surface (Doxey et al., 2015a).

C. Cyanobacteria

Among 44 cyanobacterial genomes which include the abundant and globally distributed *Prochlorococcus* (18 genomes) and *Synechococcus* (13 genomes) (respectively 14.5% and 4.2% in GOS sequences, (Rusch et al., 2007)), all but one could synthesize both B₁ and B₁₂ *de-novo*. Diazotrophic cyanobacteria (*i.e.*, *Crocospaera* and *Trichodesmium*) are also important vitamin B₁ and B₁₂ synthesizers, and their capacity to produce vitamins is independent of available nitrogen, which is an interesting feature in nitrogen-limited oligotrophic oceans

(Frischkorn et al., 2018; Sañudo-Wilhelmy et al., 2014; Walworth et al., 2018). Strikingly, 7 out of 30 B₁-requiring enzymes were found in all surveyed genomes from cyanobacteria, while *Alpha*- and *Gammaproteobacteria* taxa contain 18 and 17 B₁-requiring enzymes, respectively (Sañudo-Wilhelmy et al., 2014). These differences could have important implication for B₁ cycling in ocean since they reflect distinct metabolic dependencies toward vitamin B₁ and B₁₂ within the main representative marine microbial lineages. Although all cyanobacterial genomes previously analyzed have been shown to rather produce pseudo-B₁₂ than B₁₂ (Heal et al., 2017a; Helliwell et al., 2016), phylogenetic, metagenomic, transcriptomic, proteomic, and chemical analyses providing multiple lines of evidence that *Trichodesmium* transcribes and translates the full genomic pathway to produce B₁₂ rather than pseudo-B₁₂ (Walworth et al., 2018).

D. Rare taxa could also represent vitamin sources in the ocean

Among the rare taxa found in marine metatranscriptomes from Sapelo Island (< 4% of total community transcripts), some participated in significant way in B-vitamins synthesis, including B₁ and B₁₂ (Gómez-Consarnau et al., 2018). For example, members of *Verrucomicrobia* accounted for 13% of B₁ synthesis transcripts while accounting only for 4% of total transcripts (Gómez-Consarnau et al., 2018). Similarly, *Cyanobacteria* and *Methylococcales* accounted for 9% and 6% of B₁₂-synthesis transcripts while accounting for only 3% and 1% of total community transcripts (Gómez-Consarnau et al., 2018). This study notably suggest that vitamin biosynthesis and utilization potentials are compartmentalized among distinct microbial community members, including abundant and rare taxa (Gómez-Consarnau et al., 2018). The seasonal reoccurrence of rare archaea in the Mediterranean Sea (Hugoni et al., 2013) and rare bacteria in the Atlantic Ocean (Alonso-Sáez et al., 2015), could notably suggest that distinct rare taxa play a seasonally important role in vitamin production in these regions, as they could sometimes match with the distribution of the most abundant taxa (Galand et al., 2009).

5. Vitamin B₁ and B₁₂ influence planktonic communities' growth and structure

Several field-amendments experiments conducted both in coastal and open-ocean environments pointed to the ecological importance of B-vitamins in controlling phytoplankton growth and in shaping marine microbial community structure. Vitamin B₁₂ co-limited phytoplankton growth together with nitrogen in the North Atlantic Ocean (Koch et al., 2011, 2012), and with iron in the Ross Sea (Bertrand et al., 2007, 2015; Bertrand, Saito, Lee, et al., 2011). Enhanced growth of auxotrophic phytoplankton after B₁₂ amendments was reported in microcosms conducted with natural communities from the Gulf of Alaska (Koch et al., 2011). Picoplankton were the main contributors to B₁₂ uptake (Koch et al., 2011), as reported in other experiments conducted in the North Atlantic Ocean (Koch et al., 2012; Sañudo-Wilhelmy et al., 2006). However, heterotrophic bacteria could significantly contribute to vitamin uptake among the picoplankton (Bertrand et al., 2007, 2015; Bertrand, Saito, Lee, et al., 2011; Koch et al., 2011, 2012). This large demand of vitamins underly a strong competition between phytoplankton and bacteria.

A seasonal variability in growth responses to B₁ and B₁₂ supply was reported in experiments conducted on bacterial and phytoplankton communities from coastal and oceanic stations of the NW Iberian margin (Joglar et al., 2020; Joglar, Pontiller, et al., 2021). Microbial community response to B₁ and/or B₁₂ amendments were correlated with changes in prokaryotic community composition (Joglar et al., 2020). When B-vitamin auxotrophs were abundant *in-situ* (i.e., *Flavobacteriales* in summer), external supply of B₁₂ and/or B₁ promoted negative interactions (Joglar et al., 2020). Moreover, growth stimulation upon B₁ addition was higher on prokaryotes than on eukaryotic phytoplankton, suggesting that B₁ auxotrophy could be more prevalent in prokaryotes (Joglar et al., 2020). However, increased biomasses induced by vitamin B₁ and/or B₁₂ amendments were not accompanied by significant changes in community composition in other studies (Joglar et al., 2020; Joglar, Pontiller, et al., 2021).

6. Context and aims of the thesis

In this context, the aim of this thesis was to characterize the long-term seasonality of microbial vitamin B₁ and B₁₂ metabolisms in the coastal NW Mediterranean Sea (SOLA station). To this end, a 7-year metagenomic timeseries (monthly sampled from 2009 to 2015) was used to identify key microbial players involved in vitamin B₁ and B₁₂ cycles (producers and consumers) and analyze their inter-annual dynamics and potential interplay over time. In addition, we conducted microcosm experiments combined with metabarcoding approaches to experimentally assess the effect of vitamin B₁, B₁₂ and B₁ precursors on natural prokaryotic community structure at different times of the year. Finally, we estimated vitamin B₁, B₁₂ and B₁ precursors bioavailability in seawater along the year using bioassay experiments conducted with *Ostreococcus tauri*.

More precisely, we asked the following questions:

- Who are the producers and consumers of vitamin B₁₂ at a coastal NW Mediterranean site (SOLA station), when are they present, and which production pathways are involved? Our hypothesis is that, given the importance of vitamin B₁₂ for marine microbes and the strong seasonality of planktonic communities at SOLA station, different key prokaryotes provide vitamin B₁₂ during different seasons.
- Do the irregular environmental disturbances observed in winter impact vitamin B₁₂ metabolism? And if so, in which way? Winter floods induce changes in the physicochemical conditions of seawater, which alter the composition of planktonic communities. We therefore hypothesize that perturbations could induce changes in community functions.

- What is the prevalent metabolic pathway associated with vitamin B₁ production and salvage (including the use of exogenous precursors HMP and cHET)? Who are the major microbial players for each pathway, and when are they present? Our hypothesis is that different B₁ lifestyles coexist with distinct seasonal contributions depending on their ecological niches.
- Are vitamin B₁ and B₁₂ producers and auxotrophs interdependent? We investigated this question using a metagenomic-informed network analysis to identify potential interdependencies based on a functional complementarity for vitamin requirements between co-occurring microbes. We further tested experimentally this hypothesis in vitamin amendment experiments conducted in microcosms.

II. CHAPTER 2: Seasonality and functional redundancy in marine microbial communities: a case study for vitamin B₁₂ metabolism

1. Seasonality of microbial functions at SOLA

A 7-year metabarcoding study conducted in our coastal station of the NW Mediterranean Sea (SOLA) (**Fig. I.2, Fig. IIa.A**), showed strong and recurrent seasonal patterns of bacterial, archaeal, and phytoplanktonic communities' composition, which were mostly driven by environmental factors (*i.e.*, photoperiod and temperature) (Lambert et al., 2019).

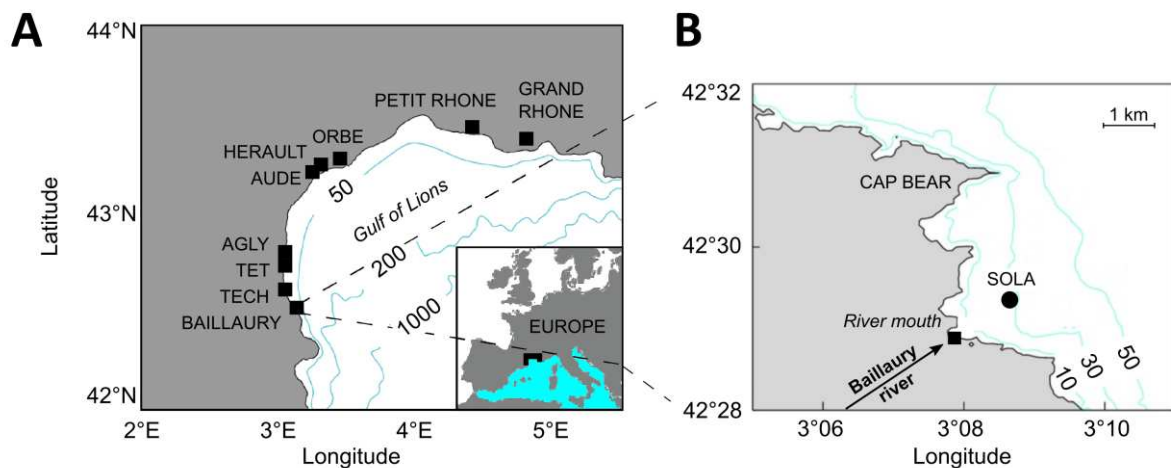


Figure IIa: Geographical localization of SOLA station. **(A)** Bathymetric map of the Gulf of Lions (black area) in the Northwestern Mediterranean Sea (colored in blue) and location of nearby rivers (black squares: river mouths). **(B)** The SOLA station (42°31'N, 03°11'E) is a shallow (24m depth) and coastal site located in front of the Baillaury river mouth', at approximately 1km off the Bay of Banyuls-sur-Mer (France). Adapted from (Guizien et al., 2007).

The high predictability of marine microbial assemblages at SOLA station leads us to ask whether this pattern is also observed at the functional level. To do this, we conducted a metagenomics analysis of the DNA samples from the 7-year metabarcoding time series (Lambert et al. 2019). In this time series associated with 90 surface water samples collected monthly from 2009 to 2015, we first recovered 8509 microbial functions identified using the KEGG database, as described in the Material and Methods section of Article 1 page 56 (**Fig. IIb.A**).

An overview of the main microbial functions recovered from the SOLA time series points to the importance of microbial metabolisms (67.6% of sequences) and notably amino acid (10.2%), carbohydrates (9.5%), terpenoids/polyketides (8.8%), energy (8.3%) and cofactors and vitamins (7.9%) metabolisms (**Fig. IIb.A**).

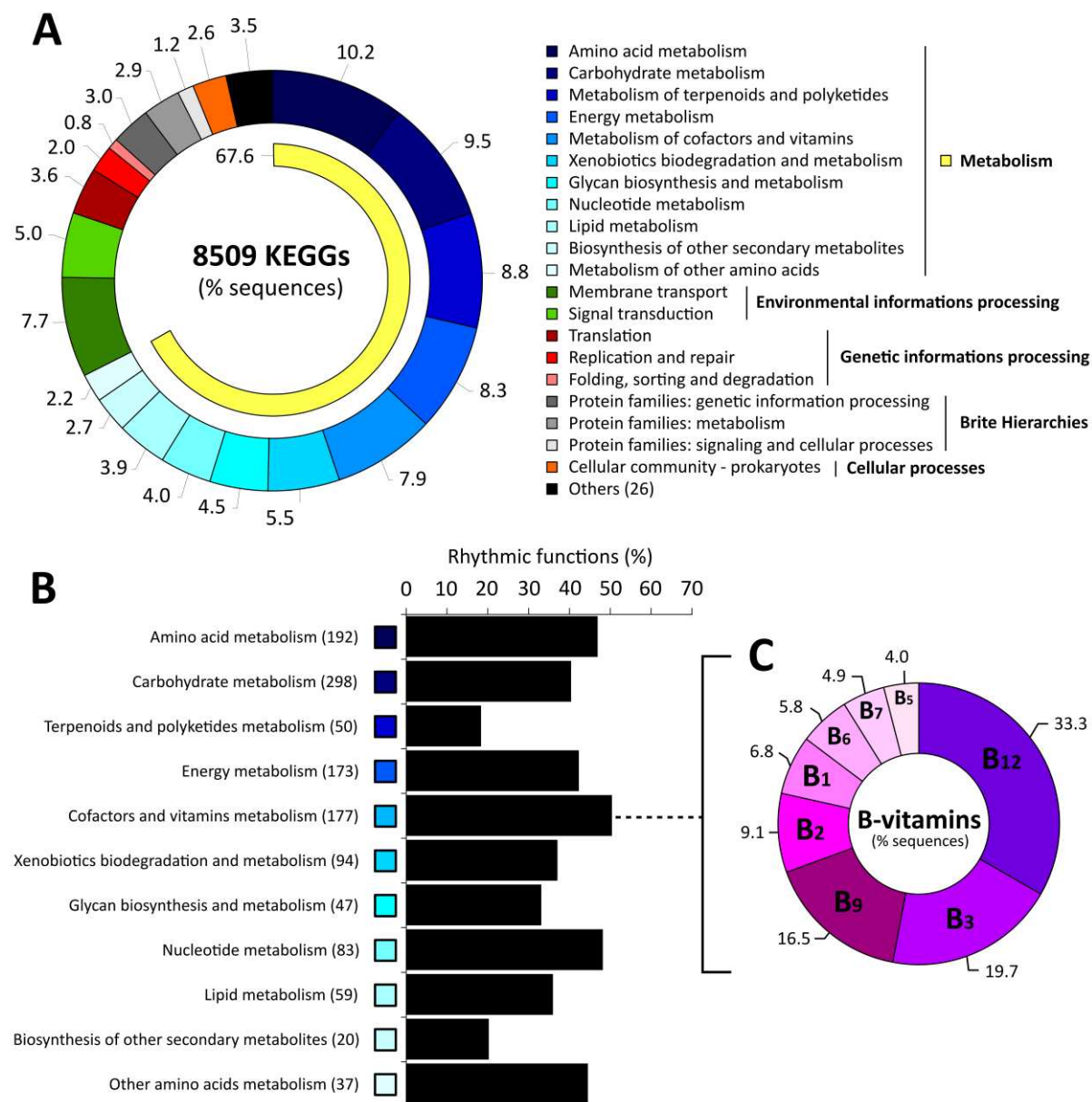


Figure IIb: Overview of 8509 microbial functions (*i.e.*, KEGGs) recovered from SOLA timeseries. **(A)** Relative abundance (% sequences) of the main functional families identified in the SOLA metagenomic time series. Functions are ranked in ascending order of abundance within the two functional levels illustrated (*i.e.*, amino acid metabolism is included in “Metabolism”). **(B)** Bar chart illustrating the number of rhythmic functions (*i.e.*, unique KEGGs with PNmax ≥ 10 according to LSP analysis) in each functional subfamily identified in “Metabolism” shown in (A). The number of rhythmic KEGGs per subfamily is shown in brackets. **(C)** Proportion of sequences of the eight B-vitamin related metabolisms among cofactor and vitamin metabolism, the most rhythmic metabolism.

We then used Lomb Scargle periodogram (LSP) analysis to identify functions associated with KEGGs presenting annual rhythms (See detailed methods in Material and Methods section of Article 1 page 56). The LSP analysis performed at the KEGG level identify many seasonal functions and showed high rhythmicity levels in major microbial metabolisms, notably those involving cofactor and vitamins (**Fig. IIb.B**). More than a half of the functions associated to vitamin metabolism were rhythmic (177 KEGGs), among which vitamin B₁₂ was the most represented metabolism (33.3% of B-vitamins-related sequences) (**Fig. IIb.B, C**). Based on these observations, we have chosen to focus on vitamin B₁₂ metabolism for subsequent analysis.

2. Seasonality and functional redundancy of vitamin B₁₂ metabolism

Vitamin B₁₂ (cobalamin), is a scarce but essential cofactor for most marine microbes, yet its production is limited to only a few prokaryotic groups in the ocean. Despite its ecological importance, the seasonal dynamics of B₁₂ metabolism and the associated microbial communities have never been studied. To address this question, we used a metagenomic approach to examine the monthly dynamic of B₁₂-related pathways and the diversity of microbial communities in the coastal NW Mediterranean Sea (SOLA station) over a 7 years timeseries. Our findings revealed a seasonal succession of distinct B₁₂ producers, each carrying different production pathways. In winter, *Thaumarchaeota* (*Nitrosopumilus*, *Nitrosopelagicus*) were the main potential B₁₂ producers through the anaerobic pathway, while *Alphaproteobacteria* such as *Roseobacter* (HIMB11) and SAR116 (UBA8309, *Puniceispirillum*) could potentially produce B₁₂ through the aerobic pathway during warmer months. In addition, cyanobacteria dominated autotrophic communities in summer and are potential pseudo-B₁₂ producers. Finally, we showed that during years with irregular environmental perturbations (*i.e.*, floods of the Baillaury river, **Fig. IIa.B**), seasonal B₁₂ producers are replaced by others carrying similar functions. Such functional redundancy provides an ecological insurance for B₁₂ production by ensuring the maintenance of this essential function despite irregular disturbances.

Article 1: Functional redundancy of seasonal vitamin B₁₂ biosynthesis pathways in coastal marine microbial communities

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Data availability statement: The catalog of prokaryotic genes associated with all the B₁₂-related KEGGs used in this study is accessible online in the Zenodo open repository: <https://doi.org/10.5281/zenodo.7409518>. The raw sequences were deposited in the NCBI Sequence Read Archive under accession number PRJEB66489.

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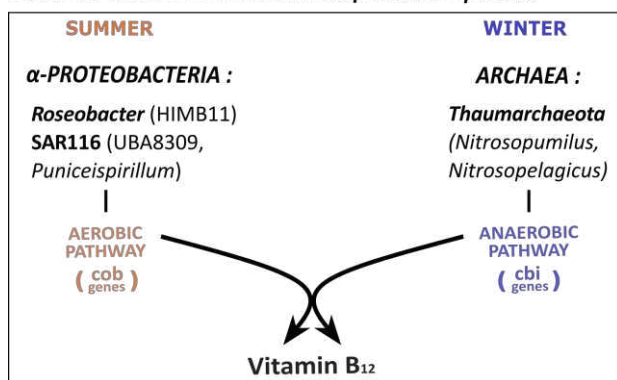
Keywords: Vitamin B₁₂, Metagenomic time series, Planktonic communities, Functional communities, Ecological insurance

Abstract

Vitamin B₁₂ (cobalamin) is a major cofactor required by most marine microbes, but only produced by a few prokaryotes in the ocean, which is globally B₁₂-depleted. Despite the ecological importance of B₁₂, the seasonality of B₁₂ metabolisms and the organisms involved in its synthesis in the ocean remain poorly known. Here we use metagenomics to assess the monthly dynamics of B₁₂-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 B₁₂ metabolisms were characterized by an annual succession of different organisms carrying distinct production pathways. During the most productive winter months, archaea (*Nitrosopumilus*, *Nitrosopelagicus*) were the main contributors to B₁₂ synthesis potential through the anaerobic pathway (*cbi* genes). In turn, *Alphaproteobacteria* (*HIMB11*, *UBA8309*, *Puniceispirillum*) contributed to B₁₂ 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 B₁₂ synthesis genes were replaced by others having the same gene, thus maintaining the potential for B₁₂ production. Such ecological insurance could contribute to the long-term functional resilience of marine microbial communities exposed to contrasting inter-annual environmental conditions.

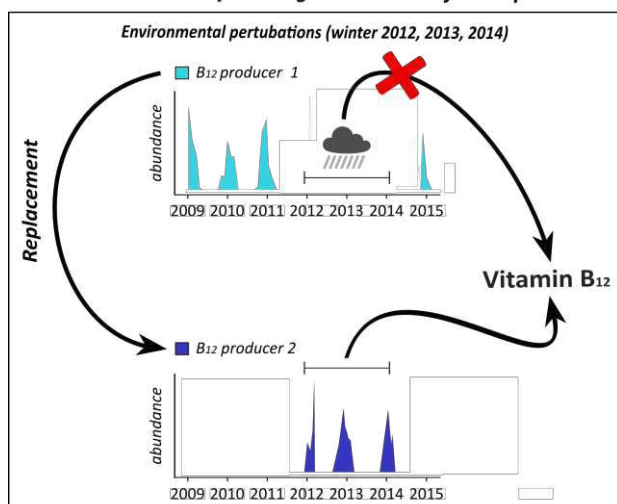
Graphical abstract

Recurrent and seasonal vitamin B₁₂ production pattern



This seven years metagenomic time series study from the NW Mediterranean Sea demonstrates a seasonal succession of distinct potential vitamin B₁₂ production pathways in summer (*i.e.*, aerobic pathway) and winter (*i.e.*, anaerobic pathway) carried by phylogenetically distant organisms (*i.e.*, bacteria and archaea, respectively).

Functional redundancy : ecological insurance for B₁₂ production



We then show that during environmental perturbations, potential B₁₂ producers were replaced by others carrying similar functions (functional redundancy), maintaining the potential for B₁₂ production even under contrasting environmental conditions (ecological insurance).

Introduction

Marine planktonic communities strongly influence global biogeochemical cycles (Falkowski et al., 2008), and it is now well established that their spatio-temporal 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., 2019b). 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 B₁₂ (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 et al., 2006). Genomic and culture-based approaches suggest, however, that most eukaryotes are auxotrophs towards B₁₂ including more than a half of phytoplankton species, compared to about 25% and 8% for thiamin (vitamin B₁) and biotin (vitamin B₇), respectively (Croft et al., 2005; Sañudo-Wilhelmy et al., 2014b). This high incidence of B₁₂ 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., 2014b; Shelton et al., 2019).

Although many microorganisms have an absolute requirement of B₁₂-related coenzymes, vitamin B₁₂ 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 B₁₂-depleted (picomolar concentrations), and that its 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., 2018b). Auxotrophic

organisms thus rely mainly on B₁₂ *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, B₁₂ *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., 2014b; Shelton et al., 2019). More precisely, targeted characterizations of B₁₂ biosynthesis genes in the Ross Sea identified members of *Gammaproteobacteria* (*i.e.*, *Oceanospirillales*) as putative B₁₂ synthesizers in the Southern Ocean surface waters (Bertrand et al., 2011, 2015). Metagenomics studies also suggests that *Thaumarchaeota* may have an important role in B₁₂ biosynthesis in the deeper ocean and in 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 B₁₂ (Zhou et al., 2020). A metatranscriptomic study carried out in the Atlantic Ocean quarterly, over one year, more precisely identified members of *Rhodobacterales* as the main potential B₁₂ producers (Gómez-Consarnau et al., 2018). Even though several vitamin B₁₂ producers have been identified punctually at different geographic locations, the extent of their diversity and dynamics over time remains to be explored.

B₁₂ biosynthesis is a complex metabolic process involving about twenty genes and thirty enzymatic steps (Balabanova et al., 2021; Fang et al., 2017; Martens et al., 2002; Raux et al., 2000) (**Fig. II.1**). Within bacteria, B₁₂ 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., 2014b; Shelton et al., 2019), while in archaea only the anaerobic one is used (Doxey et al., 2015; Fang et al., 2017) (**Fig. II.1A**).

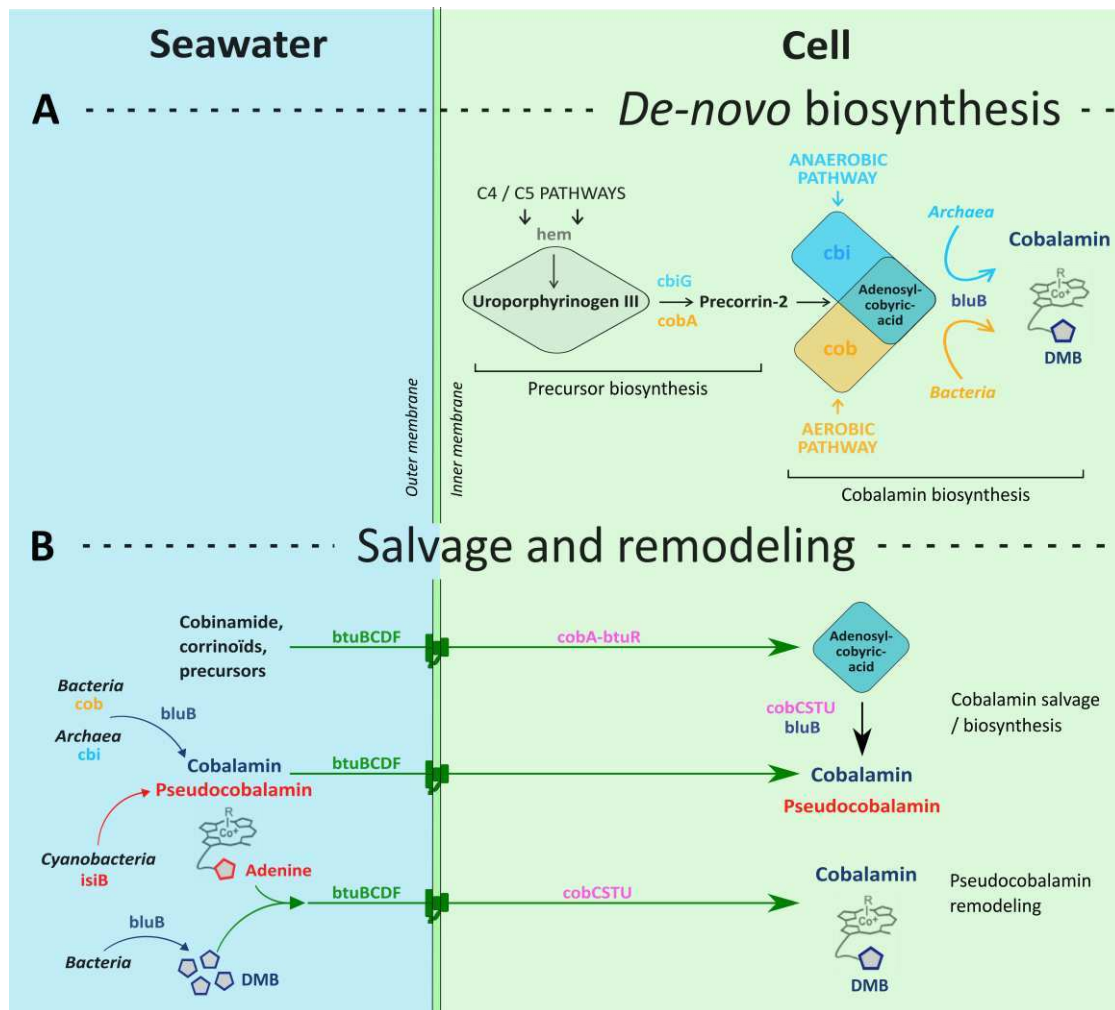


Figure II.1: Schematic overview of vitamin B₁₂ *de-novo* biosynthesis, salvage, and remodeling pathways (Balabanova et al., 2021; Fang et al., 2017; Zhou et al., 2021). **(A)** Vitamin B₁₂ *de-novo* biosynthesis occurs in the cell (right part; green) and requires the prior generation of the precursor uroporphyrinogen III via the C4 and C5 pathways, which involve the *hem* genes (grey). Depending on the organisms, *cobA* or *cysG* genes' activities catalyze the synthesis of precorrin-2 from uroporphyrinogen III, that can be metabolized in vitamin B₁₂ 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 (colored in orange and blue, respectively). Both pathways converge toward adenosylcobyrinic acid, that is finally converted into vitamin B₁₂. Archaea and bacteria produce cobalamin, a form of vitamin B₁₂ that has a dimethylbenzimidazole (DMB) molecule as a lower axial ligand, which is produced through the activity of the gene *bluB* (colored in dark blue) and then fused to the coring ring to form cobalamin. **(B)** Molecules structurally close to vitamin B₁₂ (e.g., cobinamide and other corrinoïds) can be directly scavenged from the seawater (left part; blue) through specific transporters such as *btuBCDF* in bacteria (colored in green). After translocation, these molecules are metabolized in the cell by specific enzymes which ensure the generation of adenosylcobyrinic-acid, such as ATP:co(I)rrinoid adenosyltransferases (ACATS) encoded by *cobA-btuR* gene (colored in pink). Adenosylcobyrinic-acid will integrate the biosynthesis pathway and will be transformed in vitamin B₁₂ by several enzymes encoded by the *cobCSTU* genes (colored in pink). Cyanobacteria rather produce pseudo-cobalamin (*i.e.*, pseudo-B₁₂) which have an adenine in lower axial ligand, and which is not bioactive for most marine microorganisms. The flavodoxin gene *isiB* (colored in red) is an orthologue of the cobalt reductase *fldA* found in some B₁₂-producing bacteria, and thus is supposed to be involved in pseudo-B₁₂ biosynthesis in cyanobacteria (Walworth et al., 2018). Salvaged pseudo-B₁₂ can be remodeled intracellularly to vitamin B₁₂ by some microalgae and bacteria that possess *cobCSTU* genes (e.g., *Pavlova*, *Vibrio*) (Helliwell et al., 2016; Ma et al., 2020a). In seawater, pseudo-B₁₂ remodeling requires DMB bioavailability.

In contrast to bacteria and archaea, the abundant and globally distributed marine cyanobacteria *Synechococcus* and *Prochlorococcus* rather produce pseudo-cobalamin (named pseudo-B₁₂) (Helliwell et al., 2016) (**Fig. II.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-B₁₂ remodelers can, however, remodel pseudo-B₁₂ into vitamin B₁₂ in an energy-cost-effective way through the *cobCSTU* genes (Heal et al., 2017; Helliwell et al., 2016; Ma et al., 2020b) (**Fig. II.1B**). The remodeling of pseudo-B₁₂ requires exogenous DMB (5,6-dimethylbenzimidazole), suggesting that DMB bioavailability in seawater could also impact B₁₂ cycling in the ocean, and influence the composition of microbial communities (Wienhausen et al., 2022).

Despite their ecological importance, vitamin B₁₂ 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 B₁₂ 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 B₁₂ metabolisms (biosynthesis, salvage, remodeling), and to identify the key taxonomic groups involved in these processes throughout the year. We further verify if environmental perturbations disrupt the vitamin B₁₂ seasonality.

Experimental procedures

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

Surface seawater (3m 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., 2019b).

DNA samples taken between 2009 and 2011 were shotgun sequenced using Illumina NovaSeq6000 (2 x 150 bp) at the Centre Nacional d'Anàlisi Genòmica (CNAG), while DNA samples taken between 2012 and 2015 were sequenced using Illumina HiSeq2500 (2 x 100 bp) (Galand et al., 2018b). The raw sequences were deposited in the NCBI Sequence Read Archive under accession number PRJEB66489.

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) (H. Li & Durbin, 2009). The number of counts per gene were 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 analyzed with the Genome Taxonomy Database (GTDB) using MMseqs2 and considering the last common ancestor (LCA). KEGGs involved in B₁₂ 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), and those that were used as specific markers in this study are indicated in Table II.S1 (**Table II.S1**). The catalog of prokaryotic genes associated with all the B₁₂-related KEGGs used in this study is accessible online in the Zenodo open repository: <https://doi.org/10.5281/zenodo.7409518>.

***Ostreococcus tauri* cobalamin bioassay**

The Mediterranean strain *Ostreococcus tauri* (RCC745) cultivated in the laboratory was used as model organism to evaluate the bioavailability of vitamin B₁₂ 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 B₁ and B₁₂ and has already been used to develop a vitamin B₁ bioassay (Paerl et al., 2017). Cells from a fresh culture maintained for 6 days in a B₁₂-free artificial seawater (ASW), with Streptomycin (50 µg.mL⁻¹) and Penicillin (100 µg.mL⁻¹), were inoculated in a deep well microplate (Nunc) at 5000 cells.mL⁻¹ in 1.8 mL of SOLA 0.22 µm filtered seawater supplemented with B₁₂-free Keller enrichment medium and 1 nM of vitamin B₁ (Sigma-Aldrich). Cyanocobalamin (Sigma-Aldrich) was diluted in MiliQ water at 1 mg.mL⁻¹ concentrations, aliquoted and stored at -20 °C until use.

First, the B₁₂ requirement of *O. tauri* was assessed in cultures performed in deep well microplates inoculated at 5000 cells.mL⁻¹ in 1.8 mL of B₁₂-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 cyanocobalamin (**Fig. II.S2**). In the bioassay, 0 to 25 pM of B₁₂ were added to evaluate B₁₂ bioavailability in SOLA seawaters at different time of the year.

All cultures were incubated in triplicate during 6 days under constant light conditions (35 $\mu\text{mol}\cdot\text{quanta}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). 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 B₁₂ 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 B₁₂-related KEGGs, or genes. Rhythmic means that a gene reoccurs every year at the same time of the year. The LSP is based on the Fourier transform (Lomb, 1976; Scargle, 1982) and was successfully adapted by biologists to detect periodic signals in unevenly sampled time series (Lambert et al., 2019b; 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., 2019b), 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., 2019b). 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, $p \leq 0.001$) 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 two-way crossed analysis of similarities with the ‘anosim’ function (*Anosim*, permutations = 999, $p \leq 0.001$) (Clarke, 1993; Clarke & Warwick, 2001). Correlation between environmental variables (Euclidean distances) and community dissimilarity (Bray-Curtis) were assessed using Mantel tests ($r \geq 0.5$, $p \leq 0.001$). 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_{10} 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 (NO₂, NO₃⁻, PO₄³⁻) and chlorophyll-a concentrations were higher in winter and late winter, respectively (**Fig. II.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 B₁₂ in seawater at SOLA, we used a B₁₂ bioassay based on the model organism *Ostreococcus tauri* that was previously used in vitamin B₁ bioassays (Paerl et al., 2017). Assessing the requirement for B₁₂ of our model species, maximal cell concentrations were reached at 25 pM cyanocobalamin (**Fig. II.S2**). B₁₂-limited cells incubated in SOLA filtered seawater collected at different times of the year and enriched with nitrate, phosphate, trace metals and vitamin B₁, exhibited different growth patterns (**Fig. II.2A**). *O. tauri* grew without B₁₂ addition in most water samples between April and November. Inversely, no cell growth was observed in the absence of B₁₂ in most winter water samples. B₁₂ amendment restored, however, growth or improved cell survival in a dose-dependent manner in most winter samples confirming that B₁₂ was limiting at the time. The annual pattern of the KEGG *metE*, which encodes the cobalamin independent methionine synthase was used to detect potential B₁₂ limitations (**Fig. II.2B**). *MetE* catalyzes 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 (**Fig. II.2B**). Together, figures 2A and 2B suggest that B₁₂ is limiting in SOLA seawater in winter and spring.

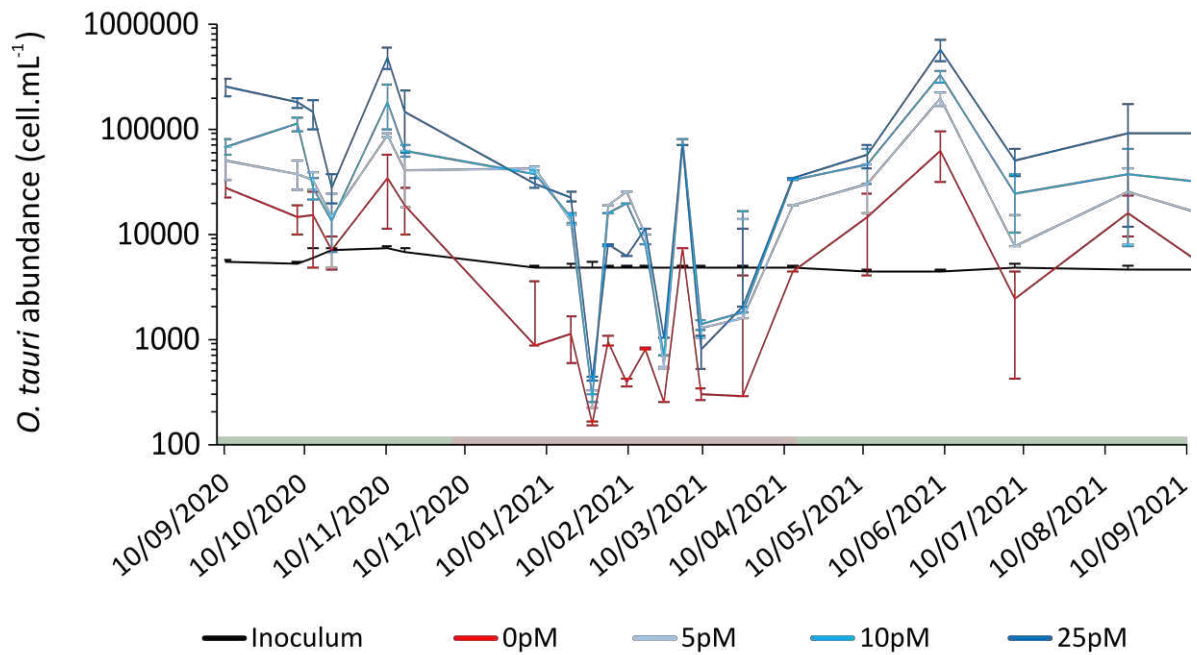
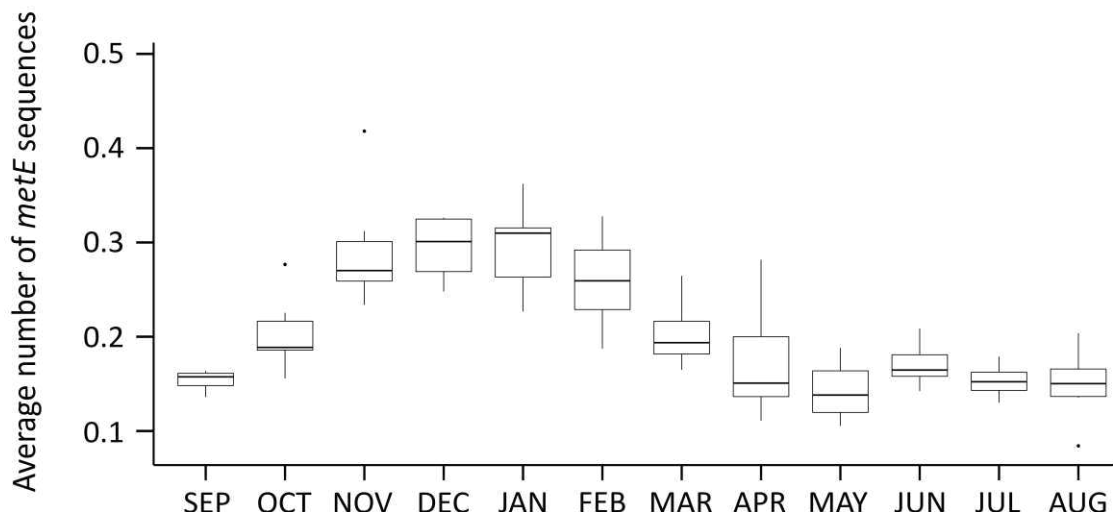
A *O. tauri* bioassay**B** *metE* annual pattern

Figure II.2: (A) Bioassay of *Ostreococcus tauri* RCC745 incubated in SOLA water collected from September 2020 to September 2021 and supplemented with increasing vitamin B₁₂ concentrations (0 pM to 25 pM). The inoculum represents the cell abundance (cell.mL⁻¹ ± SD, n=3) of the inoculate, and the periods colored in green indicate growth of *O. tauri* (i.e., B₁₂ availability in seawater) while those colored in red indicate a decline of *O. tauri* cell numbers (i.e., B₁₂ 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 B₁₂ bioavailability in SOLA seawater. *MetE* catalyzes the methionine biosynthesis in a vitamin B₁₂-independent way.

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 B₁₂ metabolisms (catabolism, production, salvage, and remodeling) (**Fig. II.1, Table II.S1**) that corresponded to 474 725 genes (**Fig. II.3A**). Among them, 30 were significantly rhythmic with a period of 365 ± 10 days as determined by the Lomb Scargle periodogram (LSP) method ($PN_{max} \geq 10$, p -values < 0.05) (**Table II.S1**). These rhythmic KEGGs comprised 68.2% of the genes associated with the 82 B₁₂-related KEGGs, and 68.6% of the sequences (**Fig. II.3A**).

A nMDS analysis based on all 82 B₁₂-related KEGGs, fitted to SOLA environmental variables (**Fig. II.S1**), showed that samples grouped significantly according to seasons (anosim, $r = 0.393$, $p = 0.001$) (**Fig. II.3B**). A nMDS focused on the 30 rhythmic KEGGs showed a stronger grouping of the samples according to seasons (anosim, $r = 0.459$, $p = 0.001$) (**Fig. II.3C**), while a nMDS based on the non-rhythmic ones showed a weaker seasonal grouping (anosim, $r = 0.165$, $p = 0.001$) (**Fig. II.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, $r = 0.2724$, $p = 0.001$) (**Fig. II.3C, D**).

When considering the genes corresponding to the B₁₂-related KEGGs, the nMDS showed that there was also a strong seasonal pattern for all genes (anosim, $r = 0.568$, $p = 0.001$) (**Fig. II.3E**), and for the ones related to the rhythmic KEGGs (anosim, $r = 0.564$; $p = 0.001$) (**Fig. II.3F**), but also for the genes from the non-rhythmic KEGGs (anosim, $r = 0.572$; $p = 0.001$) (**Fig. II.3G**). There was a stronger correlation between samples and environmental variables for the gene-based analysis than for the KEGG-based ones (**Fig. II.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, $p = 0.001$) (**Fig. II.3B – G, Fig. II.S1**).

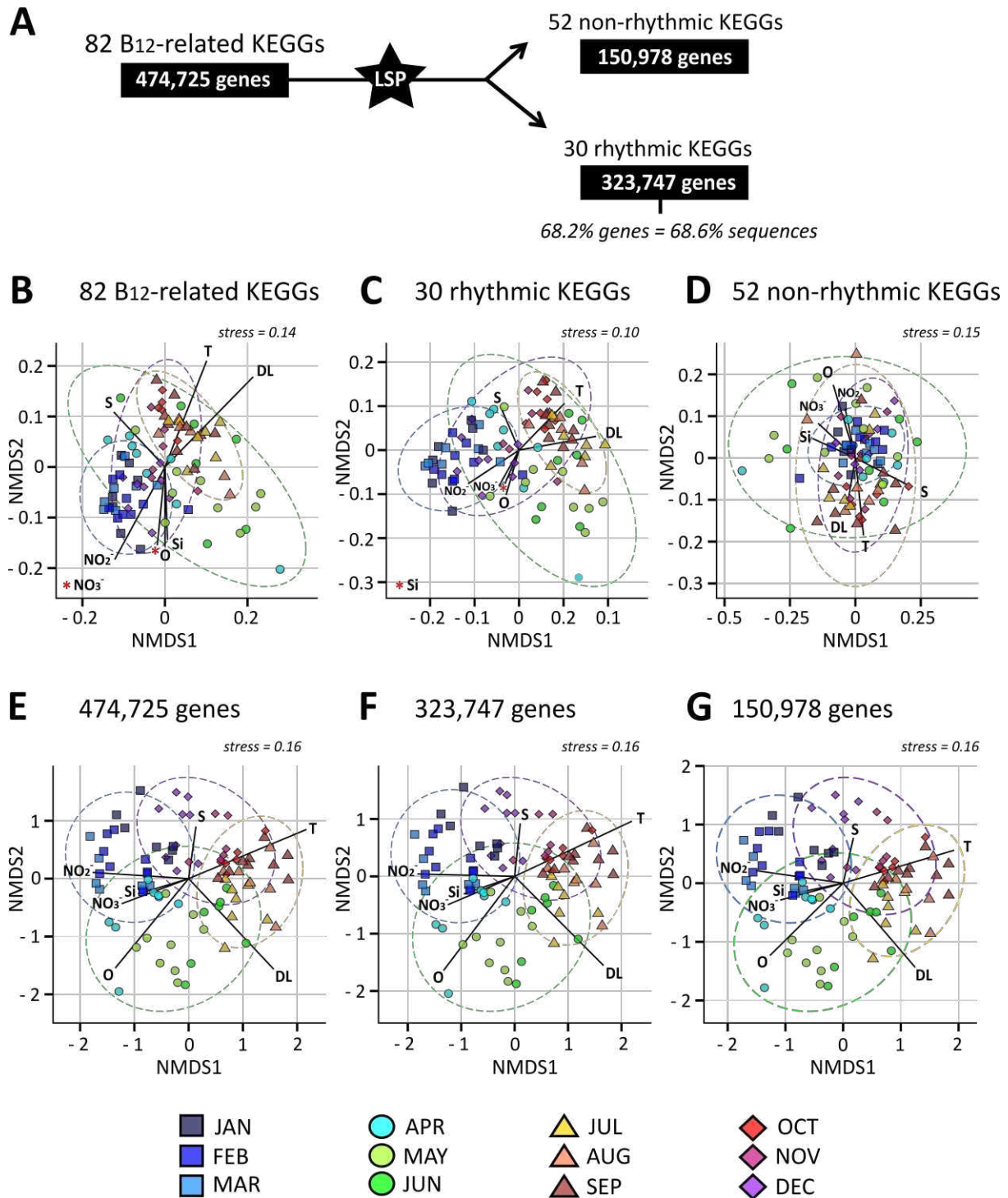


Figure II.3: Seasonality of KEGGs related to potential B₁₂ metabolisms and their associated genes. **(A)** Schematic representation of the rhythmicity analysis and the number of KEGGs and genes involved. **(B)** NMDS analysis based on all KEGGs related to vitamin B₁₂ metabolisms, **(C)** the 30 rhythmic KEGGs and **(D)** the 52 non rhythmic 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 sample's structure are fitted onto the nMDS: T: temperature; DL: day length; S: salinity; O: dissolved oxygen; Si: silicate (Si(OH)₄); NO₂⁻: nitrite; NO₃⁻: nitrate.

Seasonality of B₁₂ and pseudo-B₁₂ biosynthesis, salvage, and remodeling pathways

Among the 30 rhythmic KEGGs related to vitamin B₁₂ metabolisms (PNmax ≥ 10), we selected the 13 KEGGs specifically involved in B₁₂ or pseudo-B₁₂ biosynthesis, salvage, and remodeling pathways (**Fig. II.1, Table II.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 remodeling pathways (**Fig. II.4A**). More precisely, KEGGs markers of the anaerobic pathway (*bluB*, *cbiE*, *cbiT*, *cobY*) had peaks of occurrence in February (**Fig. II.4B, C**). Rhythmic KEGGs involved in B₁₂ *de-novo* biosynthesis via the aerobic pathway were more abundant between May and September (**Fig. II.4B**), with maximum occurrences varying from May for *cobA*, June for *cobZ*, *cobF*, and *cobG*, and August for *cobW* and *cobK-cbiJ* (**Fig. II.4B, C**). Rhythmic KEGGs involved in the salvage pathway (*cobA_btuR*) and in pseudo-B₁₂ remodeling (*cobC*) peaked in June and August, respectively (**Fig. II.4B, C**). The only potential marker of pseudo-B₁₂ production by cyanobacteria, the KEGG *isiB*, had maximum abundance in December (**Fig. II.4B, C**).

Distinct organisms are involved in seasonal vitamin B₁₂ and pseudo-B₁₂ biosynthesis

We next focused on the taxonomic diversity of the genes coding for proteins involved in vitamin B₁₂ biosynthesis. A rhythmicity analysis (LSP) performed individually on the 23,045 genes associated with the 13 rhythmic marker KEGGs related to B₁₂ and pseudo-B₁₂ biosynthesis, salvage, and remodeling identified 698 annual rhythmic genes, representing 3% of biosynthesis genes and 29% of the sequences (**Fig. II.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 (**Fig. II.S3A**). An nMDS analysis of these 13 KEGGs and their 698 rhythmic genes showed marked seasonal patterns (anosim $r = 0.716$, $p = 0.001$) and a strong influence of environmental variables, notably at the gene level (Mantel $r = 0.6879$, $p = 0.001$) (**Fig. II.S3B, C**).

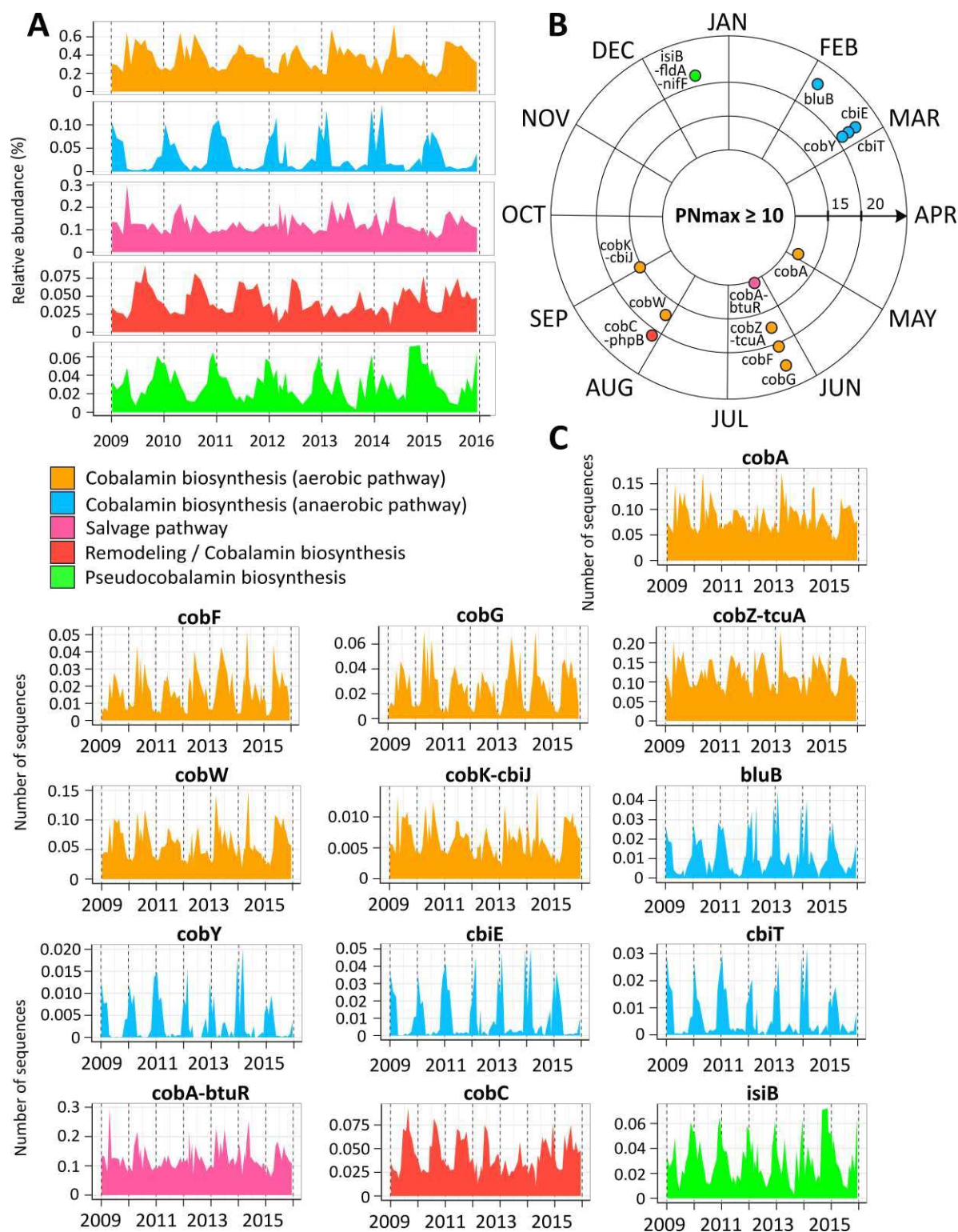


Figure II.4: Seasonal patterns of the 13 rhythmic KEGGs related to vitamin B₁₂ and pseudo-B₁₂ biosynthesis, salvage, and remodeling pathways at the SOLA station for 7 years. **(A)** Relative abundance (%) of the KEGGs summed by pathway: aerobic and anaerobic biosynthesis pathways (orange and blue, respectively), salvage pathway (pink), pseudo-B₁₂ remodeling and cobalamin biosynthesis (red), and pseudo-B₁₂ biosynthesis (green). **(B)** 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 to members of the *Nitrosopumilus* and *Nitrosopelagicus* genera, which belong to the phylum *Thaumarchaeota* (**Fig. II.5A – C**). Both genera were represented by 49 unique genes that accounted for about 94% of the archaeal sequences, which are dominated by *Nitrosopumilus* (78% of archaeal sequences) (**Fig. II.5C**). *Nitrosopumilus* and *Nitrosopelagicus* had overlapping occurrence patterns in winter, with peaks of abundance observed between December and March (**Fig. II.5A, B**). All KEGGs associated to the anaerobic biosynthesis pathway were present in these archaeal sequences, as well as *bluB*, *cobA-btuR* for the salvage pathway, and *isiB* (**Fig. II.5C**). According to our data, rhythmic archaea from the *Nitrosopelagicus* genus do not seem to possess the KEGG *cbiE* (**Fig. II.5C**). Archaeal B₁₂ biosynthesis genes sequences (*i.e.*, *cbiE* and *cbiT* sequences) were between 3 up to 9 times more abundant than bacterial B₁₂ biosynthesis genes in winter (*i.e.*, *cobG* and *cobF* sequences) (**Fig. II.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 (**Fig. II.5D – F**). Genes affiliated with *Prochlorococcus* were present in autumn between October and December, while those affiliated to *Synechococcus* were present between May and November displaying a wider annual distribution (**Fig. II.5D, E**). Both genera had the same potential toward pseudo-B₁₂ and B₁₂ synthesis or salvage, with five marker KEGGs present in various proportions: *isiB*, *cobK-cbiJ*, *cobW*, *cobA*, as well as *cobA-btuR* (**Fig. II.5F**).

For other bacteria, the most abundant genera for B₁₂ 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 SAR116 clade, *Puniceispirillum* and UBA8309

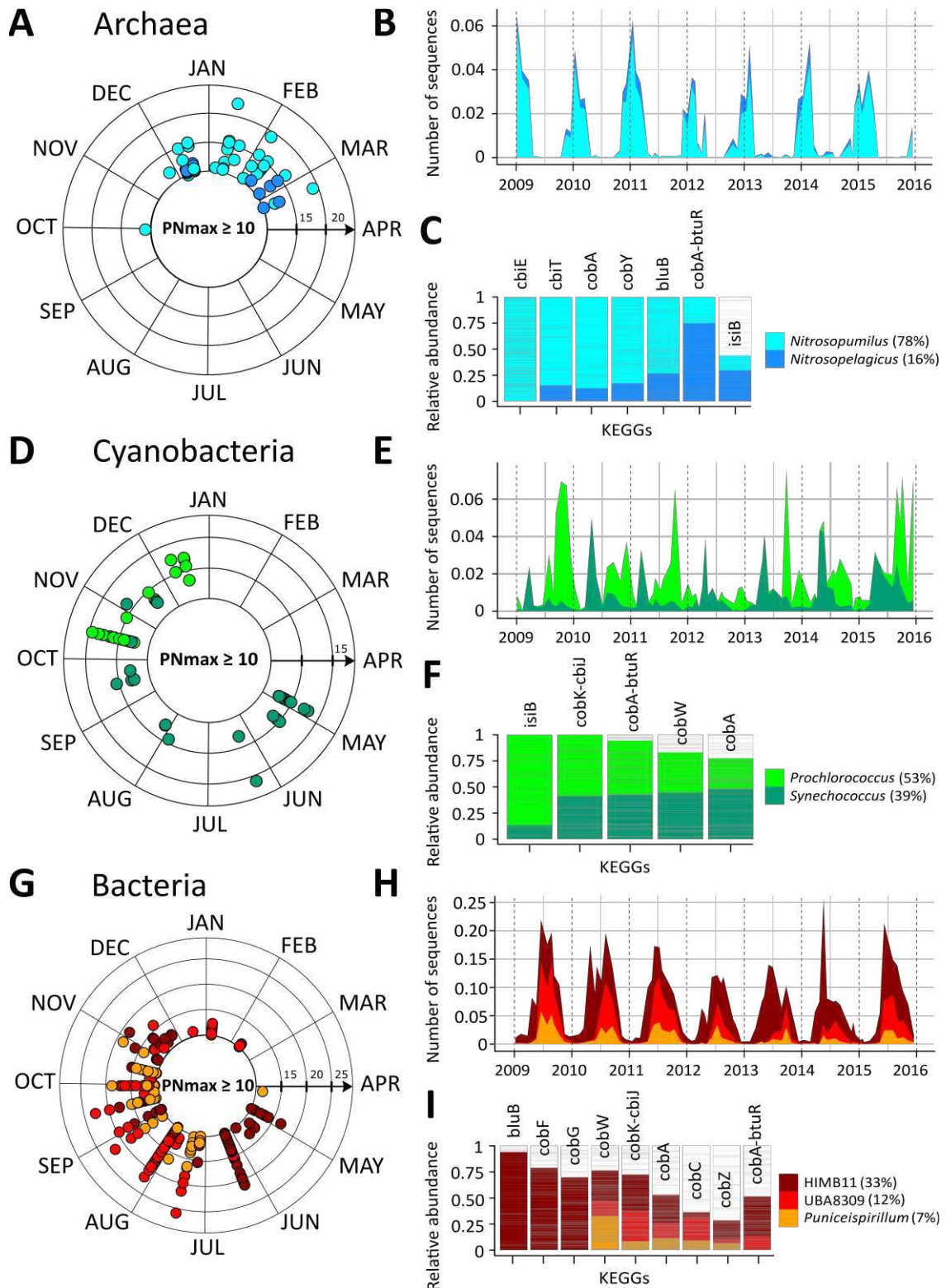


Figure II.5: Seasonal pattern and metabolic potential of the taxonomical groups involved in vitamin B₁₂ or pseudo-B₁₂ production during 7 years at SOLA. For the archaeal (**A**, **B**, **C**), cyanobacterial (**D**, **E**, **F**) and bacterial genes (**G**, **H**, **I**) which are colored by genus, the 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.

(Fig. II.5G – I). These genera represented 268 unique genes, which accounted for about 51% of bacterial sequences (Fig. II.5I). Alone, the genus HIMB11 accounted for more than a third of bacterial sequences (Fig. II.5I). The different species within these bacterial genera had maximal abundances between May and November, with overlapping occurrence patterns during this period (Fig. II.5G, H). All KEGGs associated to 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 (Fig. II.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*) (Fig. II.S5A – D). The 5 most abundant phylotypes among these dominant species accounted for more than 40% of their associated sequences, with some distinct individual seasonal patterns (Fig. II.S5A – D). The species UBA8309 *sp002457765* accounted for about 54% of the sequences related to the genus UBA8309, but other species were also abundant (Fig. II.S5E, F), and the 5 most abundant phylotypes accounted for about 60% of the sequences (Fig. II.S5E, F).

Interannual variability of vitamin B₁₂ producers

We investigated the interannual variability of vitamin B₁₂ metabolisms in SOLA by focusing on the 22 347 non-rhythmic genes (PN_{max} < 10) involved in B₁₂ biosynthesis, which represented 71% of the sequences (Fig. II.S3A, Fig. II.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 (Fig. II.S6A). We hypothesized that non-rhythmic genes, which together produced an annual rhythmic KEGG (PN_{max} ≥ 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 (**Fig. II.S6B**). The phase of five of them (*cobY*, *cbiE*, *cbiT*, *cobF*, *isiB*) was like the one initially determined when summing all the genes, or when considering only the rhythmic genes (**Fig. II.6A**). For the other KEGGs (*bluB*, *cobG*, *cobA-btuR*), the phase varied depending on the type of genes summed (**Fig. II.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 (**Fig. II.6B**), excepting for *bluB* and *cobY*, for which lower PNmax values were obtained when summing the rhythmic genes.

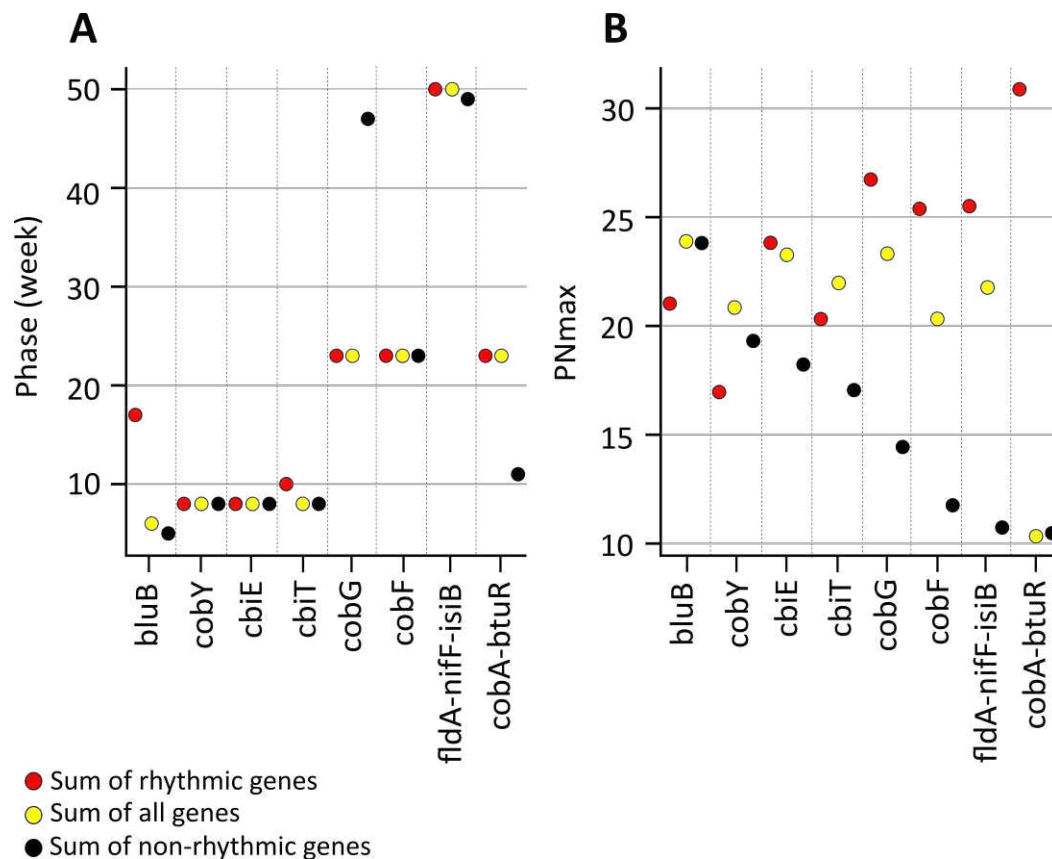
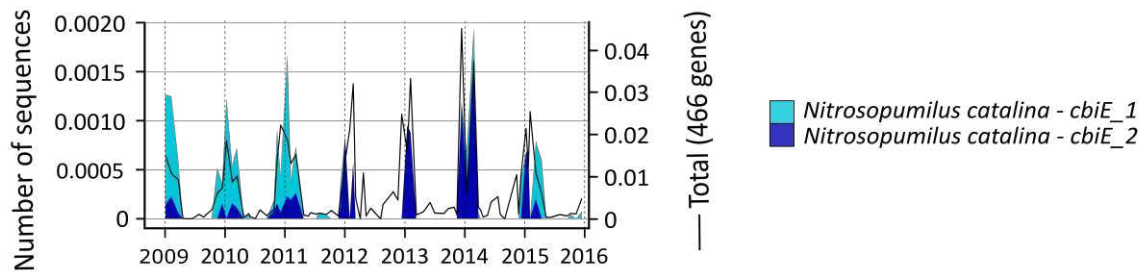


Figure II.6: (A) Week of the year during which maximal peak of abundance is observed (*i.e.*, phase) and (B) strength of rhythmicity (*i.e.*, PNmax) for the 8 KEGGs calculated after summing all gene abundances, only rhythmic genes abundances, or only non-rhythmic genes abundances. We summed together the abundances of all the genes annotated to a 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.

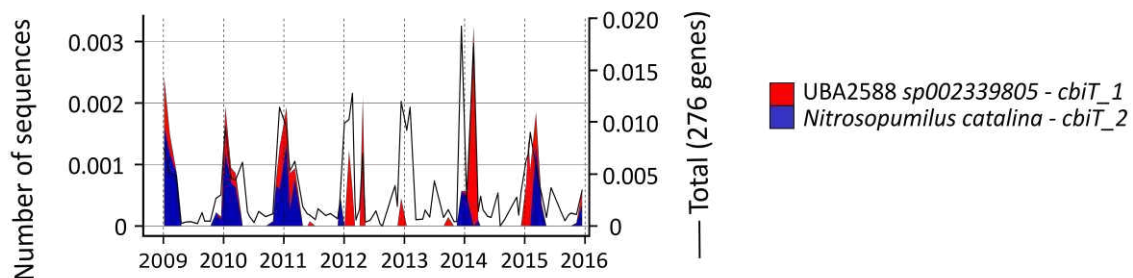
We also identified the non-rhythmic genes that were needed to get a rhythmic KEGG (*i.e.*, reaching the PNmax threshold of 10) (**Fig. II.7**, **Fig. II.S7**). Among the KEGGs involved

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 (**Fig. II.7A**).

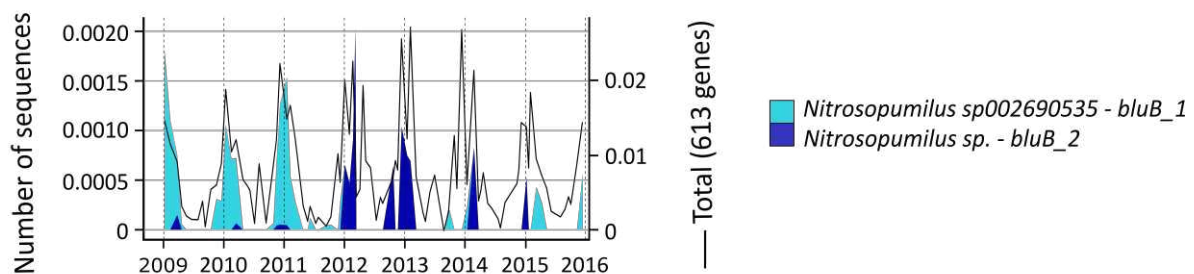
A *cbiE*



B *cbiT*



C *bluB*



D *cobY*

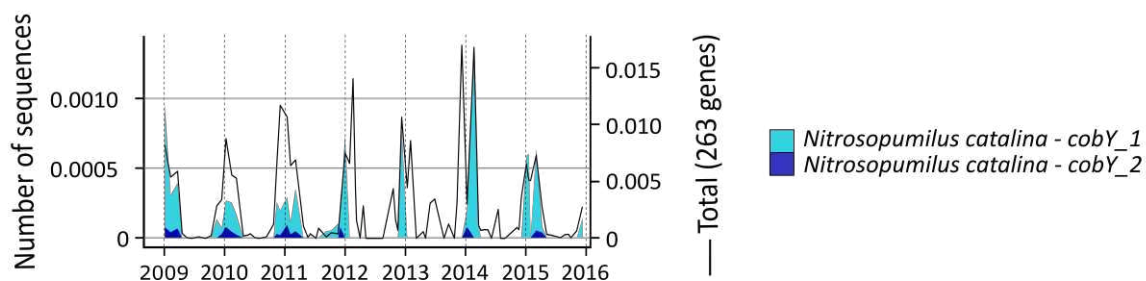


Figure II.7: Analysis of non-rhythmic genes associated to four vitamin B₁₂-biosynthesis marker KEGGs involved in the anaerobic pathway (**A:** *cbiE*, **B:** *cbiT*, **C:** *bluB*, **D:** *cobY*). Density plots show the relative abundance (number of sequences) of the first two abundant and non-rhythmic genes (PNmax < 10) sufficient to recover a rhythm at the KEGG level (PNmax ≥ 10). The black line shows the sum of all non-rhythmic genes of the respective KEGG.

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 (**Fig. II.7A**). For *cbiT*, the rhythm was recovered when *N. catalina* – *cbiE_2* was replaced by the bacteria UBA2588 *sp002339805* – *cbiT_1* (**Fig. II.7B**). For *bluB* and *cobY*, we also observed a switch between two *Nitrosopumilus* members, belonging to the same or to different species for *cobY* and *bluB*, respectively (**Fig. II.7C, D**).

For the KEGGs involved in the aerobic biosynthesis pathway (*i.e.*, *cobF* and *cobG*) and in salvage pathway (*i.e.*, *cobA-btuR*) in summer, the rhythmicity threshold was reached after summing more than 2 non rhythmic genes (**Fig. II.S7**). *CobF* and *cobG* recovered a rhythm when adding 3 and 8 most abundant non rhythmic genes, which represent 16 and 8% of the sequences, respectively (**Fig. II.S7A, B**). For both KEGGs, the genes involved in the recovered rhythm were all affiliated to the genus HIMB11 represented by 4 species: HIMB11 *sp002684375*; HIMB11 *sp001642945*; HIMB11 *sp003486095*, and HIMB11 *sp000472185* (**Fig. II.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*, *Planktomarina temperata*) and *Gammaproteobacteria* (*i.e.*, HTCC2207 *sp002469845*, HTCC2207 *sp002334915*) (**Fig. II.S7C**).

Discussion

Our long-term metagenomic time series revealed that potential vitamin B₁₂ 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 B₁₂ through the archaeal anaerobic pathway in winter, of pseudo-B₁₂ in spring and winter by cyanobacteria, and then, from spring to autumn, bacterial B₁₂ synthesis through the aerobic pathway (Fig. II.8A). This 7-year metagenomic study represents the first description of the succession of distinct B₁₂ metabolic pathways performed by phylogenetically distant organisms over the years.

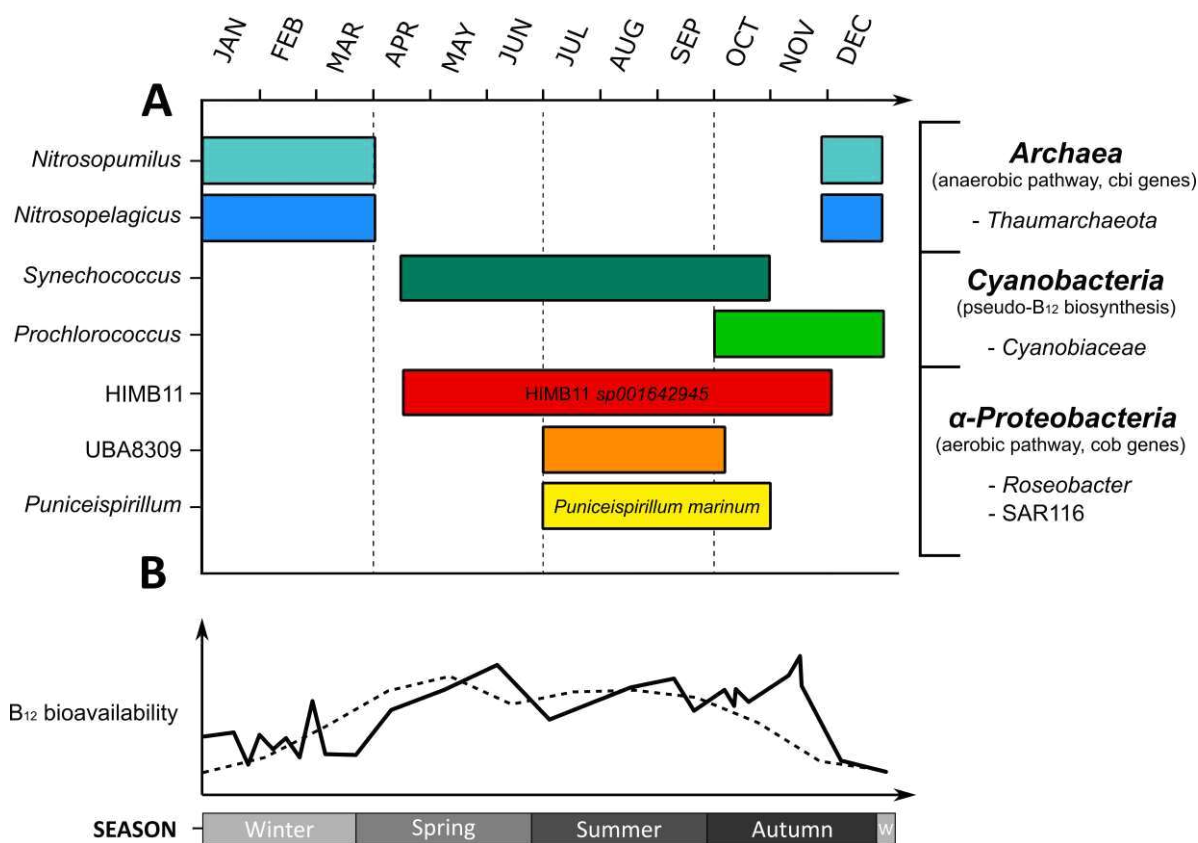


Figure II.8: General overview of the seasonality of vitamin B₁₂ and pseudo-B₁₂ potential producers and related pathways. (A) Seasonality of potential vitamin B₁₂ producers identified at the genus level and at the species level for HIMB11 and *Puniceispirillum* (the metabolic pathway used for B₁₂ biosynthesis is also mentioned). (B) Schematic overview of B₁₂ bioavailability in SOLA seawater over a year inferred from *Ostreococcus tauri* bioassay (solid line) and the inverse of *metE* relative abundances (dashed line) (Fig. II.2).

Archaea appeared to be the main potential B₁₂ producers in winter. They belonged to the phylum *Thaumarchaeota* (i.e., *Nitrosopumilus* and *Nitrosopelagicus* genera) and could use

the anaerobic pathway to produce B₁₂ through the genes *bluB*, *cbiE*, *cbiT*, and *cobY* (**Fig. II.8A**). Marine *Thaumarchaeota*, such as *Nitrosopumilus*, have been shown earlier to possess B₁₂ synthesis genes and were thus hypothesized to be a source of B₁₂ 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 to 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 B₁₂ production genes were up to 9 times more abundant than the bacterial ones in winter in the coastal NW- Mediterranean site of SOLA. *Thaumarchaeota*, that are more abundant in winter planktonic communities in temperate seas (Doxey et al., 2015; Galand et al., 2010; Lambert et al., 2019b; Pereira et al., 2021b), thus certainly play a seasonally important role in B₁₂ cycling in these areas. Remarkably, *Thaumarchaeota* were present at SOLA when B₁₂ had the lowest bioavailability, which could indicate that their activity was not sufficient to enrich the water with B₁₂. Phytoplankton cells could, however, directly use archaeal-derived B₁₂ for growth based on functional complementarity toward essential metabolite production and/or regeneration, *i.e.*, remineralization of inorganic nutrients and B₁₂ production versus algal production of carbon- or sulphur-rich organic matter and vitamin B₁ as previously reported for bacteria (Durham et al., 2014; Geng & Belas, 2010; Giovannoni, 2012; Luo & Moran, 2014). The high demand for B₁₂ by auxotrophic phytoplankton blooms (*Mamiellophyceae* and Dinoflagellates) observed in winter at SOLA could explain why B₁₂ bioavailability in water is lowest during this period (**Fig. II.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 B₁₂ 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-B₁₂ from spring to autumn, relayed by *Prochlorococcus* in winter (**Fig. II.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 their succession of these known pseudo-B₁₂ producers (Bonnet et al., 2010; Doxey et al., 2015; Helliwell et al., 2016) provides a continuous input of pseudo B₁₂ over several months. Pseudo-B₁₂ is, however, less bioavailable than B₁₂ to several microalgae (Heal et al., 2017; Helliwell et al., 2016). If DMB is provided, pseudo-B₁₂ could nevertheless be scavenged and/or remodeled 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-B₁₂ could be remodeled 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 B₁₂ via the aerobic pathway (*cobF*, *cobG*, *cobZ_tcuA*, *cobA*, *cobW*, and *cobK-cbiJ* genes) (**Fig. II.8A**). Co-occurrences between pseudo-B₁₂ producing cyanobacteria and other prokaryotic producers (*i.e.*, bacteria and archaea) suggest that pseudo-B₁₂ and B₁₂ 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 B₁₂ and pseudo-B₁₂ has been observed in the North Pacific subtropical gyre (Heal et al., 2017).

Seasonal bacterial B₁₂ production from spring to autumn was potentially performed by members of the genera HIMB11 (*Roseobacter* clade) and *Puniceispirillum* (SAR116 clade). HIMB11 had maximal abundances between May and November and could thus be an important player in the B₁₂ cycle at SOLA over several months. Most members of the marine *Roseobacter*

lineage have earlier been described as B₁₂ 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 B₁₂ production has not yet been highlighted. However, HIMB11 belongs to the Pelagic *Roseobacter* Cluster (PRC), whose members have the potential to produce B₁₂ and other vitamins like B₂ (riboflavin), and B₆ (pyridoxine), but lack genes for the synthesis of vitamins B₁ and B₇ (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 B₂, B₅ (pantothenate), B₆, B₉ (folate), and B₁₂ (Grote et al., 2011; Roda-Garcia et al., 2021). Co-culture approaches have earlier shown potential interactions based on B₁₂ 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 B₁₂ producing strains of *Roseobacter* (Luo & Moran, 2014; Newton et al., 2010), and they may be important traits for B₁₂-based microbial symbiosis between auxotrophic microalgae and B₁₂ 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 B₁₂ producers (e.g., *Thaumarchaeota*, *Rhodobacteraceae*, *Puniceispirillaceae*), and auxotrophic microalgae (e.g., *Micromonas*, *Bathycoccus*, *Ostreococcus*) (Joglar et al., 2021; Krabberød et al., 2022; Lambert et al., 2021b). A winter interaction hub associated the picoeukaryote *Bathycoccus* with a potential B₁₂ producer belonging to *Rhodobacteraceae*, in addition to archaea, which also interacts with other B₁₂ 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 B₁₂ for phytoplankton blooms. These B₁₂ 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, B₁₂-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 in fact 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 interannual

environmental conditions (Awasthi et al., 2014). Similarly, in the human gut microbiome or in the soil, functional redundancy between specific taxa maintains stability under disturbances (Y. 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, 1999b) will be lost and the functioning of the ecosystem will be impaired.

Conclusion

Our monthly 7 years metagenomic time series at the SOLA station uncovered a seasonal succession of distinct prokaryotic producers that use different biosynthesis pathways to produce vitamin B₁₂ *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 B₁₂-limited. The fact that some vitamin B₁₂ producers were replaced by others carrying the same functions during contrasted years indicates the existence of functional redundancy at a gene-level in several B₁₂-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.

List of abbreviations

B₁₂: cobalamin (vitamin B₁₂)

pseudo-B₁₂: Pseudocobalamin (pseudo-vitamin B₁₂)

DMB: 5,6-Dimethylbenzimidazole

LSP: Lomb Scargle periodogram

nMDS: non-metric Multi-Dimensional Scaling

NW Mediterranean Sea: North-Western Mediterranean Sea

PNmax: Peak Normalized power

SOLA: Service d’Observation du Laboratoire Arago

Author Contributions: MB, FYB and PEG designed the experiments and analysis. PS performed the B₁₂ bioassays. LM and RL performed the bioinformatic analysis of the metagenomic data. MB analyzed data and realized figures/tables. MB, FYB and PEG wrote the manuscript.

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Supplementary figures and table

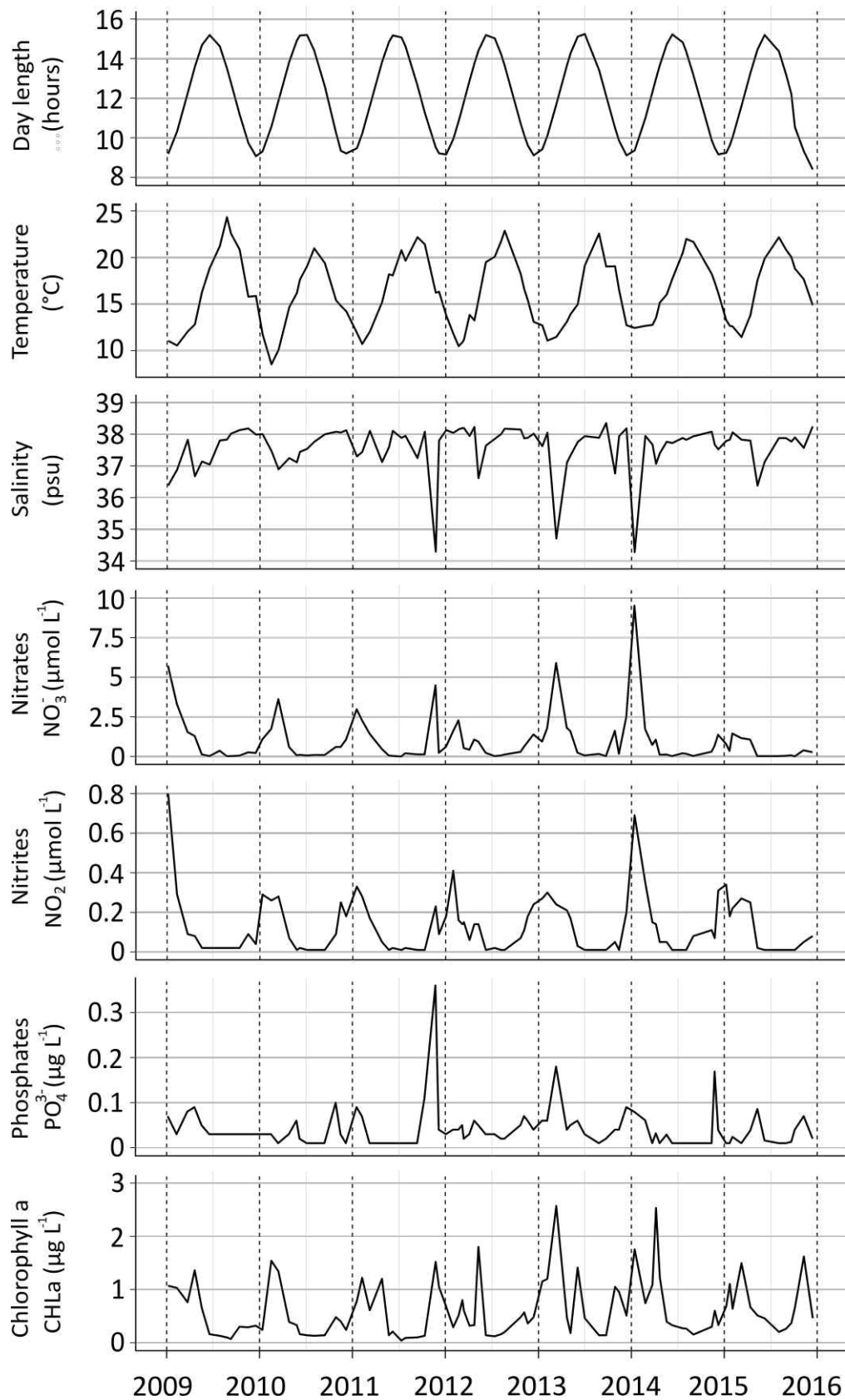


Figure II.S1: Environmental parameters measured in surface seawaters (3-meters depth) at SOLA between January 2009 and December 2015.

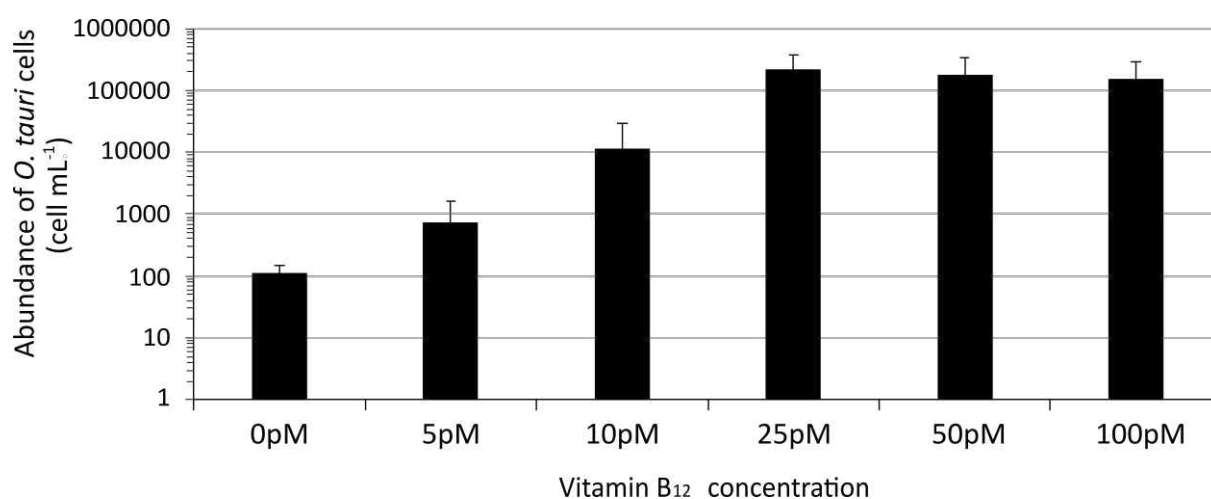


Figure II.S2: Cell abundances of *Ostreococcus tauri* RCC745 (cell.mL⁻¹ ± SD, n=3) after 6 days of incubation in fresh ASW supplemented with constant B₁ (1 nM) and increasing B₁₂ concentrations (between 0 and 100 pM). Deep well microplates were inoculated at 5000 cells.mL⁻¹.

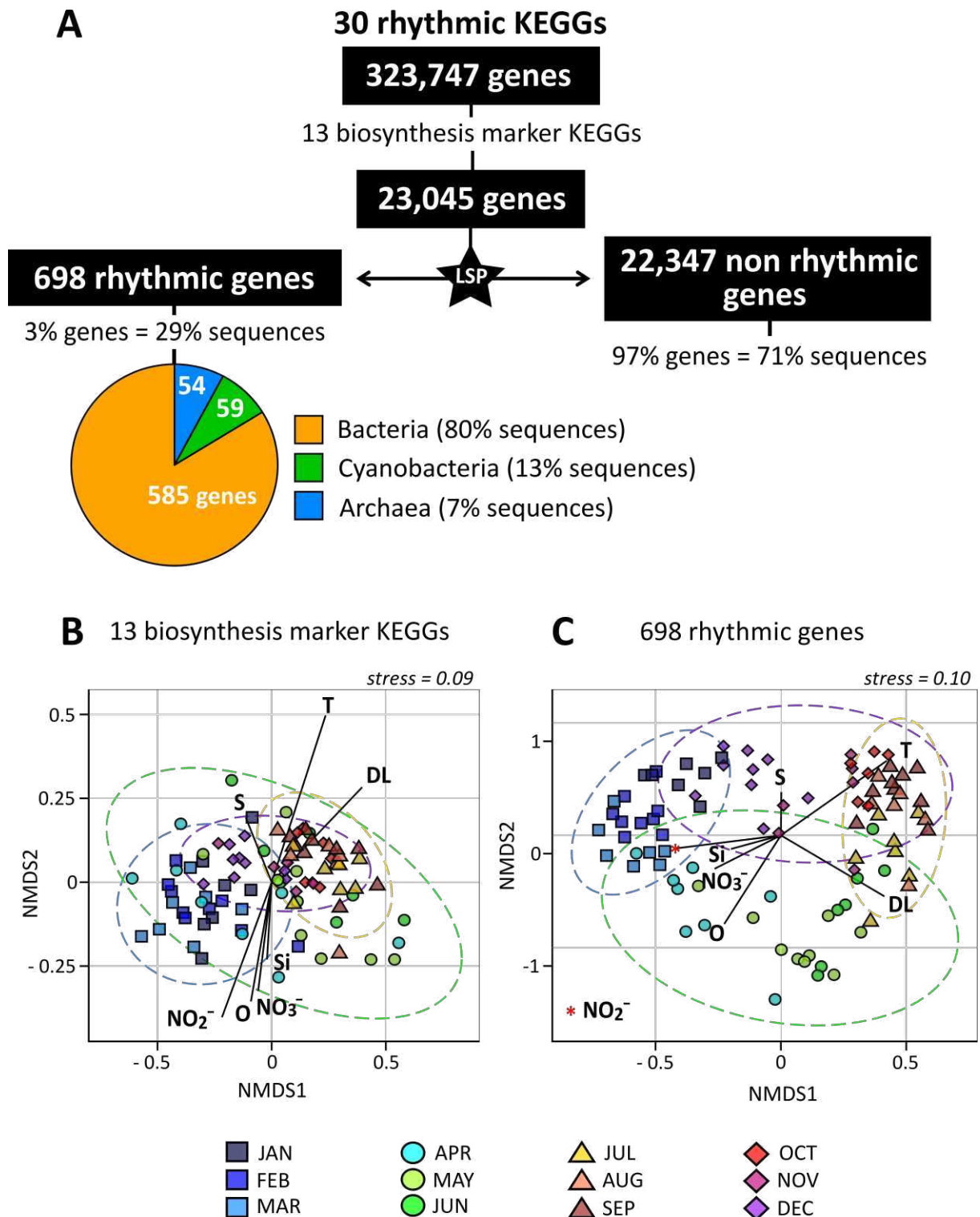


Figure II.S3: Analysis of the rhythmic KEGGs related to potential B₁₂ metabolisms and their associated genes. **(A)** Schematic of the analysis resulting in the selection of 698 rhythmic genes and their taxonomic composition. **(B)** NMDS analysis of the 13 rhythmic KEGGs related to B₁₂ biosynthesis and **(C)** of the associated 698 rhythmic genes. Covariance ellipses group the samples by seasons: winter (blue), spring (green), summer (red) and autumn (purple). Environmental variables that significantly impact sample's structure are fitted onto the nMDS: T: temperature; DL: day length; S: salinity; O: dissolved oxygen; Si: silicate (Si(OH)₄); NO₂⁻: nitrite; NO₃⁻: nitrate.

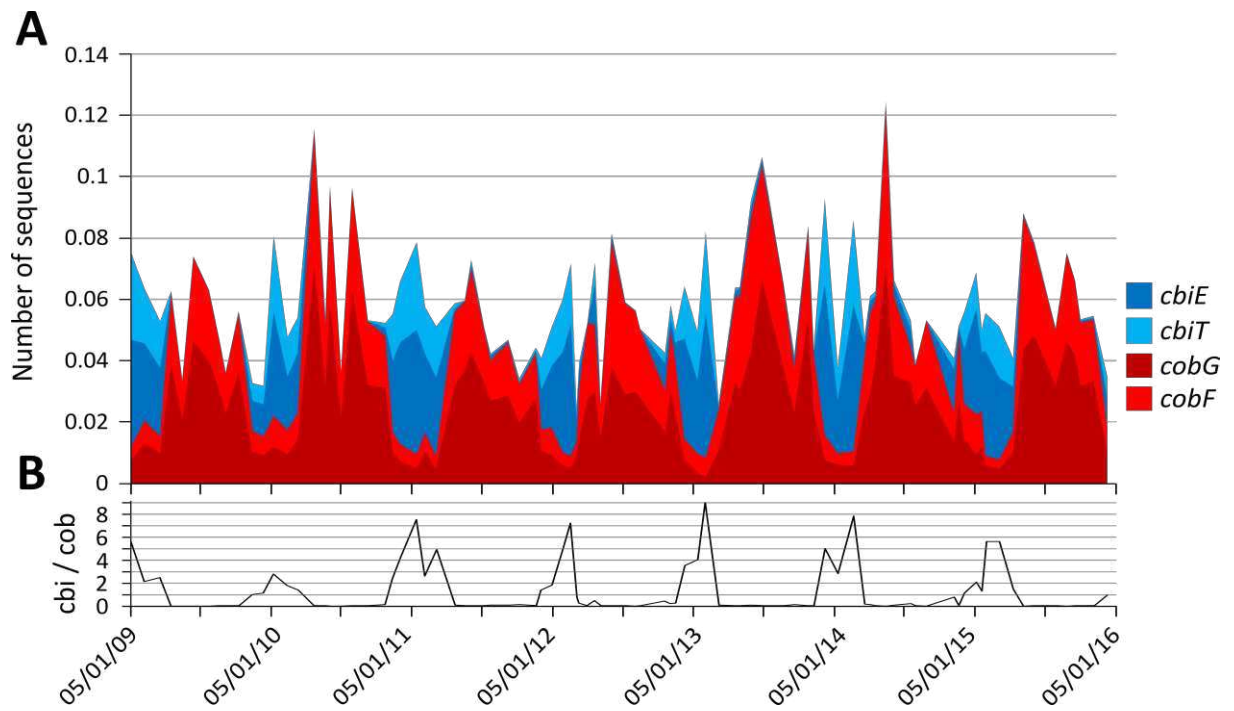


Figure II.S4: Bacterial and archaeal sequences potentially involved in vitamin B₁₂ production at SOLA in winter. **(A)** Number of sequences associated to *cobF* and *cobG* (aerobic biosynthetic pathway, red), or associated to *cbiE* and *cbiT* (anaerobic biosynthetic pathway, blue). **(B)** Ratios of archaeal *cbi* (*cbiE* + *cbiT*) to bacterial *cob* (*cobF* + *cobG*) sequence numbers between 2009 and 2015.

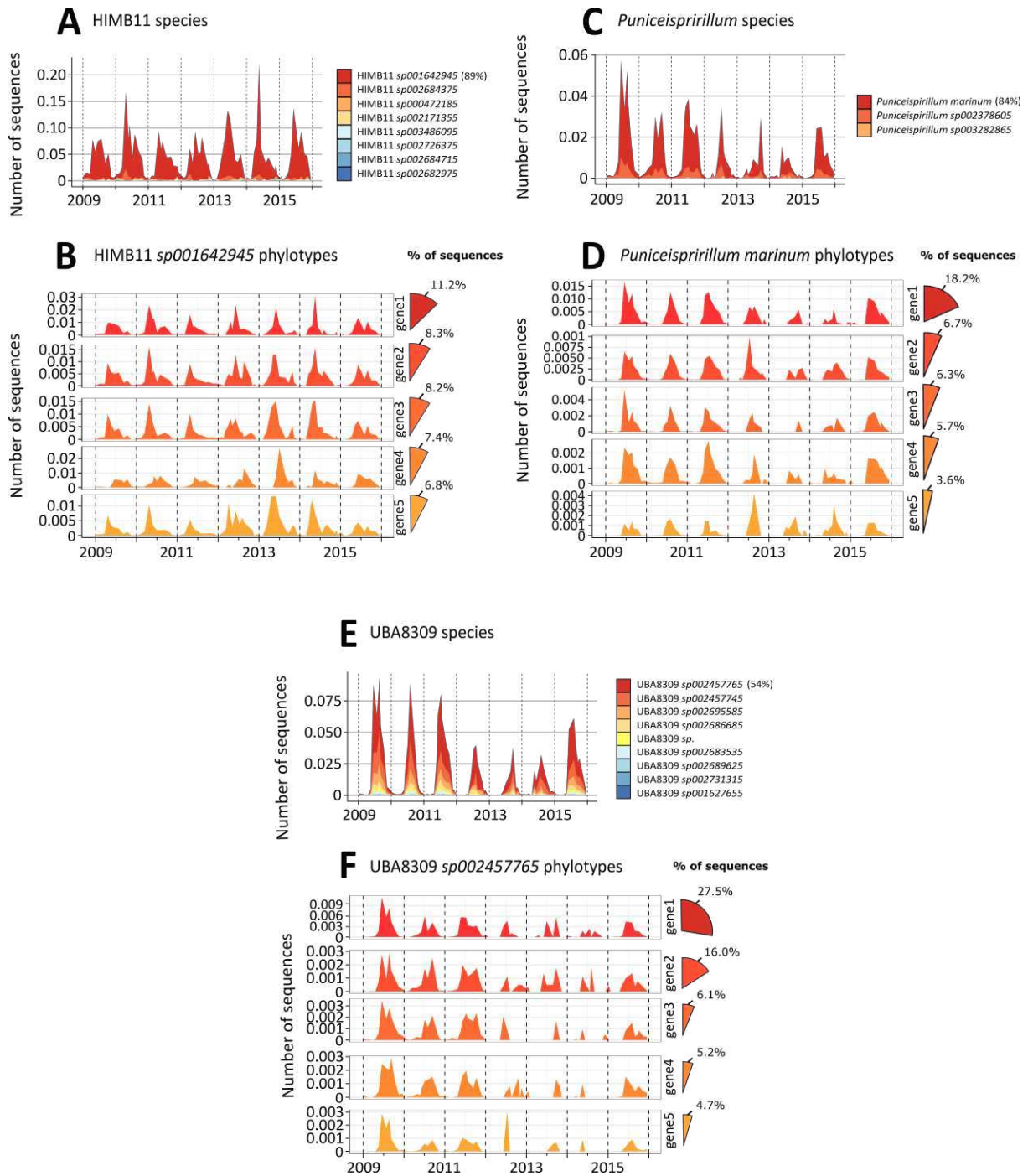


Figure II.S5: Seasonal abundance of the main bacteria potentially involved in vitamin B₁₂ production. HIMB11 (A – B); *Puniceispirillum* (C – D); UBA8309 (E – F) at the species (A, C, E) and unique gene levels (B, D, F). The proportion of sequences represented by the dominant species within each genus is shown in brackets, while the proportion of sequences represented by the 5 dominant genes within their respective species (*i.e.*, phylotypes) is shown in the pie charts in front of density plots.

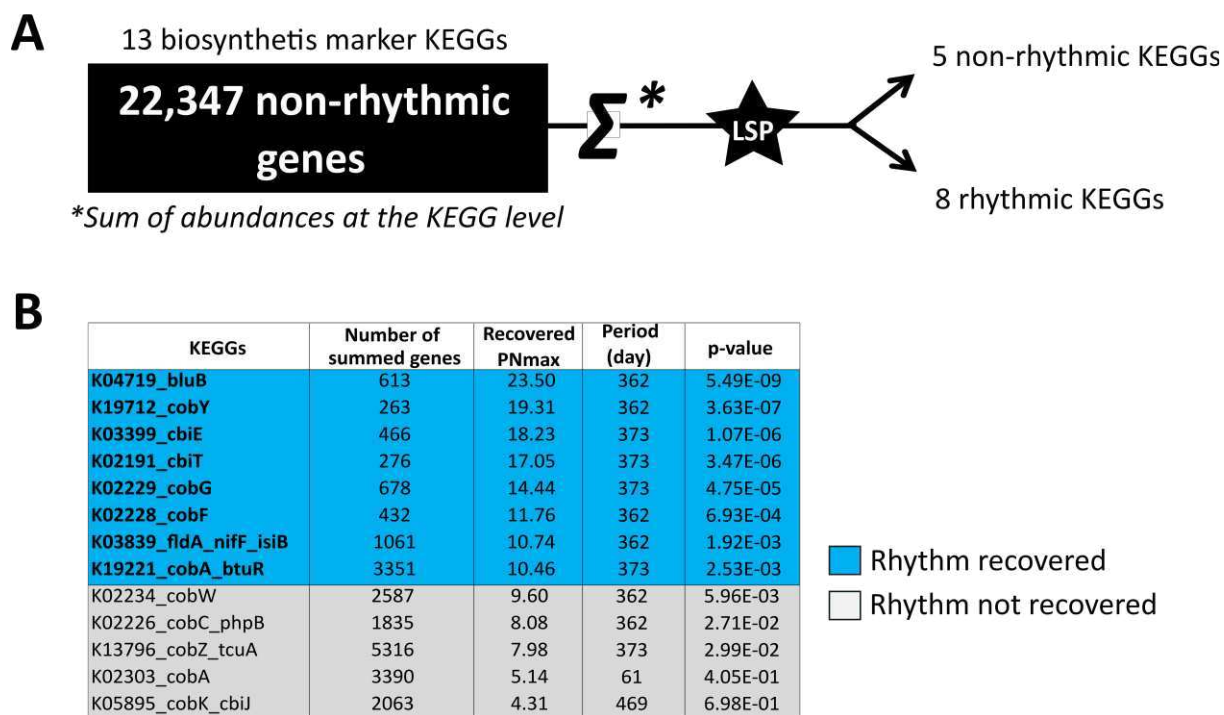
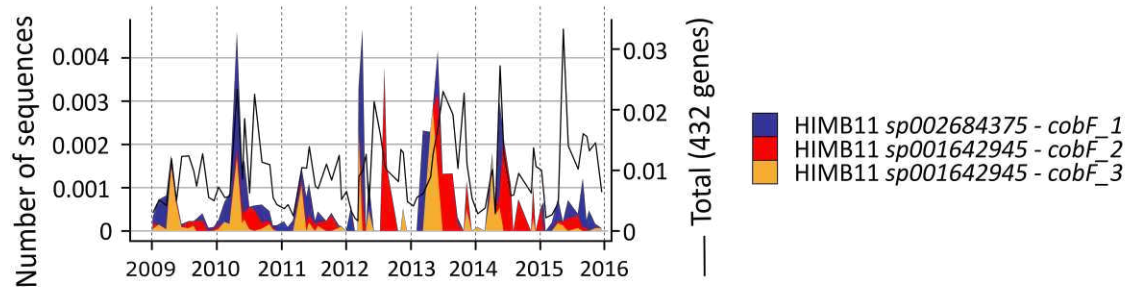
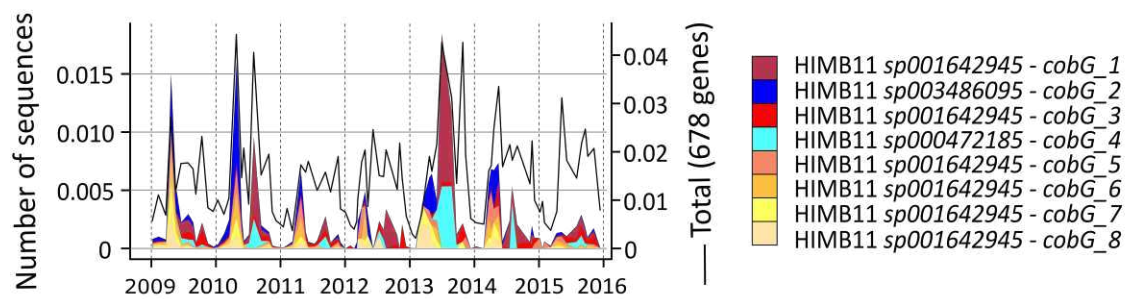


Figure II.S6: Analysis of non-rhythmic genes associated to vitamin B₁₂ biosynthesis. **(A)** Schematic overview of the analysis performed on the non-rhythmic genes related to our 13 markers. **(B)** Summary of the results from the analysis of the non-rhythmic genes (PNmax < 10). The number of genes summed for each KEGG and the PNmax they recover are shown. The period (days) and the significance level of the rhythmicity (p-value) are also indicated for KEGGs which recovered rhythm (blue) (PNmax ≥ 10) and also for those which remains non-rhythmic at the KEGG level (PNmax < 10).

A *cobF*



B *cobG*



C *cobA-btuR*

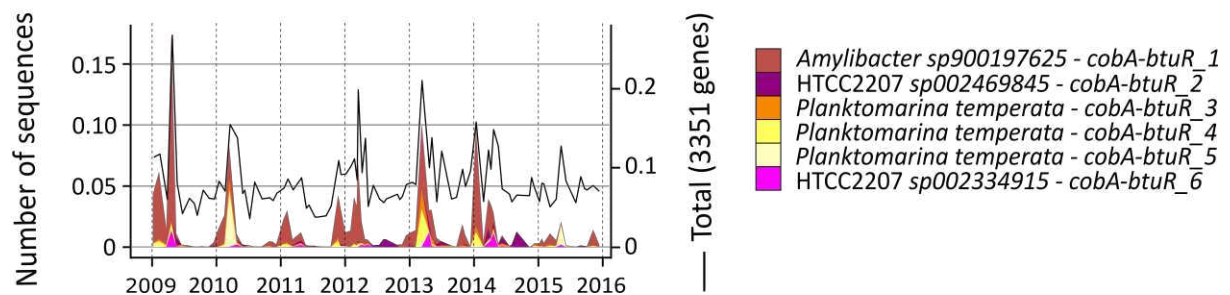


Figure II.S7: Analysis of non-rhythmic genes associated to three vitamin B₁₂-biosynthesis marker KEGGs involved in the aerobic pathway (**A**: *cobF*, **B**: *cobG*) or in the salvage pathway (**C**: *cobA-btuR*). Density plots shows the relative abundance (number of sequences) of the most abundant and non-rhythmic genes sufficient to recover a rhythm at the KEGG level. The black line shows the sum of all non-rhythmic genes of the respective KEGG.

Table II.S1: Table representing the 82 KEGGs related to vitamin B₁₂ metabolisms found at SOLA. Rhythmicity statistics are shown: rhythmicity index (PNmax), period (in days), phase (week / month) and the significance level (pvalue). KEGGs in bold are rhythmic according to LSP analysis (Lomb-Scargle Periodogram; PNmax ≥ 10). Asterisk show the KEGGs used as markers for key vitamin B12-related metabolisms. The catalog of genes associated with the B12-related KEGGs is accessible in the Zenodo open repository at <https://doi.org/10.5281/zenodo.7409518>, deposited online the 8 December 2022. The raw sequences were deposited in the NCBI Sequence Read Archive under accession number [PRJEB66489](https://doi.org/10.5281/zenodo.7409518).

Pathways	KO number	Functional gene	PNmax	Period (days)	Phase		p-values
					Week	Month	
AEROBIC COBALAMIN BIOSYNTHESIS	K02229*	cobG	23.32	373	23	JUN	6.57E-09
	K02228*	cobF	20.33	362	23	JUN	1.31E-07
	K13796*	cobZ-tcuA	17.33	362	23	JUN	2.62E-06
	K05895*	cobK-cbiJ	15.36	362	35	AUG	1.88E-05
	K02303*	cobA	11.59	362	17	APR	8.22E-04
	K00595	cobL	7.83	362	23	JUN	3.47E-02
	K02230	cobN	7.66	362	23	JUN	4.07E-02
ANAEROBIC COBALAMIN BIOSYNTHESIS	K13540	cobU	3.57	98	51	DEC	9.20E-01
	K03999*	cbiE	23.27	362	8	FEB	6.91E-09
	K02191*	cbiT	21.98	362	8	FEB	2.53E-08
	K02302	cysG	12.40	362	17	APR	3.63E-04
	K02189	cbiG	7.00	178	8	FEB	7.76E-02
	K16651	pduX	5.32	396	23	JUN	3.53E-01
	K02188	cbiD	3.97	437	17	APR	8.15E-01
COBALAMIN BIOSYNTHESIS	K02190	cbiK	3.52	95	23	JUN	9.30E-01
	K04719*	bluB	23.89	362	6	FEB	3.73E-09
	K02234*	cobW	17.26	362	31	AUG	2.83E-06
	K09882	cobS	9.74	373	39	SEP	5.22E-03
	K02231	cobP-cobU	8.35	362	23	JUN	2.07E-02
	K02224	cobB-cbiA	7.50	2534	50	DEC	4.79E-02
	K05936	cobM-cbiF	7.33	181	31	AUG	5.61E-02
	K09883	cobT	7.17	362	37	SEP	6.56E-02
	K00768	cobU-cobT	7.08	362	17	APR	7.17E-02
	K05934	cobJ-cbiH	7.07	362	23	JUN	7.26E-02
	K13541	cbiGH-cobJ	6.30	453	17	APR	1.49E-01
	K06042	cobH-cobI	4.70	453	17	APR	5.53E-01
	K02232	cobQ-cbiP	3.99	453	23	JUN	8.09E-01
	K02233	cobS-cobV	3.65	453	31	AUG	9.04E-01
	K02227	cbiB-cobD	3.61	116	34	AUG	9.12E-01
	ARCHAEAL COBALAMIN BIOSYNTHESIS	K13786	cobR	3.23	352	47	NOV
K03394		cobI-cbiL	2.86	453	23	JUN	9.94E-01
K19712*		cobY	20.85	362	8	FEB	7.82E-08
PSEUDOCOBALAMIN BIOSYNTHESIS	K03795	cbiX	3.80	155	47	NOV	8.64E-01
	K08260	cbiZ	2.01	64	1	JAN	1.00E+00
PSEUDOCOBALAMIN REMODELING/ COBALAMIN BIOSYNTHESIS	K03839*	fldA-niff-isiB	21.77	362	50	DEC	3.11E-08
SALVAGE PATHWAY / TRANSPORTERS	K02226*	cobC-phpB	20.92	362	31	AUG	7.27E-08
	K02225	cobC1-cobC	4.70	59	11	MAR	5.54E-01
METAL IONS TRANSPORT	K19221*	cobA-btuR	10.24	373	23	JUN	3.17E-03
	K16092	btuB	8.70	373	19	MAY	1.47E-02
	K06073	ABC-VB12P-btuC	4.56	79	17	APR	6.05E-01
	K06074	ABC-VB12,A-btuD	5.20	259	19	MAY	3.87E-01
	K06858	ABC-VB12,S1-btuF	3.54	58	47	NOV	9.27E-01
PORPHYRIN & CHLOROPHYLL METABOLISM (PRECURSORS BIOSYNTHESIS)	K00798	MMAB-pduO	6.23	1810	2	JAN	1.59E-01
METHIONINE SYNTHESIS	K02008	cbiQ	16.01	373	47	NOV	9.90E-06
	K02006	cbiO	14.04	373	47	NOV	7.09E-05
	K02007	cbiM	10.01	373	47	NOV	3.99E-03
	K02009	cbiN	2.24	133	51	DEC	1.00E+00
	K01749	hemC-HMBS	32.24	362	37	SEP	8.77E-13
	K01599	hemE-UROD	28.62	373	31	AUG	3.28E-11
	K01845	hemL	19.82	362	49	DEC	2.19E-07
	K01719	hemD-UROS	16.84	373	17	APR	4.29E-06
	K01698	hemB-ALAD	10.93	373	17	APR	1.58E-03
	K01885	gltX	10.14	2112	17	APR	3.49E-03
B12 DEPENDANT ENZYMES	K02492	hemA	6.43	352	17	APR	1.34E-01
	K13542	cobA-hemD	5.13	123	46	NOV	4.08E-01
	K13543	hemDX	3.24	89	47	NOV	9.71E-01
	K00549	metE	26.92	373	49	DEC	1.81E-10
	K00548	metH-MTR	10.03	362	17	APR	3.90E-03
	K01848	mcmA1	32.13	373	50	DEC	9.83E-13
	K01849	mcmA2	29.16	362	5	FEB	1.92E-11
	K12573	rnv-vacB	13.81	362	17	APR	8.87E-05
	K01699	pduC	13.64	362	17	APR	1.05E-04
	K01847	MUT	12.10	362	23	JUN	4.92E-04
	K13920	pduE	9.92	373	47	NOV	4.33E-03
	K00524	nrdJ	8.56	362	41	OCT	1.68E-02
	K03647	nrdI	7.93	373	47	NOV	3.14E-02
	K20594	genK-gacD-gntK	7.81	373	21	MAY	3.53E-02
B12 DEPENDANT ENZYMES	K04835	mal	5.37	57	51	DEC	3.39E-01
	K01846	glmS-mutS-mamA	4.62	373	31	AUG	5.82E-01
	K19268	glmE-mutE-mamB	4.10	1810	6	FEB	7.72E-01
	K03736	eutC	3.71	905	6	FEB	8.87E-01
	K01844	kamD	3.59	745	17	APR	9.16E-01
	K03735	eutB	3.45	71	50	DEC	9.43E-01
	K05368	fre-ubiB	3.06	845	47	NOV	9.86E-01
	K13919	pduD	3.99	396	46	NOV	8.09E-01
	K04032	eutT	2.28	342	37	SEP	1.00E+00
	K18011	kamE	3.40	70	49	DEC	9.51E-01
	K17898	oraE	3.11	116	35	AUG	9.82E-01
	K06122	dhbE	4.33	362	49	DEC	6.90E-01
	K06120	dhaB	1.61	218	1	JAN	1.00E+00
	K20595	genQ-gaci-gntX	2.28	576	1	JAN	1.00E+00
	K17899	oraS	3.10	83	1	JAN	9.83E-01

K000XX : B1-related KEGG | K000XX : rhythmic KEGG | *K000XX : rhythmic marker KEGG

III. **CHAPTER 3: Metabolic interdependencies between B-vitamin producers and auxotrophs in seasonal microbial communities**

The environment shapes marine microbial communities but biotic interactions are also a strong community structuring factor in the ocean. Marine microbial communities are dynamic and complex assemblages of co-occurring organisms which interact and exchange essential but scarce cofactors, such as B-vitamins. Most bacterioplankton partially or totally lack the vitamin B₁ and B₁₂ biosynthesis pathways and require an exogenous source of vitamins or precursors to grow (*i.e.*, auxotrophs). The widespread vitamin scarcity and the prevalence of auxotrophs in the ocean suggest that biotic interactions are the keystone of vitamin exchanges. In the **Chapter II**, we showed a seasonal succession of bacterial and archaeal vitamin B₁₂ producers in summer and winter, respectively. Here we study the seasonality of metabolisms related to vitamin B₁ and his related precursors (*i.e.*, cHET/HMP), as well as the interplay between B₁ and B₁₂ producers and auxotrophs over time. Through the analysis of the 7-year metagenomic time series conducted at the SOLA station, we identified **(1)** a higher contribution of seasonal B₁ producers and dual auxotrophs than previously estimated in other regions and **(2)** a strong seasonality of taxa relying on exogenous B₁ precursors in summer, dominated by HMP auxotrophs. Bioassays experiments unveiled multiple vitamin B₁ and B₁ precursors limitation periods in winter, as observed for vitamin B₁₂. Co-occurrence networks analysis revealed strong and recurrent co-occurrences between vitamin auxotrophs and producers which could complement each other vitamin or precursor requirements. These metabolic complementarities exhibited distinct patterns depending on the season, suggesting different potential interactions. The functional analysis of microcosms experiments showed different impacts of vitamin and precursor additions on prokaryotic community composition between winter and spring, confirming that metabolic interplay varies over the year, and that marine microorganisms could be interdependent for their vitamin and precursors needs.

Article 2: Functional complementarity between vitamin B₁ and B₁₂ metabolisms in marine microbial community

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Data availability statement: The raw sequences were deposited in the NCBI Sequence Read Archive under the accession numbers [PRJEB66489](#) and [PRJEB26919](#).

Conflict of interest disclosure: The authors declare that they have no conflict of interest.

Keywords: Vitamin B₁ and B₁₂, Metagenomic time series, Functional complementarity, Metabolic interdependencies

Abstract

The environment shapes marine microbial assemblages that impact biogeochemical cycles, but biotic interactions also drive community diversity and metabolisms through the exchange of rare but essential metabolites, such as B-vitamins. Vitamin B₁₂ and vitamin B₁ are scarce in ocean, but many microbial planktons including most eukaryotes require them to grow, although they can't produce them *de-novo* (*i.e.*, auxotrophs). Vitamin B₁₂ availability and production pathways are known to vary seasonally, but vitamin B₁ temporal variations remains poorly known. In addition, the seasonality of the potential complementarity between vitamin B₁ and B₁₂ auxotrophs and producers (*i.e.*, prototrophs), on which they depend, is not known. Here, we assess the seasonal dynamics and the diversity of B₁-related prokaryotic communities in a Mediterranean coastal site over 7-years and study their co-occurrences with B₁₂-related prokaryotic communities. Our unique metagenomic time series shows that B₁ production and consumption are seasonal at SOLA, and that B₁ prototrophs and dual cHET/HMP auxotrophs were much more abundant within SOLA communities than previously estimated in other oceanic regions. We demonstrate in microcosm experiments that vitamin B₁, and B₁ precursors, impacted community composition, and that different ASVs were selectively promoted by vitamin or precursor additions depending on the time of the year. Finally, potential functional complementarities for vitamin B₁, precursors and/or vitamin B₁₂ requirements were highlighted between co-occurring microorganisms. These putative metabolic interdependencies showed distinct potential interaction patterns involving different organisms during summer and winter seasons. This first long-term analysis of B₁- and B₁₂-related genes over a 7-year metagenomic time series provide novel insights into vitamin auxotrophy-induced interactions and their occurrences over time in coastal temperate waters of the Mediterranean Sea.

Introduction

Marine microbial communities are composed of a tremendous diversity of organisms that define the physicochemical properties of the environment, influence nutrient and biogeochemical cycling, and biomass production at the base of the marine food web (Falkowski et al., 2008; Sunagawa et al., 2015). They are linked through complex metabolic networks (Dart et al., 2023; Fuhrman et al., 2015; Krabberød et al., 2022; Lambert et al., 2021; Lima-Mendez et al., 2015; Parada & Fuhrman, 2017) and biotic factors are important drivers of community composition and dynamics (Ahlgren et al., 2019; Arandia-Gorostidi et al., 2022; Auladell et al., 2022; Bolaños et al., 2021; Lambert et al., 2019a; Lima-Mendez et al., 2015). Among biotic relationships that imply microscale interactions (symbiosis or cross-feeding), B-vitamins exchanges are of particular interest in marine microbial ecology. Vitamin B₁ (thiamin) and B₁₂ (cobalamin) are key enzymatic cofactors involved in the central metabolism of most marine planktons, including Krebs cycle (B₁, B₁₂), pentose phosphate pathway (B₁), and methionine synthesis (B₁₂) (Helliwell, 2017; Monteverde et al., 2017). These vitamins are also major growth regulators of photosynthetic planktonic communities, as shown experimentally with vitamin-supplemented cultures (Bertrand et al., 2007; Koch et al., 2011, 2013; Paerl et al., 2017; Paerl, Sundh, et al., 2018; Panzeca et al., 2006; Sañudo-Wilhelmy et al., 2006; Tang et al., 2010), or *via* co-culture experiments (Cooper et al., 2019; Croft et al., 2005, 2006; Durham et al., 2015, 2017; Grant et al., 2014; Kazamia et al., 2012). However, global oceanographic surveys have revealed large B₁ and B₁₂-depleted areas with low picomolar concentrations changing with longitude, latitude, depth, and seasons (Barber-Lluch et al., 2021; Bonnet et al., 2013; Fiala, 1982; Heal et al., 2017; Joglar et al., 2021; Koch et al., 2012; Sañudo-Wilhelmy et al., 2012; Suffridge et al., 2018, 2020). In the Mediterranean Sea, B₁ precursors and B₁₂ isoforms also vary over time and space (Suffridge et al., 2018). Omics and culture-based studies have highlighted that B₁ and B₁₂ auxotrophy (*i.e.*, obligatory requirement of an exogenous

source of vitamin) is widespread within many phytoplankton (Croft et al., 2005; Sañudo-Wilhelmy et al., 2014) and prokaryotes (Gómez-Consarnau et al., 2018; Sañudo-Wilhelmy et al., 2014; Shelton et al., 2019), and could be randomly distributed across taxonomic levels (Helliwell et al., 2013; Tang et al., 2010). These studies also showed that most eukaryotic and prokaryotic planktons lack at least one B-vitamin biosynthesis pathway (Gómez-Consarnau et al., 2018; Sañudo-Wilhelmy et al., 2014; Zoccarato et al., 2022). The scarcity of vitamin B₁ and B₁₂ in the ocean, coupled to the prevalence of B-vitamin auxotrophy among marine microbes, raises the question of which are the main producers of B-vitamins in the ocean.

Auxotrophs could depend on obligatory interactions with neighboring microbes producing the vitamins or precursor that they need (*i.e.*, auxotrophy-induced interactions) (Johnson et al., 2020), and these organisms may in turn depend on molecules that they cannot produce. Interdependencies may therefore be an important mechanism shaping microbial community composition and dynamics. Such microbial interactions involving B₁ and pantothenate have recently been identified in bioreactor experiments (Hessler et al., 2023). Even though several cocultivation experiments have highlighted metabolic exchanges between bacteria and phytoplanktonic cells involving exchanges of B-vitamins and growth factors, the existence of such mutualistic interdependencies in marine microbial ecosystems remains unknown, as does the impact of these ecological associations on the composition of marine microbial assemblages (Amin et al., 2015; Cooper et al., 2019; Romine et al., 2017).

In the coastal Mediterranean, vitamin B₁₂ metabolism is characterized by an annual succession of different organisms using distinct pathways to produce B₁₂, associated to a variable B₁₂ bioavailability in seawater (Beauvais et al., 2023). Thus, different suppliers adapted to different seasons provide vitamins to auxotrophs. For vitamin B₁, there is little information to date, but the few studies on the topic indicate that auxotrophy is prevalent and widespread (Paerl, Sundh, et al., 2018). Auxotrophs require an exogenous source of vitamin B₁ (*i.e.*, intact

auxotroph), or pyrimidine-related precursors (HMP) (*i.e.*, HMP auxotroph), thiazole-related precursors (cHET/HET) (*i.e.*, cHET auxotroph) or both precursors (*i.e.*, dual auxotroph) (Paerl, Sundh, et al., 2018). Abundant marine bacterial lineages such as SAR86 and SAR11, require an exogenous source of HMP to complete B₁ synthesis (Carini et al., 2014; Dupont et al., 2011; Sañudo-Wilhelmy et al., 2014). In the Baltic Sea, HMP-auxotrophs and intact B₁-auxotrophs dominated bacterial metagenomes and coexisted with dual auxotrophs, while the only B₁ producers were rare and belonged to *Verrucomicrobia* (Paerl, Sundh, et al., 2018). In the Sargasso Sea as well as in most open ocean stations from Tara Oceans, most bacteria lacked the gene *thiC* (*i.e.*, HMP auxotrophs) (Carini et al., 2014; Paerl, Sundh, et al., 2018; Tully, Graham, et al., 2017). Weekly and monthly changes in B₁-related genes abundances were also identified in the Baltic Sea, highlighting that exogenous B₁ and precursors bioavailability could be dynamic over short timescales (Paerl, Sundh, et al., 2018), as observed earlier for HMP (Carini et al., 2014). The widespread presence of auxotrophs in the ocean therefore raises the question of the origin of vitamin B₁. It could be produced by different organisms that become seasonally abundant during short periods of time, but no study has yet looked at the repartition and the long-term dynamics of B₁ metabolisms in oceans.

The first objective of this study was to identify the long-term seasonal dynamics of vitamin B₁ metabolic pathways, including precursors, and to identify associated microbial taxa. The second goal was to detect potential vitamin B₁ and B₁₂ interdependencies among marine microbes and to test how vitamin and their precursors influence community composition. To do this, we combined the analysis of a 7-year monthly time series of metagenomics data from the north-western Mediterranean coastal sea (SOLA station) with bioassay based on model organisms, and microcosm amendment experiments for vitamin B₁₂/B₁ and their precursors.

Methods

Seawater sampling, and DNA extraction and sequencing

Seawater samples were collected monthly at 3m depth at the Banyuls Bay microbial observatory (SOLA station; 42°31'N, 03°11'E; Northwestern Mediterranean Sea, France) from January 2009 to December 2015 (7 years). Sampling protocol, seawater filtration, DNA extraction and shotgun-sequencing methods have been described elsewhere (Hugoni et al., 2013; Lambert et al., 2019a)(Beauvais et al., 2023). The raw sequences were deposited in the NCBI Sequence Read Archive under accession numbers [PRJEB66489](#) and [PRJEB26919](#).

Functional and taxonomic identification of prokaryotic sequences

The methods used for the generation of the abundance table related to our metagenomic timeseries was described previously (Beauvais et al., 2023). Briefly, metagenomic reads were trimmed with CUTADAPT v1.16 (Martin, 2011) and 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) and 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 using BWA (Burrows-Wheeler Alignment tool) (H. Li & Durbin, 2009), and the number of counts per gene were calculated with HTSeq (Anders et al., 2015) and subsequently normalized within samples by gene length, and among samples by a set of single-copy core gene (Salazar et al., 2019). The taxonomic affiliations of functional genes were done against the Genome Taxonomy Database (GTDB) considering the last common ancestor (LCA).

We carried out exhaustive literature research of key markers involved in B₁ and B₁₂ metabolisms and associated pathways (Balabanova et al., 2021; Fang et al., 2017; Helliwell, 2017; Jurgenson et al., 2009; Maupin-Furlow, 2018; Zhou et al., 2021) (**Table III.S1**).

Co-assembly & reconstruction of SOLA Metagenome assembled genomes (MAGs)

We aimed at co-assembling groups of metagenomes that had comparable information content. To define those groups, we determine pairwise metagenome similarity using *simka* v1.5.2 (Benoit et al., 2016), with a k-mer size = 21, minimum shannon-index = 1.5, minimum read size = 70, and Bray Curtis dissimilarities. Samples from each group were co-assembled using *MegaHIT* v1.2.9 (D. Li et al., 2015) with presets *meta-large*. Before the binning step, and to obtain contig abundances across samples, the metagenomes were back-mapped to the four co-assemblies with *BWA-mem2* v.2.2.1 (Burrows-Wheeler Aligner) (H. Li & Durbin, 2009) in default mode. Unmapped reads, secondary hits, and reads with an alignment quality below 10 were removed using *Samtools* v1.9 (H. Li et al., 2009). The resulting BAM files were used as input to *MetaBAT* v2.12.1 (Kang et al., 2019), ran in default mode with a minimum contig length of 2.5 kb, to generate four different sets of metagenome assembled genomes (MAGs). The contig depth values per metagenome from *MetaBAT*, along with the original BAM files, were given as input to two other metagenomic bidders to generate additional MAGs: *Concoct* v0.4.2 (Alneberg et al., 2014) and *MaxBin2* v.2.2.5 (Wu et al., 2014), both ran in default mode and using a minimum contig length of 2.5 kb. Then, to further improve the quality and accuracy of the predicted genomes, all MAGs from the 3 bidders were mixed and refined with the *MetaWrap* v1.3-4bf5f8a pipeline (Uritskiy et al., 2018) in default mode. Only refined MAGs with completeness $\geq 50\%$ and contamination $\leq 10\%$ were kept (Tully, Sachdeva, et al., 2017). A total of 2,098 MAGs were obtained (568, 356, 527, and 647 MAGs for G1, G2, G3, and G4, respectively), which were taxonomically annotated with *GTDB-Tk* v2.1.1 (Chaumeil et al., 2020) in default mode with the *classify_wf* workflow and using *GTDB-Tk* reference data version r207. To remove redundancy, a genomic de-replication at 99% Average Nucleotide Identity (ANI) was done with *dRep* v3.2.2 (Olm et al., 2017) for all MAGs, independently of the original group of samples. In the end, a total of 1,243 non-redundant MAGs were generated, including 641 high quality MAGs (HQ MAGs, completeness $> 80\%$, contamination $< 10\%$)

(Table III.S4). Based on their abundance, 356 MAGs representing more than 95% of the sequences of HQ MAGs were selected for further analysis. Gene predictions and functional annotation were carried out with EnrichM v0.5.0's database v10 (Boyd, 2020).

Abundance and Horizontal Coverage across samples

To get the abundances across all datasets and to reduce the number of incorrectly mapped reads, we performed a competitive mapping approach. First, all MAGs were concatenated into a single fasta file. Second, all the clean reads were mapped with BWA-mem2 v2.2.1 against the concatenated fasta file. Only reads with identity > 95% and alignment coverage > 80% were kept. Third, individual BAM files for each MAG and sample were extracted from the BAM files generated in the second step using Samtools 1.8. Last, RPKG values (mapped reads per kb of Genome per Gb of metagenome) were computed for each metagenomic sample by extracting the number of mapped reads with Samtools 1.8 view function (-c -F 4 options).

Rules for the assignment of functional capacities associated with vitamin B₁ and B₁₂

Vitamin B₁: A total of 16 KEGGs were used for the identification of B₁-related functional capacities (Table III.S1). In the gene table, sequences associated with these 16 markers were summed at the genus level and the 50 most abundant genera representing about 80% of normalized sequences were kept for further analysis. A KEGG was considered as present in a genus when it represented more than 1% of the total number of sequences in the genus (Table III.S2). These markers were also used for the assessment of the MAG functional capacities (presence/absence) (Table III.S4).

Genera or MAGs which had at least *thiC* and *thiG* or *thi4-thi1* were considered as B₁ prototrophs. Those with *thiD*, *thiG* and at least one component of HMP transporters (*thiX*, *thiY* or *thiZ*) were considered as HMP auxotrophs, while those with *thiM* but lacking *thiG* are

considered as dual auxotrophs. Those with no genes involved in *de-novo* synthesis or exogenous precursor utilization (*thiC*, *thiD*, *thiM*, *thiG*, *thi4-thi1*) and at least *thiN-TPK1* or two B₁ transporters (*thiB*, *thiP*, *thiQ*, *VB1X-A*, *VB1X-P* or *VB1X-S*) were considered as B₁ auxotrophs. Other B₁ functional capacities which could not be clearly identified were categorized as “uncomplete pathway”. Here and throughout the study, we use the term cHET (5-(2-hydroxyethyl)-4-methyl-1,3-thiazole-2-carboxylic acid) to refer to all thiazole-related precursors.

Vitamin B₁₂: For the determination of B₁₂ functional capacities, we identified the 50 most abundant genera, representing 77% of normalized sequences, associated with 26 markers genes of B₁₂ metabolisms (Beauvais et al. 2023) (**Table III.S3**). In the gene table, genera with no biosynthesis genes and at least the *btuB* transporter, or at least 2 genes involved in nucleotide loop assembly, B₁₂ remodeling, or B₁₂ salvage, were considered as B₁₂ auxotrophs (**Table III.S3**). Otherwise, genera with at least 5 biosynthesis genes were considered as B₁₂ prototrophs, while other functional capacities which could not be clearly identified were categorized as “uncomplete pathway” (**Table III.S3**). As we expected a better pathway completeness with the MAG-driven approach, 3 KEGGs involved in nucleotide loop assembly, B₁₂ remodeling, or B₁₂ salvage, were required for a MAG without *btuB* to be considered as B₁₂ auxotrophs (instead of 2 in the gene table).

TARA Ocean data analysis

To make comparisons between SOLA timeseries and TARA Ocean stations, we extracted the abundances related to the core B₁-biosynthesis and salvage genes *thiC* (PF01964), *thiD* (PF08543), *thiG* (PF05690) and *thiM* (PF02110) in the TARA Oceans Microbiome Reference prokaryotic Gene Catalog (v1) available online at <https://tara-oceans.mio.osupytheas.fr/ocean-gene-atlas/> (**Table III.S5**).

Co-occurrence graph inference and clustering

To account for the compositional (or relative) nature of the metagenomics data, we applied the Centered Log-Ratio (CLR) transformation. Co-occurrences were computed with a proportionality metric, denoted as ρ and ranging from -1 and 1, to quantify the global strength of associations. Proportionality has emerged as a valid measure for detecting associations for compositional data, as it accounts for the logarithmic relationship between species relative counts (Erb & Notredame, 2016; Lovell et al., 2015). A false discovery rate (FDR) combined with a bootstrap approach was used to determine the positive ρ threshold (Quinn et al., 2017). The FDR was less than 0.01 for ρ greater than 0.35, resulting in 163 439 significant positive pairs out of 770 662.

***Ostreococcus tauri* bioassay**

A bioassay was used to evaluate the B₁ and precursors bioavailability in SOLA seawaters sampled 15 times between 16/12/2016 and 21/03/2017. The bioassay is based on the ability of the cultivated picoalgae *Ostreococcus tauri* to grow on B₁ alone or in the presence of the precursors cHET (5-(2-hydroxyethyl)-4-methyl-1,3-thiazole-2-carboxylic acid) and HMP (4-amino-5-hydroxymethyl-2-methylpyrimidine) (Paerl, Bertrand, et al., 2018; Paerl et al., 2017). Cells from a fresh culture of *O. tauri* RCC745 maintained for 6 days in B₁-free artificial seawater with Streptomycin (50 $\mu\text{g}\cdot\text{mL}^{-1}$) and Penicillin (100 $\mu\text{g}\cdot\text{mL}^{-1}$) were refreshed in a deep well microplate (Nunc) at 5000 cells. mL^{-1} in 1.8 mL of SOLA 0.22 μm filtered seawater supplemented with Keller enrichment medium containing vitamin B12 (1 nM) but lacking vitamin B₁ and precursors (Beauvais et al., 2023). *O. tauri* cells were incubated in different vitamin and precursor combinations. At day 6, 66 μl of cultures were fixed with 2 μL of ultrapure glutaraldehyde (Sigma-Aldrich) for 15 min at 4 °C prior to cell counting using an Accuri C6 flow cytometer (Beckman Coulter). To estimate vitamin B₁ and precursors limitations, a negative control was realized without vitamin and precursor addition. Vitamin B₁

amendment at 1 nM concentrations was used as positive control. In the absence of growth in this control, the bioassay was considered as inconclusive as described earlier (Beauvais et al. 2023). Compared growth between HMP and cHET amendments at 1 nM were used to estimate the bioavailability of precursors, HMP + cHET amendment being considered as the positive control. Thiamin, and HMP were purchased from SIGMA Aldrich, cHET was chemically synthesized (Paerl, Bertrand, et al., 2018). These compounds were diluted in MiliQ water at 1 mg.mL⁻¹ concentrations, aliquoted and stored at -20 °C until use.

In-situ abundances of picoeukaryotes at SOLA along the study period were also estimated by flow cytometry, as well as bacterial abundance at D0 for samples used in microcosms experiments.

Microcosms experiments

Microcosm experiments were conducted in triplicates using 3µm filtered seawater from SOLA collected from 09/12/2017 to 23/03/2017. SOLA natural microbial communities filtered through 3 µm pore-size polycarbonate filters (Merck-Millipore, Darmstadt, Germany) supplemented with trace metals, nitrate (5 µM), phosphate (0.2 µM) and devoid of vitamins, were incubated under different vitamin B₁, B₁₂ and precursor (cHET, HMP) conditions at 1 nM concentrations in 500 mL plastic bottles for 5 days. The different conditions tested were addition of vitamin B₁ and B₁₂ alone (B₁, B₁₂) or together (B₁+B₁₂), and similarly addition of HMP and cHET alone (H, C) or together (H+C). At the end of the experiment, bacterial and picophytoplankton were counted by flow cytometry as described above and the microbial biomass was collected on 0.22-µm pore-size GV Sterivex cartridges (Merck-Millipore). DNA was then extracted, and microbial community composition described using 16S rRNA metabarcoding as described earlier (Lambert et al., 2021). Briefly, DNA was amplified using specific primers 515F-Y [50-GTGYCAGCMGCCGCGGTAA] and 926R [50-CCGYCAATTYMTTTRAGTTT] (Parada et al., 2016) that target V4-V5 regions of the

bacterial 16S rRNA gene. Amplicons were sequenced using Illumina HiSeq 2000 at the Genotoul platform (Toulouse, France).

The standard pipeline of the DADA2 package (v1.6) (Callahan et al., 2016) in ‘R’ was used to do the analysis of the raw sequences as detailed in (Lambert et al., 2021). A total of 1177 ASVs were recovered from the 34 microcosm samples (**Table III.S6**).

Statistics

The Lomb Scargle periodogram (LSP) in the ‘lomb’ package in the ‘R’ software (v.4.0.3) was used to determine if annual rhythmic patterns were present in B₁₂-related KEGGs, or genes. KEGGs or genes were considered as rhythmic when they had a PNmax index ≥ 10 with a significant p-value ($p \leq 0.001$) as previously reported (Lambert et al., 2019b), and are considered as annual if their period was 365 ± 10 days. Upset diagrams were created using the R package “upsetR” (Conway et al., 2017) on presence/absence matrix of selected markers genes. Heatmaps was constructed with the R package "pheatmap" from normalized z-score abundances. To identify significant differences between the *thiC / thiG*, *thiC / thiD* and *thiM / thiG* ratios of SOLA and TARA oceans, non-parametric Wilcoxon tests were performed as the conditions for applied parametric tests were not met (Shapiro-Wilk test, Bartlett test; $p < 0.05$). For differences between SOLA ratios averaged by month, non-parametric Kruskal-Wallis tests were performed. Results from statistical analysis were regrouped in Supplementary Table III.S7 (**Table III.S7**).

Results

Relative abundance and seasonality of genes involved in vitamin B₁ production and salvage

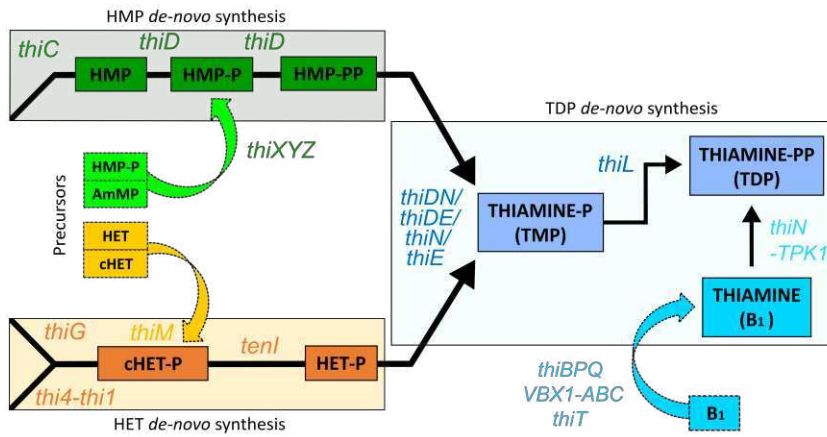
Within the SOLA metagenomic time series, we identified 22 marker genes coding for the different steps of the pathways of vitamin B₁ production and salvage (**Fig. III.1a, Table III.S1**). Among these genes, *thiL*, *thiE*, *thiG*, *thiD* and *thiC*, which encode the main functions involved in the *de-novo* production of thiamine diphosphate (TDP, vitamin B₁), had highest sequence abundance (**Fig. III.1a, b**). The genes *thiG* and *thiD* showed no seasonal pattern (**Fig. III.1c**), while *thiL* had a peak of maximal abundance in June, *thiE* in September and *thiC* in December (**Fig. III.1c, d**).

The gene *thiM*, which is involved in the use of exogenous cHET, had lower relative abundance compared to *thiN-TPK1* and *thiD*, involved in exogenous B₁ and HMP utilization (respectively), which indicate that B₁ and HMP auxotrophs were more common than cHET or dual auxotrophs (**Fig. III.1a, b**). *ThiM* had a seasonal pattern with highest relative abundance in summer (August), while *thiN-TPK1* peaked in winter (December) (**Fig. III.1c, d**).

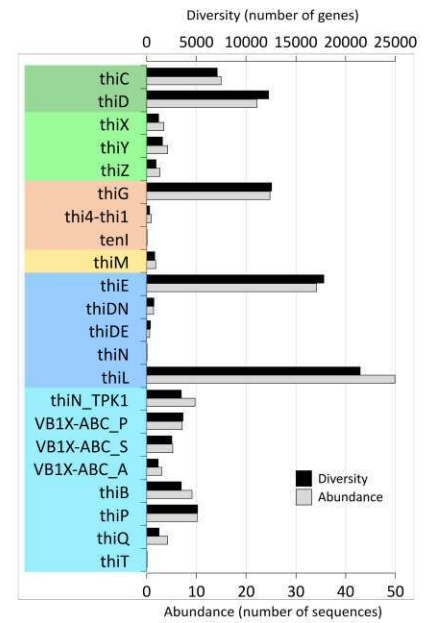
Regarding transporters, HMP (*thiXYZ*) and B₁ transporters (*thiBPQ*, *VBIX-APS*) were overall more abundant than *ThiM*, which confirms that that B₁ and HMP auxotrophs are prevalent at SOLA compared to cHET or dual auxotrophs (**Fig. III.1a, b**). The genes *thiXYZ* and *VBIX-APS* had a strong seasonal pattern with highest relative abundance in summer (from July to August) (**Fig. III.1c, d**).

Thi4-thi, which encode a thiazole synthase for cHET production in archaea, had overall a much lower relative abundance than its bacterial equivalent *thiG*, but was strongly seasonal with a peak of relative abundance in February (**Fig. III.1a - c**). These observations could indicate that cHET is mostly produced throughout the year by bacteria or cyanobacteria via *thiG*, while archaea use *thi4-thi1* in winter (**Fig. III.1b - d**).

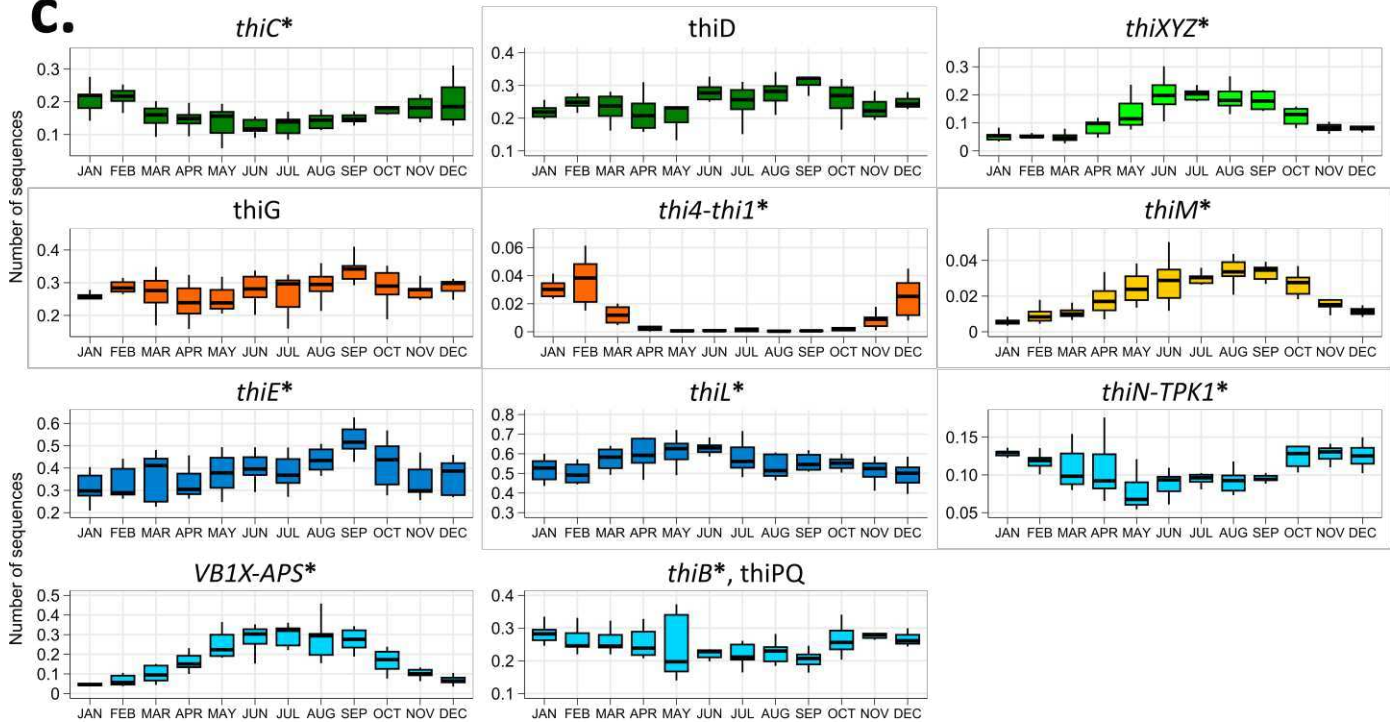
a.



b.



c.



d.

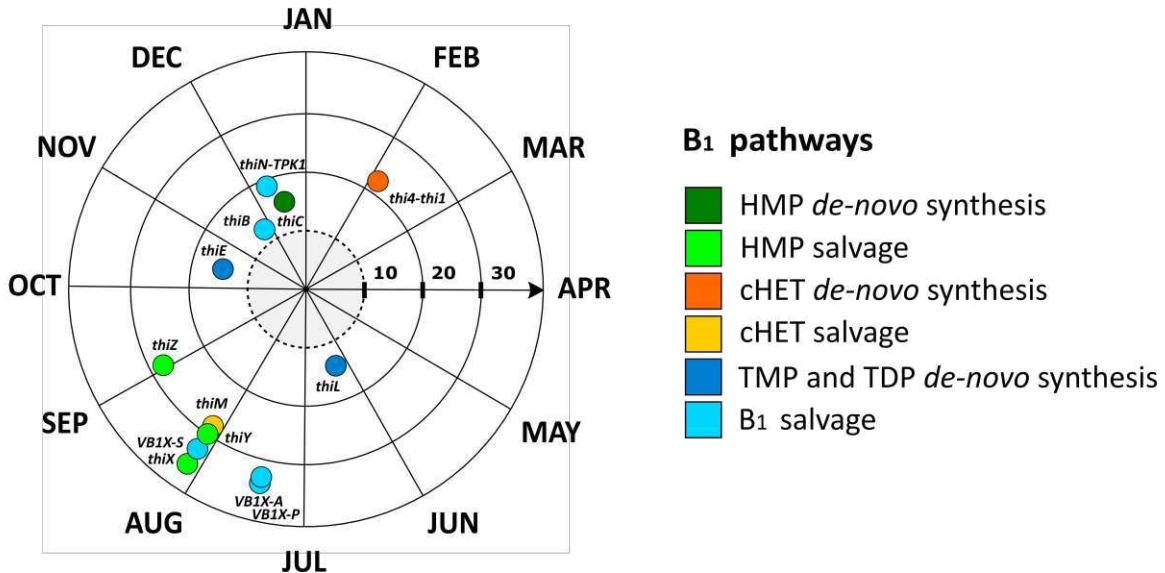


Figure III.1: Schematic overview of vitamin B₁ (thiamine) *de-novo* biosynthesis and salvage pathways, and temporal dynamics of related functions at SOLA station. (a) B₁ *de-novo* biosynthesis require the prior generation of two different precursors synthesized by distinct routes: the 4-amino-5-hydroxymethyl-2-methylpyrimidine (HMP) produced via the HMP synthase encoded by the gene *thiC* (in green) and the 5-(2-hydroxyethyl)-4-methyl-1,3-thiazole-2-carboxylic acid (cHET) produced *via* the thiazole synthase encoded by the gene *thiG* in bacteria, or *thi4-thi1* in archaea (in orange). Both precursors are then condensed in thiamine monophosphate (TMP, in blue) by different genes depending on the organisms considered. TMP is then phosphorylated by the TMP kinase encoded by *thiL* to finally produce the active form of vitamin B₁, thiamine diphosphate (TDP). Thiamine and related precursors can also be recovered from the environment *via* specific transporters, such as those encoded by *thiXYZ* genes for HMP (light green) or by *thiBPQT* genes for B₁ (cyan). In the cell, these precursors are phosphorylated by specific enzymes encoded by the gene *thiM* for cHET (light orange) or by the gene *thiD* for HMP (green), to be then condensed and phosphorylated in TDP *via* the same route. TenI encoded a thiazole tautomerase involved in the conversion of cHET into 4-methyl-5-thiazoleethanol (HET). Thiamin salvagers use a specific diphosphorylase encoded by the gene *thiN-TPK1* to produce TDP. (b) Barchart showing the abundance distributions of the 22 marker KEGGs related to HMP (green), cHET (orange), and TDP (blue) metabolisms at SOLA station over 7 years (in number of genes, top axis in black; in number of sequences, bottom axis in gray). (c) Monthly abundance and (d) seasonality of the 17 most abundant markers associated with each B₁ metabolisms. Abundances (in number of sequences) were averaged per month over 7 years and rhythmic KEGGs (PNmax > 10) are indicated in italics with an asterisk. Abundances related to each component of B₁ and precursors transporters were summed together in the boxplots (*i.e.*, *thiXYZ*, *thiBPQ*, *VBIX-APS*). *VBIX-ABC* is a putative thiamin ABC transporter composed of 3 components: -P (permease), -S (substrate-binding protein) and -A (ATP-binding protein). In the polarplot, which include only rhythmic KEGGs, the seasonality of each function is evaluated according to their peak phase (month) and to the strength of their rhythmicity pattern (PNmax). Adapted from (Helliwell, 2017; Jurgenson et al., 2009; Maupin-Furlow, 2018; Paerl, Sundh, et al., 2018).

An analysis of the relative abundance of *thiG*, *thiD*, *thiC* and *thiM* in 62 Tara Ocean stations revealed that *thiG* and *thiD* were overall more abundant than *thiC* and *thiM* in the global open ocean (**Fig. III.S1a**), confirming that HMP auxotrophy is more common than cHET or dual auxotrophy. The mean values of *thiC* / *thiG*, *thiC* / *thiD* and *thiM* / *thiG* ratios were higher at SOLA compared to TARA stations, even though they were always below 1 (Wilcoxon tests, *p*-values < 0.01) (**Fig. III.S1b**, **Table III.S5**, **Table III.S6**). These observations suggests that B₁ prototrophs and dual auxotrophs are more abundant at the SOLA station than in the global open ocean, and that most samples from both datasets were dominated by HMP auxotrophs (**Fig. III.S1b**).

Vitamin B₁-related community dynamics and diversity

Based on the taxonomic annotation of the vitamin B₁ marker genes in the metagenomes, we identified the prokaryotes involved in the different pathways of B₁ production and salvage at SOLA. We then used a hierarchical clustering to group together, at the genus level, the prokaryotes that potentially carried the same set of genes. We thus identified the microorganisms that corresponded to 4 different B₁ functional capacities (**Fig. III.2a**). The largest group (37.5% of taxa corresponding to 28.6% of the sequences) was composed of B₁ auxotrophs that had at least the gene *thiN-TPK1* or two components of the B₁ transporter *thiBPQ* (**Fig. III.2a**). Within this group, the most abundant taxa were HIMB11, *Puniceispirillum* and *Amylibacter*, which represented 5.1%, 4.0% and 1.9% of the sequences, respectively. The second most abundant group was composed predominantly of B₁ prototrophs (29.2% of the taxa and 16.2% of the sequences), which possess at least *thiC* and *thiG*, or *thi4-thi1* (**Fig. III.2a**). B₁ prototrophs included bacteria, archaea, and cyanobacteria, such as *Pseudothioglobus* and MB11C04 (2.6% and 1.8% of the sequences respectively), *Nitrosopumilus* and *Nitrosopelagicus* (each representing 0.5% of the sequences), and

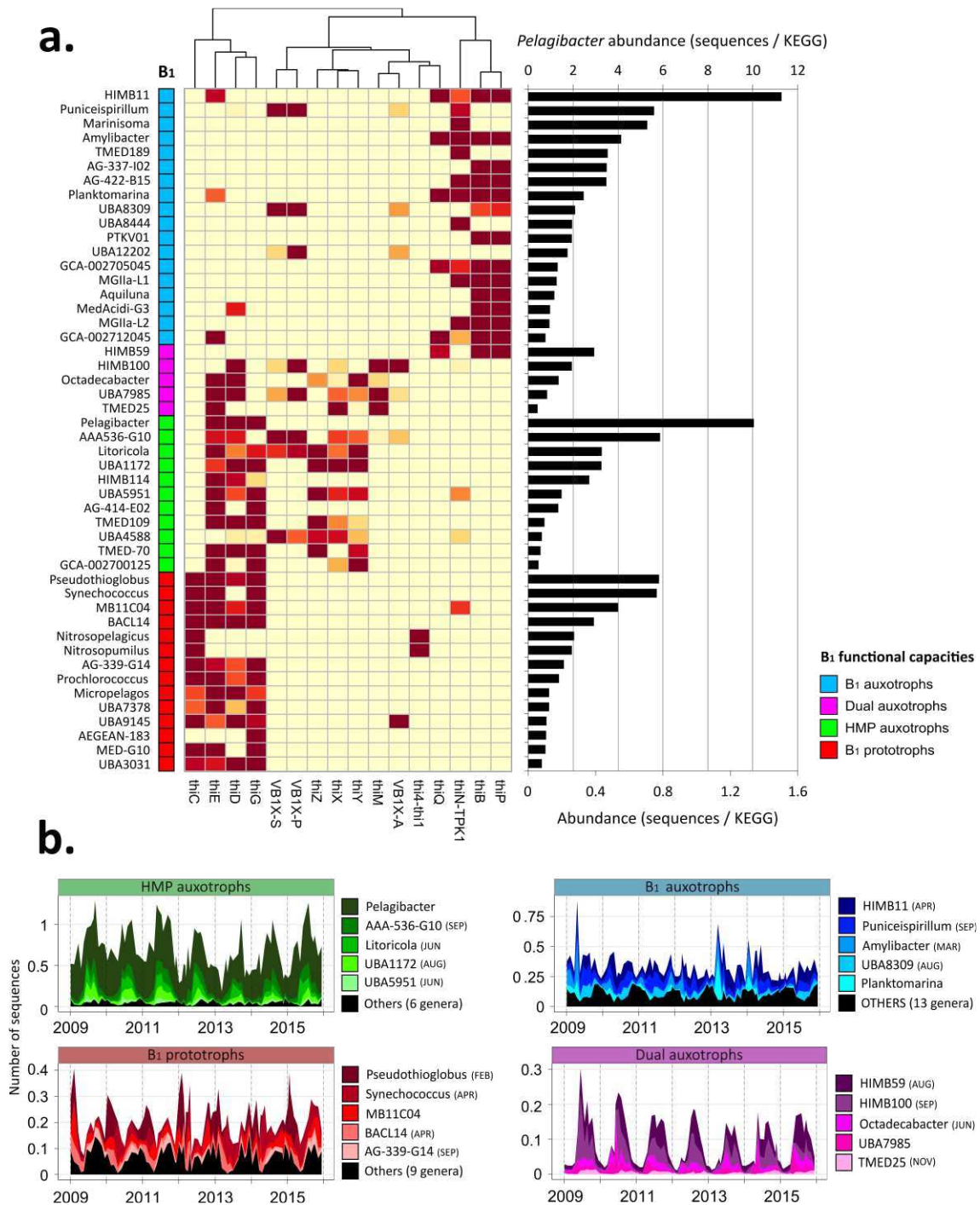


Figure III.2: B₁ functional capacities, abundance, and seasonality of 48 abundant genera identified in SOLA metagenomic sequences. **(a)** Heatmap showing the completeness of B₁ metabolic pathways in these 48 genera based on 16 marker KEGGs (left panel) (Table III.S1). Abundances used for clustering (in number of sequences) were normalized to z-scores from 0 to 1 per genus. The number of sequences per KEGG per genus is shown in the bar chart in the right panel (black bar, top axis), arranged in ascending order for HMP auxotrophs (green circle), B₁ auxotrophs (blue circle), B₁ prototrophs (red circle) and dual auxotrophs (pink circle). Since *Pelagibacter* is the most abundant genera in our dataset, its abundance is indicated by the second x axis (grey bar, bottom axis). **(b)** Seasonality of the 5 most abundant genera within each B₁ functional capacities calculated using a LSP analysis performed on the metagenomic sequences over the timeseries. Temporal patterns of abundances are shown in number of sequences per genus. The rhythmicity strength (PNmax) of each genus is indicated in brackets, and the peak phase (month) is also indicated for rhythmic ones. Other PNmax and peak phase values calculated after summing all the sequences by functional capacities are shown above their respective density plots.

Synechococcus and *Prochlorococcus* (2.1% and 0.6% of the sequences respectively). The two last groups included HMP auxotrophs (22.9% of the taxa corresponding to 51.2% of the sequences) and dual auxotrophs (10.4% of the taxa corresponding to 4% of the sequences) that carried genes involved in precursor transporters or scavenging (**Fig. III.2a**). *Pelagibacter* followed by AAA-536-G10 and *Litoricola* were the most abundant HMP auxotroph in this group representing 5.3% and 2.9% of the total sequences. Dual auxotrophs were dominated by the genera HIMB59 (1.9%), HIMB100 (1.6%) and *Octadecabacter* (0.7%) (**Fig. III.2a**).

Prokaryotic taxa identified as HMP and dual auxotrophs showed the strongest rhythmicity peaking between June and September (**Fig. III.2b**, PN_{max} = 23.9 and 32.5 respectively). Overall, the B₁ auxotroph genus summed together had no seasonal rhythm, but individual genus did display annual rhythms with different phases (**Fig. III.2b**, **Fig. III.S2**). For B₁ prototrophs, a main peak of abundance dominated by *Pseudothioglobus* and *Nitrosopumilus* was observed during winter, and smaller peaks with other bacteria were seen in April (*Synechococcus*, BACL14) and September (AG-339-G14) (**Fig. III.2b**, **Fig. III.S2**).

Analysis of high-quality MAGs

We selected the 356 most abundant HQ MAGs, representing more than 95% of the HQ MAG. Among them we identified 200 MAGs that had at least one vitamin B₁ marker gene, representing 52.4% of HQ MAGs sequences (**Fig. III.S3a, b**), similarly to observations from metagenomic sequences (**Fig. III.1b**). Each MAG was associated to a putative B₁ functional capacity based on its set of B₁ marker genes (**Fig. III.S3b**). The most abundant functional capacity was B₁ auxotrophy with a total of 48 MAGs representing 37.9% of the sequences, then B₁ prototrophs representing 28.8% of sequences with 74 MAGs (**Fig. III.S3b**). Putative HMP auxotrophs represented 13% of the sequences (18 MAGs), and dual auxotrophs representing 7.9% of sequences (13 MAGs) (**Fig. III.S3c**).

The abundance ratios between prototrophs and auxotrophs deduced from MAGs showed a strong seasonal pattern ($PN_{max} = 20.5$) with highest values from October to February, and lowest from May to September (Kruskal-Wallis tests, Dunn test, p -values < 0.05) (**Fig. III.3a, Table III.S7**). The most significant differences were found between January/February and May, June, July, August, and September (**Table III.S7**). It indicates that prototrophs were more abundant in winter, although they remained less abundant than auxotrophs (ratios < 1), which are dominant all year round, especially in summer. Ratios calculated directly from the metagenomes (gene abundance table) showed a similar pattern (**Fig. III.3a**).

The most abundant rhythmic MAGs involved in B₁ production included prototrophic bacteria and archaea, such as *Pseudothioglobus* and *Nitrosopumilus*, which were abundant in winter (**Fig. III.3b, c**). Among prototrophs, different MAGs belonging to the same genus had different seasonal patterns, such as MB11C04 and *Synechococcus*, which span a period from May to December (**Fig. III.3b, Fig. III.S4a**). Auxotrophic organisms (*i.e.*, B₁ consumers) were dominated by *Alphaproteobacteria*, such as *Amylibacter*, and UBA8309 which peaked in winter and summer, respectively (**Fig. III.3b, c, Fig. III.S4b**). Several B₁ and dual auxotrophs associated with the same genus also showed distinct seasonal patterns with peaks staggered between a few weeks to 6 months (*i.e.*, UBA8309, UBA7985) (**Fig. III.3b, Fig. III.S4b**).

To assess the homogeneity of the B₁ functional capacities within genus, we compared the capacities obtained from the gene table against those identified with the HQ MAGs. Among the 50 most abundant genus shown in the gene table (**Table III.S2**), 25 had a least one representative HQ MAGs, within which only four showed a different B₁ functional capacities between MAGs and metagenomes (**Fig. III.3d**). They were, however, always assigned to auxotrophs (either B₁ or dual auxotrophs) (**Fig. III.3d**).

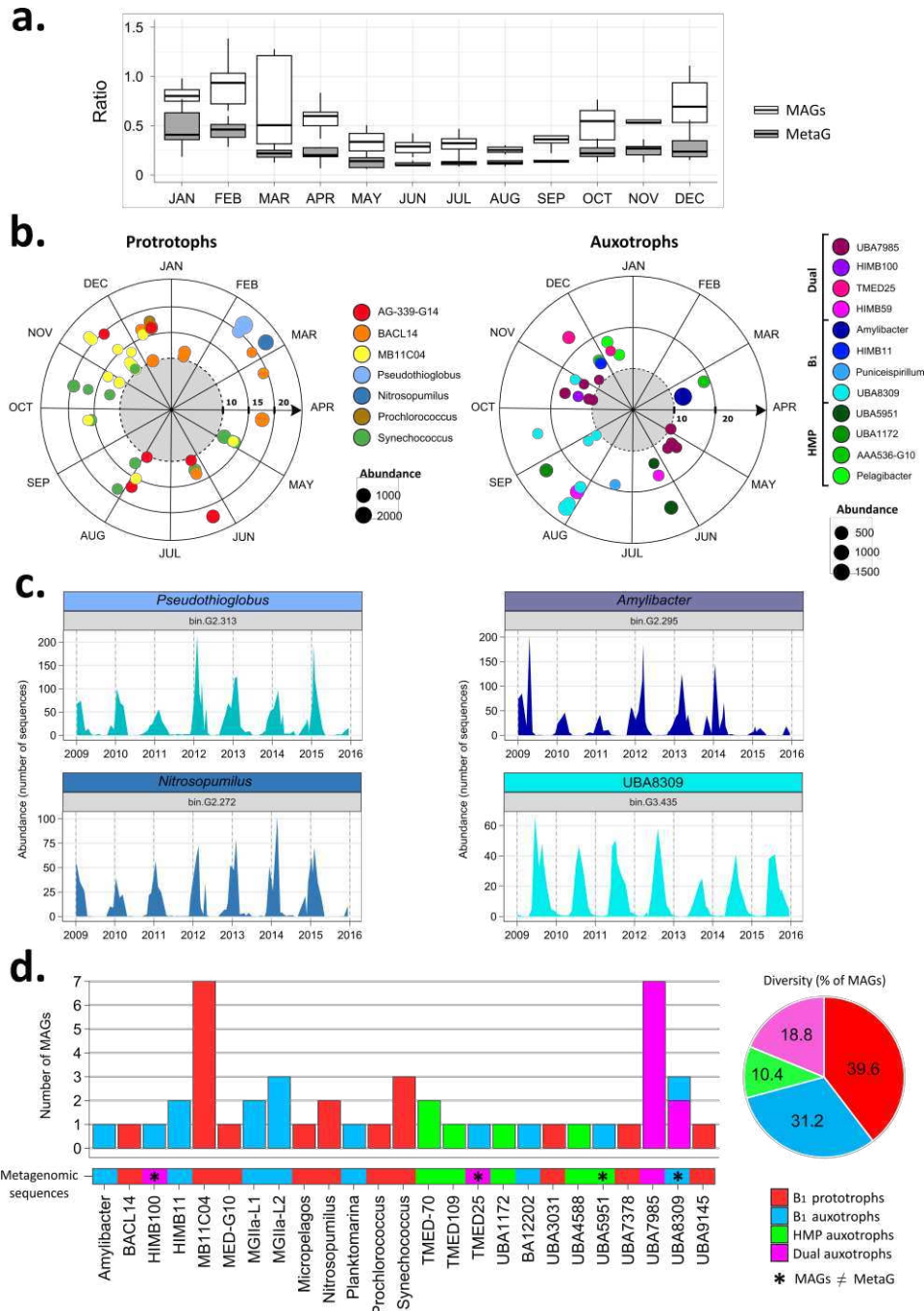


Figure III.3: Comparison of B₁ functional capacities from MAGs and metagenomic data. **(a)** Ratio of prototrophs to auxotrophs abundances over time. The grey boxes correspond to the sum of metagenomic sequences associated with genera identified as B₁ prototroph or auxotroph, while the black boxes correspond to the sum of abundances associated with MAGs identified as B₁ prototroph or auxotroph. Abundances were averaged by months, and auxotrophs considered included both B₁ and precursors auxotrophs. **(b)** Seasonality of rhythmic MAGs (PNmax ≥ 10) associated with the most abundant genera of B₁ producers (left panel) and B₁/precursor auxotrophs (right panel) identified in SOLA metagenomic sequences. The size of the point is proportional to the relative abundance of the MAG considered, and auxotrophs were colored as followed: B₁ auxotrophs (blue), HMP auxotrophs (green), dual auxotrophs (pink). **(c)** Temporal dynamics of the most abundant prototrophic and auxotrophic MAGs. **(d)** Assessment of B₁ functional capacities at the genus level in HQ MAGs from SOLA. The genera represented are among the 50 most abundant genera associated with the 16 markers of B₁ metabolism with: B₁ auxotrophs (blue), HMP auxotrophs (green), dual auxotrophs (pink) and B₁ prototrophs (red). Asterisks underlie differences between the identified functional capacities identify with both approaches.

Correlations between vitamin B₁ and B₁₂ metabolisms

To study the potential relationship between B₁ and B₁₂ metabolisms, we first assessed the B₁₂ functional capacities of the 50 most abundant genera related to 16 markers of B₁₂ metabolisms (**Table III.S2, Fig. III.S5a**). Our analysis indicates that B₁ and HMP auxotrophies are prevalent within B₁₂-related communities, while dual auxotrophy is the rarest (**Fig. III.S5b**). We then annotated the MAGs for their potential capabilities in vitamin B₁₂ related metabolisms (**Table III.S3**). The most abundant capability among genus and MAGs was B₁ prototrophy combined with B₁₂ auxotrophy (**Table III.S4, Fig. III.S5b**).

Correlation networks were then used to generate hypothesis about possible vitamin B₁ and B₁₂ interdependencies. We first constructed a network based on the abundance of all the MAGs that was separated in two seasonal sub-networks containing 282 and 173 MAGs for summer/autumn and winter/spring, respectively (**Fig. III.S6a**). Representations based on B₁/B₁₂ MAGs only (23 MAGs in summer and 16 MAGs in winter), showed that there were more interconnected MAGs with a larger number of connections in the summer network than in the winter network (85 versus 25, respectively), reflecting distinct B₁/B₁₂ co-occurrence patterns depending on the season (**Fig. III.4a, b**).

We then counted the number of interactions for all the combinations of B₁/B₁₂ functional capacities (**Fig. III.4b**). Potential double interdependencies for vitamin B₁ and B₁₂ were more prevalent in summer (29% of the MAG correlations) than in winter (16%) (**Fig. III.4b**). Among them, most correlations involved organisms that were potentially B₁ prototrophs and B₁₂ auxotrophs versus organisms that were B₁ auxotrophs and B₁₂ prototrophs (**Fig. III.4b**). Potential interdependencies between B₁ prototrophs and B₁/precursor auxotrophs were mainly detected in winter, and the most common was between organisms that were B₁ prototrophs and B₁₂ prototrophs versus others that were B₁ auxotrophs and B₁₂ prototrophs (**Fig. III.4**). Moreover, potential interdependencies between B₁₂ auxotrophs and prototrophs were also more prevalent in winter (12% of correlations) than in summer (1.18%) (**Fig. III.4a, b**).

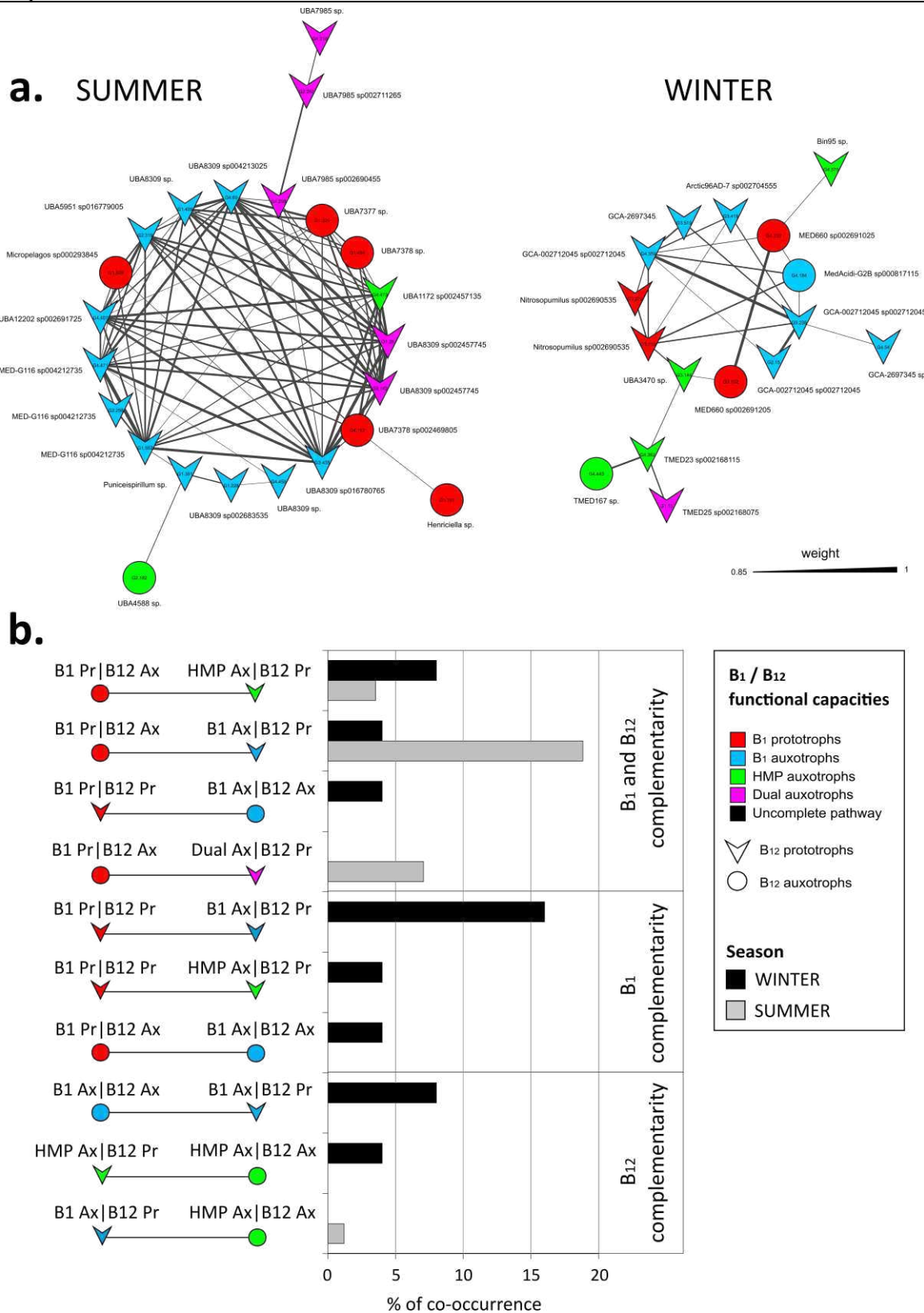


Figure III.4: (a) Co-occurrence networks between HQ MAGs from SOLA time series in summer (left panel) and winter (right panel). HQ MAGs for which B₁ and/or B₁₂ functional capacities were not identified were deleted from the analysis, and only strongly weighted edges were kept (weight > 0.85). B₁ and B₁₂ functional capacities are indicated as followed: HMP auxotrophs (in green), B₁ auxotrophs (in blue), B₁ prototrophs (in red), dual auxotrophs (in pink), B₁₂ auxotrophs (circles) and B₁₂ prototrophs (rafters). **(b)** Prevalence of each type of co-occurrence (% of co-occurrence) per type of vitamin complementarity depending on the season (summer or winter).

We also detected many correlations that did not involve potential vitamin interdependencies. In summer, 69.4% of the correlations did not involve potential vitamin interdependencies, and in winter 48% (**Fig. III.S6c**). To verify our network, we did a hierarchical clustering based on Spearman rank correlations which also revealed two main clusters of co-occurrences which occurs in winter and summer (**Fig. III.S5c, Additional information Fig. III.S5**).

Bioavailability of vitamin B₁ and precursors in natural seawater and effect of vitamin B₁ and B₁₂ amendments on natural microbial communities

We experimentally assessed the bioavailability of vitamin B₁, HMP and cHET in SOLA seawater collected at different time of the year by conducting bioassays with the microalgae *Ostreococcus tauri* RCC745 as previously described (Paerl et al., 2017). *O. tauri* cells grown in B₁ depleted artificial seawater were incubated in winter/spring SOLA seawater supplemented with vitamin B₁₂, nitrate, phosphate and trace metals and amended or not with vitamin B₁ or its precursors (HMP and cHET), alone or in combination. Growth was observed upon addition of B₁ or HMP+cHET (**Fig. III.5a**). When B₁ or precursor were not added, a strong decrease in cell abundance was seen in the beginning of February and beginning of March, indicating a low *in-situ* bioavailability of vitamin and precursor at these periods (**Fig. III.5a, Fig. III.S7a**). These sampling times notably corresponded to natural picophytoplankton bloom observed *in-situ* (**Fig. III.S7b**). Moreover, the addition of a single precursor was not sufficient to mitigate vitamin and precursors limitations observed at these times (**Fig. III.S7a**).

We also conducted microcosms experiments to assess the effect of vitamin B₁, HMP, cHET and vitamin B₁₂ amendments on the composition of natural microbial communities collected at SOLA between December 2016 and July 2017 (**Fig. III.5b, Fig. III.S8, Table III.S6**). A comparison of prokaryotic community structure in our microcosms based on Bray-Curtis dissimilarities showed that different vitamin/precursor amendments had different effects on community composition depending on the month, with overall a stronger effect of the

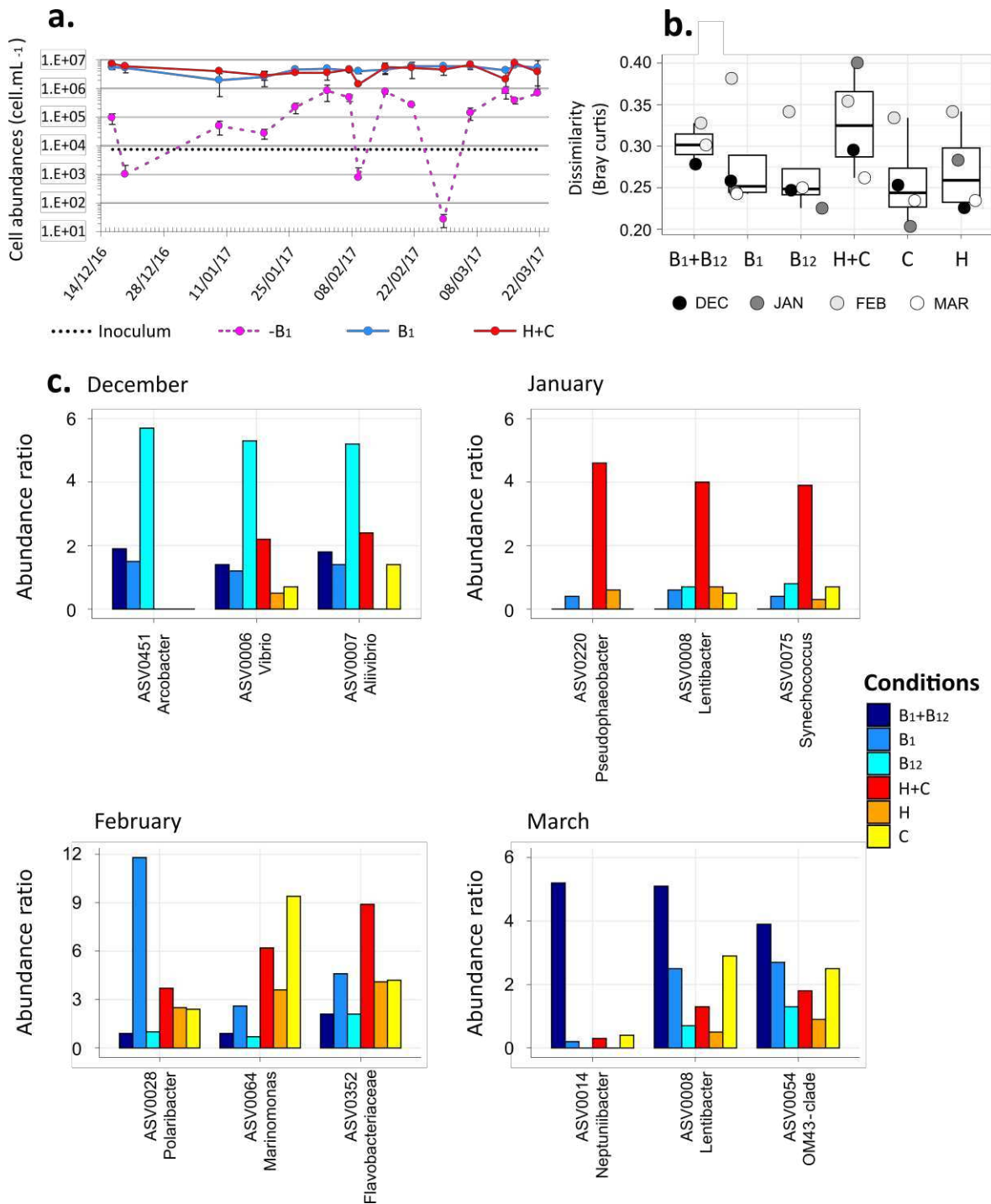


Figure III.5: Assessment of B₁ and precursors *in-situ* bioavailability at SOLA from December to March and impact of vitamins or precursors amendments on community composition. **(a)** Bioassay of *Ostreococcus tauri* RCC745 incubated in SOLA seawater collected at different times of the year (from December to March 2017) and supplemented with vitamin B₁ (blue) or not (dotted pink) or both precursors (red, H+C; HMP + cHET) at 1 nM. The inoculum represents the cell abundance of the inoculate (7629 +/- 609 cell.mL⁻¹ ± SD, n=3), and the growth of *O. tauri* is evaluated according to this baseline to identify B₁ and/or precursors limitation in the natural seawater. The growth of *O. tauri* in control indicate that B₁ and/or precursors are available in the seawater, while *O. tauri* cultures decline show B₁ and/or precursor limitations. **(b)** Bray-Curtis distances calculated between prokaryotic communities of the control versus each condition tested at day 5 in the microcosm experiments performed in December (DEC, dark), January (JAN, grey), February (FEB, light grey) and March (MAR, white). The 3 ASVs with the greatest change in abundance relative to control after 4 days of incubation are shown in **(c)** for December, January, February, and March in each condition tested: B₁+B₁₂ (darkblue), B₁ (blue), B₁₂ (cyan), H+C (red), C (orange) et H (yellow).

addition of H+C (**Fig. III.5b, Table III.S6**). The February community was always the most impacted by all the amendments, except for H+C in January. The simultaneous addition of B₁ and B₁₂ had more impact on community composition than the addition of B₁ or B₁₂ alone in all month but February (**Fig. III.5b**). Unlike in other months, in March the prokaryotic community did not respond to vitamins amendments (**Fig. III.5b, Fig. III.S7c**). Instead, we observed an increase of photosynthetic picoeukaryotes associated to a decrease in bacteria cell numbers in all microcosms suggesting that picoeukaryotes outcompeted prokaryotes (**Fig. III.S7c**).

We then identified the 3 ASVs that increased the most in abundance under the addition of the different amendments tested at each month (**Fig. III.5c, Fig. III.S8, Table III.S6**). Our results show that the ASVs that changed the most were not the same from month to month (**Fig. III.5c**). In December, B₁₂ had the strongest effect on the 3 ASVs that changed the most. In January, H+C had the greatest effect (**Fig. III.5c**). In February, different amendment had different effects of the ASVs that varied the most. In March, the combined addition of B₁ and B₁₂ had the strongest effect but as mentioned above, this was not linked to bacterial growth (**Fig. III.5c, Fig. III.S7c**). The ASVs that varied the most were different from month to month. Regarding their metabolic capabilities, there was sometimes a link between the vitamin's requirements inferred from metagenomics and the vitamin effect in microcosms amendment, but not always. In December, the addition of B₁₂ favored B₁₂ auxotrophs/B₁ producers (ASV0451 *Arcobacter* and ASV0006 *Vibrio*), and a B₁ auxotroph (ASV0007 *Aliivibrio*) (**Fig. III.5c**). In January, the addition of H+C favored a B₁₂ auxotroph (ASV0008 *Lentibacter*), but also a B₁₂ prototroph (ASV0075 *Synechococcus*) (**Fig. III.5c**). In March, the addition of B₁ and B₁₂ promoted a double B₁/B₁₂ auxotroph (ASV008 *Lentibacter*), and also B₁ prototrophs (ASV0005 and ASV0001 *Tenacibaculum*) (**Fig. III.5c, Fig. III.S8**), but as mentioned above, no bacterial growth was detected in March microcosm (**Fig. III.5c, Fig. III.S7c**).

Discussion

Our results show that B₁ prototrophs are more abundant in the northwestern Mediterranean Sea than previously estimated in other oceanic regions (overall 16% to 30% of the B₁-related prokaryotes based on metagenomic or MAG approach respectively). Conversely, Paerl and colleagues (2018) reported the absence or very low relative abundance of *thiC*, marker of prototrophy, in the Baltic Sea, the North Sea and from the coastal eastern Pacific Ocean (Paerl, Sundh, et al., 2018). Our comparisons with TARA Ocean metagenomes further confirmed that *thiC* was more prevalent at SOLA compared to global open ocean stations. The low abundance of prototrophs seen earlier may relate to the sampling season. The abundance of B₁ producers at SOLA varied significantly with seasons with an overall higher abundance in winter. The only temporal study to date showed higher abundances in summer during a 9-month survey in the Baltic Sea (Paerl, Sundh, et al., 2018), certainly due to cyanobacteria bloom (Legrand et al., 2015), indicating that different marine ecosystems may have different dynamics of B₁ producers. A good understanding of the importance of B₁ producers in the ocean thus needs long term seasonal samplings at a good temporal resolution to avoid missing important processes.

We could identify diverse putative prototrophs at SOLA that could seasonally produce B₁ and precursors at different time of the year. Most B₁ producing *Gammaproteobacteria*, such as *Pseudothioglobus*, *BACL14*, and UBA9145, were present in winter, while one, AG-339-G14, was in summer. *Verrucomicrobiota* (MB11C04) was prevalent in autumn, cyanobacteria (*Synechococcus*, *Prochlorococcus*) from spring to autumn, and archaea (*Nitrosopumilus*, *Nitrosopelagicus*) in winter. Members of *Gammaproteobacteria*, *Verrucomicrobiota*, cyanobacteria and archaea have been noted earlier as potential vitamin providers in cold marine ecosystems (Bertrand et al., 2015; Bertrand, Saito, Jeon, et al., 2011; Doxey et al., 2015; Paerl, Sundh, et al., 2018), and our study extend these observations to temperate coastal waters, for which data are lacking notably at fine taxonomic scale. These B₁ producers may represent

keystone organisms (*i.e.*, « core microbes ») that have maintained essential functions, in this case B₁ production, despite their metabolic costs (Morris, 2015; Morris et al., 2012). Energetically expensive metabolisms may be more prone to strong selective pressure in free-living microbes, which leads to genome reduction (*i.e.*, genome streamlining) (Giovannoni et al., 2005, 2014; Roda-Garcia et al., 2023), together with loss of some functions, as proposed with the Black Queen Hypothesis (Morris, 2015; Morris et al., 2012). The microorganisms that we identified maintain key functions are thus essential for ecosystem functioning and community structuring.

Although B₁ vitamin producers were more abundant in winter, our bioassays showed that our model organism could not grow in sea water collected between December and March. This indicates that the bioavailability of B₁ and its precursors was too low to promote growth, and therefore that vitamin B₁ is a limiting factor in SOLA seawater in winter. Nevertheless, this period corresponds to large seasonal blooms of auxotrophic picoeukaryotes belonging to the *Mamiellophyceae* (*i.e.*, *Micromonas*, *Bathycoccus*) (Lambert et al., 2019a). These microorganisms are thus able to meet their B₁ requirements probably with the help of the producers that we have identified, and in particular archaea. Vitamin B₁ could be transferred directly over short cell to cell distances to consumers, which may explain why it's not freely available in seawater.

Alternating periods of vitamin production and consumption, carried out by distinct prokaryotes from one month to the next, could lead to variations in the bioavailability of vitamins and precursors in the water column, which may influence community composition. We were able to demonstrate in our microcosms that vitamins have a significant impact on microbial communities. The vitamins or the precursors changed community composition, and the magnitude of change varied from month to month, with overall the strongest effect in February. In addition, the vitamin or precursors with the greatest influence were not the same

from one month to the next. During the winter to spring transition, B₁₂, H+C, and B₁+B₁₂ had successively the most influence. Finally, the bacteria that grew after addition of vitamins were not the same from one month to the next. This may indicate that vitamin or precursor limitations vary in time, so their addition had greater effect at times when limited in nature. Interestingly, the addition promoted the growth of both prototrophs and auxotrophs. This demonstrates that microbial communities are structured by complex interdependencies. The growth of certain bacteria leads to the production of micronutrient that indirectly stimulated the growth of others. In addition to these mechanisms of complementarity, changes in community composition may be due to prototrophs switching from a costly vitamin production pathway to a cost-effective salvage pathway to meet their demand. Vitamins and precursors have already been suggested as potential drivers of microbial community composition in the oligotrophic Mediterranean Sea, where both pyrimidine- and thiazole-related precursors have been detected and showed variable distribution patterns depending on depth and location (Suffridge et al., 2018). B₁ and precursor also shaped the composition of synthetic microbial co-cultures (Sathe et al., 2022), and a link between B₁ uptake and the proton motive force generated by proteorhodopsin under light was identified in auxotrophic *Flavobacteria* (Gómez-Consarnau et al., 2016). Vitamins are also emerging as key factors controlling phytoplankton growth and species succession (Koch et al., 2011, 2012, 2013), as they can co-limit phytoplanktonic growth with nitrogen (Koch et al., 2012) or iron (Bertrand et al., 2007, 2015; Bertrand, Saito, Lee, et al., 2011), and hypothetically indirectly influence bacterial community composition (Suffridge et al., 2020). Our data provide the first clear evidence of the direct impact of vitamin on entire prokaryotic community composition.

Although our results demonstrate that B₁ producers are more abundant than previously thought, the data confirmed the commonness of B-vitamin auxotrophy in the global ocean and revealed that the types of auxotrophy varied with seasons. Auxotrophic organisms, which

represented more than 84% of the B₁ related prokaryotes, were overall dominated by HMP (*Pelagibacter*, *AAA536-G10*, *Litoricola*) and intact B₁ auxotrophs (HIMB11, *Puniceispirillum*, *Amylibacter*). Interestingly cHET auxotrophs were rare at the SOLA station. Precursor auxotrophs (*i.e.*, HMP and dual cHET/HMP auxotrophs) showed strong seasonal patterns with maximal abundances observed in summer, suggesting that precursors were produced at that time of the year. Intact B₁ auxotrophs were overall present all year long, but different organisms carried the metabolisms at different times of the year. Dual cHET/HMP auxotrophs were the less abundant group at SOLA and were represented by *Rhodobacterales*, *Puniceispirillales* and *Rhizobiales* orders (*Alphaproteobacteria*). Although dual auxotrophy was already reported in *Rhodobacterales*, which include the pelagic *Roseobacter* clade (PRC) (Paerl, Sundh, et al., 2018), our results show that important PRC members which are B₁₂ providers (*i.e.*, HIMB11) are intact B₁ auxotrophs (Billerbeck et al., 2016).

It is noteworthy that all bacteria, identified in the metagenomic sequences, that can produce B₁ were unable to produce B₁₂ (*i.e.*, *Pseudothioglobus*, MB11C04, BACL14), while cyanobacteria (*Synechococcus*, *Prochlorococcus*) and archaea (*Nitrosopumilus*, *Nitrosopelagicus*) can produce both vitamins. Similarly, most bacteria identified as putative B₁₂ producers were auxotrophic to B₁ (*i.e.*, HIMB11, *Puniceispirillum*, *Amylibacter*), to HMP (UBA1172, UBA5951, UBA4588), or to both cHET and HMP (*Octadecabacter*, UBA7985, TMED25). It raises the question of the potential interdependency of B₁ and B₁₂ producers. Our results show that B₁- and B₁₂- community networks had distinct correlation patterns in summer versus winter, suggesting that potential complementarities for vitamins and precursors depend on the season. Many co-occurrences implied simple or double functional complementarity between auxotrophic organisms for B₁, precursors and/or for B₁₂, also known as « auxotrophic mutualism » (Johnson et al., 2020). In summer, community members were more interconnected than in winter, and there were more intact B₁ and precursor auxotrophs, which could produce

B₁₂, co-occurring with B₁ prototrophs, which are B₁₂ auxotrophs. It could be a common strategy of interdependency between these metabolisms. In winter, simple complementarity for intact B₁ or precursors, or for B₁₂, were more common. At that time of the year, co-occurrences also involved B₁ or HMP auxotrophs that cannot produce B₁₂, and B₁ producers which were mostly B₁₂ auxotrophs (excepting archaea and cyanobacteria). Mutual exchanges between double vitamin prototrophs (*i.e.*, archaea), and double vitamin auxotrophs in winter, would imply that auxotrophs must produce a valuable compound for archaea, such as vitamin B₇, siderophores or amino acids. These observations suggest that auxotrophs are linked with other microbial community members through a complex web of metabolic dependencies. Thus, trophic interactions for vitamins supply could have important implications for the spatio-temporal dynamic of microbial communities (Gralka et al., 2020).

The coupling of two complementary approaches, both quantitative (*i.e.*, metagenomics) and qualitative (*i.e.*, MAG assembly), enabled us to limit the lack of resolution of the first and the assembly constraints of the other, while leading to convergent observations. The abundance of HMP auxotrophs was underestimated in MAGs, since abundant genera (*i.e.*, *Pelagibacter*, *Litoricola*, *AAA-536-G10*) were missing from the reconstructed MAGs. Nevertheless, the genus-level approach based on metagenomics allowed us to affiliate a precise B₁ functional capacity to some genera and consider those abundant genera in the analysis. We also show that several MAGs associated with a same genus could have distinct seasonal patterns while most of them possess the same B₁ functional capacity than the one identified at the genus level, whether in bacteria, cyanobacteria, and archaea.

List of abbreviations

B₁: thiamine (vitamin B₁)

B₁₂: cobalamin (vitamin B₁₂)

Pseudo-B₁₂: pseudocobalamin (pseudo-vitamin B₁₂)

HMP: 4-amino-5-hydroxymethyl-2-methylpyrimidine

cHET: 5-(2-hydroxyethyl)-4-methyl-1,3-thiazole-2-carboxylic acid

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Supplementary figures and tables

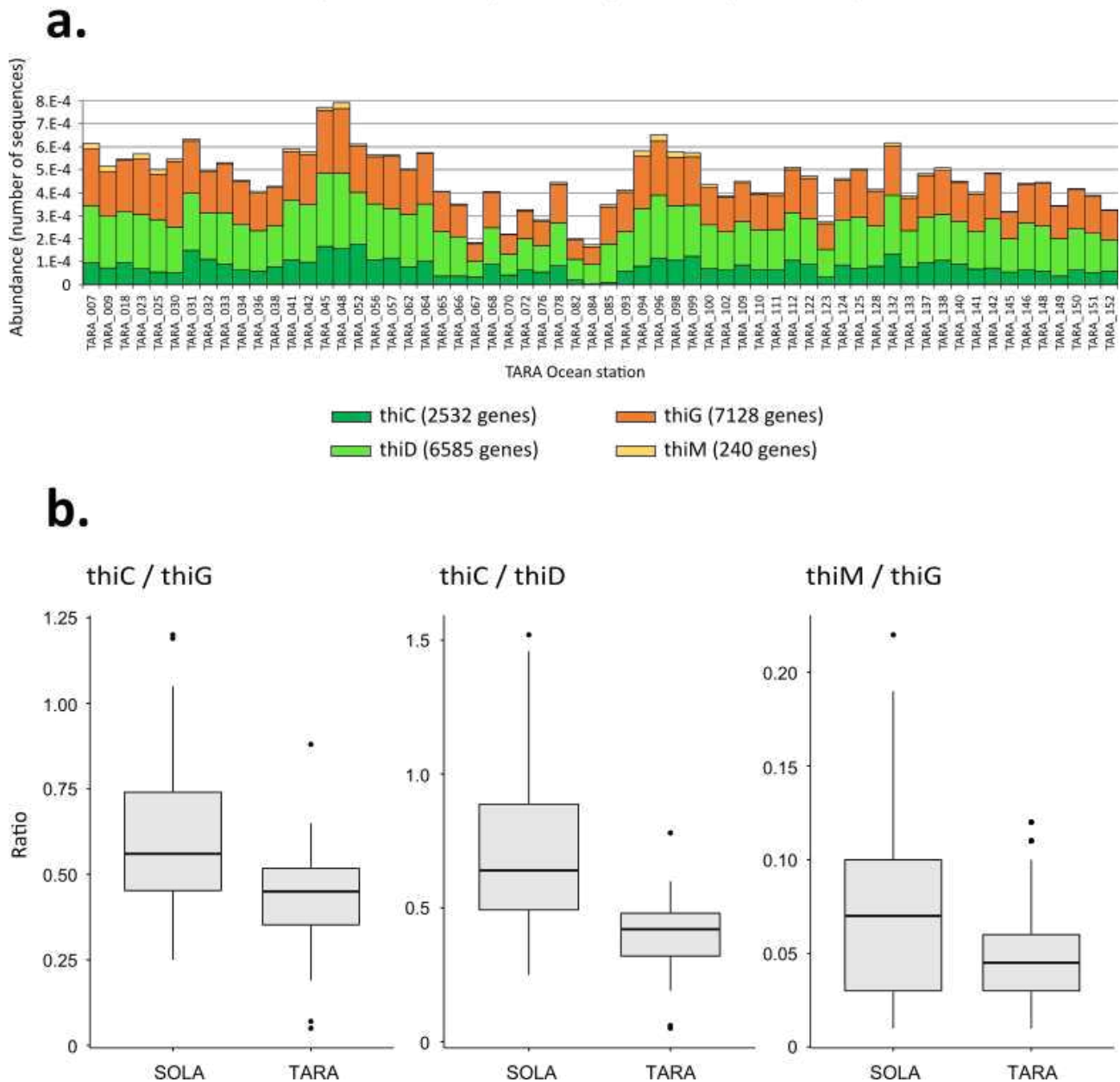


Figure III.S1: (a) Abundance of key B₁ metabolism genes in TARA Ocean stations. Abundances of *thiC* (dark green), *thiD* (light green), *thiG* (orange) and *thiM* (yellow) are shown in the 62 TARA Ocean stations. Data are available at: <https://tara-oceans.mio.osupytheas.fr/ocean-gene-atlas/>. **(b)** Comparison of *thiC* / *thiG*, *thiC* / *thiD* and *thiM* / *thiG* ratios at SOLA (grey) and in TARA stations (black).

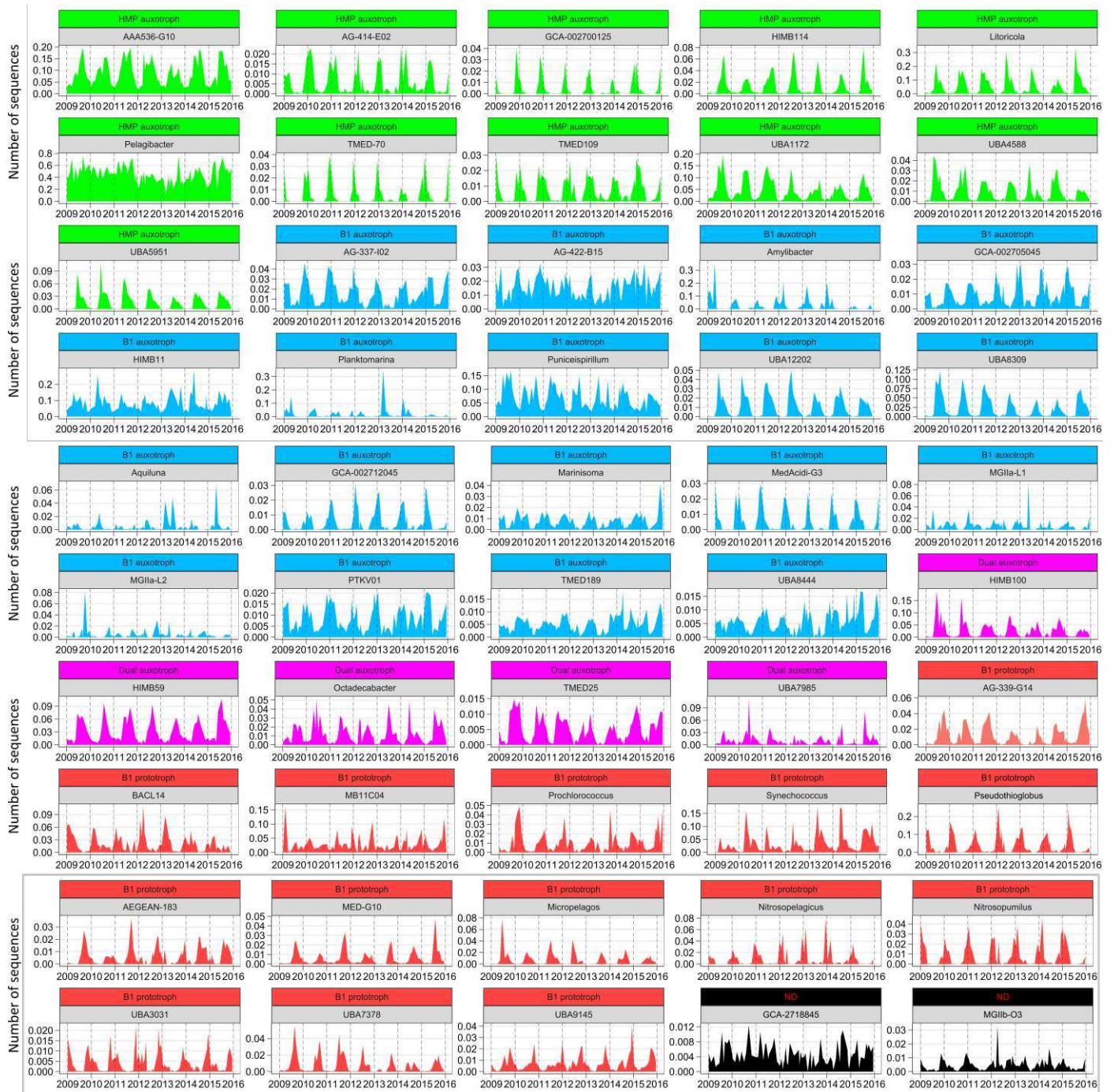


Figure III.S2: Temporal dynamics of the 50 most abundant genera which are represented in the heatmap (Fig. III.2). The 7-year dynamics of ‘OTHER’ genera are shown for HMP auxotrophs (in green), B₁ auxotrophs (in blue) and for B₁ prototrophs (in red), with their respective genus indicated above. The B₁ functional capacities of two genera (the bacteria GCA-2718845 and the archaea MGIIb-O3) cannot be identified, so they are not represented in the heatmap.

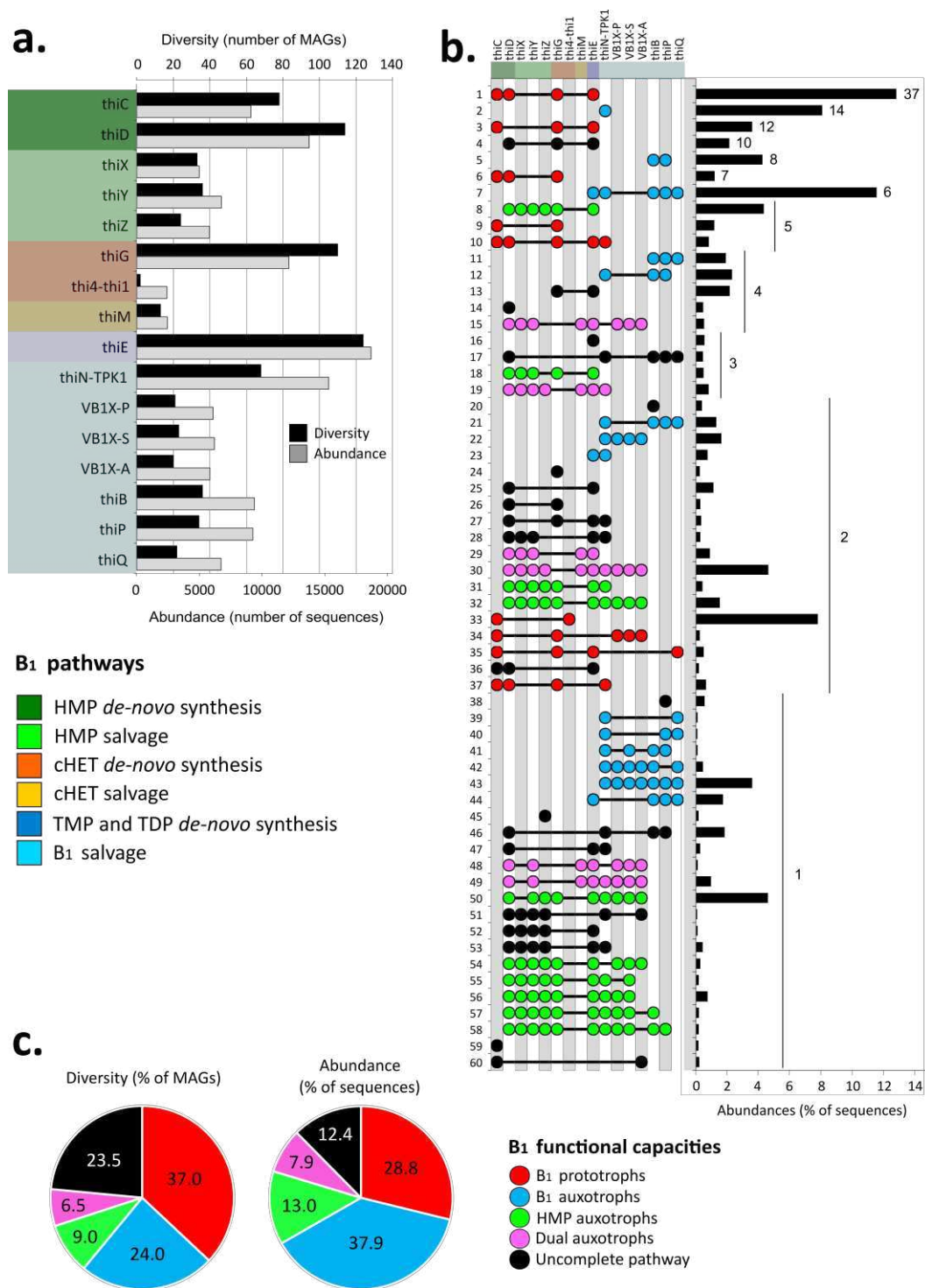


Figure III.S3: Abundance distribution of 16 marker KEGGs related to B₁ metabolisms among 200 high-quality MAGs (HQ MAGs) at SOLA station. **(a)** The barchart shows the abundance distributions of the functional markers related to HMP (green), cHET (orange), and TDP (blue) metabolisms, in number of MAGs (top axis, in black) and in number of sequences (bottom axis, in gray). **(b)** The upset diagram on the left show 60 intersections with their relative abundance indicated in the barchart (% sequences), as well as the number of HQ MAGs contained in these intersections in front of each vertical bars. A B₁ functional capacity was assigned to each intersection for which it was possible: HMP auxotrophs (green circle), B₁ auxotrophs (blue circle), B₁ prototrophs (red circle) and dual auxotrophs (pink circle). Those for which no functional capacity was identified were referred as "uncomplete pathway" (black circle). The proportion of each functional capacity within the 200 HQ MAGs are indicated in the piecharts below **(c)**.

a.



Figure III.S4a: Temporal dynamics of rhythmic MAGs represented in the polarplots in Figure III.3 (Fig. III.3). The abundance of each MAG within each genus identified as putative B₁ prototrophs are shown. The color code used in Figure 3 is maintained for each genus.

b.

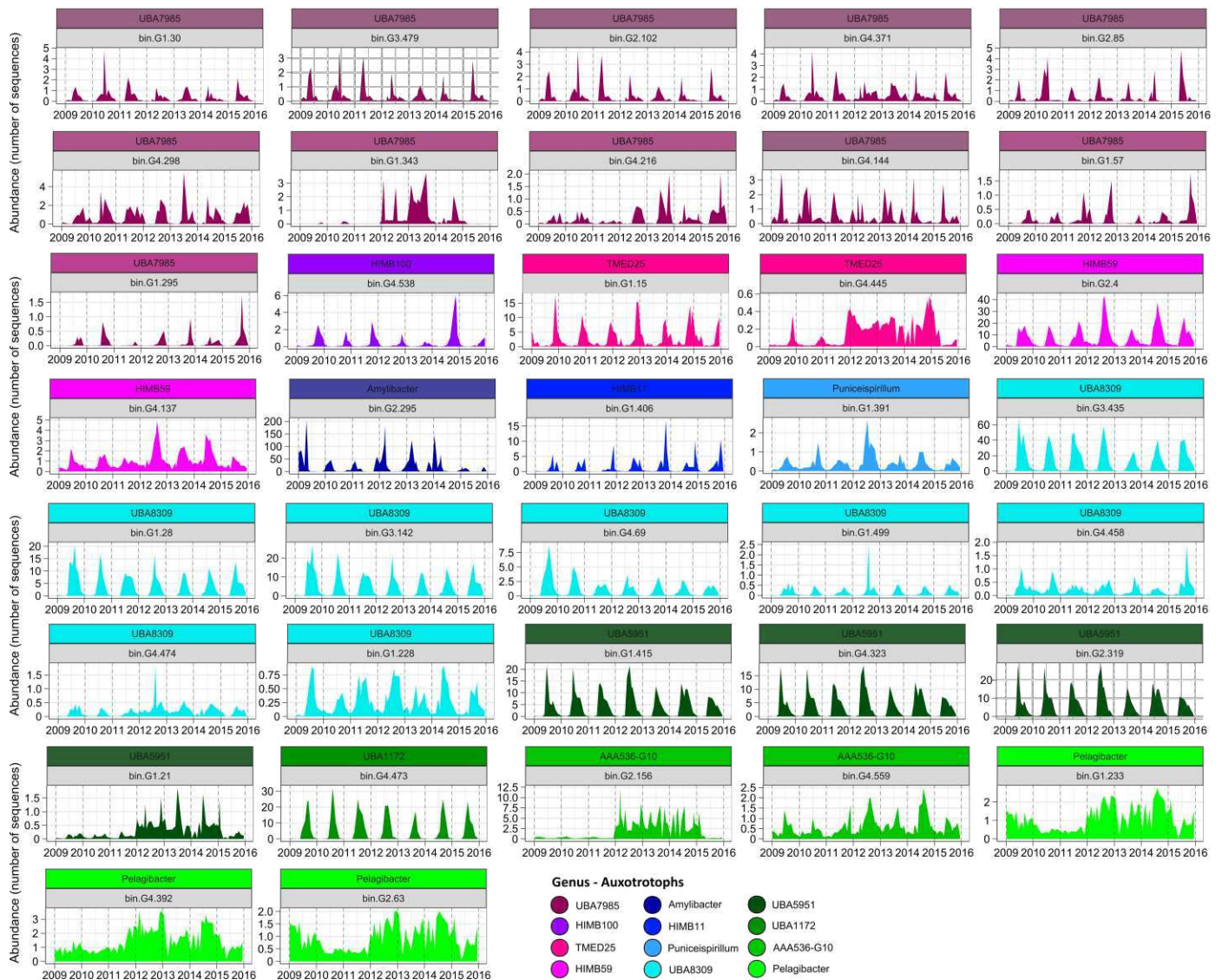


Figure III.S4b: Temporal dynamics of MAGs represented in the polarplots in Figure III.3 (Fig. III.3). The abundance of each MAG within each genus identified as B₁ or precursor auxotrophs are shown. The color code used in Figure 3 is maintained for each genus.

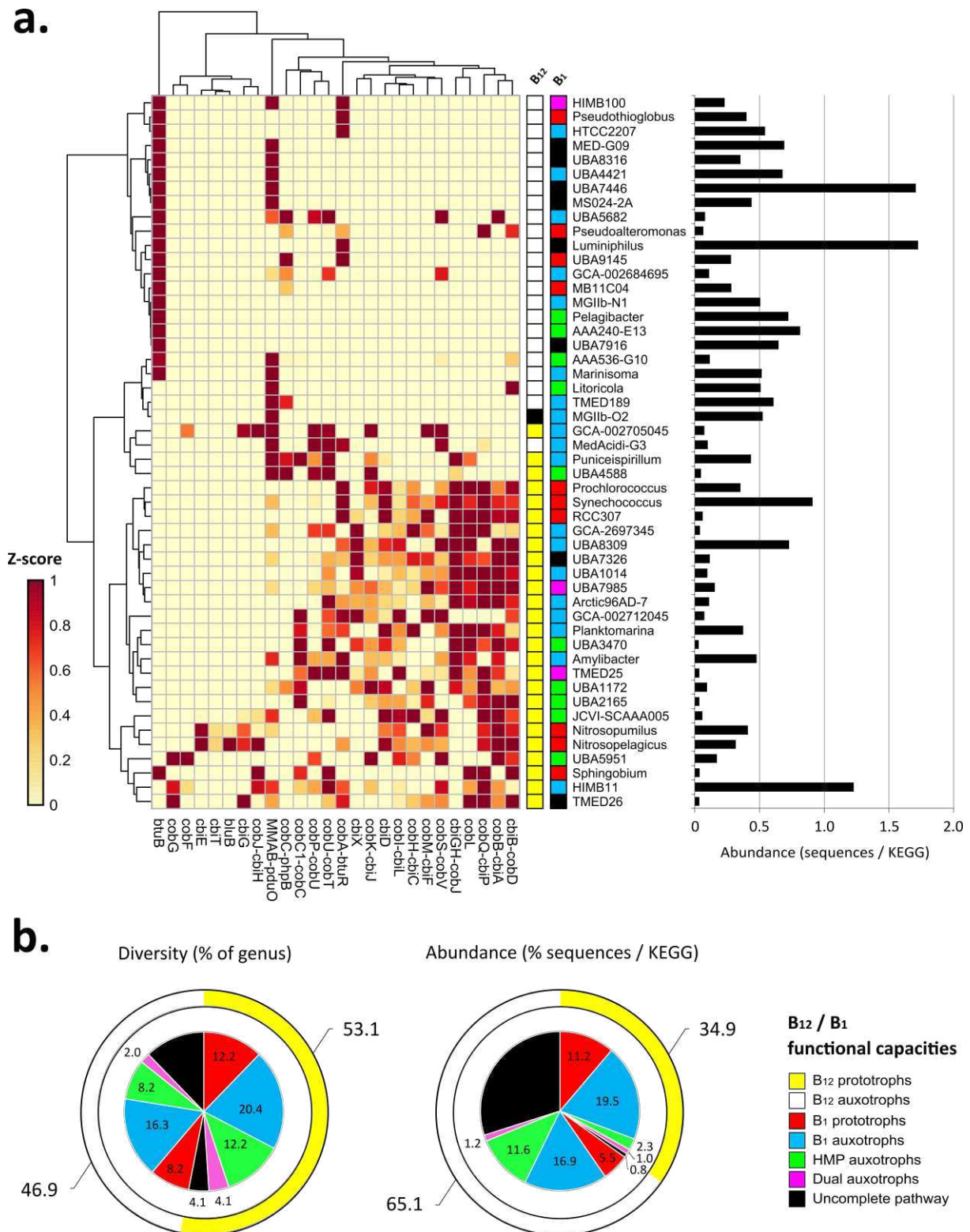


Figure III.S5a, b: Functional capacities, abundances, and seasonality of the 50 most abundant genera identified in SOLA metagenomic sequences according to 26 markers related to B₁₂ metabolisms. **(a)** Heatmap (left panel) showing the completeness of B₁₂ metabolic pathways and related functional capacities: with B₁₂ auxotrophs (empty circle) and B₁₂ prototrophs (yellow circle). Abundances used for clustering (in number of sequences) were normalized to z-scores from 0 to 1 per genus. The number of sequences per KEGG per genus is shown in the barchart in the right panel. **(b)** Prevalence of each B₁ functional capacity within B₁₂ auxotrophy and prototrophy in terms of diversity (% of genus, left panel) and abundances (% of sequences, right panel) for the 41 genera shown on the heatmap.

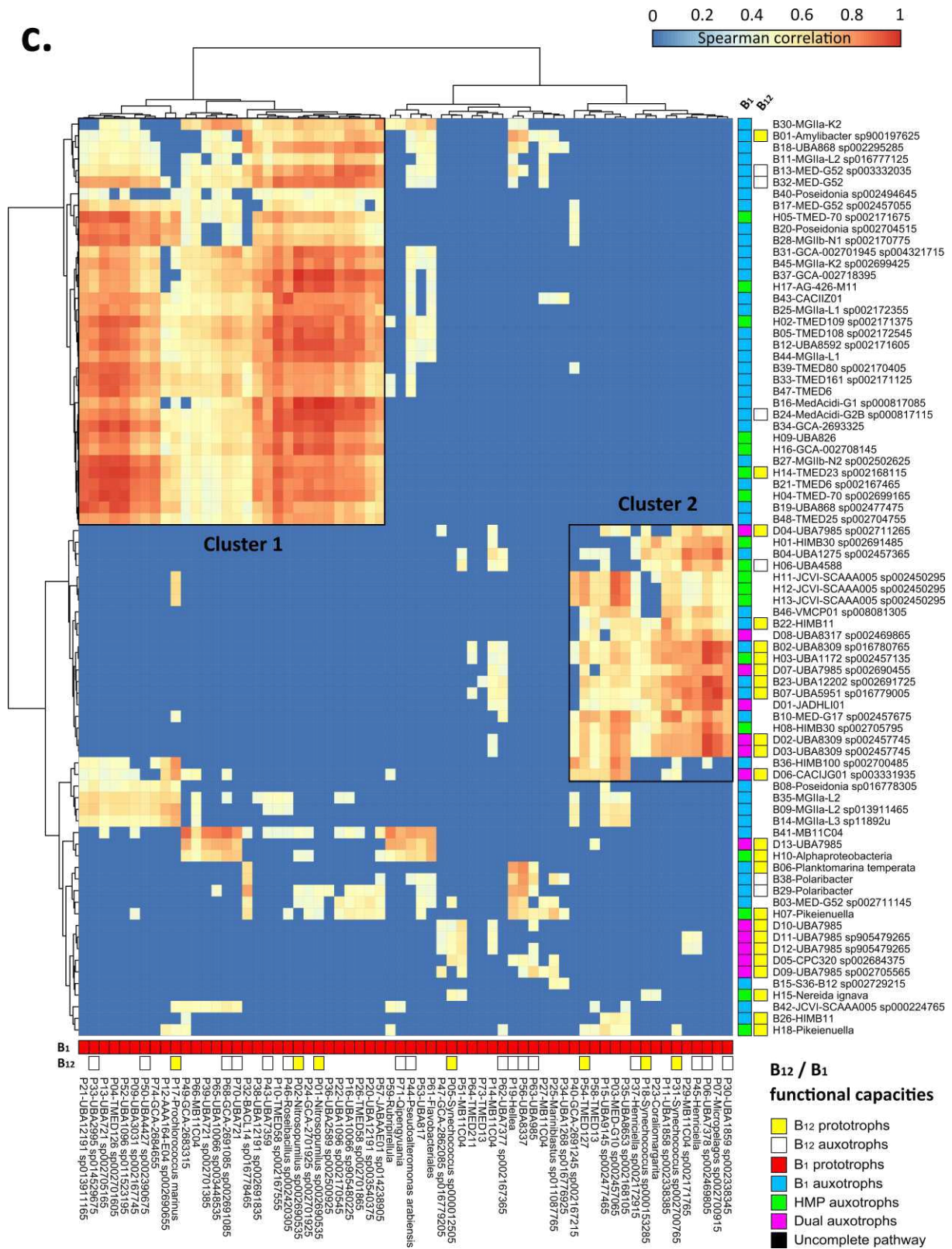


Figure III.S5c: Co-occurrences between B₁ prototrophs (x-axis) and B₁/precursor auxotrophs (y-axis) over 7 years at SOLA station. The heatmap show correlations between high quality MAGs which are abundant at SOLA (> 95% sequences summed) based on Spearman coefficients calculated over the 7-year time series (only significant correlations > 0.4 are shown). The B₁ and B₁₂ functional capacities of each MAG are indicated in front of each MAGs as colored squares, with B₁ auxotrophs in blue, B₁ prototrophs in red, HMP auxotrophs in green, dual auxotrophs in pink, B₁₂ auxotrophs in white and B₁₂ prototrophs in yellow.

Additional information on Supplementary Figure III.5a, b, c

The 50 most abundant genera associated with 26 markers of B₁₂ metabolisms are represented by diverse organisms such as *Alphaproteobacteria* (*i.e.*, *HIMB11*, *UBA8309*, *Amylibacter*, *Planktomarina*) which are mostly B₁ or precursors auxotrophs, while cyanobacteria (*Synechococcus*, *Prochlorococcus*, *RCC307*) and archaea (*Nitrosopumilus*, *Nitrosopelagicus*) are B₁ prototrophs (**Fig. III.S5a**). All bacteria which are putative B₁₂ producers were B₁, HMP, or dual auxotrophs (excepting *Sphingobium*) (**Fig. III.S5a**). B₁₂ auxotrophs are mainly represented by *Gammaproteobacteria* which are B₁ auxotrophs (*i.e.*, *UBA4421*, *HTCC2207*) or *Alphaproteobacteria* which are HMP auxotrophs (*i.e.*, *Pelagibacter*, *AAA240-E13*) (**Fig. III.S5a**). Several B₁₂ auxotrophic bacteria can however potentially produce B₁ *de-novo*, such as *Pseudothioglobus*, *Pseudoalteromonas*, *UBA9145* and *MB11C04* (**Fig. III.S5a**).

To study the co-occurrences between B₁ and B₁₂ metabolisms, we selected 153 HQ MAGs for which a B₁ functional capacity was identified (*i.e.*, 74 prototrophs, 48 B₁ auxotrophs, 18 HMP auxotrophs, 13 dual auxotrophs) (**Fig. III.S3b**). A hierarchical clustering based on Spearman rank correlations revealed two main clusters of co-occurrences which occurs at different seasons (**Fig. III.S5a**). The main cluster 1 contains 35 MAGs which are B₁ (28 MAGs) or HMP auxotrophs (7 MAGs) that co-occurred with B₁ prototrophs in winter (30 MAGs), with a potential B₁₂ complementarity in some cases (**Fig. III.S5a**). All B₁ prototrophs but archaea (*Nitrosopumilus sp002690535*) and cyanobacteria (*Prochlorococcus marinus*) are B₁₂ auxotrophs, but *Amylibacter* (B₁ auxotroph) and *TMED23* (HMP auxotroph) can also potentially produce B₁₂ at this time (**Fig. III.S5a**). The cluster 2 include 22 MAGs which are B₁, HMP or dual auxotrophs (8, 7 and 7 MAGs, respectively), which co-occurred with B₁ prototrophs (16 MAGs) in summer and fall (**Fig. III.S5a**). At this period, most B₁ prototrophs cannot produce B₁₂ excepting cyanobacteria (*Synechococcus sp00015328* and *sp002700765*, *RCC307*) and *TMED127 sp.* (*Alphaproteobacteria*), (**Fig. III.S5a**). However much more B₁ and precursor auxotrophs could produce B₁₂ in summer compared to winter (**Fig. III.S5a**).

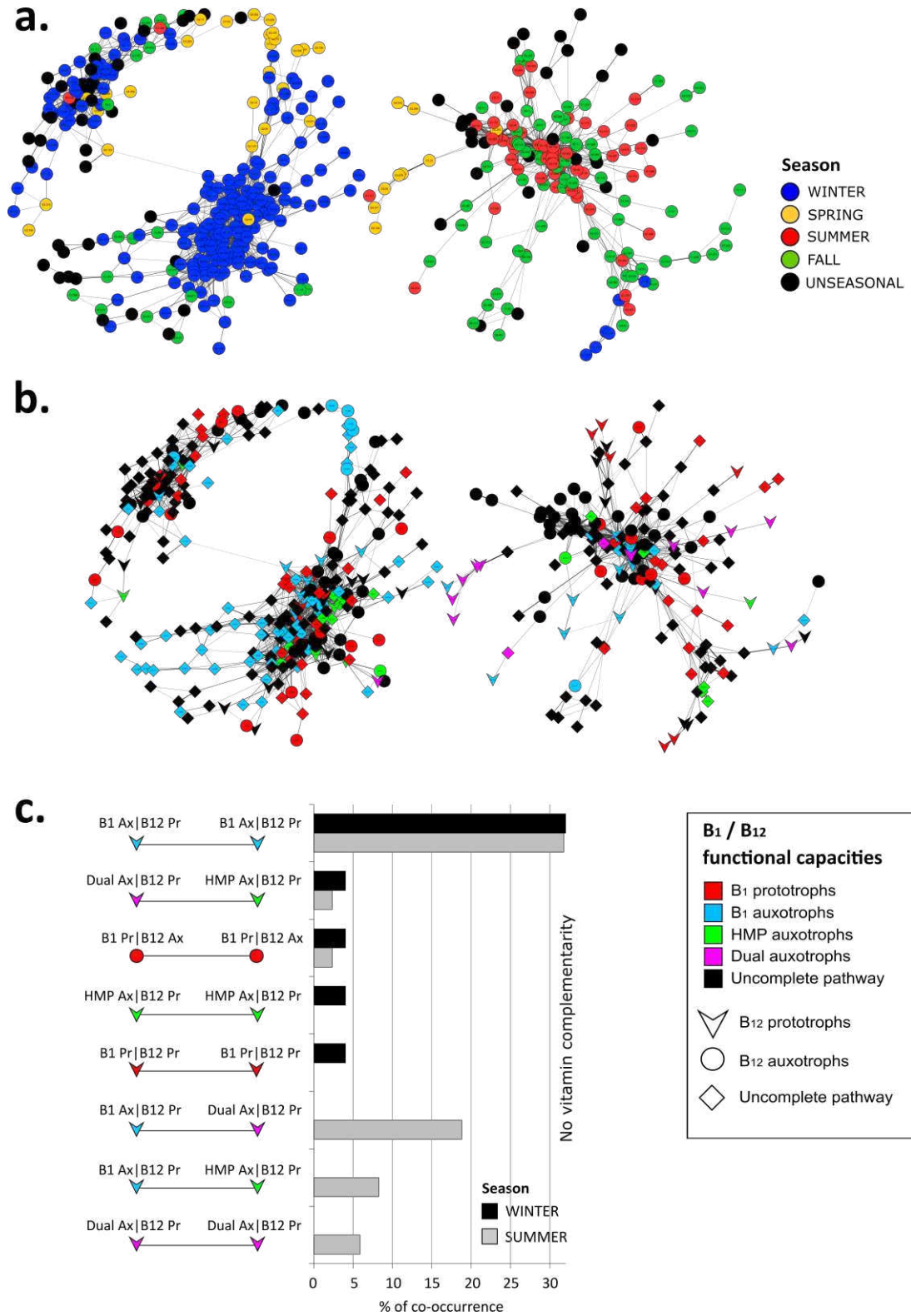


Figure III.S6: Co-occurrence networks between HQ MAGs from SOLA time series according to **(a)**, the season during which each rhythmic MAGs peak and **(b)** their B₁ and B₁₂ functional capacities. B₁ and B₁₂ functional capacities are indicated as followed: HMP auxotrophs (green), B₁ auxotrophs (blue), B₁ prototrophs (red), dual auxotrophs (pink), B₁₂ auxotrophs (circles) and B₁₂ prototrophs (rafters). MAGs for which no B₁ and/or B₁₂ functional capacities was identified were referred as "uncomplete pathway" (black diamonds). **(c)** Prevalence of each type of co-occurrence (% of co-occurrence) with no vitamin complementarity depending on the season (summer or winter).

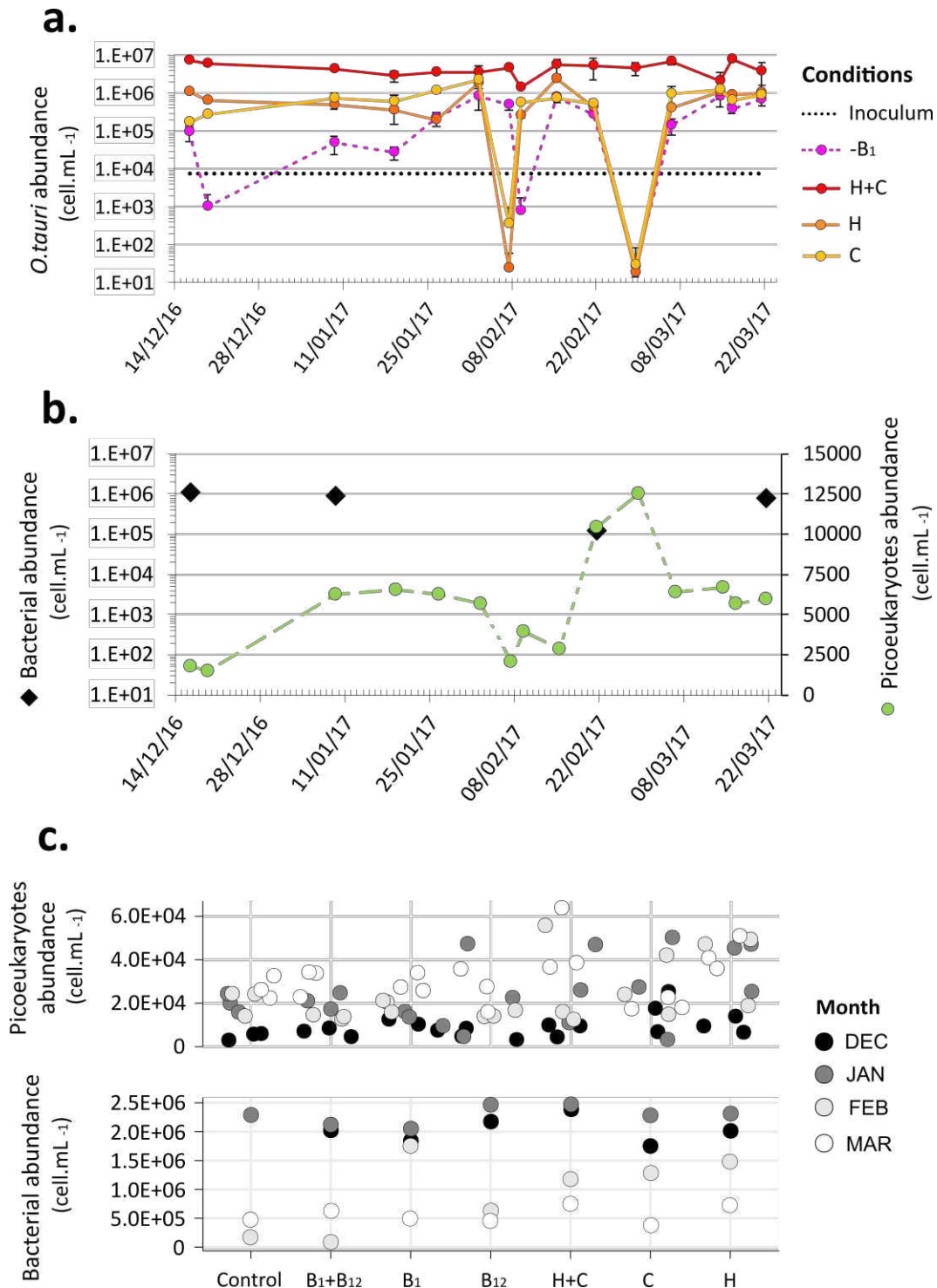
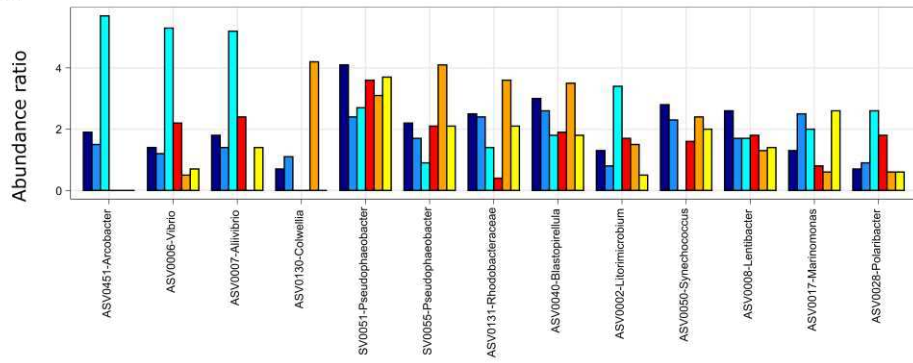
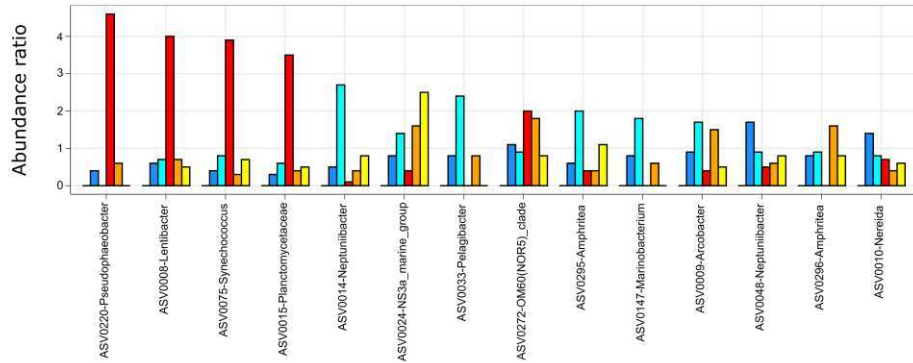


Figure III.S7: (a) Bioassay of *Ostreococcus tauri* RCC745 incubated in SOLA seawater collected at different times of the year (from December to March 2017) and supplemented with single or both precursors at 1 nM (H: HMP; C: cHET; H+C: HMP + cHET) or without vitamin (control). The inoculum represents the cell abundance of the inoculate ($7629 \pm 609 \text{ cell.mL}^{-1} \pm \text{SD}$, $n=3$), and the growth of *O. tauri* is evaluated according to this baseline to identify B₁ and/or precursors limitation in the natural seawater. The growth of *O. tauri* in control indicate that B₁ and/or precursors are available in the seawater, while *O. tauri* cultures decline show B₁ and/or precursor limitations. (b) Abundance of bacteria in microcosms at D0 (black diamonds) and *in-situ* abundance of photosynthetic picoeukaryotes at SOLA (green circles). (c) Cytometry counts for picoeukaryotes (top panel) and for bacteria (bottom panel) are shown for each condition of each month after 4 days of incubations in microcosms performed in December (DEC, dark), January (JAN, grey), February (FEB, light grey) and March (MAR, white). Due to cultural problems, bacterial counts from February experiments were taken at D3.

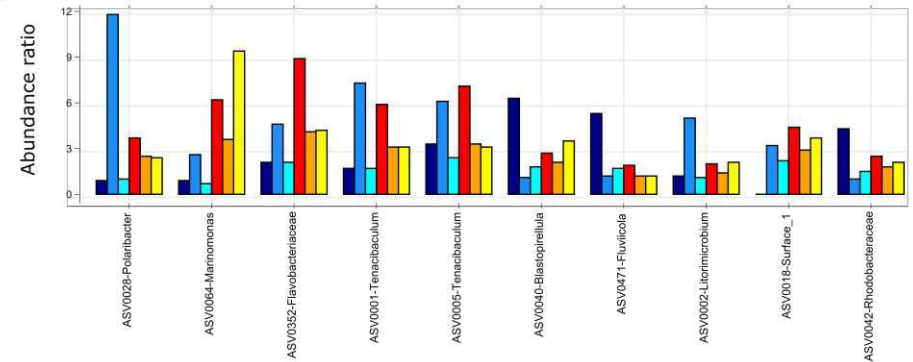
a. December



b. January



c. February



d. March

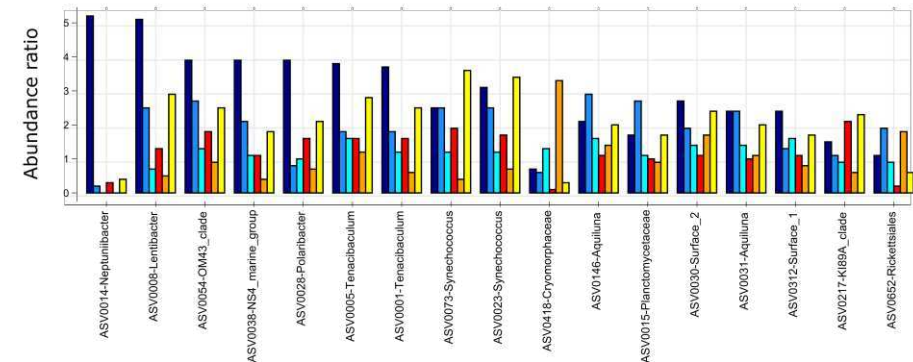


Figure III.S8: Impact of vitamins or precursors amendments on SOLA communities. The ASVs whose abundances increase the most after 5 days of incubation under each conditions tested are indicated for seawater collected in (a) December, (b) January, (c) February and (d) March. The ratio between relative abundance at D0 (indicated above each ASV) and D4 is plotted as a function of the conditions tested: B₁+B₁₂ (darkblue), B₁ (blue), B₁₂ (cyan), H+C (red), C (orange) et H (yellow).

Table III.S1: Table representing the 22 marker KEGGs related to vitamin B₁ metabolisms recovered from SOLA time series. The strength of rhythmicity is indicated by the value of the PNmax index (PNmax), and for rhythmic KEGGs (in bold, PNmax ≥ 10), the period (in days), the peak phase (in week, month) and the significance level of the rhythm (p-value) are also indicated. Asterisks show the 16 KEGGs used as markers in this study based on their abundance in the gene table (Fig. III.1b).

Pathways B ₁	KO number	Functional gene	PNmax	Period (days)	Phase		Sig.level
					Week	Month	
HMP <i>de-novo</i> synthesis	*K03147	<i>thiC</i>	15.4	362	50	DEC	1.81E-05
	*K00941	<i>thiD</i>	9.2				
HMP salvage	*K15599	<i>thiX</i>	36.1	362	31	JUL	1.88E-14
	*K15598	<i>thiY</i>	29.9	362	31	JUL	8.87E-12
	*K15600	<i>thiZ</i>	27.6	362	35	AUG	8.71E-11
cHET <i>de-novo</i> synthesis	*K03149	<i>thiG</i>	6.7				2.05E-08
	*K03146	<i>thi4_thi1</i>	22.2	362	5	JAN	
	K10810	<i>tenI</i>	4.4				
cHET salvage	*K00878	<i>thiM</i>	28.3	362	31	JUL	4.58E-11
TMP and TDP synthesis	*K00788	<i>thiE</i>	14.6	2534	41	OCT	4.01E-05
	K21219	<i>thiDN</i>	15.5	362	8	FEB	1.69E-05
	K14153	<i>thiDE</i>	3.7				
	K21220	<i>thiN</i>	13.5	373	46	NOV	1.27E-04
	K00946	<i>thiL</i>	14.0	373	23	JUN	7.04E-05
Thiamine (B ₁) salvage	*K00949	<i>thiN, TPK1</i>	18.8	373	49	DEC	5.94E-07
	*K05778	<i>ABC-VB1X_P</i>	34.1	362	28	JUL	1.38E-13
	*K05777	<i>ABC-VB1X_S</i>	33.0	362	31	JUL	4.12E-13
	*K05779	<i>ABC-VB1X_A</i>	33.0	362	28	JUL	4.11E-13
	*K02064	<i>thiB</i>	12.4	373	47	NOV	3.48E-04
	*K02063	<i>thiP</i>	5.2				
	*K02062	<i>thiQ</i>	4.5				
	K16789	<i>thiT</i>	11.3	362	49	DEC	1.05E-03

Table III.S3: Table of B₁₂ functional capacity assignments for the 50 most abundant genera present in the gene table associated with our 26 markers (77.1% of sequences). The diversity (in number of genes) and abundances associated with each genus are shown in the first part of the table (in number of sequences, percentage of sequences, number of KEGGs, and number of sequences per KEGG). B₁₂ functional capabilities have been assigned according to the completeness of markers associated with each biosynthesis, nucleotide loop assembly, salvage, or remodeling pathways (see *Methods* page 105). A KEGG is considered representative of a genus when its abundance exceeds 1% of the sequences associated with the genus. The genera are arranged in ascending order of abundance according to their B₁₂ functional capacity, with B₁₂ auxotrophs (blue), B₁₂ prototrophs (red) and unknown (black). The color scale in the KEGG abundances represents the distribution of KEGGs within a genus, and abundances written in red indicate observations below our 1% threshold, as these KEGGs are not considered as representative of the genus.

GENUS	Diversity		Abundances			Functional capacity	De-novo biosynthesis															Nucleotide loop assembly, B ₁₂ remodeling, salvage											
	Genes	Seq	%Seq	KEGGs	Seq / KEGG		cbiX	cbiG	cbiD	cbiE	cbiT	cobI_cbiL	cobG	cbiGH_cobI	cbiH_cbiH	cobM_cbiF	cobF	cobK_cbiJ	cobL	cobH_cbiC	cobL_cbiA	cobQ_cbiP	cbiB_cobD	cobP_cobU	cobS_cobV	cobC1_cobC	cobC_phpB	cobU_cobT	bluB	cobA_btur	MMAB_pduO	btuB	
*Luminiphilus	1161	5.2	3.0%	3	1.72	B₁₂ auxotrophs	2%															29%											
UBA7446	264	3.4	2.0%	2	1.71																	37%											
AAA240-E13	1038	0.8	0.5%	1	0.81																	100%											
Pelagibacter	1751	1.4	0.8%	2	0.72																	2%											
MED-G09	86	1.4	0.8%	2	0.69																	45%											
UBA4421	559	1.4	0.8%	2	0.68																	31%											
UBA7916	226	0.6	0.4%	1	0.65																	100%											
Actinomarina	237	1.2	0.7%	2	0.61																	84%											
HTCC2207	394	1.1	0.6%	2	0.54																	16%											
Marinisoma	180	1.0	0.6%	2	0.52																	45%											
HIMB30	160	1.0	0.6%	2	0.51																	66%											
MGIIb-N1	508	1.0	0.6%	2	0.51																	31%											
MS024-2A	263	0.9	0.5%	2	0.44																	69%											
*Pseudothioglobus	633	1.2	0.7%	3	0.40																	3%											
UBA8316	207	0.7	0.4%	2	0.35																	2%											
MB11C04	308	0.6	0.3%	2	0.28																	47%											
UBA9145	242	0.8	0.5%	3	0.28																	17%											
HIMB100	166	0.9	0.5%	4	0.23																	21%											
**AAA536-G10	1062	1.6	0.9%	14	0.11																	2%											
GCA-002684695	482	0.5	0.3%	5	0.11																	2%											
MedAcidi-G3	149	0.7	0.4%	7	0.10																1%												
*UBA868	239	0.5	0.3%	7	0.08																1%												
Pseudoalteromonas	153	0.5	0.3%	8	0.06																2%												
HIMB11	5088	24.5	14.1%	20	1.23	B₁₂ prototrophs	3%															4%											
Synechococcus	6303	13.6	7.8%	15	0.91		4%															8%											
UBA8309	5034	11.6	6.7%	17	0.68		7%															6%											
Amylibacter	494	8.1	4.7%	17	0.48		3%															5%											
Punicispirillum	2951	5.6	3.2%	13	0.43		2%															4%											
Nitrosopumilus	748	6.2	3.5%	15	0.41		7%															6%											
Planktomarina	1131	6.3	3.6%	17	0.37		4%															9%											
Prochlorococcus	3235	5.3	3.0%	15	0.35		1%															8%											
Nitrosopelagicus	3708	4.7	2.7%	15	0.32		7%															7%											
UBA5951	551	2.2	1.3%	13	0.17		3%															8%											
UBA7985	1198	2.5	1.4%	16	0.16		6%															5%											
UBA7326	1120	1.9	1.1%	17	0.11		9%															5%											
Arctic96AD-7	1857	1.8	1.0%	16	0.11		5%															5%											
UBA1014	1518	1.5	0.8%	15	0.10		8%															4%											
UBA1172	259	1.6	0.9%	17	0.10		6%															7%											
GCA-002712045	217	0.7	0.4%	10	0.07		16%															5%											
GCA-002705045	783	0.9	0.5%	13	0.07		8%															11%											
RCC307	1103	0.9	0.5%	15	0.06		5%															8%											
JCVI-SCAAA005	686	0.9	0.5%	15	0.06		4%															9%											
UBA4588	239	0.5	0.3%	12	0.05		8%															1%											
GCA-2697345	499	0.6	0.4%	16	0.04	8%															1%												
Sphingobium	249	0.6	0.4%	17	0.04	1%															5%												
TMED26	362	0.6	0.4%	18	0.03	8%															2%												
TMED23	612	0.5	0.3%	16	0.03	4%															9%												
TMED25	222	0.6	0.3%	17	0.03	4%															3%												
UBA3470	863	0.5	0.3%	18	0.03	6%															6%												
MGIIb-O2	8	0.5	0.3%	1	0.53																100%												

* Luminiphilus, Pseudothioglobus and UBA868 were assigned to putative B₁₂ auxotrophs although they possess one biosynthesis gene

** AAA536-G10 was assigned to putative B₁₂ auxotroph, although it possesses 4 biosynthesis genes in low proportion. 92% of the sequences are associated with nucleotide loop assembly, salvage, or remodeling.

IV. **CHAPTER 4: Discussion**

1. **Metagenomic time series: identification of vitamin functional capacities and interactions through the metagenomics lens**

Quantitation of vitamin B₁ and B₁₂, coculture experiments, microcosm amendments, as well as *in-silico* genomic analysis of B₁ and B₁₂ functional capacities, have suggested that B₁ and B₁₂ are important metabolites for structuring microbial communities in the global ocean (Cooper & Smith, 2015; Croft et al., 2005; Gómez-Consarnau et al., 2018; Koch et al., 2011, 2012; Sañudo-Wilhelmy et al., 2012, 2014; Seymour et al., 2017; Suffridge et al., 2018, 2020; Zoccarato et al., 2022). Still, we lack a global view of the importance of vitamin B₁ and B₁₂ in structuring seasonal microbial communities in the sea (Wienhausen, Bittner, et al., 2022), while the influence of environmental factors is well described (Bunse & Pinhassi, 2017).

Our incomplete understanding of the role of B-vitamins in metabolic interactions between marine microbes is due to the lack of functional data. Large-scale measurements of key metabolites in the seawater, such as B-vitamins, represent both a technical and financial challenge. Even though coculture experiments have highlighted metabolites tradeoff between bacteria, or bacteria and phytoplankton, most marine bacteria such as those from the dominant SAR11-clade, and others from the domain archaea, are difficult to cultivate or uncultivable, limiting the scope for coculture experiments in the laboratory. For example, our metagenomic approach suggest that *Thaumarchaeota* (*Nitrospumilus* and *Nitrosopelagicus*) may be the main vitamin B₁₂ providers in winter, but the validation of this hypothesis remains difficult using cultural approaches. In addition to cultural limitations, the well-known “bottle effect” often leads to the selection of a few species during microcosm experiments, making vitamin amendment experiments difficult to interpret, such as those described in **Chapter III** (Domingues et al., 2023; Ferguson et al., 1984; Nogueira et al., 2014; Zobell & Anderson, 1936). The application of metagenomic approaches to marine timeseries offers unique

opportunities to identify potential metabolic interactions in dynamic seasonal microbial communities. In **Chapter II** and **III**, we show that the seasonality observed in planktonic community composition (Lambert et al., 2019, 2021) is also reflected at the functional level, with seasonal functions associated with vitamin B₁ and B₁₂ metabolisms (*i.e.*, production, uptake) as well as seasonal MAGs carrying different B₁ and B₁₂ production potential. Seasonal organisms exhibiting recurrent annual patterns were both represented by vitamin-producing or auxotrophic organisms, including bacteria, cyanobacteria, and archaea. These recurrent co-occurrence patterns between abundant organisms which are complementary for their vitamin B₁ and/or B₁₂ needs underlie potential metabolic interdependencies. It should be noted that the dynamics of the main eukaryotic phytoplankton groups at SOLA, and their functional capacities for B₁ and B₁₂, were not considered in our studies due to difficulties in the functional annotation of the eukaryotic metagenomic dataset. However, based on the functional capacities for B₁ and B₁₂ inferred from the literature including culture-based and genomic surveys, we integrated the most abundant seasonal phytoplanktonic eukaryotes in the global picture (**Fig. IV.1**).

2. Seasonality of B₁ and B₁₂ metabolisms at SOLA

A. Winter

The productive winter months during which vitamin B₁ and B₁₂ limitations are observed are dominated by eukaryotic seasonal phytoplankton, predominantly *Mamiellophyceae* and *Dinophyceae* (Lambert et al., 2019), which are important primary producers for predator/prey linkages in the marine food web (Massana, 2011) (**Fig. IV.1**). These highly predictable seasonal blooms are usually followed by increases in *Bacilliarophyta*, which are prevalent in the large size fraction (< 3µm) (Lambert et al., 2019, 2021). Winter phytoplankton communities are therefore composed of three main groups within which a high prevalence of vitamin B₁ and B₁₂ auxotrophy has been widely reported (Croft et al., 2005, 2006; Helliwell, 2017; Paerl et al., 2017; Sañudo-Wilhelmy et al., 2014; Tang et al., 2010). Furthermore, our data suggest that

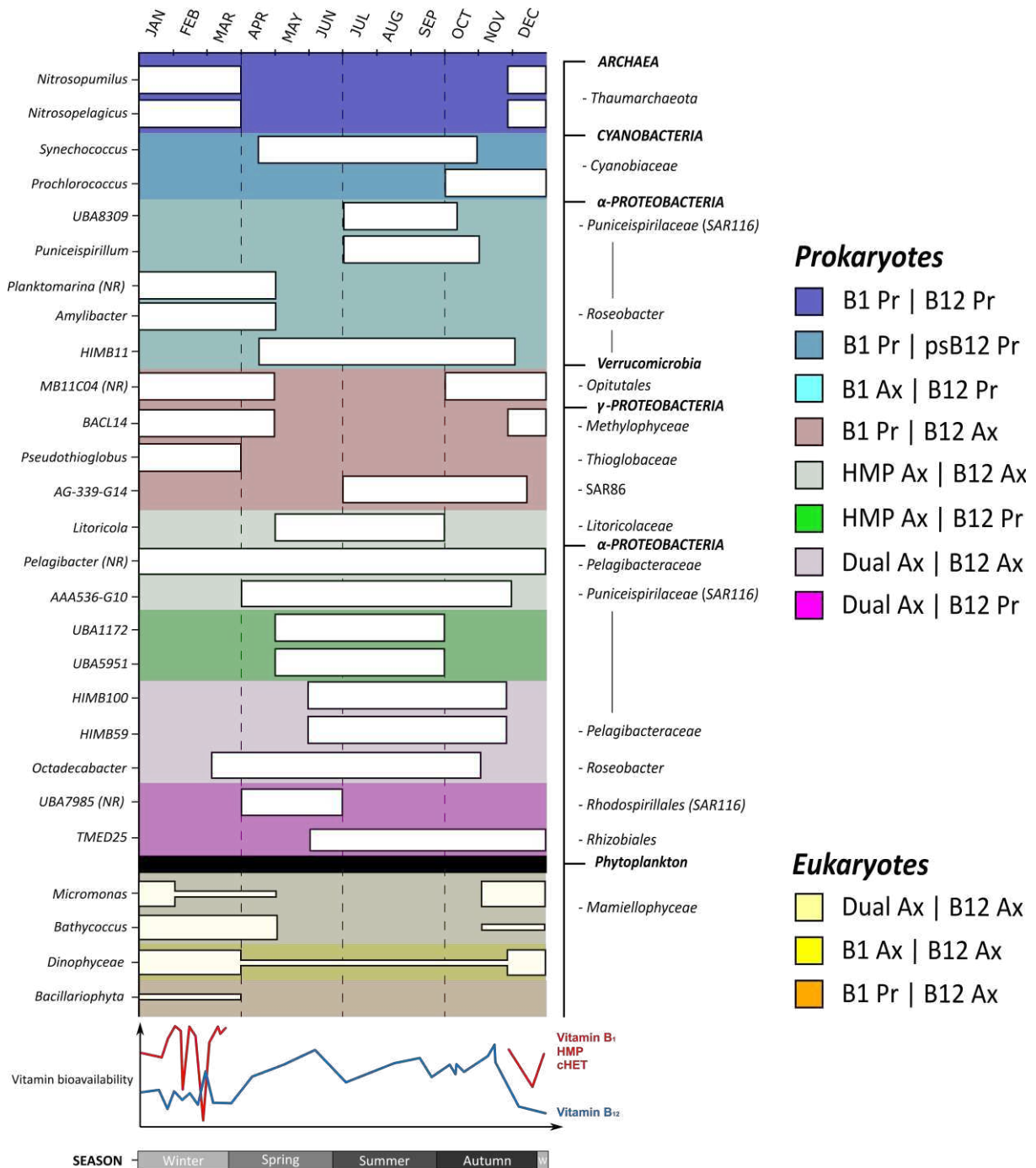


Figure IV.1: General overview of the main actors involved in vitamin B₁ and B₁₂ metabolisms at the SOLA station and their co-occurrence over a year. The horizontal bars represent the most frequent period of occurrence of each microbial genus inferred from their dynamics observed in the 7 years metagenomic time series (Chapter II, III). Each genus is grouped by color according to their vitamin B₁ and B₁₂ functional capacities, and their associated taxonomy is indicated on the right side of the figure. Phytoplanktonic abundances were inferred from Lambert et al., 2019, 2021 (Lambert et al., 2019, 2021). Vitamin B₁, HMP, cHET and B₁₂ bioavailability (bottom panel) were inferred from *Ostreococcus tauri* bioassays conducted with SOLA seawater collected during different seasons. Only the period from December 2016 to March 2017 was covered to assess vitamin B₁ and precursors bioavailability. Vitamin B₁ and B₁₂ functional capacities are indicate as: B₁ Pr (B₁ prototroph); B₁₂ Pr (B₁₂ prototroph); psB₁₂ Pr (pseudo-B₁₂ prototroph); B₁ Ax (B₁ auxotroph); B₁₂ Ax (B₁₂ auxotroph); HMP Ax (HMP auxotroph) and Dual Ax (dual HMP/cHET auxotroph).

vitamin B₁ and B₁₂ are limiting at this period, suggesting that these auxotrophs depend largely on prokaryotic vitamin producers to bloom. Remarkably, drops in vitamin bioavailability was concomitant with picoeucaryotes blooms characterized by a succession between *Bathycoccus* and *Micromonas* (Lambert et al., 2019), suggesting that these microalgae and bloom associated bacteria are important vitamin consumers in winter. The maximal occurrence of the KEGG *metE* in winter confirms the B₁₂ limitations identified by our bioassays and suggest that the metabolically costly B₁₂-independent methionine synthase is a strategy for certain prokaryotes to compensate vitamin B₁₂ limitation in winter. This strategy has also been identified in diatoms (Cohen et al., 2017), which could indicate its widespread occurrence in marine genomes.

In winter *Thaumarchaeota* (*i.e.*, *Nitrosopumilus*, *Nitrosopelagicus*) represented the main potential vitamin B₁₂ synthesizers (**Chapter II**) and to some extent B₁ production (**Chapter III**). Although *Thaumarchaeota* are known to be less abundant than bacteria and cyanobacteria in surface waters (Church et al., 2003; Karner et al., 2001; Massana et al., 2000) they were supposed to be major B₁₂ producers in other temperate (Doxey et al., 2015a) and polar (Heal et al., 2017a) regions. *Prochlorococcus* could also produce both vitamin B₁ and B₁₂ but is unlikely to be a major B₁₂ provider since, as other cyanobacteria, it rather produces pseudo-B₁₂ (Helliwell et al., 2016). Although B₁₂ and pseudo-B₁₂ coexist in the surface ocean, they have been shown to support different enzymatic demands, highlighting that cyanobacteria rely on a distinct cofactor from eukaryotes and prokaryotes, unless the latter could remodel it (Grossman, 2016; Heal et al., 2017a; Helliwell et al., 2016; Ma et al., 2020).

Only few bacterial lineages, such as those belonging to *Roseobacter* (*Amylibacter*) and the SAR116-clades (*Planktomarina*) had the potential to produce B₁₂ in winter but they were putative B₁ auxotroph (**Figure IV.1**). Conversely, vitamin B₁ producers belonging to *Gammaproteobacteria* (*Pseudothioglobus*, BAACL14) and *Verrucomicrobia* (MB11C04) were B₁₂ auxotrophs. *Verrucomicrobia*, have been identified as potential vitamin B₁ providers in cold

marine ecosystems (Paerl, Sundh, et al., 2018), and MB11C04 is seasonally observed during summer in the Atlantic Ocean (Alonso-Sáez et al., 2015), suggesting that they may also play an important role in B₁ synthesis in this region. Recurrent cooccurrences between functionally complementary organisms (e.g., putative B₁₂ producer auxotroph to B₁ co-occurs with a putative B₁ producer auxotroph to B₁₂) unveil potential metabolic interdependencies for vitamin and precursor requirements, since both metabolisms could complement each other.

In summary, the winter productive month are characterized by strong vitamin B₁, B₁₂ and precursor limitations, with only a few potential producers belonging to *Thaumarchaeota*. The low vitamin bioavailability suggests that either the vitamin fluxes between producers are high with efficient uptake systems and/or that vitamins exchanges involved cell vicinity. Positive correlations were indeed observed between archaea and phytoplankton belonging to *Mamiellophyceae* and *Dinophyceae*, further suggesting that these types of interactions may exist between eukaryotic phytoplankton and archaea (Lambert et al., 2019). Co-occurrence network analysis of a high frequency timeseries (3 to 7 days of resolution from 2015-2017) unveiled potential interactions such as those between *Bathycoccus* and *Rhodobacterales*, which vary from one year to another (Lambert et al., 2021). These observations further support that functional redundancy may exist between vitamin producers (**Chapter II**).

B. Summer

In summer SOLA is characterized by a stratified water column, the thermocline being around 15 m depth. In our study and earlier ones, sampling was performed at 3 m depth corresponding to more oligotrophic conditions associated to low phosphate and 10°C higher temperature than below the thermocline. Eukaryotic phytoplankton is virtually absent from the upper part of the water column which is dominated by cyanobacteria belonging to *Synechococcus*. At SOLA, B₁₂ bioavailability is higher in summer than in winter (**Chapter II**), either because B₁₂ consumers were less abundant and/or producers more abundant. Phototrophs in summer are

dominated by *Synechococcus* cells which are B₁₂ prototrophs. However, even though *Synechococcus* had higher cell quotas of cobalamin analogs than other B₁₂-producing prokaryotes including *Prochlorococcus*, they produce pseudo-B₁₂ which is not bioavailable to most other consumers (Heal et al., 2017a). Unlike winter, which is dominated by archaeal producers of vitamin B₁₂, in summer, many potential producers of vitamin B₁₂ are bacteria, dominated by *Alphaproteobacteria* belonging to *Roseobacter*-clade (HIMB11), SAR116-clade (UBA8309, *Puniceispirillum*, UBA1172, UBA5951), *Rhodospirillales* (UBA7985) and *Rhizobiales* (TMED25). Overall, the higher B₁₂ bioavailability in the seawater and the lower demand from autotrophs, suggest that B₁₂ may be less limiting than nutrients such as phosphate.

Compared to B₁₂, the picture of B₁ metabolism is less clear in summer than in winter mainly because we lack data on B₁ bioavailability in the water column. Except for *Pelagibacter*, which was detected throughout the year in the metagenomic dataset, HMP auxotrophs and HET/HMP dual auxotrophs had maximal abundances in summer (**Chapter III**). The fact that these organisms need an exogenous source of B₁ or precursors to grow suggests their sufficient bioavailability in seawater at this period. HMP and other pyrimidine-related compounds have been reported to be more stable than B₁, and the predominance of HMP auxotrophs in summer may be linked to the higher UV irradiance and temperature in summer compared to winter (Carlucci et al., 1969; Gold, 1968). Noteworthy, the main potential B₁₂ producers in summer are also auxotrophic to vitamin B₁ (HIMB11, UBA8309 *Puniceispirillum*) or B₁ precursors; HMP for UBA1172, UBA5951) or cHET and HMP for UBA7985, TMED25. *Roseobacter* members are known to provide B₁₂ to auxotrophic phytoplankton in dual co-culture experiments (Cooper et al., 2019; Durham et al., 2015, 2017) and both cultural and genome-based studies suggest their important role in B-vitamin production and community interaction in the ocean (Newton et al., 2010; Seyedsayamdost et al., 2011; Seymour et al., 2017), notably concerning B₁₂ (Cooper et al., 2019; Luo & Moran, 2014; Sañudo-Wilhelmy et al., 2014).

Our microcosm experiments further suggest that in summer, metabolic complementarities exist between B₁ prototrophs and B₁₂ prototrophs, which are auxotrophs to B₁₂ and B₁/HMP/cHET, respectively (**Chapter III**). As described in winter, additional interactions may be detected in summer co-occurrence network analysis in the high frequency time series conducted during three years at SOLA (Lambert et al., 2021).

3. Limits and advantages of KEGG and MAG-driven approaches

As mentioned above, experimental assessment of vitamin functional capacities is challenging and time consuming due to technical limitations (*e.g.*, measurements of vitamins in the seawater) and because most marine bacteria are not cultivable. Metagenomics offer a unique opportunity to build the landscape of vitamin potentialities in such complex communities. However, one should keep in mind that although powerful, this approach has also bias and limits. For this reason, we decided to work on the gene abundance table, *i.e.*, KEGGS, as well as on the Metagenome Assembled Genomes (MAGs). Each of the two methodological approaches has its own advantages and inconveniences.

Gene abundance table analysis enabled us to decipher the seasonal dynamics of functions and associated organisms at the genus level. However, the assignment of a B₁ and B₁₂ functional capacity from KEGGs was difficult in some cases for several reasons: **(1)** the sequences were relatively short, and the taxonomic assignment was not always possible, especially at the species level due to high sequence conservation; **(2)** when considering a genus, there could be heterogeneity in the functional capacity between species. To limit this bias, we removed the rare KEGGs (below 1%) and compared the metabolic potential of gene table and MAG-driven approaches (**Chapter III**); and **(3)** we had to set up rules and thresholds to assign vitamin functional capacities, resulting in potential errors notably when two species from the same genus have a different potential. For example, 5 biosynthesis genes were sufficient to be considered as B₁₂ prototroph, while their absence is required to be considered as B₁₂ auxotroph.

Conversely, MAGs provided a better picture of metabolic potentials, enabling more precise assignment of B₁ and B₁₂ functional capacities, even though many MAGs remained incomplete. In addition, a MAG often represent a chimeric assemblage of different closely related genomes due to bias in the assembling process. For example, *Pelagibacter* was absent from MAGs, even though it was the most abundant in the gene table. Consequently, HMP auxotrophs were far less abundant in the MAGs while dominating the gene table. Furthermore, double B₁ and B₁₂ auxotrophs are abundant in MAGs but very poorly represented in gene table, where abundant B₁ auxotrophs are mostly B₁₂ producers. Despite the differences between both approaches 34 of the 50 most abundant genera in our metagenomic dataset (> 75% sequences) were represented in the MAGs and found in cooccurrences network at SOLA, such as *Amylibacter*, *Prochlorococcus*, *Nitrosopumilus*, BAACL14 in winter, and *Synechococcus*, MB11C04, HIMB11, UBA8309, UBA5951, UBA1172 in summer.

In summary the KEGG and MAG driven approaches have each their advantage and limits, but they are highly complementary to identify seasonal patterns of metabolic potential, and cooccurrences and potential interdependencies between vitamin producers and consumers.

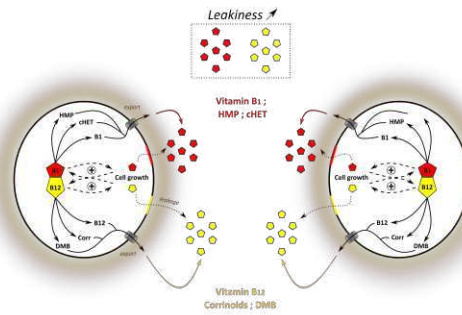
4. Ecological interpretation of vitamin-based interactions

Decades of genomic and cultural efforts have shown that B₁ and especially B₁₂ production pathways are restricted to certain microbial groups in ocean (Gómez-Consarnau et al., 2018; Sañudo-Wilhelmy et al., 2014; Shelton et al., 2019), which could be partially explained by the genome streamlining theory (Giovannoni et al., 2005, 2014). However, the Black Queen Hypothesis suggests that some core species would maintain vitamin biosynthesis pathways, although they are costly, because they are essential to ecosystem functioning (Morris, 2015; Morris et al., 2012). An exhaustive analysis of 77 studies which experimentally demonstrated metabolite cross-feeding in bacterial isolates reported about 135 different interactions mostly involving carbon, nitrogen, amino acids, nucleotides, and vitamins (D'Souza et al., 2018).

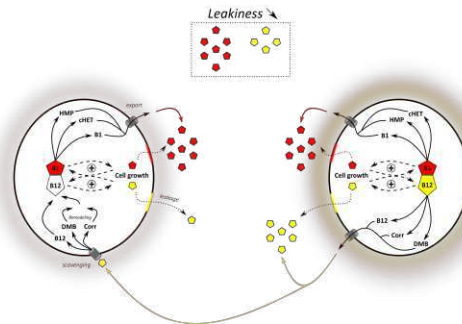
Competition experiments with amino acids showed that auxotrophic bacteria gain a significant growth advantage relative to their prototrophic counterpart when the metabolite is sufficiently available (D'Souza & Kost, 2016). Although vitamin scavenging mechanisms require energy (Jaehme & Slotboom, 2015; Rodionov et al., 2009), the complexity of vitamin biosynthesis pathways, and results from culture-based studies also point to the energy benefits of salvaging strategies, notably for B₁ precursors (Gutowska et al., 2017; Jurgenson et al., 2009; Paerl, Bertrand, et al., 2018). Given that HMP auxotrophs dominate surface ocean (Carini et al., 2014; Paerl, Sundh, et al., 2018) and that precursors can be more abundant than B₁ in the ocean (Suffridge et al., 2020) while promoting higher growth yields in phytoplankton (Gutowska et al., 2017), salvaging strategies could have successfully emerged within eukaryotes and prokaryotes. These energy-saving strategies could be keystone for inter-species syntrophic relationships between vitamin scavenging auxotrophs and producers in ocean (Estrela et al., 2016; Yamagishi et al., 2021).

Interesting evolutionary mechanisms have been proposed to explain the rise, the evolution, and the maintenance of metabolic interdependencies between marine microbes (D'Souza et al., 2018) (**Fig. IV.2**). The fact that many B-vitamin (including B₁₂) syntheses are thermodynamically realizable without enzyme intervention implies that life emerging on Earth first benefited from an abundant supply of vitamins ("prebiotic stock") which was generated by abiotic processes, such as lightning (autocatalytic formation) (Monteverde et al., 2017). This high vitamin leakiness may have encouraged the loss of vitamin biosynthesis genes to avoid their metabolic costs (genome streamlining) and favor salvaging strategies (**Fig. IV.2**).

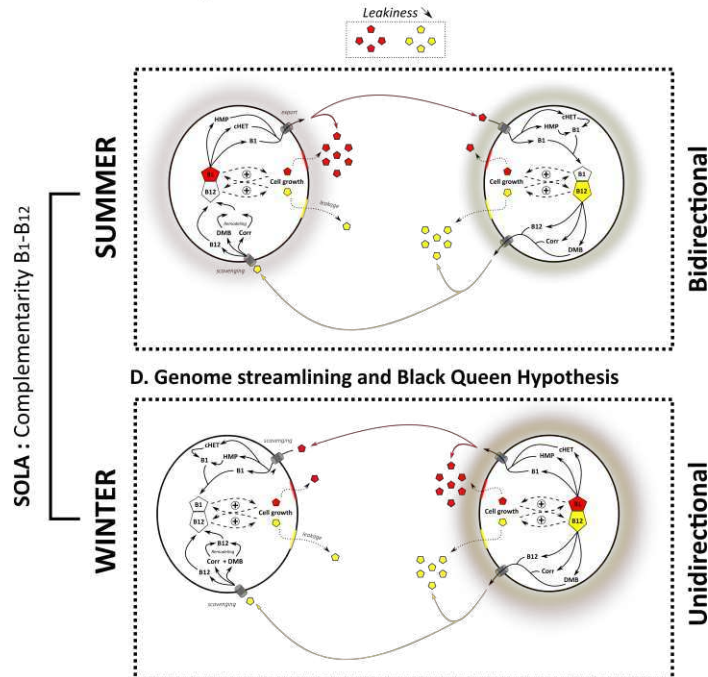
A. Metabolic independency and metabolite leakage/export



B. Emergence of dependencies through adaptive gene loss



C. Emergence of mutual interdependencies



D. Genome streamlining and Black Queen Hypothesis

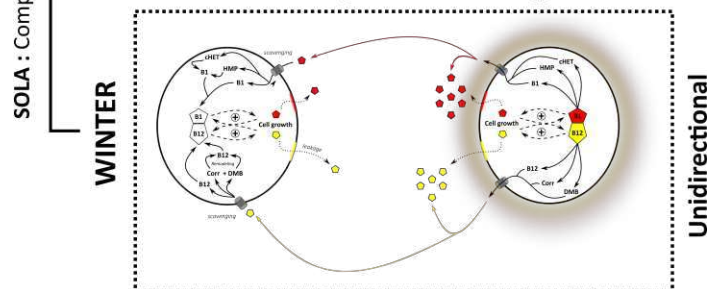


Figure IV.2: Hypothetical model to explain the emergence of metabolic interdependencies considering vitamin B₁ and B₁₂ and their occurrence at SOLA. **(A)** Two co-occurring organisms may initially synthesize both vitamins that are already present in the water column through prebiotic formation. In addition to the *de-novo* biosynthesis used for their own cell growth, a faction of vitamins is passively (leakage) or actively (excretion) released into the environment, becoming available to neighboring microbes and increasing metabolite leakiness. During the evolution, the loss of the vitamin biosynthesis genes in relation to the level of metabolite leakiness can provide a selective advantage for auxotrophs, and lead to the initial establishment of **(B)** unidirectional metabolic interactions, and then potentially, **(C)** bidirectional interactions. **(D)** According to the genome streamlining theory and the Black Queen Hypothesis, marine taxa tend to lose key functions such as vitamin synthesis genes (left panel), while other few taxa must maintain these essential functions (right panel), potentially leading to multiple but unidirectional complementarities. At SOLA, simple but mutual complementarity for B₁ and B₁₂ **(C)** was prevalent in summer, while double but unidirectional B₁ and B₁₂ complementarity **(D)** was prevalent in winter. Adapted from (D’Souza et al., 2018; Morris, 2015; Morris et al., 2012)

Subsequently to gene loss, auxotrophs could develop different interactions strategies to acquire their vitamins, from unidirectional dependencies to mutual interdependencies (D'Souza et al., 2018) (**Fig. IV.2**). Mathematical models notably suggested that adaptation to metabolite leakiness is a valuable strategy to establish symbiotic relationships and is a key factor for the emergence of community interdependencies (Estrela et al., 2016; Yamagishi et al., 2021). The directionality of these interactions is important to consider, as they can have distinct impacts on the stability, diversity, and productivity of microbial communities (Johnson et al., 2020).

Potential syntrophic interactions for vitamin B₁ and B₁₂ were highlighted at SOLA and showed distinct, but recurrent patterns involving different organisms depending on the seasons (**Chapter III**) (**Fig. IV.2C, D**). These interdependencies may have been differentially selected by winter and summer conditions associated with distinct microbial communities, suggesting that abiotic factors can influence the way marine organisms interact. Nevertheless, we assume that our model is very simplistic, given that we are considering only two key metabolites and two organisms, whereas marine microbial communities are extremely complex and diverse.

5. Functional redundancy improves ecosystem stability under perturbations

We have shown that marine microbial community composition exhibits strong recurrences in the coastal Northwestern Mediterranean Sea, even under irregular environmental perturbations (Lambert et al., 2019, Beauvais et al., 2023). However, changes in their taxonomic composition were also identified in response to flood events using a higher sampling frequency (Lambert et al., 2021) or by looking at the finer taxonomic level (*i.e.*, phylotypes) (Beauvais et al., 2023).

In the **chapter III**, we showed that changes in taxonomic composition induced by three consecutive flood events in winter 2012, 2013 and 2014, did not impact the functional potential associated with vitamin B₁₂ production (Beauvais et al., 2023). The level of partial functional redundancy in B₁₂ biosynthesis functions led to an effective ecological insurance for winter

communities at SOLA in response to irregular perturbations, improving the long-term functional resilience and the stability of the ecosystem (Beauvais et al., 2023). These observations are consistent with the "insurance hypothesis" which stated that a high genetic diversity insure the ecosystem against functional loss, as many species might better maintain functions if others fail (Yachi & Loreau, 1999). Such functional redundant organisms (*i.e.*, *Nitrosopumilus* ecotypes) could be seen has potential “Black Queens” which maintain their essential role despite environmental perturbations. Consistent with a previous study conducted on archaeal communities from SOLA (Hugoni et al., 2013), *Nitrosopumilus* ecotypes could be seen as a seed bank of rare taxa which thrive in distinct ecological niches. Others key taxa, such as HIMB11 ecotypes, rather followed the seasonal variations of environmental conditions in summer but remain the main suppliers of vitamin B₁₂ at this period. Since vitamin B₁ and B₁₂ are essential but scarce cofactors, it's not surprising to observe such safety mechanisms, as vitamin production impairing would critically impact ecosystem functioning. Given that auxotrophic phytoplankton seasonally dominate microbial communities in winter at SOLA, we suggest an overriding importance of functional redundancy for B₁₂ production during winter in case of diversity loss (Lambert et al., 2021).

Here we provide answers to the plankton paradox (Hutchinson, 1961), as the maintenance of taxonomically diverse and functionally redundant organisms would ensure the stability of marine microbial assemblages under perturbations. Our observations are consistent with previous studies which showed a high level of functional redundancy in marine ecosystems (Louca et al., 2017; Sunagawa et al., 2015), but are challenged at SOLA were a shift in the composition altered the overall functional attributes of the communities (Galand et al., 2018). These different observations from the same site reflect the distinct levels of redundancy that can be considered (*i.e.*, strict, and partial). Then, the pattern showing that the overall community functions are directly associated to community composition (Galand et al., 2018) could be

moderated by the partial redundancy of specific key functions for ecosystem functioning, such as B-vitamin production (Beauvais et al., 2023). This pattern was notably observed at the finest taxonomic level, with the replacement of functionally redundant ecotypes, and at the highest taxonomic level, with the replacement of phylogenetically distant organisms (Beauvais et al., 2023). Our study thus provides interesting insights into the links between strict and partial redundancy over a long timescale, notably in response to environmental perturbations.

However, the level of functional redundancy may vary depending on the pathways considered, as some functions are widespread in marine microbial lineages (*i.e.*, nitrate reduction, fermentation, aerobic chemoheterotrophy) (Louca et al., 2016), while others are restricted to certain microbial taxa, such as vitamin B₁ and B₁₂ production (Gómez-Consarnau et al., 2018; Sañudo-Wilhelmy et al., 2014; Shelton et al., 2019). In future studies, it will be necessary to determine the level of partial functional redundancy in key metabolic processes, which is a critical point to understand the response of marine microbial communities to future environmental changes. Indeed, structural, and functional shifts will have a significant impact on downstream microbial processes, which remain largely unpredictable as the pace and intensity of global change remain predictive. In this context, long-term time series studies provide a comprehensive overview of a well-defined environment over time, which is a powerful tool to improve our integrated understandings of microbial community dynamics and evolution in response to climate change.

V. **CHAPTER 5: Perspectives**

1. **Experimental testing of B-vitamins functional capacity and potential biotic interactions**

A. **Coculture experiments**

In order to confirm the vitamin-based complementary interactions identified by our genomic approaches, complementary experiments using cultural approaches could be used to validate their ecological relevance. Specific strains associated with taxa identified as key producers of vitamins B₁ and B₁₂ could be isolated and then cocultured with auxotrophic phytoplankton such as *Ostreococcus* or *Bathycoccus*. Co-cultures between prokaryotes that are functionally complementary for their vitamin needs could also be used to validate the putative metabolic interdependencies we showed. To check whether vitamin functional capacities are similar across taxonomic levels, different strains associated with the same species and different species from the same genus (etc.) could also be tested together. However, given that only a tiny proportion of marine microbes are cultivable, the isolation stage could prove tedious. B₁₂-producing *Roseobacter* strains, which are commonly used in co-culture experiments, and an isolate of the archaea *Nitropumilus maritimus*, would be good candidates to test.

B. **Microcosm experiments**

We have used microcosm experiments to assess the importance of vitamins in structuring microbial community and test the interdependence between vitamin B₁ and B₁₂ producers and consumers. During the first six months, my thesis involved a systematic approach, setting up microcosms at different times of the year, testing different vitamin and precursor conditions. I tried to identify changes in community structure in response to *in-situ* bioavailability of these metabolites, without much success. Of the 17 experiments launched between October and March, only one, corresponding to pre-bloom conditions, resulted in picoeukaryotes growth. Although I tested numerous parameters, the cause of these deaths remains unexplained.

Thus, we used a dataset for microcosm experiments that was produced before the beginning of this PhD. Overall, we showed that microbial communities strongly responded to vitamin B₁, B₁₂ and B₁ precursors additions at some time but not at others. Vitamin amendment was often the most efficient when the vitamin was limiting *in-situ*. Several conclusions can be drawn. The addition of vitamin such as B₁ can induce the growth of B₁ auxotroph that produce B₁₂, supporting the growth of B₁₂ auxotrophs, and the emergence of different type of vitamin interdependencies at different time of the year, consistently with cooccurrence networks realized with *in-situ* communities over 7 years. Although microcosms are the only functional evidence we have of metabolic interdependencies, they do not reflect the reality of microbial interactions in the sea, as most marine microbes are not cultivable and their selection during incubations is due to the bottle effect. Another limitation of the microcosm experiment is that it was restricted to community composition with a metabarcoding approach. It would be interesting to use a metagenomic approach to identify the metabolic potential of microcosm communities as a function of various vitamin amendments.

2. Assessing functional redundancy over space and time in ocean

A. Quantification of partial functional redundancy

The level of partial redundancy of key functions in ocean are critically lacking over time, and simple a snapshot of genomes collected at the same location is not a faithful representation of partial functional redundancy. In this context, it would be interesting to assess the level of partial functional redundancy in marine time series and notably at SOLA : **(1)** on a wider range of functions associated with B₁₂ biosynthesis, to determine whether the redundancy is strict (whole pathway) or partial (some functions); **(2)** on other key functions such as other B-vitamins production pathways, to evaluate the extent of the ecological assurance we highlighted for B₁₂; and **(3)** in other oceanic and coastal regions of the global ocean, to establish whether our local but long term observations made at SOLA station can be transposed to other oceanic regions.

There is no standardized method to assess the level of redundancy within a function and define a metric could be a first step forward. In this study, we used an original method that considers the abundances of the most abundant non-rhythmic taxa associated with B₁₂-biosynthesis functions, of which they represented a significant proportion. Nevertheless, we omitted both the rhythmic taxa and the rare non-rhythmic taxa, which may also play a role in the partial redundancy. Thus, our method remains an incomplete description and should be improved to better assess the level of partial redundancy in marine microbial communities.

B. Co-occurrence networks to study functional redundancy

Co-occurrence networks were recently and successfully used to illustrate potential interplays between marine microbes from the temperate Mediterranean Sea (Arandia-Gorostidi et al., 2022b; Deutschmann et al., 2023; Krabberød et al., 2022; Lambert et al., 2021). They structure notably showed strong responses to environmental disturbances in winter at SOLA station (Lambert et al., 2021), implying deep structural and maybe, functional shifts.

Although we showed that an effective ecological insurance allows the maintenance of B₁₂ biosynthesis functions during the winter perturbations at SOLA (Beauvais et al., 2023), we only consider B₁ and B₁₂ production, and we don't know to what extent our co-occurrence networks vary over time and in response to these extreme events. It would be interesting to realize seasonal functional co-occurrence networks year by year to establish if the structure of microbial interplay changes from one year to another and in response to disturbances. To refine our hypotheses, other functional traits that are important in establishing biotic interactions could be further considered in our networks, such as the ability to synthesize other B-vitamins (B₃, B₇, B₉) or metabolites (growth factors), motility (flagella), cell communication (quorum sensing) and secretion systems (Zoccarato et al., 2022). Adding complexity to these ecological interactions, which definitely are, also facilitates to distinguish co-occurrences due to ecological niche sharing, from those implying recurring mutualistic associations. An integrated view of

the functions that can be mobilized in such associations will improve the ecological significance of our observations and enable us to assess whether the level of functional redundancy is sufficient to provide an insurance for key metabolite exchanges from a more global perspective.

3. Improving SOLA time series

Although the 7-year time series at SOLA station has proven its robustness for studying the seasonality of microbial diversity and functions, the functional redundancy and ecological insurance hypothesis, as well as the syntrophic relationships between vitamin auxotroph and producers, the monthly resolution does not capture dynamic changes at a fine temporal scale, such as those induced by perturbation. To match dynamic changes in microbial functions, metagenomic analysis of the high-frequency (weekly / bi-weekly) timeseries could be carried out, as has already been done by metabarcoding over 3 years (Lambert et al., 2021). In addition, the coupling of "omics" data to metagenomics such as metatranscriptomics, metaproteomics and metabolomics could significantly improve the ecological relevance of our observations. Coastal zones of the NW Mediterranean are often limited in vitamins B₁ and B₁₂ which exhibit patchy distributions patterns in the water column. Biotests using *Ostreococcus tauri* allowed to detect vitamin and precursor limitations, but true quantifications could be performed using available methods (Heal et al., 2014; Suffridge et al., 2017). In addition, sediments can be a source of vitamin B₁ and B₁₂ (Monteverde et al., 2015; Zhu et al., 2011) and can be resuspended during winter storms at SOLA. Apply the multidisciplinary approaches presented with the already available deep water (24 m) and sediment samples could improve our understanding of the coupling between water column and sediments for vitamin supply. Finally, the integration of all these biological, physical, and chemical data into complex interaction networks or robust mathematical models will significantly improve our overall understanding the role of vitamins in the marine microbial ecosystem (Boza et al., 2023; Estrela et al., 2016; Hessler et al., 2023; Yamagishi et al., 2021; Zelezniak et al., 2015).

VI. References

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Abstract

Marine microbial communities drive ocean biogeochemical cycles and harbor a tremendous diversity of organisms and functions, which are linked through a complex web of interactions and metabolic dependencies. Vitamins B₁ and B₁₂ are scarce in the ocean and have emerged as essential cofactors for phytoplankton and bacteria, as most cannot produce them *de-novo* (*i.e.*, auxotrophs). These observations suggest that biotic interactions are essential for vitamin exchanges in ocean, but how auxotrophs interact with producers and how these interactions evolve over time remains unknown. To address this question, we conducted a 7-year metagenomic time-series analysis at a coastal station of the NW Mediterranean Sea (SOLA), coupled with bioassay and microcosm experiments. We showed a seasonal and recurrent succession of different potential vitamin B₁₂ producers, dominated by archaea in winter and bacteria in summer, which could use the anaerobic and aerobic vitamin B₁₂ production pathways, respectively. Functional redundancy was observed in vitamin B₁₂ production pathways, providing an ecological insurance against environmental perturbations. We also observed a strong seasonality in metabolisms related to the use of vitamin B₁ precursors in summer (*i.e.*, HMP/cHET), dominated by HMP auxotrophs and to a lesser extent, dual auxotrophs. We reported a higher proportion of vitamin B₁ producers and dual auxotrophs at SOLA than previously estimated in other oceanic regions. Finally, a metagenomic-informed network analysis showed recurrent patterns of co-occurrence between organisms which are complementary for their vitamin B₁ and B₁₂ needs. These metabolic complementarities changed over the seasons, suggesting different patterns of interactions. Finally, vitamin B₁₂, B₁ and precursor amendment experiments conducted in microcosms demonstrated their role in structuring microbial communities and confirmed the existence of changing metabolic complementarities along the year. By exploring the long-term patterns of B-vitamins and precursors seasonality and complementarities, our work constitutes a first step towards understanding the nature of vitamin-based mutualistic interactions and their role in structuring seasonal microbial communities in the ocean.

Keywords: Metagenomic | Time series | Functional communities | Vitamin B₁ & B₁₂ | Metabolic interdependencies

Résumé

Les communautés microbiennes marines sont le moteur des cycles biogéochimiques et abritent une immense diversité d'organismes et de fonctions liés dans un réseau complexe d'interactions et de dépendances métaboliques. Les vitamines B₁ et B₁₂ sont rares dans l'océan et sont des cofacteurs essentiels pour le phytoplancton et les bactéries, car la plupart ne peuvent pas les produire *de-novo* (ils sont auxotrophes). Ces observations suggèrent que les interactions biotiques sont essentielles aux échanges de vitamines dans l'océan, mais la manière dont les auxotrophes interagissent avec les producteurs et comment ces interactions évoluent au fil du temps demeure inconnue. Pour répondre à cette question, nous avons réalisé une analyse métagénomique d'une série temporelle menée sur 7 ans dans une station côtière de Méditerranée Nord-Ouest (SOLA), couplée à des bio-essais et des expériences de microcosmes. Nous avons montré une succession saisonnière et récurrente de différents producteurs potentiels de B₁₂, dominés par les archées en hiver et les bactéries en été, qui pourraient utiliser respectivement les voies de production anaérobie et aérobie de B₁₂. Une redondance fonctionnelle a été observée dans les voies de production de B₁₂, assurant une assurance écologique contre les perturbations environnementales. Nous avons également observé une forte saisonnalité des métabolismes liés à l'utilisation de précurseurs de B₁ en été (*i.e.*, HMP/cHET), dominés par les auxotrophes au HMP et en une moindre mesure, par les doubles auxotrophes aux HMP et cHET. Nous avons observé une proportion élevée de producteurs de B₁ et d'auxotrophes aux cHET et HMP auxotrophes à SOLA par rapport à d'autres régions océaniques. Enfin, une analyse des réseaux basés sur les métagénomomes a montré des cooccurrences récurrentes entre des organismes complémentaires pour leurs besoins en vitamines B₁ et B₁₂. La nature de ces complémentarités métaboliques change au cours des saisons, ce qui suggère différents schémas d'interactions potentiels. Enfin, des expériences d'amendement en vitamines B₁₂, B₁ et en précurseurs menés en microcosmes ont démontré leur rôle dans la structuration des communautés microbiennes et ont confirmé l'existence de complémentarités métaboliques changeantes au cours de l'année. En explorant les schémas à long terme de la saisonnalité et des complémentarités des vitamines B et de leurs précurseurs, nos travaux constituent un premier pas vers la compréhension de la nature des interactions mutualistes basées sur les vitamines et de leur rôle dans la structuration des communautés microbiennes saisonnières dans l'océan.

Mots clefs : Métagénomique | Séries temporelles | Communautés fonctionnelles | Vitamines B₁ et B₁₂ | Interdépendances métaboliques
