Availability of vitamin B$_{12}$ and its lower ligand intermediate \(\alpha\)-ribazole impact prokaryotic and protist communities in oceanic systems

Gerrit Wienhausen, Leon Dlugosch, Renè Jarling, Heinz Wilkes, Helge-A. Giebel and Meinhard Simon

INTRODUCTION

The cofactor vitamin B$_{12}$ (cobalamin, called B$_{12}$ herein) is widely used among prokaryotes and eukaryotes for numerous metabolic functions. Based on genomic sequences to-date, only about one-third of all prokaryotes including Thaumarchaeota, Cyanobacteria and alpha- and gammaproteobacterial lineages are predicted to produce B$_{12}$ [1–4]. Among these prokaryotic groups biosynthesis of cobalamin varies greatly [3, 4]. In oceanic systems Rhodobacterales and Rhizobiales are the main alphaproteobacterial B$_{12}$ producers, whereas the abundant alphaproteobacterial SAR11 clade, 99% of Flavo- and Sphingobacteria and the euryarchaeotal Marine Group II are B$_{12}$ auxotrophic [1, 5–7]. In contrast to the rather limited proportion of prokaryotes predicted or shown to produce B$_{12}$, almost all marine prokaryotic and more than half of all marine eukaryotic microbes are known or genomically predicted to possess B$_{12}$-dependent enzymes [1, 3, 4, 8]. Thus, all marine B$_{12}$-dependent eukaryotic and the majority of marine prokaryotic organisms rely on exogenous supply of this pivotal cofactor. This disparity between dependency and supply of B$_{12}$ results in close microbial interactions [8–12].

In pelagic marine ecosystems, concentrations of B$_{12}$ greatly vary, ranging from highest concentrations of \(~90\) pM to below the detection limit of \(<1\) pM in surface waters [11, 13–15]. B$_{12}$ has been shown to be growth-limiting for distinct phytoplankton groups such as diatoms and dinoflagellates and thus crucial in shaping phytoplankton community composition [12, 16–19]. However, despite the fact that many marine bacteria are B$_{12}$ auxotrophic no information exists whether growth and composition of prokaryotic microbial communities in pelagic marine systems are also affected by the availability of B$_{12}$.

Cobamides summarise a group of coenzymes of the B$_{12}$-family, consisting of a corrin ring centred around a cobalt ion to which a lower axial ligand, consisting of a phenolic, purine, or benzimidazole derivative, is attached. The chemical form of the lower ligand alters the cobamide structure and thereby affects the cofactor binding and consequently the coenzyme catalysis [4, 20–23]. Availability of dissolved exogenous cobamide variants differ greatly, depending on the ambient prokaryotic communities [3, 4, 12, 24]. Cobalamin, the most common and bioavailable cobamide cofactor in near-surface marine ecosystems, comprises 5,6-dimethylbenzimidazole (DMB) as lower ligand (Fig. 1A), whereas pseudocobalamin encompasses adenine as lower ligand and is synthesised by Cyanobacteria and less bioavailable to most other organisms [20, 25]. Notably, some prokaryotic as well as
eukaryotic microorganisms acquired an adaptive strategy to remodel unusable cobamides in the presence of suitable lower ligands [23, 25]. In particular, \( \alpha \)-ribazole, the activated form of DMB, has been shown to be taken up by a bacterium, enabling the synthesis of cobalamin, thus complementing the genetic deficiency of the biosynthesis of this lower ligand intermediate [26]. Based on comparative genome analyses, 17% of all bacterial B\( _{12} \) auxotrophs feature partial biosynthetic pathways of cobamides and thus presumably possess the capability to salvage B\( _{12} \) building blocks [3, 27]. Lower ligand salvaging towards the most common and bioactive cobalamin is mostly tested by targeting DMB which has been detected in soil, a freshwater creek and the intestine of animals [24, 28]. Nothing is known about its presence in marine waters. However, there is some information available that the lower ligand intermediate \( \alpha \)-ribazole is present in marine waters. A molecular formula matching that of \( \alpha \)-ribazole has been detected in marine dissolved organic matter (DOM) and within the exometabolome of several marine prokaryotes [29–32]. Life of the majority of marine microorganisms depends on the availability of B\( _{12} \), however only a minority of marine prokaryotes holds the genetic apparatus for its de novo synthesis. Hence we hypothesise that uptake of \( \alpha \)-ribazole, thus salvaging its biosynthetic deficiency, is a pathway to overcome B\( _{12} \) auxotrophy and to function as crucial element in microbial vitamin networks, especially for prokaryotic communities. To test whether growth and composition of pelagic marine prokaryotic communities are affected by the availability of B\( _{12} \) or \( \alpha \)-ribazole we supplemented mesocosms in the Pacific Ocean with these compounds and monitored growth activity and composition of the prokaryotic communities over several days.

**MATERIAL AND METHODS**

**Location, sampling, hydrography and set-up of mesocosm experiments**

Mesocosm experiments were carried out in three different biogeographic regions of the Pacific Ocean, the South Pacific Subtropical Gyre (SPSG; station 2, M1), the Pacific North Equatorial Current (PNEC; station 7, M2) and the North Pacific Polar Frontal region (NPPF; station 7, M3). At these stations, temperature and salinity profiles and concentrations of chlorophyll a were determined. They are part of a transect between 30°S and 59°N investigated for hydrographic, biogeochemical and microbial variables during a cruise with RV Sonne (SO248) from May 3\textsuperscript{rd} to June 1\textsuperscript{st} 2016. Further details are described elsewhere [33, 34]. Here we present data of the depth of sampling for the mesocosm experiments, 20 m at stations 2 and 14, and 75 m at station 7 (Table 1). We collected water from station 2, M1), the Pacific North Equatorial Current (PNEC; station 7, M3) were conducted. The graphic was created using ocean data view. For further details of stations see Table 1.

**Mesocosm experiments**

The Nalgene bottles were first washed twice with acidified ultrapure water (MilliQ, pH 2) and then rinsed with the respective mesocosm water. The bottles were filled to a volume of 23 l and amended with FeSO\( _{4} \) (5 mM), Na\( \text{H}_{2}\text{PO}_{4} \) (1 mM), Na\( \text{SiO}_{3} \) (32 mM), NaNO\( _{3} \) (16 mM) and CoCl\( _{2} \) (500 pM) to avoid a B\( _{12} \) co-limitation (Supplementary Table S1) as observed by previous studies [13, 14, 16]. All experiments were conducted in triplicates with supplements of B\( _{12} \) (cyanocobalamin; 100 pM; Sigma-Aldrich, St. Louis, MO, USA) or \( \alpha \)-ribazole (100 pM; own preparation, see below) and a negative control. Incubation for six days was performed at the respective in situ temperature in a temperature controlled room (Table 1) and illuminated in a daylight of 12:12 hours (15–20 \text{µmol} \text{photons} m\(^{-2}\) s\(^{-1}\)).

Subsamples were withdrawn periodically to assess prokaryotic cell numbers, chlorophyll autofluorescence and heterotrophic prokaryotic production (HPP). Subsamples for analysing the composition of the pro- and eukaryotic microbial communities and metatranscriptomic analyses were withdrawn throughout the experiment. Metatranscriptomic analyses were only conducted for experiments M1 and M2.

**Preparation and purity verification of \( \alpha \)-ribazole**

Alpha-ribazole is not commercially available. Therefore it was prepared by alkaline hydrolysis of B\( _{12} \) and purified as described in detail in the Supplementary Methods (see also Supplementary Fig. S1).

**Prokaryotic abundance, chlorophyll autofluorescence and HPP**

Prokaryotic abundance, chlorophyll autofluorescence of eukaryotic pico- and nanophytoplankton were measured immediately after sampling on board using a BD Accuri C6 cytometer (BD Biosciences, Heidelberg, Germany) as described elsewhere [33, 34]. Briefly, abundance of heterotrophic prokaryotes was determined after staining with SybrGreen I and that of autofluorescent pico- and nanophytoplankton by re-gating data from a plot of fluorescence FL3 (red, >670 nm) vs. FL2 (orange, 585 ± 20 nm) to a cytogram of FL4 (red, 675 ± 12.5 nm) vs. forward scattered light.
Table 1. Location, hydrographic and microbial features at the mesocosm stations.

| Province, mesocosm ID | Station no. | Longitude | Latitude | Depth (m) | Date (2016) | Temperature (°C) | Salinity | Chl a | Prokaryote cells | HPP (ng DFAA (µg) | Growth rate (day−1) |
|-----------------------|-------------|-----------|----------|-----------|-------------|------------------|---------|------|-----------------|------------------|------------------|
| SPSG (M1)             | Station 1   | 178.21°E | 26.99°S | 20        | 04 May      | 23.22            | 35.64   | 0.27 | 4.5             | 34.32            | 0.02             |
| PNEQ (M2)             | Station 2   | 179.40°E | 04.65°N | 60        | 14 May      | 28.71            | 34.32   | 0.10 | 4.4             | 382.1            | 0.14             |
| NPB (M3)              | Station 7   | 128.75°E | 45.00°N | 20        | 24 May      | 58.64            | 33.31   | 0.72 | 9.7             | 93.0             | 0.13             |
| NPB (M4)              | Station 14  | 128.75°E | 45.00°N | 20        | 24 May      | 58.64            | 33.31   | 0.72 | 9.7             | 93.0             | 0.13             |

Given are the biogeographic provinces (SPSG: South Pacific Subtropical Gyre; PNEQ: Pacific North Equatorial Current; NPB: North Pacific Equatorial Frontal Current), ID of the mesocosm experiments, station number, date of sampling, depth of sampling, 75 m, are not available. For further details of provinces see Giebel et al. [33].

Microbial community analysis

The composition of the prokaryotic and eukaryotic microbial communities was analysed in all experiments three hours after the onset and at days 3 and 6. Five hundred ml of water were withdrawn from the mesocosms and concentrated by vacuum filtration on a 0.2 µm polycarbonate filter (Millipore, Burlington, MA, USA), immediately deep-frozen in liquid nitrogen and stored at –80°C. DNA and RNA were extracted simultaneously as described elsewhere [36].

Prokaryotic and eukaryotic microbial communities were analysed using a taxonomic marker gene sequencing. For prokaryotes the V3-V4 variable region of the 16S rRNA gene was targeted and for eukaryotes the V9 variable region of the 18S rRNA gene was used. PCR amplification of the extracted DNA and sequencing of the purified amplicon libraries on a MiSeq sequencer (Illumina, San Diego, USA) were carried out as described in the Supplementary Methods. Additionally, for targeting the active prokaryotic communities, cDNA of the V3-V4 region of the 16S rRNA was sequenced after preparation from RNA extracts as described in the Supplementary Methods. DNA reads in the demultiplexed sequencing files were trimmed for quality using Trimmomatic v. 0.32 [39] with the settings SLIDINGWINDOW:5:3 and MINLEN: 51. The trimmed reads were dereplicated and formatted for use in the UPARSE workflow [41]. The dereplicated reads were clustered using the usearch v. 7.0.1090 -cluster_otus command with default settings, and the resulting OTU abundances were estimated using the usearch v. 7.0.1090 -usearch_global command with id 0.97 -maxaccepts 0 -mamarejects 0. Taxonomy was assigned using the RDP classifier [42] as implemented in the parallel_assign_taxonomy_rdp.py script in QIIME [43], using –confidence 0.8 and the SILVA database, release 132 [44].

The results were analysed in R v. 3.5.0 (R Core Team, 2017) through the Rstudio IDE using the ampvis package v.2.3.16 [45]. Alpha diversities were estimated after rarefaction using the R-packages ‘picante’, ‘cairo’, ‘vegan,’ and ‘ape’. Rarefaction curves and statistical evaluation data are presented in Supplementary Fig. S2 and Supplementary Data 1. The 14 most abundant OTUs, from their mean relative abundance in a given mesocosm, are presented as Box-Whisker plots and significant differences between the treatments compared to the control were calculated running two-sample Student’s t test (see Supplementary Data 1). Rarefaction curves showed that sampling efforts in the mesocosm experiments varied and that in M3 saturation was almost reached (Supplementary Fig. S2). All sequencing and bioinformatic analyses were carried out by DNASense (Aalborg, Denmark).

Metagenome and metatranscriptome analyses

Metagenome and metatranscriptome library preparation and sequencing was carried out as described in detail in the Supplementary Methods on a HiSeq and NovaSeq system (Illumina), respectively by DNASense (Aalborg, Denmark). Illumina reads were quality checked and low-quality regions as well as adaptor sequences were trimmed using Trimmomatic 0.36 [39] (ADAPTER:2:30:10 SLIDINGWINDOW:4:25 MINLEN:100). The high quality (HQ) reads were assembled using metaSPAdes 3.11.1 [46] using k-mer sizes 21, 33, 55 and 77. Contigs smaller than 210 bp and average coverage <2 were discarded. Gene-coding sequences of the assembled contigs were predicted using Prodigal 2.6.2 in meta-mode [47]. Genes shorter than 210 bp and longer than 4500 bp were discarded resulting in 6.83 M unique gene sequences.
Gene catalogue generation

The gene catalogue was generated as previously described [48] clustering at 95% identity, resulting in 1.89 Mio cluster centroids. These were taxonomically classified using Kaiju 1.6 ([49]) against NCBI nr database (downloaded 2018-05-29) and the proGenomes database [50] (downloaded 2019-07-26). Genes were functionally classified against the Kyoto Encyclopedia of Genes and Genomes (KEGG) as previously described [48]. 39% of all sequences were assigned to a KEGG orthologue (KO). Both mesocosm M1 and M2 sequencing efforts were near saturation as shown by rarefaction (Supplementary Fig. S3).

Transcriptome read abundance and normalisation

Reads were filtered for ribosomal RNA using SortMeRNA version 4.0.0 [51] matching against concatenated SILVA 90% database (165, 185 and 235 rRNA) [44] (release 132) and the rfn 5s database [52]. HQ Illumina reads longer than 75 bp were mapped to the representative gene sequences using bowtie2 [53] 2.3.5 (--very-sensitive-local mode). SAMtools [54] version 1.9-S8-gbd1a409 was used to convert the SAM alignment file to read abundance tables, discarding reads that did not map to any non-redundant sequence. Normalisation and scaling factors were calculated and applied as previously described [48, 55]. Analysed transcripted genes were normalised to the gene abundance of the taxonomic group under investigation.

Metatranscriptome evaluation

For each prokaryotic annotated gene transcript, the log2-fold change and p value was calculated, treatments (B12 or α-ribazole addition) with negative control on each sampling point (mesocosm 1 and 2, 3 h, days 1 and 3). Volcano plots were generated using the R-package ‘cairo’. The majority of gene transcripts with log2-fold change >2 and p < 0.05 were associated to key proteins of photosystem I and II and carbon fixation of Prochlorococcus (Supplementary Fig. S4), ammonium and amino acid transport and metabolism of Pelagibacteraceae as well as ammonium transport, B12 transport and cell motility of Gammmaproteobacteria. Therefore, we grouped protein coding transcripts involved in cellular functions of respective taxonomic groups. The considered genes of the investigated cellular functions are listed in Supplementary Data 1. In order to understand the effect of B12 and α-ribazole supply on the metabolism of the B12 pathway and the B12 outer membrane permease, encoded by btuB in the prokaryotic community, we grouped respective gene transcripts. Differentially expressed genes between treatments and negative control were tested by two-sample Student’s t test (p < 0.05; Supplementary Data 1).

Data deposition

Sequence data of the microbial community, metagenome and metatranscriptome analyses were deposited in the European Nucleotide Archive (ENA) at EMBL-EBI with accession numbers PRJEB43936, PRJEB43939, PRJEB43941, PRJEB43944 and PRJEB43946, using the data brokerage service of the German Federation for Biological Data (GFBio, [56]), in compliance with the Minimal Information about any (X) Sequence (MIXS) standard [57].

RESULTS

During a cruise across the Pacific Ocean from New Zealand to Alaska we carried out mesocosm experiments in three biogeochemical provinces, SPSG, PNEQ and NPPF, to test for the effect of B12 and α-ribazole on growth and related changes in microbial community composition (Fig. 1B). These biogeochemical provinces differ greatly regarding hydrographic, biogeochemical, biotic and microbial features [33, 34]. SPSG encompasses the most oligotrophic and permanently stratified region of the Pacific Ocean with water temperatures of 26°–30°C. In the upper 100 m, we encountered nitrate concentrations close to or below the detection limit (0.3 µM), concentrations of chlorophyll a below 0.35 µg l–1, except at the deep chlorophyll maximum (DCM) around 90 m depth, and rates of HPP not exceeding 30 ng C l–1 h–1. PNEQ adjacent to the equatorial upwelling is more productive. The upper 100 m are as warm as SPSG and concentrations of inorganic nutrients, chlorophyll a, POC and rates of HPP were also low in the upper 40 m but increased at 60 m and below. At these depths, inorganic phosphate concentrations reached 0.23 µM, well above the detection limit of 0.04 µM, and HPP 382 ng C l–1 h–1. Chlorophyll a concentrations remained as low as in SPSG with a maximum at the DCM at 105 m. Prokaryotic cell numbers were also as low as in SPSG, but bulk growth rates were much higher and reached 0.71 d–1, reflecting the metabolically highly active heterotrophic prokaryotic communities. The cold and nutrient-rich NPPF exhibited highest concentrations of chlorophyll a of 0.7–1.1 µg l–1 and highest numbers of prokaryotes (up to 1.8 x 108 cells ml–1) but lower rates of HPP than in PNEQ, 90–140 µg C l–1 h–1. Highest values consistently occurred at 20 m depth and continuously decreased below. Further details of the biogeochemical and microbial features of these provinces, stations and depths of sampling are published elsewhere [33, 34] and shown in Table 1.

Microbial abundance and HPP

In experiment M1 in SPSG abundance of heterotrophic prokaryotes increased continuously and similarly in both treatments and the control (Fig. 2A). In experiment M2 in PNEQ abundance of heterotrophic prokaryotes increased only until day 3 and then remained constant (Fig. 2B). In the B12 treatment, numbers remained lower onwards from day 1 than in the α-ribazole treatment and the control. This reduced prokaryotic abundance is consistent with the hypothesis of enhanced protist grazing in the B12 treatment. Relative proportions of the uncultured marine stramenopiles (MAST) group 1 and “other” protist groups, comprising more than 45% of total eukaryotic reads, were enhanced in this treatment relative to the control and the α-ribazole treatment on days 3 and/or 6 (Supplementary Fig. S5). As these MAST lineages comprise heterotrophic protists which can graze on nanoplankton and bacteria [58, 59] we hypothesise that an enhanced grazing pressure in the B12 treatment led to reduced numbers of prokaryotes. In experiment M3 in NPPF with the highest initial prokaryotic abundance, cell numbers decreased slightly from the beginning until day 3 and more so thereafter. Those in the B12 treatment were lower than in the control and partially also than in the α-ribazole treatment (Fig. 2C). As “other” protist and the MAST 1 groups in M3 constituted more than 50% of total eukaryotic reads and significantly higher values in the B12 treatment relative to the control at day 6 these data are also consistent with the protist grazing hypothesis (Fig. 2C, Supplementary Fig. S6). Initial abundances of eukaryotic picoplankton and nanoplanckton were highest in M3, in line with the highest concentrations of chlorophyll a (Table 1). Abundances decreased in all experiments continuously after day 1 whereas initially in experiment M2 they increased and in M3 remained constant (Supplementary Fig. S7). In M1 data for hour 3 were not recorded. Rates of HPP exhibited positive responses to the supply of B12 and α-ribazole in all three mesocosm experiments. In experiment M1, HPP increased until day 3 and exhibited twofold higher values in the B12 treatment at days 3 and 6 and 1.5-fold higher values in the α-ribazole treatment at day 3 relative to the control (Fig. 2D). In experiment M2, rates of HPP in the α-ribazole and B12 treatments were two- and threefold higher than in the control already at the initial sampling at hour 3 (Fig. 2E). At this station and in this experiment, prokaryotic growth activities were very high, also in the control. This is evident from the very high rates of HPP and prokaryotic growth at 60 m depth, in fact the highest value at this depth over the entire transect (Table 1 and reference [34]). At hour 3 we observed in both treatments and at day 2 in the B12 treatment significantly higher rates of HPP than in the control and recorded an up and down pattern throughout the experiment (Fig. 2E and Supplementary Data 1). In experiment M3 in NPPF, rates of HPP were lowest (Fig. 2F). Highest values occurred on day 3 and decreased thereafter, in line with decreasing prokaryotic abundance. Addition of B12 yielded a
significantly higher HPP rate at most sampling points throughout this experiment. Similarly, α-ribazole supply yielded significantly higher rates at days 4 and 5 than the control (Supplementary Data 1).

Composition of microbial communities
In experiments M1 and M2, the initial prokaryotic communities were largely dominated by Prochlorococcus and two sub-lineages of the SAR11 clade. In M2, the SAR86 clade affiliated to Oceansiophillales, Rhodospirillaceae and euryarchaeotal Marine Group II (Thermoplasmatales) constituted 5–7%. Other lineages, including SAR16, Flavobacteriaceae, Rhodobacteraceae, Alteromonas and Marine Group III, constituted less than 3% each at the beginning of both experiments (Supplementary Figs. S8 and S9). In experiment M3, the prokaryotic community initially was largely dominated by Alphaproteobacteria, predominantly various lineages of the SAR11 clade, and Flavobacteria, mostly sub-lineages of the NS2, NS4 and NS5 groups, constituting 58% and 21% of the total. Gammaproteobacteria, predominantly the SAR86 clade, constituted 11% and other lineages including Cyanobacteria and Thermoplasmatales < 3% each (Supplementary Fig. S10). The initial eukaryotic communities in M1 and M2 were dominated by Dinoflagellata, Protalveolata and Retaria (Supplementary Figs. S11 and S5) whereas in M3 it was more diverse and dominated by Ochrophyta, including diatoms, and MAST-1, representing 21% and 10% of the total, respectively (Supplementary Fig. S6). In all initial communities, “other” eukaryotic groups, representing accumulated lineages of < 3% each, constituted large proportions. The initial composition of the mesocosm communities was very similar to that of the ambient communities at the stations and depth ranges at which the water for the experiments was collected (Supplementary Fig. S12).

During the course of all experiments and as response to the B12 or α-ribazole supply the composition of the prokaryotic as well as eukaryotic communities changed substantially. The global effects of these observations are visualised by a non-metric multidimensional scaling (NMDS) analysis based on the V3-V4 fragment of the 16S rRNA gene and its transcript (cDNA) and of the V9 fragment of the 18S rRNA gene (Fig. 3). The clustering exhibits differences not only over time but also reflecting the effect of the B12 and α-ribazole supply on the composition of the prokaryotic and eukaryotic communities. Details of the temporal differences of the major prokaryotic groups including Prochlorococcus, the SAR11 and SAR86 clades, Rhodospirillaceae, Pseudonalteromonas, Flavobacteriaceae and Marine Group II are shown in Supplementary Figs. S8, S9 and S10. The different treatments led also to pronounced and significant changes in single prokaryotic groups. In M1, the accumulated OTUs affiliated to Oceansiophillales increased in the B12 treatment relative to the other treatment and control at day 3 and 6 with a highly significant increase on the latter day (Fig. 4C). In M2, the percentages of Marine Group III (Thermoplasmatales), Alteromonas, one family of the SAR86 clade and Rhodospirillaceae increased in either the B12 or α-ribazole treatment or both relative to the control on days 3 and/or 6 (Fig. 4F, Supplementary Fig. S9). In M3, significant changes of the prokaryotic community were predominantly caused by enhanced proportions in the B12 treatment of the SAR11 clade and Oceansiophillales at day 6, similar to M1, and reduced proportions of flavobacterial lineages (Fig. 4G, Supplementary Fig. S10).

The composition of the eukaryotic communities also changed as a result of the B12 and α-ribazole supply. In M1, the community composition among the two treatments and the control remained rather similar except for reduced fractions of Ochrophyta in the B12 treatment (Supplementary Fig. S11). In M2, lineages MAST 1 and “other” significantly increased on day 6 in the B12 treatment relative to the control whereas Protalveolata decreased (Supplementary Fig. S5 and Supplementary Data 1). In M3, shifts in the eukaryotic community correlated with increased proportions of the heterotrophic lineages MAST-1, MAST-7 and Picconamidada and reduced proportions of Ochrophyta and Prymnesiades at days 3 and/or 6 in the B12 treatment relative to the control (Supplementary Fig. S6). The strong relative increases of the heterotrophic flagellates MAST-1 and MAST-7 in the B12 treatment are consistent with the hypothesis of a grazing-induced decrease of prokaryotic cell numbers in this treatment.

Fig. 2  Prokaryotic abundance and heterotrophic prokaryotic production (HPP) in the course of the mesocosm experiments. A–C Prokaryotic abundance of the treatments with additions of α-ribazole and B12 and an untreated control. Shown are means of triplicates and standard deviations. Significant differences of the means of a treatment and control (T-test, *p < 0.05, **p < 0.01, ***p < 0.001).
The major effects of the B12 and α-ribazole supply on prokaryotic abundance, HPP, responsive prokaryotic and eukaryotic lineages are summarised in Fig. 5.

**Metatranscriptomic responses**

The prokaryotic communities exhibited transcriptional responses predominantly to the B12 treatment in experiments M1 and M2 in which metatranscriptomic analyses were conducted. We focused our analyses on identified genes affiliated to the major prokaryotic groups in both experiments which exhibited high transcriptional responses and/or encode proteins which are potentially affected by B12. As in experiment M1 transcriptional responses were much more pronounced than in M2 we focus mainly on the former. For results of M2 see Supplementary Fig. S13. Transcription of *btuB*, encoding the outer membrane permease for B12, for subsequent import into the cell [27], was downregulated in the B12 treatment relative to the control at hour 3 and day 3 (Fig. 6). Transcripts affiliated almost exclusively to *Oceanospirillales* of Gammaproteobacteria, indicating that this order directly took advantage of the B12 supply by reducing energetic costs for the biosynthesis of its importer. We did not find a transcriptional downregulation of genes associated to B12 biosynthesis pathways in both treatments relative to the control. Similarly, we did not find a transcriptional upregulation of genes encoding enzymes requiring B12 as cofactor such as the B12-dependent methionine synthase *metH*. As often few transcripts of a single gene and taxon were detected as significantly upregulated at one sampling point we pooled transcripts of individual taxa to major prokaryotic groups. Significant metabolic responses were found for *Prochlorococcus*, *Oceanospirillales* and *Pelagibacteraceae*. Transcripts affiliated to *Prochlorococcus* constituted 23.3 ± 4.4% in M1 and 14.2 ± 2.3% in M2, respectively. *Prochlorococcus* also constituted proportions of 15-45% of the prokaryotic communities in both experiments until day 3 (Fig. 4, Supplementary Fig. S9). In M1, already three hours after the B12 supply, the number of transcripts of genes encoding various functions related to the light and dark reaction of photosynthesis significantly increased relative to the control and the α-ribazole treatment (Fig. 6). Upregulated transcripts included genes encoding key features of photosystem I and II, cytochrome b, the magnesium chelatase, a key enzyme of chlorophyll biosynthesis, ATP synthase, CO2-fixation via the Calvin Benson cycle and monosaccharide metabolism via the pentose phosphate pathway. This pathway is closely linked to the Calvin Benson cycle in *Prochlorococcus* [60]. Further, a gene encoding a sulfate permease which mediates also the uptake of nitrate [61] and genes encoding the reduction of nitrate to nitrite (*nirA*) and further to ammonium (*nitA*) were upregulated (Fig. 6). Nitrate reduction was recently shown to be an important trait of several lineages of *Prochlorococcus* [62, 63]. Some of these transcriptional upregulations were still present at day 3 (Fig. 6). For *Oceanospirillales*, in addition to the transcriptional downregulation of B12 import, the gene encoding ammonium transport (*amt*) was upregulated at hour 3 whereas genes encoding motility were downregulated at this time point and also at day 3 (Fig. 6). For *Pelagibacteraceae*, the genes encoding ammonium transport (*amt*) and binding to glutamate (*gltB*, *gltD*) were also upregulated at hour 3 whereas others encoding transport of branched chain amino acids (livK), propanoate (adh1, acuL) and methionine metabolism were downregulated at this time point and/or at day 3 (Fig. 6). For a complete list of regulated genes see Supplementary Data 1. The major effects of the B12 and α-ribazole supply on the transcriptional response of genes or gene families are summarised in Fig. 5.

**DISCUSSION**

Supply of B12 and α-ribazole enhanced HPP and prokaryotic growth and led to changes in the composition of prokaryotic and eukaryotic communities, visualised globally by NMDS analyses.
(Fig. 3), in all three mesocosm experiments in distinctly different biogeographic provinces of the Pacific Ocean. Effects of B12 were greater than of α-ribazole. Supply in particular of B12 also affected expression of genes of the major groups of the prokaryotic community predominantly in experiment M1 and to a lesser degree in M2. Surprisingly, Prochlorococcus yielded the most pronounced transcriptional response even though most known cyanobacteria, including Synechococcaceae, produce and use pseudo-B12 [12, 25]. These findings add important information to previous observations that supply and availability of B12 affects growth of various eukaryotic phytoplankton groups in different oceanic regions [8, 16–19].

**Effects of B12 and α-ribazole on heterotrophic prokaryotes**

Genomic and metatranscriptomic analyses predict that B12 is produced only by about one-third of prokaryotes, mainly comprising Thaumarchaeota, Cyanobacteria and alphaproteobacterial lineages including Rhodobacteriales, Rhizobiales and 80% of Rickettsiales [1, 3, 5, 12]. These analyses further predict B12 auxotrophy for the alphaproteobacterial lineage SAR11, marine Flavobacteria and Sphingobacteria and for 54% of Oceanospirillales [1, 3, 5]. For the SAR86 clade, affiliated also to Oceanospirillales but not included in the mentioned analyses, genomic information on B12-related metabolism is scarce. In the only sequenced genome of subclade B, B12 biosynthesis is
encoded whereas in the only sequenced genome of subclade A it is not and for subclades C and D no information is available [64]. Genomic data further show that most Alteromonadales are B12 auxotrophic [1] but a few lineages such as Alteromonas macleodii encode a B12-dependent (metH) and a B12-independent methionine synthase (metE) [65]. For E. coli it was shown that the former is >100-fold more efficient than the latter [66] and for Vibrio cholerae metH was shown to be operational when B12 is available and metE knocked out [67]. These findings indicate that the simple division into B12-auxo- and prototrophic organisms may not be sufficient to provide a comprehensive understanding of the B12 requirements in a given ecosystem. Thus, it is also conceivable that A. macleodii uses the metH-encoded methionine synthase when B12 is available and that Alteromonadales are responsive to B12 supply irrespective of auxo- or prototrophy. Our results corroborate the genomic predictions because in all three experiments one or several of these lineages or sublineages responded to the addition of B12 by enhancing their proportions in transcription of genes encoding different proteins in Gammaproteobacteria (mainly SAR86 clade), Pelagibacteraceae and Prochlorococcus. -: decrease; +: increase. d1, d2, d3, d4, d6: day 1, 2, 3, 4 and 6; h3: hour 3. ++ or – indicate strong changes. When no + or no – is given no significant change between a treatment and the control was detected. M3 was not analysed for metatranscriptomics (accentuated in grey). For further details and specific responses of individual lineages see Figs. 4 and 6 and Supplementary Figs. S5 and S7 to S12. For other abbreviations see text.

Fig. 5  Environmental and biotic features of the locations of mesocosm experiments M1, M2 and M3 and major effects of the supply of B12 and α-ribazole in the course of the experiments. Treatment effects include changes in the composition of prokaryotic and eukaryotic communities and changes in transcription of genes encoding different proteins in Gammaproteobacteria (mainly SAR86 clade), Pelagibacteraceae and Prochlorococcus. ++: increase; –: decrease; d1, d2, d3, d4, d6: day 1, 2, 3, 4 and 6; h3: hour 3. ++ or – indicate strong changes. When no + or no – is given no significant change between a treatment and the control was detected. M3 was not analysed for metatranscriptomics (accentuated in grey). For further details and specific responses of individual lineages see Figs. 4 and 6 and Supplementary Figs. S5 and S7 to S12. For other abbreviations see text.
consistent with our findings. This suggest that Oceanospirillales benefitted from available B12, resulting in an enhanced abundance in experiment M1. This order presumably outcompeted the SAR11 clade in the uptake of B12, as we identified no downregulated transcripts of btuB affiliated to the SAR11 clade. It is unknown, though, whether transcription of btuB in the genome-streamlined SAR11 clade is regulated by B12. Downregulated gene transcripts encoding enzymes of methionine metabolism, as observed at day 3 (Fig. 6), also suggest a lower availability of B12 to SAR11. Oceanospirillales and the SAR11 clade presumably initially benefitted from ammonium released by Prochlorococcus. Evidence for this is provided by the upregulated nitrate metabolism in this primary producer and the upregulation of amt transcript in both heterotrophic lineages and the downregulated expression of genes encoding transporters of branched-chain amino acids. Further, transcription of genes encoding motility were downregulated in Oceanospirillales. We are not aware of any direct functional link between motility and B12 availability and speculate that this downregulation may have been an indirect consequence of the altered supply by organic compounds during the incubation. The relative increase of the abundance of the SAR11 clade in experiment M3 is consistent with its genomically encoded B12 auxotrophy [1, 64]. However, another possible positive effect on the SAR11 clade in M3 as a result of B12 supply, about which we can only speculate, could be an increased availability of methionine. Bacteria of the SAR11 clade I require for their growth exogenous reduced sulphur compounds, which can be partly obtained from methionine [69]. Its synthesis in turn depends on B12 availability in most bacteria [1]. Hence, an enhanced supply of methionine by other bacteria and eukaryotes to SAR11 due to a relaxed control of methionine synthesis and release into the surrounding water could also explain the relatively enhanced proportion of SAR11. Oceanospirillales also responded in this experiment to the B12 supply but much less than in experiment M1. These differences between both experiments may be explained by the presence of different SAR86 subclades in both biogeographic provinces diverging in their B12-biosynthesis traits. In experiment M2, Marine Group III responded most significantly to B12 supply by enhancing its proportions. This euryarchaeotal group has been studied little so far and there is no genomic information on its potential for vitamin biosynthesis [70]. The only genomic information on Thermoplasmatales is available from a metagenome assembled genome of Marine Group II indicating that the biosynthetic pathway for B12 is not encoded whereas proteins for transport of B12 are encoded [6]. If these traits are a general genomic feature of Thermoplasmatales this may explain why Marine Group III responded positively to the B12 supply in experiment M2. In this case, growth of Marine Group II, however, would have been controlled differently as it did not respond to B12 supply (Supplementary Fig. S9). Hence, these observations provide some evidence for the B12 auxotrophy of Marine Group III.

Our findings indicate that growth of the major marine prokaryotic lineages or sublineages including the SAR86 clade of Oceanospirillales; SAR11 and possibly Marine Group III is limited by the availability of B12. Responses of individual groups vary in the different oceanic regions. The environmental and biotic conditions and the interacting microbial communities may also affect the responses to B12 availability. In the only other study, which examined a response of B12 supply to natural prokaryotic communities in a pelagic system, the Southern Ocean, and applied metatranscriptomic analyses, only one unidentified Gammaproteobacterium exhibited a downregulation of a transcript of a single gene, cobC, encoding the protein which catalyses the final step in the B12 biosynthesis [17].
As indicated by increasing or constant prokaryotic cell numbers in experiments M1 and M2 the responding groups raised their proportions of the prokaryotic communities by enhanced growth. In experiment M3 with generally decreasing cell numbers the responding groups increased their proportions by a relatively slower temporal decrease than the other groups by a more sustained growth due to supply of B12. In this experiment heterotrophic protists such as MAST-1, "other" protists and Protalveolata increased from ~10% to >35% thus covarying inversely with prokaryotic abundance. This inverse correlation is consistent with the hypothesis that grazing by these protozoans led to the continuous reduction of prokaryotic cell numbers (see above, Supplementary Fig. S6). A further indication of the relatively enhanced proportions of the mentioned prokaryotic lineages in the B12 treatment of the M3 experiment were the lower cell numbers relative to the other treatment and the control, also consistent with the hypothesis of enhanced grazing on the actively growing prokaryotes in this treatment. Our findings indicate that availability and use of B12 needs to be explored much better as a growth-controlling factor of oceanic prokaryotic communities.

In addition to the effect of B12 our results also showed that supply of its lower ligand intermediate α-ribozale affected the composition of prokaryotic communities in the course of the mesocosm experiment. These global effects were evident from the NMDS analyses (Fig. 3) but effects on growth of individual lineages such as the SAR11 and SAR86 clades and of an Alteromonas sublineage were non-significant. The findings imply that if α-ribozale or possibly also DMB are available in oceanic marine systems these compounds should be considered as another factor affecting growth of pelagic prokaryotic communities.

B12 occurrence and producing and remodelling prokaryotes
Concentrations of B12 in the mixed layer of the oceans range between <1 and about 20 pM and >30 pM at 200 m and below [14, 15, 71]. Our B12 additions of 100 pM thus raised the ambient concentrations by an unknown factor. All other experiments, which tested the effect of B12 supply on pelagic prokaryotic and eukaryotic microbial communities, applied concentration of 90–200 pM [16–19]. So the concentration of our B12 supply is well in the range of those even though this concentration is well above in situ concentrations. In situ concentrations of α-ribozale or lower ligand derivatives are unknown. However, a mass formula matching that of α-ribozale has been detected in the exometabolome of various Rhodobacteraceae, Nitrosopumilus maritimus and in marine DOM [29–32] and a gene enabling transport of α-ribozale into a bacterium was identified [26]. Hence, it is highly probable that lower ligands occur in one or the other form in oceanic systems as a release product of prokaryotes and are available to and used by prokaryotes.

Looking for the supply of B12 to auxotrophic members of oceanic microbial communities Rhodobacteraceae, Rhizobiales and sublineages of Rhodospirillales, Oceanospirillales and Pseudomonadales are the most important candidates as genomic analyses of the great majority of the members of the two former and about 50% of the latter lineages predict them to be B12 prototrophic [1, 12]. In fact, a metatranscriptomic and a metaproteomic study in neritic ecosystems identified active B12 producers, including Rhodobacteraceae, Rhizobiales and also Cyanobacteria [5, 7]. Several Rhodobacteraceae are well known to produce and provide phytoplankton algae with B12 and constitute 2 to 15% of pelagic prokaryotic communities [72–75]. Usually, also at the stations of our experiments, members of these lineages constitute small fractions of the prokaryotic communities [34]. Hence it is reasonable to speculate that these members act as black queens for supply of B12 [76] to auxotrophic prokaryotes and possibly to grazing protists. Another phylum of cobamide suppliers are Cyanobacteria but most of them including Prochlorococcus and Synechococcus produce pseudo-B12 which differs from B12 in that adenine is the lower ligand instead of DMB. Some heterotrophic prokaryotes also produce and use pseudo-B12 [77, 78]. Whereas pseudo-B12 can be used by several prokaryotes its availability to other pro- and eukaryotes may require its remodelling to B12 [12, 25, 67]. Remodelling depends on the availability of the lower ligand DMB as has been shown for Vibrio cholerae [67]. Hence, when DMB, or possibly α-ribozale, and appropriate prokaryotes are present remodelling of pseudo-B12 is possible and this pathway may contribute to salvaging B12 requirements by prokaryotes as well as eukaryotes. A compound with a mass formula identical to that of α-ribozale was identified as an exometabolite of various prokaryotes and in marine DOM (see above) but no information is available on DMB in marine pelagic systems. DMB has been detected in soil, freshwater and the intestine of animals [24, 28]. This provides some evidence that remodelling of pseudo-B12 by using α-ribozale could be another yet unexplored pathway to salvage B12 requirements of marine microbes.

Effects of B12 and α-ribozale on Prochlorococcus
Unexpectedly, Prochlorococcus responded to the B12 addition in experiment M1 by enhancing transcription of genes encoding key proteins of photosynthesis, carbon fixation and nitrate uptake and reduction (Fig. 6). Principally there are two possible explanations for this response; i) a direct response of B12 on the functions encoded by these genes; ii) an indirect response via a direct effect of B12 supply on other prokaryotes favouring photosynthesis and carbon fixation of Prochlorococcus by other factors. Even though it is well established that Cyanobacteria including Prochlorococcus produce, use and release pseudo-B12 as cofactor [12, 25, 79], a direct effect by B12 cannot be ruled out. It has been shown that Salmonella enterica synthesises pseudo-B12 but can readily use pseudo-B12 as well as B12 [80]. If B12 can be used in a similar way by Prochlorococcus, its strong transcriptional response could be a direct result of the B12 supply. As considered for the effect of B12 on the photosynthesis of a diatom [81], a B12 deficiency could lead to reduced availability of phyloquinone (vitamin K1), an electron carrier of photosystem I, because its synthesis requires B12-dependent 5-adenosyl methionine [82]. Phyloquinone also serves as electron carrier in photosystem I of cyanobacteria. Hence, the enhanced supply of B12 could explain the upregulation of gene transcripts encoding photosystem I and the energy metabolism of Prochlorococcus we observed.

There is, however, also experimental evidence for the second explanation. It has been reported that when Prochlorococcus was growing in co-culture with an A. macleodii strain in particular transcripts of genes encoding pseudo-B12 biosynthesis, photosystem I and biosynthetic pathways were upregulated even though growth was not enhanced relative to an axenic control culture [65]. The authors speculate that supply of pseudo-B12 to A. macleodii (and subsequent remodelling to B12) favoured growth of this organism which responded in supplying Prochlorococcus with unknown goods favouring energy conservation via photosystem I, excessive release of DOM and possibly biosynthesis of pseudo-B12. The elevated nitrate concentration in our experiments may have further enhanced these effects as genes encoding proteins for nitrate uptake and reduction were upregulated. Nitrate uptake can be mediated via a sulfate permease [61] and the gene encoding this permease was upregulated in experiment M1. Nitrate assimilation and reduction is encoded in quite a few Prochlorococcus sublineages [63] and growth yield of Prochlorococcus is significantly reduced when using nitrate as nitrogen source as compared to ammonium [62].

We are unable to decide which of both explanations better support our observations on the transcriptional effects of B12 on Prochlorococcus and these explanations are even not mutually

G. Wienhausen et al. 2011
exclusive. In any case, our findings indicate that availability of B12 can effect photosynthesis and energy acquisition of Prochlorococcus and should be considered as a yet unexplored factor regulating growth of this globally important cyanobacterium. Future in-depth studies need to elucidate the details of how B12 availability affects photosynthesis and growth of Prochlorococcus.

Effects of B12 and α-ribazole on eukaryotes
The eukaryotic communities in all three experiments were also affected by supply of B12 and α-ribazole as shown by the NMDS analyses (Fig. 3) and the shifts in the community composition with enhanced proportions mainly of heterotrophic protists (Supplementary Figs. S5 and S6). In previous studies, a positive effect of B12 on growth of phytoplankton algae has been shown in subpolar and polar seas [16–18]. Our experiments provide good evidence that growth of heterotrophic protists in oceanic microbial communities is also stimulated by supply of B12. In particular, the uncultured lineages MAST-1 and MAST-7 were stimulated even though to varying extent in the different experiments and regions. Lineages of these groups have been shown to be abundant in the Pacific Ocean and to graze on bacteria [58, 83]. So far only scarce information is available on the effect of B12 on growth of heterotrophic protists [84, 85] and none from marine environments. It has been shown that an amoeba requires a B12 prototrophic bacterium for growth and that bacterial remodelling of pseudo-B12 can also meet the B12 requirements of this amoeba [85]. From our experiments, we cannot disentangle whether B12 had a direct or indirect effect via ingested B12-enriched prokaryotic cells on growth of the protists or by directly taking up B12. Based on the scarce available information we assume that protists benefitted from grazing on B12-enriched prokaryotic cells. However, irrespective of the mode of action of B12, our findings shed new light on the significance this growth factor has for growth of heterotrophic protists in marine ecosystems. The availability of this growth factor does not only affect growth of phytoplankton algae, as shown in previous studies, but also of heterotrophic protists and prokaryotes in marine pelagic systems. The different effects of B12 availability on protists in the experiments in different oceanic regions may reflect the different partitioning of prokaryotes, pico- and nano-eukaryotes in the food for the heterotrophic protists.

CONCLUSION
Often the effect of B12 is considered in a rather narrow biochemical context of methionine biosynthesis in prokaryotic species, despite its known role as a cofactor also in other biochemical reactions. We have shown that effects of B12 and α-ribazole go far beyond these species-specific biochemical reactions: Availability of B12 and α-ribazole affects growth and related changes in the composition of prokaryotic and eukaryotic microbial communities in oceanic regions of different trophic state. Both growth factors but more so B12 favour growth of members of the major lineages of pelagic marine prokaryotes such as SAR11, Oceanospirillales/SAR86 and the euryarchaeotal Marine Group III, and several heterotrophic protist groups, in particular of the uncultured MAST-lineages. Surprisingly also Prochlorococcus responded positively to supply of B12, suggesting that it also benefits from availability of this growth factor. Our findings show in particular the significance of B12 for controlling growth of and complex interactions among hetero- and autotrophic prokaryotes and eukaryotes in oceanic systems. The positive effect of α-ribazole on altering the composition of prokaryotic communities, together with the detection of a mass formula similar to this compound in previous studies, suggests that this lower ligand intermediate is available in oceanic systems and plays a role in salvaging B12 requirements by distinct prokaryotic lineages.

REFERENCES
1. Sañudo-Wilhelmy SA, Gómez-Consarnau L, Suffridge C, Webb EA. The role of B vitamins in marine biochemistry. Annu Rev Mar Sci. 2014;6:339–67.
2. Doxey AC, Kurtz DA, Lynch MD, Sauder LA, Neufeld JD. Aquatic metagenomes implicate *Thaumarchaeota* in global cobalamin production. ISME J. 2015;9:961–71.
3. Shelton AN, Seth EC, Mok KC, Han AW, Jackson SN, Haft DR, et al. Uneven distribution of cobamide biosynthesis and dependence in bacteria predicted by comparative genomics. ISME J. 2019;13:789–804.
4. Sokolovskaya OM, Shelton AN, Taga ME. Sharing vitamins: cobamides unveil microbial interactions. Science. 2020;369:eaba0165.
5. Gómez-Consarnau L, Sachdeva R, Gifford SM, Cutter LS, Fuhrman JA, Sañudo-Wilhelmy SA, et al. Mosaic patterns of B-vitamin synthesis and utilization in a natural marine microbial community. Environ Microbiol. 2018;20:2809–23.
6. Iverson V, Morris RM, Frazar CD, Berthiaume CT, Morales RL, Armbrust EV. Untangling genomes from metagenomes: revealing an uncultured class of marine *Euryarchaeta*. Science. 2012;335:587–90.
7. Isaac A, Francis B, Arnall RI, Amin SA. Tight Adherence (Ted) Pilus genes indicate putative niche differentiation in phytoplankton bloom associated Rhodobacter-ales. Front Microbiol. 2021;12:718297.
8. Croft MT, Lawrence AD, Raux-Deery E, Warren MJ, Smith AG. Algale acquire vitamin B12 through a symbiotic relationship with bacteria. Nature. 2005;438:90–93.
9. Cruz-López R, Maske H. The vitamin B1 and B12 required by the marine dino-flagellate *Lingulodinium polyedrum* can be provided by its associated bacterial community in culture. Front Microbiol. 2017;6:560.
10. Grant MA, Kazamia E, Cicuta P, Smith AG. Direct exchange of vitamin B12 is demonstrated by modelling the growth dynamics of algal–bacterial cocultures. ISME J. 2014;8:1418–27.
11. Sañudo-Wilhelmy SA, Gobler CJ, Okbamichael M, Taylor GT. Regulation of phytoplankton dynamics by vitamin B12. Geophys Res Lett. 2006;33:L04604.
12. Heal KR, Qin W, Ribalet F, Bertagnolli AD, Coyote-Maestas W, Hmelo Jr, et al. Two distinct pools of B12 analogs reveal community interdependencies in the ocean. Proc Natl Acad Sci USA. 2017;114:364–69.
13. Menzel DW, Spaeth JP. Occurrence of vitamin B12 in the Sargasso Sea. Limnol Oceanogr. 1962;7:151–54.
14. Suffridge CP, Gómez-Consarnau L, Monteverde DR, Cutter LS, Aristegui J, Alvarez-Salgado XA, et al. B Vitamins and their congeners as potential drivers of microbial community composition in an oligotrophic marine ecosystem. J Geophys Res Biogeosciences 2018;123:890–907.
15. Suffridge C, Cutter L, Sañudo-Wilhelmy SA. A new analytical method for direct measurement of particulate and dissolved B-vitamins and their congeners in seawater. Front Mar Sci. 2017;4:e11.
16. Bertrand EM, Saito MA, Rose JM, Riesselman CR, Lohan MC, Noble AE, et al. Vitamin B12 and iron colimitation of phytoplankton growth in the Ross Sea. Limnol Oceanogr. 2007;52:1079–93.
17. Bertrand EM, McCrow JP, Moustafa A, Zheng H, McQuaid JB, Delmont TO, et al. Phytoplankton–bacterial interactions mediate micronutrient colimitation at the coastal Antarctic sea ice edge. Proc Natl Acad Sci USA. 2015;112:9938–43.
18. Koch F, Marcoalva MA, Panzeca C, Bruland KW, Sañudo-Wilhelmy SA, Gobler CJ. The effect of vitamin B12 on phytoplankton growth and community structure in the Gulf of Alaska. Limnol Oceanogr. 2011;56:1023–34.
19. Browning TJ, Achterberg EP, Rapp I, Engel A, Bertrand EM, Tagliabue A, et al. Nutrient co-limitation at the boundary of an oceanic gyre. Nature. 2017;551:242–46.
20. Crofts TS, Seth EC, Hazra AB, Taga ME. Cobamide structure depends on both lower ligand availability and codSubstrate specificity. Chem Biol. 2013;20:1265–74.
21. Mok KC, Taga ME. Growth inhibition of *Sporosarcina ovata* by incorporation of benzimidazole bases into cobamides. J Bacteriol. 2013;195:1902–11.
22. Stupperich E, Steiner I, Eisinger HJ. Substitution of Co α-(5-hydroxy-5-methylindolyl)cobamide (factor III) by vitamin B12 in Methanobacterium thermoautotrophicum. J Bacteriol. 1987;169:3076–81.
23. Yi S, Seth EC, Men Y-J, Stabler SP, Allen RH, Alvarez-Cohen L, et al. Versatility in corinoid salvaging and remodeling pathways supports corinoid-dependent metabolism in *Dehalococcoides mccartyi*. Appl Environ Microbiol. 2012;78:7745–52.
24. Lu X, Heal KR, Ingalls AE, Doxey AC, Neufeld JD. Metagenomic and chemical characterization of soil cobalamin production. ISME J. 2020;14:53–66.
25. Heal KR, Lawrence AD, Holzer A, Kudahl UJ, Sasso S, Krüttler B, et al. Cyanobacteria and eukaryotic algae use different chemical variants of vitamin B12. Curr Biol. 2016;26:999–1008.
26. Gray MJ, Escalante-Semerena JC. A New Pathway for the synthesis of α-ribazole-phosphate in *Listeria innocua*. Mol Microbiol. 2010;77:1429–38.
27. Lundrigan MD, Koster W, Kaderi RJ. Transcribed sequences of the Escherichia coli btoB gene control its expression and regulation by vitamin B12. Proc Natl Acad Sci USA. 1991;88:1479–83.
28. Crofts TS, Men Y, Alvarez-Cohen L, Taga ME. A bioassay for the detection of benzimidazoles reveals their presence in a range of environmental samples. Front Microbiol. 2014;5:592.
29. Johnsen WM, Kido MI, Riviere MC, Kujawiński EB. Evidence for quorum sensing and differential metabolite production by a marine bacterium in response to DMSP. ISME J. 2016;10:2304–16.
30. Romano S, Dittmar T, Bondarev V, Weber RJM, Viant MR, Schulz-Vogt HN. Exo-metabolome of Pseudovibrio sp. FO-BEG1 analyzed by ultra-high resolution mass spectrometry and the effect of phosphate limitation. PLoS One. 2014;9:e96038.
31. Wienhoven G, Nöriege-Ortega BE, Niggemann J, Dittmar T, Simon M. The exometabolome of two model strains of the Roseobacter group: a marketplace of microbial metabolites. Front Microbiol. 2017;8:1985.
32. Law KP, He W, Tao J, Zhang C. Characterization of the exometabolome of Nitroporus marinus SCM1 by liquid chromatography–ion mobility mass spectrometry. Front Microbiol. 2021;12:1355.
33. Balmonte JP, Simon M, Giebel H-A, Arnosti C. A sea change in microbial enzymes: heterogeneous latitudinal and depth-related gradients in bulk water and particle-associated enzymatic activities from 30° to 59°N in the Pacific Ocean. Limnol Oceanogr. 2021;66:3489–507.
34. Giebel H-A, Arnosti C, Badewien TH, Bakenhus I, Balmonte JP, Billerbeck S, et al. Microbial growth and organic matter cycling in the Pacific Ocean along a latitudinal transect between subarctic and subantarctic waters. Front Mar Sci. 2021;8:1888.
35. Giebel H-A, Wolterink M, Brinkhoff T, Simon M. Complementary energy acquisition via aerobic anoxygenic photosynthesis and carbon monoxide oxidation by Planktomarina temperata of the Roseobacter group. FEMS Microbiol Ecol. 2019;95:e5050.
36. Schneider D, Wemheuer F, Pfeiffer B, Wemheuer B. Extraction of total DNA and RNA from marine filter samples and generation of a cDNA as universal template for marker gene studies. In: Streit WR, Daniel R, editors. Metagenomics: methods and protocols. 2017. New York, NY: Springer; p. 13–22.
37. Sundberg C, Al-Soud WA, Larsson M, Alm E, Yekta SS, Svensson BH, et al. 454 pyrosequencing analyses of bacterial and archaeal richness in 21 full-scale biogas digesters. FEMS Microbiol Ecol. 2013;85:612–26.
38. Amaral-Zettler LA, McIlmome EA, Dukelow HW, Huse SM. A method for studying protistan diversity using massively parallel sequencing of V9 hypervariable regions of small-subunit ribosomal RNA genes. PLoS One. 2009;4:e6372.
39. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics. 2014;30:2114–20.
40. Magot T, Salzberg SL. FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinforma Oxf Engl. 2011;27:2957–63.
41. Edgar RC. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat Methods. 2013;10:996–9.
42. Wang Q, Garrity GM, Tiedje JM, Cole JR. Naïve Bayesian classifier for rapid assignment of RNA sequences into the new bacterial taxonomy. Appl Environ Microbiol. 2007;73:5268.
43. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, et al. QIIME allows analysis of high-throughput community sequencing data. Nat Methods. 2010;7:335–6.
44. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 2013;41:D590–600.
45. Albertsen M, Karst SM, Ziegler AS, Kirkegaard RH, Nielsen PH. Back to basics – the influence of DNA extraction and primer choice on phylogenetic analysis of activated sludge communities. PLoS One. 2015;10:e0132783.
46. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, et al. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol. 2012;19:455–77.
47. Hyatt D, Chen G-L, LoCascio PF, Land ML, Larimer FW, Hauser LJ. Prodigal: prokaryotic gene recognition and translation initiation site identification. BMC Bioinformatics. 2010;11:119.
48. Dlugosch L, Poehlein A, Wemheuer B, Pfeiffer B, Badewien TH, Daniel R, et al. Significance of gene variants for the functional biogeography of the near-surface Atlantic Ocean microbiome. Nat Comm. 2022;13:456.
49. Menzel P, Ng K, Kuo A, Miotto R, Mihaishi F, Heo J, et al. Multiple B-vitamin depletion in large areas of the coastal ocean. Proc Natl Acad Sci USA. 2012;109:19441–45.
50. Wagner-Döllner I, Ballhausen B, Berger M, Brinkhoff T, Buchholz I, Bunk B, et al. The complete genome sequence of the algal symbiont Dinoroseobacter shibae: a hitchhiker’s guide to life in the sea. ISME J. 2010;4:61–77.
51. Segov E, Wyche TP, Kim KH, Petersen J, Ellebracht C, Vlamakis H, et al. Dynamic metabolic exchange governs a marine algal-bacterial interaction. eLife. 2016;5:e17473.
52. Voget S, Wemheuer B, Brinkhoff T, Vollmers J, Dietrich S, Giebel H-A, et al. Adaptation of an abundant Roseobacter RCA organism to pelagic systems revealed by genomic and transcriptomic analyses. ISME J. 2015;9:37–49.
53. Voget S, Wemheuer B, Brinkhoff T, Vollmers J, Dietrich S, Giebel H-A, et al. Adaptation of an abundant Roseobacter RCA organism to pelagic systems revealed by genomic and transcriptomic analyses. ISME J. 2015;9:37–49.
54. Billerbeck S, Wemheuer B, Voget S, Poehlein A, Giebel H-A, Brinkhoff T, et al. Biogeography and environmental genomics of the Roseobacter-affiliated pelagic CHAB-I-S lineage. Nat Microbiol. 2016;1:1–8.
55. Morris JJ, Lenski RE, Zenser ER. The Black Queen hypothesis: evolution of genetic cooperation through adaptive gene loss. mBio. 2012;3:e00036–12.
56. Stupperich E, Kräutler B. Pseudo vitamin B12 or 5-hydroxybenzimidazolyl-cobamide are the coenzymes found in methanogenic bacteria. Arch Microbiol. 1988;149:268–71.
ACKNOWLEDGEMENTS

We thank the master L. Mallon and the crew of RV Sonne for their most valuable support on shipboard, T. Badewien and R. Henkel for the hydrographic data and technical assistance in sampling. We are grateful to F. Milke, M. Wolterink, and B. Kuezel for their support in sampling and sample analysis on shipboard and to M. Wolterink also for his contribution to the logistics of this cruise. Sequencing was carried out by DNAsense (Aalborg, Denmark), with professional support in data processing by Mads Albertsen and his team. The study was supported by Deutsche Forschungsgemeinschaft within the Collaborative Research Center TRR51 Roseobacter and by grant BacGeoPac 03G0248A awarded to MS from the German Ministry of Education and Research (BMBF).

AUTHOR CONTRIBUTIONS

GW designed the experiment, carried out most of the data analysis and wrote large parts of the manuscript; LD carried out the basic bioinformatic evaluation of the metatranscriptomic analyses; RH synthesised α-ribozale; HW supervised the synthesis of α-ribozale; HAG carried out the flow cytometric analysis and enumeration of the prokaryotic and eukaryotic microbial communities; MS contributed to designing the experiments, carried out the HPP measurements, advised data evaluation and wrote parts of and finalised the manuscript. All authors revised carefully the manuscript.

FUNDING

Open Access funding enabled and organized by Projekt DEAL.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41396-022-01250-7.

Correspondence and requests for materials should be addressed to Meinhard Simon.

Reprints and permission information is available at http://www.nature.com/reprints

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2022