Unravelling microalgal-bacterial interactions in aquatic ecosystems through 16S rRNA gene-based co-occurrence networks

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Abstract

Microalgae and bacteria are the most widespread and dominant planktonic organisms of aquatic ecosystems. Their interactions can directly influence the global carbon and nutrient cycling and therefore aquatic ecosystem productivity. A large number of microalgae inhabit the earth but most associations with bacteria are unknown. Compiling knowledge on such associations through conventional methods is labour intensive and impractical. Environmental sequencing data coupled with network analysis can generate microbial-association networks which can reveal taxon co-occurrence patterns. Thus, co-occurrence networks have potential to explore microalgal-bacterial associations in nature. The 16S rRNA marker gene can be used to assess bacteria and

microalgae, as it is present in algal chloroplasts. Here, we used 16S rRNA gene

metabarcoding data from 10 Earth microbiome projects to investigate potential

interactions between microalgae and bacteria. Co-occurrence patterns were

readily revealed, including several previously known associations and a range of

potentially new associations such as frequent co-occurrences of *Bacillariophyta*

with uncultured *Planctomycetes OM190* and *Deltaproteobacteria order NB1-j*.

In accordance with previous work, *Proteobacteria* and *Bacteroidetes* were the

bacterial phyla most commonly associated with microalgae. We also detected

Planctomycetes and *Verrucomicrobia* co-occurring with an array of microalgal

groups implying successful associations with microalgae. Moreover, there was

no clear taxonomic pattern visible among the co-occurring microalgae and

bacteria. Our results also indicate that both intrinsic (metabolic functional

potential) and extrinsic (geography) factors may influence microalgal-bacterial

associations. This study reports a range of associations between microalgae and

bacteria based on co-occurrences networks in aquatic ecosystems. Overall, we

demonstrated the potential of co-occurrence networks to identify microalgal-

bacterial associations and argue that this technique can serve as a hypothesis-

generating framework to guide more focused research.

Keywords: microalgae, bacteria, microalgal-bacterial associations, co-

occurrence networks, 16S rRNA gene

Background

Microalgae (photosynthetic micro-eukaryotes) and bacteria are the most widespread and dominant planktonic organisms in aquatic ecosystems. As primary producers, microalgae form the foundations of the food web and directly influence global carbon and nutrient cycling, as well as the energy flow in aquatic ecosystems. A wide spectrum of associations between microalgae and bacteria have been reported, predominantly related to nutrient exchange, with important bottom-up effects on primary production. Associations include increased bioavailability of vitamins (e.g.B12 [1, 2]), metals (e.g. iron [3, 4]) and growth promoting hormones [5] by bacteria in exchange for organic carbon from the algae. Apart from many beneficial interactions, some bacteria can have negative influences on algae by being algicidal and opportunistic pathogens [6, 7]. Thus, algal-bacterial interactions can range from mutualistic to parasitic, and a complex array of interactions can be hypothesised. For instance, previous work suggests that strong associations may exist between communities of microalgae and bacteria over a large geographical range in the open ocean [8]. However, the knowledge we have so far about specific microalgal-bacterial interactions represents only a small fraction of what potentially occurs in nature. Microalgae are highly diverse and innumerable, therefore gathering knowledge on their associations with equally ubiquitous and diverse bacteria is challenging.

High-throughput DNA sequencing such as 16S and 18S rRNA gene

metabarcoding has been used to deliver insights into bacterial and eukaryotic

community composition of diverse ecosystems. Microbial community abundance

data can be used to identify associations between community members via

microbial association networks [9]. A network consists of nodes representing taxa

and edges representing the associations between taxa. Microbial association

networks targeting positive correlations are termed co-occurrence networks. A

positive relationship is presumed when taxonomically relevant units co-occur or

exhibit similarity in their compositions over multiple samples [10] and

biologically meaningful groups or communities of a network can be identified

with network clustering [11]. Also, network metrics such as node degree,

closeness centrality for example, can be used for quantitative description of

communities and identify ecologically important taxa [12, 13]. Various network

analyses have reported co-occurring microbial taxa in different environments,

identified clusters of microbes representing metabolic consortia, defined

keystone species, and documented recurrent microbial modules [[14–16].

Overall, networks provide insights into the structure of a community and taxon

co-occurrence patterns, offering perspectives to use them to generate hypotheses

about interactions that can be investigated with more focused studies. Thus,

environmental sequencing data combined with network analysis can be a

convenient addition to the toolkit for deriving interactions between organisms

across myriads of environments.

Studies targeting 16S rRNA marker genes are generally focused on the bacterial communities and often disregard the chloroplast sequences that are also amplified. The shared origin of the chloroplasts of all oxygenic photosynthetic micro-eukaryotes and cyanobacteria [17] enable the use of 16S rRNA marker gene to concurrently estimate relative abundances of microalgae and bacteria across samples, facilitating the construction of correlation networks to identify co-occurring taxa and speculate on their potential interactions. Robust co-occurrences can be detected through analysis of a sufficient number of samples, ideally covering temporal and spatial gradients, as these provide adequate variability in taxon abundances [9]. This can be coupled with stringent network building steps such as filtration of low prevalence organisms to reveal significant co-occurrences [18]. The ever-expanding sequence databases can be a great starting point to investigate such correlations. Analysis of Tara Ocean dataset based on interaction networks has shown that environment and geography can only explain 18% of plankton community variation, while 95% of models based on Operational Taxonomic Units (OTU) composition alone were more accurate [19]. Similar observations have been shown in phytoplankton blooms where environmental parameters were insignificant in influencing the community structure of plankton [20]. These results indicate that biological interactions are more influential in determining the community structure than environmental factors. Thus, taxon-taxon co-occurrence networks built on taxon compositions

alone could capture potential interactions in nature. Our work is based on the

premise that unknown associations between microalgae and bacteria may be

discovered at large scales from existing 16S rRNA gene-based metabarcoding

datasets using co-occurrence networks.

We investigated taxon-taxon co-occurrence networks to discover potential

microalgal-bacterial interactions using 16S rRNA gene metabarcoding datasets

from the Earth Microbiome Project (EMP) that have sampled aquatic

environments (both marine and freshwaters including water column and sediment

samples) and analysed them individually to recover co-occurring microalgae and

bacteria using co-occurrence networks. Network modules, which represent

ecological communities, were used to identify tight co-occurrences and in some

instances were coupled with network metrics to evaluate the ecological relevance

of taxa of interest. Using this approach, co-occurring taxa were recovered,

representing both known and unknown associations. Discussion of some of these

new discoveries are provided in detail to set the stage for future research.

Methods

Data Acquisition

Publicly available EMP datasets [21] were screened using the Qiita portal [22]

and 10 studies (4 marine and 6 Freshwater environments) targeting aquatic

environments were selected. Since the goal of this study was to individually

analyse samples representing a particular environment and reveal co-occurrences,

studies targeting any aquatic samples (water and sediment samples) were

selected. To our knowledge, based on study and sample information provided on

qiita portal for the selected studies, no size fractionation was carried out on the

samples (water samples) provided to EMP. The data analysed in this study have

amplified the same 16S rRNA gene V4 hypervariable region using 515F-

'GTGCCAGCMGCCGCGGTAA' and 806R-

'GGACTACHVGGGTWTCTAAT' primer pair [23, 24] and sequenced on

Illumina Hiseq2000. Detailed information on the selected EMP projects can be

found in Supplementary material (Table S1) and can also be further explored

using Qiita ids provided using the Qiita portal.

Sequencing Data Workflow

Raw demultiplexed reads were downloaded using the EBI accessions (provided

in Qiita portal) from the European Nucleotide Archive and analysed individually

employing a single bioinformatic pipeline built on Qiime2 version 2019.10 [25].

Primer sequences attached to all reads were trimmed using cutadapt [26].

Sequence denoising, chimera checking and dereplication was performed in

DADA2 [27] to correct sequencing errors and remove low quality bases. Reads

were truncated based on a median quality score of 30. The final outputs of

DADA2, an abundance table of Amplicon Sequence Variants (ASVs) and fasta

sequences of the ASVs were further processed as described hereafter. Taxonomy

was assigned against Silva v132[30] 16S rRNA gene sequences trained with a

Naive Bayes classifier [28]. Chloroplast sequences were filtered using the Silva

taxonomy file by identifying sequences assigned as "chloroplast". These

chloroplast sequences were then classified again using a Qiime2-compatible

version of PhytoRef[29] database accessed at [30]. All the algal identities with

PhytoRef had a confidence score of 0.7 or higher. Due to varying taxonomic

confidence of each algal node, taxonomic rank Class will be used for their

taxonomic identities. Where relevant, lower taxonomic affiliations will be

provided.

The DADA2 abundance table was filtered to create a bacteria-only abundance

table (by removing mitochondrial, Archaeal and chloroplast ASVs) and a

chloroplast-only abundance table (by retaining only the ASV ids assigned as

chloroplast). The bacteria-only table was then collapsed at the genus level to

reduce weak links in downstream network building [31] and was merged with the

chloroplast-only table to create the final abundance table. ASVs identified as

microalgae were not collapsed since the 16S rRNA gene marker is highly

conserved in algae and ASVs likely correspond to species or higher-level taxa

[29]. This final abundance table was filtered to remove low prevalence organisms

present in less than 10% of samples to prevent them from introducing artefacts in

network inference.

Correlation analysis and network construction

We used a compositional data analysis tool, FastSpar [32], which is a rapid and

parallelizable implementation of the SparCC algorithm [33] to compute

correlations. FastSpar quantifies the correlation between all ASVs and assesses

the statistical significance (p-values) of the inferred correlations using a bootstrap

procedure. The correlation and p-value matrices created by FastSpar were used

to create a network of significant correlations for each study separately. Co-

occurrence networks were created using the igraph package [34] in R studio

version 1.4.1106. Undirected weighted networks were created using statistically

(p<0.05) significant correlations >0.5 or higher (0.5 for freshwater and 0.6 for

marine datasets). Coefficient cut-off of 0.5 was selected for freshwater datasets

as a value above that resulted in fewer algal nodes in networks compared to 0.5.

The idea behind the coefficient cutoff selection was to preserve as many links as

possible with microalgae in resulting networks while choosing a considerably

higher coefficient cut off to report stronger links. Networks were visualised in

Cytoscape version 3.8 [35].

Network Analysis and module detection

The Network Analyzer plugin [36] in Cytoscape was used to compute global

network properties of each network to get an overview of node specific and edge-

specific attributes. Networks were checked for the scale free nature by identifying

the presence of highly connected nodes coexisting with nodes with fewer links

[37] using the node attributes. In order to identify communities, networks were

clustered using the Cytoscape plugin clusterMaker [38] using the MCL clustering

algorithm [39] with an inflation value of 2.0. Co-occurring microalgae and

bacteria were identified using resulting modules (modules with nodes > or = 4).

These co-occurring taxa (identified beyond the taxonomic rank Phylum) were

recorded for each environment and summarised in heatmaps using Pheatmap

v1.0.12 [40] in R.

Results and Discussion

16S rRNA gene co-occurrence networks can recover microalgal-bacterial

associations

Our data show clear evidence for co-occurrences between microalgae and

bacteria in aquatic habitats through network analysis of 16S rRNA metabarcoding

data (Figure 1 and 2). From the 10 co-occurrence networks generated, we

observed highly connected nodes coexisting with nodes with fewer links. This

indicates the scale-free nature of the networks, a characteristic in real world

networks [41, 42]. The clustered networks (modules/communities) comprised

nodes representing microalgae or bacteria and the edges between them were

instances of co-occurrence. Analysis of the modules identified 40 algal nodes

associated with at least one of 76 bacterial nodes in marine environments (Figure

1, Table S2) and 112 algal nodes with at least one of 311 bacterial nodes in

freshwater environments (Figure 2, Table S3).

These bacterial nodes were represented by different phyla such as

Proteobacteria, *Bacteroidetes*, *Planctomycetes* and *Chloroflexi* (Figure S1). The

summary of inferred associations showed that *Proteobacteria* and *Bacteroidetes*

were the most common bacterial phyla associated with microalgae. Previous

studies, using different techniques, have also found these two phyla to be the

major bacterial groups affiliated with microalgae [43–45]. The microalgal nodes

recovered too represented diverse phyla such as *Cryptophyta*, *Ochrophyta*,

Haptophyta, *Chlorophyta, Streptophyta* and *Euglenophta* representing 17

different taxonomic classes.

We then investigated if this network approach can recover known associations.

The inferred associations were investigated at a lower taxonomic level to identify

known bacterial associates of microalgae proven using other techniques. We

recognized known bacterial associates of microalgae such as *Flavobacteriales*

[46, 47], *Rhizobiales* [48, 49],*Cytophagales* [50, 51] and *Rhodobacterales* [52]

.Previously reported associations of microalgal genera with specific bacterial

groups were also recovered in the analysis, including that between *Bacteroidetes*

and the cosmopolitan *Prymnesiophyceae* genus *Phaeocystis* [53–55] (Table S2)

and that of diatoms (*Bacillariophyta*) with the genus *Flavobacterium* (Table S3)

[56–58]. The ability to identify previously known microalga-bacterial

associations suggest that the network approach used in this study can yield

biologically meaningful results and can be further used to identify potential

interactions which were previously unknown.

Emerging microalgal-bacterial associations that can guide functional studies

Our co-occurrence network analysis identified potential new interactions that

have not been previously described. For instance, uncultured *Deltaproteobacteria*

order *NB1-j* was identified as consistently co-occurring with *Bacillariophyta* in

marine environments. In one environment where the *NB1-j-Bacillariophyta* link

was observed (Surface water samples from Western English Channel, EBI

accession-ERP016541), *Bacillariophyta* was the only microalgal node directly

interacting with *NB1-j*. In another environment (Seawater metagenome samples

from Catlin arctic survey (2010 expedition), EBI accession ERP020022), *NB1-j*

was identified as the hub node with the highest node degree (43), in an algal-

bacterial module consisting of 77 nodes and 817 edges. Node degree is the

number of edges connected to a node. Hub nodes represent highly connected

species and are usually considered as keystone species [59]. This *NB1-j* node also

had the highest closeness centrality [0.69]. Closeness centrality measures how

close a node is to another node and helps to identify centrally positioned taxa in

the network [18]. Other than this *NB1-j* node, there were 3 more *NB1-j* nodes in

this community. All together these 4 *NB1-j* nodes were directly interacting

(edges) with 57 nodes (out of 73 other nodes) of which 25 were representing

microalgal nodes (Figure 3). Out of the 25 microalgal nodes, 13 represented the

taxonomic class *Bacillariophyta*. Some of these *Bacillariophyta* were

taxonomically identified at genus level as *Chaetoceros* and *Fragilariopsis*.

Interestingly, except for one, all of these Bacillariophyta nodes represented top

hub nodes of the community with node degrees > 26. Most connected taxa are

believed to have ecological relevance to the community as their removal causes

the highest impact on many associations [60].

Functional roles of *NB1-j* in marine ecosystems are largely unknown. It is

believed to be involved in hydrocarbon degradation [61] and has been reported

mostly from marine sediments [62, 63], mud volcanoes [61], sponges [64] and

cyanobacterial mats [65]. To the best of our knowledge, this *Deltaproteobacteria*

group is not reported in algal microbiomes. A predictive metagenomic approach

based on sponge samples originating from reef sites in West Java (Indonesia)

suggest that *NB1-j* may be involved in nitrogen metabolism [64]. It was found

that *NB1-j* was responsible for the elevated predictive gene count corresponding

to N-cycling genes such as those encoding nitric oxide reductase (*norB)*,

nitrogenase (*nifD*) and hydroxylamine reductase. The available information

indicates that the nitrogen cycling capacity of *NB1-j* may underly its association

with algae, potentially facilitating the nitrogen needs of the algae while benefiting

from algal organic carbon. However, a repertoire of interactions, as often

expected from a keystone species, can be hypothesised between *NB1-j* and

microalgae in a community.

Bacillariophyta (*Fragilariopsis, Chaetoceros* and many other diatoms

unidentified at lower taxonomic levels) also showed frequent co-occurrences

with an uncultured clade of *Planctomycetes*, *OM190* (SILVA taxonomy) in both

freshwater and marine environments. In the Catlin arctic survey samples

(ERP02022), *OM190* demonstrated frequent associations with *Bacillariophyta*.

Three *OM190* nodes were observed with high node degrees (29, 30 and 39) and

these were directly connected to 59 nodes out of a total of 74 other nodes in the

community. From the total of 35 microalgal nodes in this community, *OM190*

were directly connected to 27 nodes out of which 15 nodes were represented by

Bacillariophyta (Figure S2A). Similarly in a freshwater environment (Lake

Superior, Michigan, EBI accession: ERP016492), *OM190* was identified as the

6th most connected node with a node degree of 89 and was directly connected to

32 microalgal nodes including *Bacillariophyta* (Figure S2B). As mentioned

previously, highly connected nodes act as hub nodes in the community. Multiple

OM190 acting as top hub nodes within their habitats indicate that they have high

co-occurrences with both microalgae and bacteria in their communities.

OM190 shows deep branching within the *Planctomycetes* group and is usually

found in different environments such as soil and seawater. Members of this clade

are usually considered to be associated with macroalgae [66], such as red [67]

and brown algae [68]. Since *OM190* is yet uncultured, information on its

metabolism is scarce. A metagenome-assembled-genome (MAG) for *OM190*

(Likely *OM190*) with a rich diversity of secondary metabolite potential has been

reported [69]. The production of secondary metabolites by *OM190*, including

antimicrobial compounds may be one of the underlying reasons for their

association with algae as they could protect the alga from undesired microbes. In

a recent study [70], it was shown that diatoms produce fucose-containing sulfated

polysaccharides (FCSP) which can be hypothesised as an energy source for

OM190. Many *Planctomycetes* have abundant sulfatases, but these were not

confirmed in the existing *OM190* MAG.

More generally speaking, *Planctomycetes* often associate with macroalgae and

favour a biofilm lifestyle [68]. Protein clusters that may be involved in

Planctomycetes symbiosis or biofilm maintenance have been reported [71], and

production of sulfated polysaccharides by the alga that serve as the substrate for

the abundant sulfatases produced by the *Planctomycetes* contributes to the

reasons for successful associations between them [66, 71]. Although successful

associations are known between macroalgae and *Planctomycetes*, interactions

with microalgae are largely unknown. Apart from their associations with diatoms,

our network analysis identified taxa representing *Planctomycetes* (c_*Phycisphaerae*, c_*Planctomycetacia*) co-occurring with a range of microalgal groups. In a previous study, *Planctomycetes* closely related to *Pirellula* were identified as one of the dominant lineages associated with diatom blooms [72]. Results of our network analysis demonstrate that associations between *Planctomycetes* and microalgae may be as common as those they maintain with macroalgae. The role of *Planctomycetes* bacteria in global nitrogen, carbon and sulphur cycles is gaining attention [71, 73]. Thus, microalgal-*Planctomycetes* associations may be playing a crucial role in the global environmental cycles.

Members of the Gammaproteobacterial order *Cellvibrionales* were commonly associated with microalgal groups including *Prymnesiophyceae*, *Bacillariophyta*, *Cryptophyceae* and *Dictyochophyceae* in both marine and freshwater environments. The *Cellvibrionales* were taxonomically identified as *Halieaceae* and *Porticoccaceae*. They are considered oligotrophs and include strains capable of photoheterotrophic growth using light harvesting pigments such as proteorhodopsin [74]. As photoheterotrophs, they use light to generate energy and organic carbon compounds from the environment to satisfy their carbon needs [75]. *Cellvibrionales* benefit from associations with microalgae like *Mychonastes* [76]. They can assimilate organic carbon sources such as starch and glucose, suggesting they may benefit from the association by satisfying their carbon requirements to support the photoheterotrophic growth [77]. In return,

microalgae may be benefiting from the products of organic carbon

remineralisation. The associations inferred from our network analysis showcases

the ability of this bacterial group to potentially interact with a wide range of

microalgae.

Verrucomicrobia were also consistently associated with an array of microalgae

in fresh and marine environments indicating that they may be common associates

of microalgae. Here in our study, all the Verrucomicrobial members co-occurring

with microalgae in microalgal-bacterial communities were represented by the

taxonomic class *Verrucomicrobiae*. Among the genus-specific associations

revealed in our network analysis is that of the Verrucomicrobial genus

Lentimonas and microalgal genera *Pyramimonas* (c_*Prasinophyceae*),

Phaeocystis, *Tisochrysis*, *Haptolina*, and *Chrysochromulina*

(c_*Prymnesiophyceae*). A recent study showed hundreds of *Lentimonas* enzymes

able to digest brown algal fucoidan [78]. Although fucose-containing sulphated

polysaccharides were regarded as a macroalgal polysaccharide, microalgae such

as diatoms have the potential to produce them [70]. Our work suggests that other

microalgae might produce these sulfated polysaccharides as well, or that

members of *Lentimonas* may have a broader palate than just FCSP. Although

widely distributed, functional roles of *Verrucomicrobia* in the aquatic ecosystem

are not well understood. Primary reason for this is the lack of many cultured

strains [79]. Some members of *Verrucomicrobia* have been shown to consume

algal extracellular polymeric substances [80–82] but little is known about other

factors contributing to the associations between *Verrucomicrobia* and algae.

Both Intrinsic and Extrinsic factors influence microalgal-bacterial associations

There was no clear taxonomic pattern observed among the co-occurring

microalgae and bacteria. In accordance with previous work on phytoplankton-

bacteria co-occurrences [8], taxonomically diverse bacteria co-occurred with

different microalgal groups. Since 16S rRNA gene is highly conserved in algae,

and the sequenced fragment is short, most of the recovered algal nodes could not

be identified at the species level. Therefore, we observed the co-occurring

bacterial taxa of the same algal genus within and between environments and did

not identify similar patterns. Moreover, except for a few, there was no recurrence

of bacterial genera (the lowest taxonomic identity of bacterial nodes used in this

study) across microalgal-bacterial communities of different environments. A

plausible explanation for such observation could be the sample origin or

geography. Interestingly, a recent study [83] has provided evidence for

biogeographic differentiation of algal microbiomes, showing ecological

boundaries driven by differences in environmental conditions altering the spatial

scaling of the algal microbiomes.

To understand if there is any factor driving general patterns of interactions, some

insights may be gleaned from known functions of the co-occurring bacteria. We

noted that general patterns of interactions could be observed at a functional level.

Our observation was that functional similarities are usually seen among the

bacterial associates in different microalgal communities although they are

taxonomically different. For instance, microalgae co-occur with bacterial groups

equipped with specific metabolic functional potentials such as algal

polysaccharide degradation and provision of vitamins. Co-occurrences with

taxonomically diverse members of *Bacteroidetes* [55] and *Verrucomicrobia* [80]

with polysaccharide degradation ability and *Rhizobiales* [84], *Rhodobacterales*

[85] and *SAR116* [86] with Vitamin B12 synthesis ability can be cited as

examples. As a summary, these results suggest that both intrinsic (functional) and

extrinsic (geography) factors influence microalgal-bacterial associations.

Conclusion

Our study identified a range of interactions between microalgae and bacteria

including both known and unknown associations. Network analysis indicated that

there is no clear taxonomic pattern with regard to microalgal-bacterial

associations. General patterns of interactions were observed at a functional level

indicating the influence of intrinsic factors on these associations. The

identification of previously known microalgal-bacterial associations suggests that

the network approach used in this study yields biologically meaningful results

and identifies a broad range of previously unknown algal-bacterial interactions.

Our study illustrates the promise of using 16S rRNA gene-based co-occurrence

networks as a hypothesis-generating framework to guide focused research in

future to clarify the functional nature of these potential interactions. The

recovered associations will help enrich our understanding of the microalgal-

bacterial interactions in aquatic ecosystems.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

No data was generated in this study and all the data analysed are publicly

available. The EMP datasets analysed are available in European Nucleotide

Archive and EMP Qiita portal under the Accession numbers and Qiita ids,

respectively, provided in Supplementary material TableS1. FastSpar script used

for the correlation analysis, R script used in generating networks and the

networks files generated in Cytoscape can be found at

https://melbourne.figshare.com/projects/Unravelling_microalgal-

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Competing interests

The authors declare that they have no competing interests

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Authors' contribution

UP, HV and AW formulated the project. UP analysed the data and wrote the

manuscript with inputs from all the authors. HV, AW and KT contributed ideas

and helped with analysis. All authors read and approved the final version of the

manuscript.

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References

1. Croft MT, Lawrence AD, Raux-Deery E, Warren MJ, Smith AG. Algae

acquire vitamin B12 through a symbiotic relationship with bacteria.

Nature. 2005. https://doi.org/10.1038/nature04056.

2. Kazamia E, Czesnick H, Nguyen TT Van, Croft MT, Sherwood E,

Sasso S, et al. Mutualistic interactions between vitamin B12-dependent

algae and heterotrophic bacteria exhibit regulation. Environ Microbiol.

2012. https://doi.org/10.1111/j.1462-2920.2012.02733.x.

3. Butler A. Acquisition and utilization of transition metal ions by marine

organisms. Science. 1998.

4. Amin SA, Green DH, Hart MC, Küpper FC, Sunda WG, Carrano CJ.

Photolysis of iron-siderophore chelates promotes bacterial-algal

mutualism. Proc Natl Acad Sci U S A. 2009.

https://doi.org/10.1073/pnas.0905512106.

5. Amin SA, Hmelo LR, Van Tol HM, Durham BP, Carlson LT, Heal KR,

et al. Interaction and signalling between a cosmopolitan phytoplankton

and associated bacteria. Nature. 2015.

https://doi.org/10.1038/nature14488.

6. Mayali X, Azam F. Algicidal bacteria in the sea and their impact on

algal blooms. In: Journal of Eukaryotic Microbiology. 2004.

7. Bagwell CE, Abernathy A, Barnwell R, Milliken CE, Noble PA, Dale T,

et al. Discovery of bioactive metabolites in biofuel microalgae that offer

protection against predatory bacteria. Front Microbiol. 2016.

https://doi.org/10.3389/fmicb.2016.00516.

8. Milici M, Deng ZL, Tomasch J, Decelle J, Wos-Oxley ML, Wang H, et

al. Co-occurrence analysis of microbial taxa in the Atlantic ocean reveals

high connectivity in the free-living bacterioplankton. Front Microbiol.

2016;7 MAY:1–20.

9. Barberán A, Bates ST, Casamayor EO, Fierer N. Using network

analysis to explore co-occurrence patterns in soil microbial communities.

ISME J. 2012;6:343–51.

10. Faust K, Raes J. Microbial interactions: From networks to models.

Nat Rev Microbiol. 2012;10:538–50.

11. Chun SJ, Cui Y, Lee JJ, Choi IC, Oh HM, Ahn CY. Network analysis

reveals succession of Microcystis genotypes accompanying distinctive

microbial modules with recurrent patterns. Water Res. 2020.

https://doi.org/10.1016/j.watres.2019.115326.

12. Huang S. Back to the biology in systems biology: What can we learn

from biomolecular networks? Briefings Funct Genomics Proteomics.

2004. https://doi.org/10.1093/bfgp/2.4.279.

13. Ma'ayan A. Introduction to network analysis in systems biology.

Science Signaling. 2011.

14. Williams RJ, Howe A, Hofmockel KS. Demonstrating microbial co-

occurrence pattern analyses within and between ecosystems. Front

Microbiol. 2014;5 JULY:1–10.

15. Fuhrman JA, Cram JA, Needham DM. Marine microbial community

dynamics and their ecological interpretation. Nature Reviews

Microbiology. 2015.

16. Chafee M, Fernàndez-Guerra A, Buttigieg PL, Gerdts G, Eren AM,

Teeling H, et al. Recurrent patterns of microdiversity in a temperate

coastal marine environment. ISME J. 2018;12:237–52.

17. Bennke CM, Pollehne F, Müller A, Hansen R, Kreikemeyer B,

Labrenz M. The distribution of phytoplankton in the Baltic Sea assessed

by a prokaryotic 16S rRNA gene primer system. J Plankton Res.

2018;40:244–54.

18. Berry D, Widder S. Deciphering microbial interactions and detecting

keystone species with co-occurrence networks. Front Microbiol. 2014.

https://doi.org/10.3389/fmicb.2014.00219.

19. Lima-Mendez G, Faust K, Henry N, Decelle J, Colin S, Carcillo F, et

al. Determinants of community structure in the global plankton

interactome. Science (80-). 2015.

https://doi.org/10.1126/science.1262073.

20. Needham DM, Fuhrman JA. Pronounced daily succession of

phytoplankton, archaea and bacteria following a spring bloom. Nat

Microbiol. 2016. https://doi.org/10.1038/nmicrobiol.2016.5.

21. Thompson LR, Sanders JG, McDonald D, Amir A, Ladau J, Locey

KJ, et al. A communal catalogue reveals Earth's multiscale microbial

diversity. Nature. 2017. https://doi.org/10.1038/nature24621.

22. Gonzalez A, Navas-Molina JA, Kosciolek T, McDonald D, Vázquez-

Baeza Y, Ackermann G, et al. Qiita: rapid, web-enabled microbiome

meta-analysis. Nat Methods. 2018. https://doi.org/10.1038/s41592-018-

0141-9.

23. Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Lozupone CA,

Turnbaugh PJ, et al. Global patterns of 16S rRNA diversity at a depth of

millions of sequences per sample. Proc Natl Acad Sci U S A. 2011.

https://doi.org/10.1073/pnas.1000080107.

24. Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Huntley J,

Fierer N, et al. Ultra-high-throughput microbial community analysis on

the Illumina HiSeq and MiSeq platforms. ISME J. 2012.

https://doi.org/10.1038/ismej.2012.8.

25. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith

GA, et al. Reproducible, interactive, scalable and extensible microbiome

data science using QIIME 2. Nature Biotechnology. 2019.

26. Martin M. Cutadapt removes adapter sequences from high-

throughput sequencing reads. EMBnet.journal. 2011.

https://doi.org/10.14806/ej.17.1.200.

27. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA,

Holmes SP. DADA2: High-resolution sample inference from Illumina

amplicon data. Nat Methods. 2016. https://doi.org/10.1038/nmeth.3869.

28. Bokulich NA, Kaehler BD, Rideout JR, Dillon M, Bolyen E, Knight R,

et al. Optimizing taxonomic classification of marker-gene amplicon

sequences with QIIME 2's q2-feature-classifier plugin. Microbiome.

2018. https://doi.org/10.1186/s40168-018-0470-z.

29. Decelle J, Romac S, Stern RF, Bendif EM, Zingone A, Audic S, et al.

PhytoREF: A reference database of the plastidial 16S rRNA gene of

photosynthetic eukaryotes with curated taxonomy. Mol Ecol Resour.

2015;15:1435–45.

30. Major oceanic 16S/18S databases in qiime2 format.

https://github.com/ndu-invitae/Oceanic_database/tree/master/PhytoRef.

Accessed 20 Oct 2019.

31. Hemprich-Bennett DR, Oliveira HFM, Le Comber SC, Rossiter SJ,

Clare EL. Assessing the impact of taxon resolution on network structure.

Ecology. 2021. https://doi.org/10.1002/ecy.3256.

32. Watts SC, Ritchie SC, Inouye M, Holt KE. FastSpar: Rapid and

scalable correlation estimation for compositional data. Bioinformatics.

2019. https://doi.org/10.1093/bioinformatics/bty734.

33. Friedman J, Alm EJ. Inferring Correlation Networks from Genomic

Survey Data. PLoS Comput Biol. 2012.

https://doi.org/10.1371/journal.pcbi.1002687.

34. Csardi, G., & Nepusz T. The igraph software package for complex

network research. InterJournal, Complex Systems. igraph Softw Packag.

2006.

35. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, et

al. Cytoscape: A software Environment for integrated models of

biomolecular interaction networks. Genome Res. 2003.

https://doi.org/10.1101/gr.1239303.

36. Assenov Y, Ramírez F, Schelhorn SESE, Lengauer T, Albrecht M.

Computing topological parameters of biological networks. Bioinformatics.

2008. https://doi.org/10.1093/bioinformatics/btm554.

37. Barabási AL. Scale-free networks: A decade and beyond. Science.

2009.

38. Morris JH, Apeltsin L, Newman AM, Baumbach J, Wittkop T, Su G,

et al. ClusterMaker: A multi-algorithm clustering plugin for Cytoscape.

BMC Bioinformatics. 2011. https://doi.org/10.1186/1471-2105-12-436.

39. Van Dongen S, Abreu-Goodger C. Using MCL to extract clusters

from networks. Methods Mol Biol. 2012. https://doi.org/10.1007/978-1-

61779-361-5_15.

40. Raivo K. Pheatmap: pretty heatmaps. R Pacakage Version. 2012.

41. Albert R, Jeong H, Barabási AL. Diameter of the world-wide web.

Nature. 1999. https://doi.org/10.1038/43601.

42. Eisenberg E, Levanon EY. Preferential attachment in the protein

network evolution. Phys Rev Lett. 2003.

https://doi.org/10.1103/PhysRevLett.91.138701.

43. Jones KM, Kobayashi H, Davies BW, Taga ME, Walker GC. How

rhizobial symbionts invade plants: The Sinorhizobium - Medicago model.

Nature Reviews Microbiology. 2007.

44. Goecke F, Thiel V, Wiese J, Labes A, Imhoff JF. Algae as an

important environment for bacteria - Phylogenetic relationships among

new bacterial species isolated from algae. Phycologia. 2013;52:14–24.

45. Krohn-Molt I, Wemheuer B, Alawi M, Poehlein A, Güllert S,

Schmeisser C, et al. Metagenome survey of a multispecies and alga-

associated biofilm revealed key elements of bacterial-algal interactions

in photobioreactors. Appl Environ Microbiol. 2013.

https://doi.org/10.1128/AEM.01641-13.

46. Barbeyron T, L'Haridon S, Corre E, Kloareg B, Potin P. Zobellia

galactanovorans gen. nov., sp. nov., a marine species of

Flavobacteriaceae isolated from a red alga, and classification of

[Cytophaga] uliginosa (ZoBell and Upham 1944) Reichenbach 1989 as

Zobellia uliginosa gen. nov., comb. nov. Int J Syst Evol Microbiol. 2001.

https://doi.org/10.1099/00207713-51-3-985.

47. Nedashkovskaya OI, Kim SB, Han SK, Lysenko AM, Rohde M,

Zhukova N V., et al. Mesonia algae gen. nov., sp. nov., a novel marine

bacterium of the family Flavobacteriaceae isolated from the green alga

Acrosiphonia sonderi (Kütz) Kornm. Int J Syst Evol Microbiol. 2003.

https://doi.org/10.1099/ijs.0.02626-0.

48. Kim BH, Ramanan R, Cho DH, Oh HM, Kim HS. Role of Rhizobium,

a plant growth promoting bacterium, in enhancing algal biomass through

mutualistic interaction. Biomass and Bioenergy. 2014.

https://doi.org/10.1016/j.biombioe.2014.07.015.

49. Rivas MO, Vargas P, Riquelme CE. Interactions of Botryococcus

braunii Cultures with Bacterial Biofilms. Microb Ecol. 2010.

https://doi.org/10.1007/s00248-010-9686-6.

50. Cole JJ. Interactions between bacteria and algae in aquatic

ecosystems. Annu Rev Ecol Syst Vol 13. 1982.

https://doi.org/10.1146/annurev.es.13.110182.001451.

51. Fulbright SP, Robbins-Pianka A, Berg-Lyons D, Knight R, Reardon

KF, Chisholm ST. Bacterial community changes in an industrial algae

production system. Algal Res. 2018.

https://doi.org/10.1016/j.algal.2017.09.010.

52. Zhou J, Richlen ML, Sehein TR, Kulis DM, Anderson DM, Cai Z.

Microbial community structure and associations during a marine

dinoflagellate bloom. Front Microbiol. 2018.

https://doi.org/10.3389/fmicb.2018.01201.

53. Simon M, Glöckner FO, Amann R. Different community structure and

temperature optima of heterotrophic picoplankton in various regions of

the Southern Ocean. Aquat Microb Ecol. 1999.

https://doi.org/10.3354/ame018275.

54. Brussaard CPD, Mari X, Van Bleijswijk JDL, Veldhuis MJW. A mesocosm study of Phaeocystis globosa (Prymnesiophyceae) population dynamics: II. Significance for the microbial community. Harmful Algae. 2005. https://doi.org/10.1016/j.hal.2004.12.012.

55. Janse I, Zwart G, Van der Maarel MJEC, Gottschal JC. Composition of the bacterial community degrading Phaeocystis mucopolysaccharides in enrichment cultures. Aquat Microb Ecol. 2000. https://doi.org/10.3354/ame022119.

56. Grossart HP, Levold F, Allgaier M, Simon M, Brinkhoff T. Marine diatom species harbour distinct bacterial communities. Environ Microbiol. 2005. https://doi.org/10.1111/j.1462-2920.2005.00759.x.

57. Sapp M, Schwaderer AS, Wiltshire KH, Hoppe HG, Gerdts G, Wichels A. Species-specific bacterial communities in the phycosphere of microalgae? Microb Ecol. 2007. https://doi.org/10.1007/s00248-006-9162-5.

58. Sapp M, Wichels A, Gerdts G. Impacts of cultivation of marine diatoms on the associated bacterial community. Appl Environ Microbiol. 2007. https://doi.org/10.1128/AEM.02274-06.

59. Wang H, Wei Z, Mei L, Gu J, Yin S, Faust K, et al. Combined use of network inference tools identifies ecologically meaningful bacterial

associations in a paddy soil. Soil Biol Biochem. 2017;105:227–35.

60. Proulx SR, Promislow DEL, Phillips PC. Network thinking in ecology

and evolution. Trends in Ecology and Evolution. 2005.

61. Coelho FJRC, Louvado A, Domingues PM, Cleary DFR, Ferreira M,

Almeida A, et al. Integrated analysis of bacterial and microeukaryotic

communities from differentially active mud volcanoes in the Gulf of

Cadiz. Sci Rep. 2016;6 September:1–10.

62. Queiroz LL, Bendia AG, Duarte RTD, das Graças DA, da Costa da

Silva AL, Nakayama CR, et al. Bacterial diversity in deep-sea sediments

under influence of asphalt seep at the São Paulo Plateau. Antonie van

Leeuwenhoek, Int J Gen Mol Microbiol. 2020.

https://doi.org/10.1007/s10482-020-01384-8.

63. Dell'Anno F, Rastelli E, Tangherlini M, Corinaldesi C, Sansone C,

Brunet C, et al. Highly Contaminated Marine Sediments Can Host Rare

Bacterial Taxa Potentially Useful for Bioremediation. Front Microbiol.

2021. https://doi.org/10.3389/fmicb.2021.584850.

64. de Voogd NJ, Cleary DFR, Polónia ARM, Gomes NCM. Bacterial

community composition and predicted functional ecology of sponges,

sediment and seawater from the thousand islands reef complex, West

Java, Indonesia. FEMS Microbiol Ecol. 2015.

https://doi.org/10.1093/femsec/fiv019.

65. Vigneron A, Cruaud P, Mohit V, Martineau MJ, Culley AI, Lovejoy C,

et al. Multiple strategies for light-harvesting, photoprotection, and carbon

flow in high latitude microbial mats. Front Microbiol. 2018.

https://doi.org/10.3389/fmicb.2018.02881.

66. Lage OM, Bondoso J. Planctomycetes diversity associated with

macroalgae. FEMS Microbiol Ecol. 2011. https://doi.org/10.1111/j.1574-

6941.2011.01168.x.

67. Longford SR, Tujula NA, Crocetti GR, Holmes AJ, Holmström C,

Kjelleberg S, et al. Comparisons of diversity of bacterial communities

associated with three sessile marine eukaryotes. Aquat Microb Ecol.

2007. https://doi.org/10.3354/ame048217.

68. Bengtsson MM, Øvreås L. Planctomycetes dominate biofilms on

surfaces of the kelp Laminaria hyperborea. BMC Microbiol. 2010.

https://doi.org/10.1186/1471-2180-10-261.

69. Ludington WB, Seher TD, Applegate O, Li X, Kliegman JI, Langelier

C, et al. Assessing biosynthetic potential of agricultural groundwater

through metagenomic sequencing: A diverse anammox community

dominates nitrate-rich groundwater. PLoS One. 2017.

https://doi.org/10.1371/journal.pone.0174930.

70. Vidal-Melgosa S, Sichert A, Francis T Ben, Bartosik D, Niggemann

J, Wichels A, et al. Diatom fucan polysaccharide precipitates carbon

during algal blooms. Nat Commun. 2021;12:1–13.

71. Faria M, Bordin N, Kizina J, Harder J, Devos D, Lage OM.

Planctomycetes attached to algal surfaces: Insight into their genomes.

Genomics. 2018. https://doi.org/10.1016/j.ygeno.2017.10.007.

72. Morris RM, Longnecker K, Giovannoni SJ. Pirellula and OM43 are

among the dominant lineages identified in an Oregon coast diatom

bloom. Environ Microbiol. 2006. https://doi.org/10.1111/j.1462-

2920.2006.01029.x.

73. Woebken D, Teeling H, Wecker P, Dumitriu A, Kostadinov I, Delong

EF, et al. Fosmids of novel marine Planctomycetes from the Namibian

and Oregon coast upwelling systems and their cross-comparison with

planctomycete genomes. ISME J. 2007.

https://doi.org/10.1038/ismej.2007.63.

74. Spring S, Scheuner C, Göker M, Klenk HP. A taxonomic framework

for emerging groups of ecologically important marine

gammaproteobacteria based on the reconstruction of evolutionary

relationships using genome-scale data. Front Microbiol. 2015.

https://doi.org/10.3389/fmicb.2015.00281.

75. Zubkov M V. Photoheterotrophy in marine prokaryotes. J Plankton

Res. 2009. https://doi.org/10.1093/plankt/fbp043.

76. Sörenson E, Capo E, Farnelid H, Lindehoff E, Legrand C.

Temperature Stress Induces Shift From Co-Existence to Competition for

Organic Carbon in Microalgae-Bacterial Photobioreactor Community –

Enabling Continuous Production of Microalgal Biomass. Front Microbiol.

2021. https://doi.org/10.3389/fmicb.2021.607601.

77. Bryson S, Li Z, Chavez F, Weber PK, Pett-Ridge J, Hettich RL, et al.

Phylogenetically conserved resource partitioning in the coastal microbial

loop. ISME J. 2017. https://doi.org/10.1038/ismej.2017.128.

78. Sichert A, Corzett CH, Schechter MS, Unfried F, Markert S, Becher

D, et al. Verrucomicrobia use hundreds of enzymes to digest the algal

polysaccharide fucoidan. Nat Microbiol. 2020.

https://doi.org/10.1038/s41564-020-0720-2.

79. Bohórquez J, McGenity TJ, Papaspyrou S, García-Robledo E, Corzo

A, Underwood GJC. Different types of diatom-derived extracellular

polymeric substances drive changes in heterotrophic bacterial

communities from intertidal sediments. Front Microbiol. 2017.

https://doi.org/10.3389/fmicb.2017.00245.

80. Martinez-Garcia M, Brazel DM, Swan BK, Arnosti C, Chain PSG,

Reitenga KG, et al. Capturing single cell genomes of active

polysaccharide degraders: An unexpected contribution of

verrucomicrobia. PLoS One. 2012.

https://doi.org/10.1371/journal.pone.0035314.

81. Landa M, Cottrell MT, Kirchman DL, Kaiser K, Medeiros PM,

Tremblay L, et al. Phylogenetic and structural response of heterotrophic

bacteria to dissolved organic matter of different chemical composition in

a continuous culture study. Environ Microbiol. 2014.

https://doi.org/10.1111/1462-2920.12242.

82. Orsi WD, Smith JM, Liu S, Liu Z, Sakamoto CM, Wilken S, et al.

Diverse, uncultivated bacteria and archaea underlying the cycling of

dissolved protein in the ocean. ISME J. 2016.

https://doi.org/10.1038/ismej.2016.20.

83. Martin K, Schmidt K, Toseland A, Boulton CA, Barry K, Beszteri B, et

al. The biogeographic differentiation of algal microbiomes in the upper

ocean from pole to pole. Nat Commun. 2021.

https://doi.org/10.1038/s41467-021-25646-9.

84. Newton RJ, Griffin LE, Bowles KM, Meile C, Gifford S, Givens CE, et

al. Genome characteristics of a generalist marine bacterial lineage.

ISME J. 2010. https://doi.org/10.1038/ismej.2009.150.

85. Sañudo-Wilhelmy SA, Gómez-Consarnau L, Suffridge C, Webb EA.

The role of B vitamins in marine biogeochemistry. Ann Rev Mar Sci.

2014. https://doi.org/10.1146/annurev-marine-120710-100912.

86. Durham BP, Sharma S, Luo H, Smith CB, Amin SA, Bender SJ, et

al. Cryptic carbon and sulfur cycling between surface ocean plankton.

Proc Natl Acad Sci U S A. 2015.

https://doi.org/10.1073/pnas.1413137112.

Figure legends

Figure 1: Summarised interactions in marine environments

Heatmap of co-occurring microalgae (columns) and bacteria (rows) in marine

environments. "Taxonomy" represents the taxonomic affiliations of microalgal

nodes at class level. "Taxonomy2" represents the taxonomic affiliations of

bacteria (p_, c_, o_, f_ and g_ represent Phylum, Class, Order, Family and

Genus, respectively). "Project" represents the accession numbers of EMP

projects and indicate which project the microalgal and bacterial nodes were

recovered from.

Figure 2: Summarised interactions in Freshwater environments

Heatmap of co-occurring microalgae (columns) and bacteria (rows) in

freshwater environments. "Taxonomy" represents the taxonomic affiliations of

microalgal nodes at class level. "Taxonomy2" represents the taxonomic

affiliations of bacteria (p_, c_, o_, f_ and g_ represent Phylum, Class, Order,

Family and Genus, respectively). "Project" represents the accessions of EMP

projects and indicate which project the microalgal and bacterial nodes were

recovered from.

Figure 3: Interactions of the Deltaproteobacterial order *NB1-j* with

microalgae in a marine environment.

Figure shows the first neighbours (directly interacting taxa) including

microalgal taxa co-occurring with Deltaproteobacterial order *NB1-j*. Nodes are

labelled at the order level (If unclassified at the order level, higher taxonomic

affiliations are provided). The edge width and node size are continuously

mapped to edge weight (correlation strength) and node degree, respectively.

Supplementary Data

Figure S1: Major bacterial Phyla associated with microalgae in freshwater

and marine environments

Bars represent the number of bacterial nodes affiliated with each phylum. The

total number of bacterial nodes identified to be co-occurring with microalgae in

marine environments were 76 and freshwater environments were 311.

Figure S2: *Planctomycetes* class *OM190* interacting with microalgal taxa in

a marine (A) and freshwater (B) environment.

Networks (A, B) created from a microalgal-bacterial module by selecting the

first neighbours of the *OM190* to showcase the complex interactions with the

neighbouring microalgal taxa. The edge width and node size are continuously

mapped to edge weight and node degree. Marine and Freshwater environments

represent EMP project ERP020022 and ERP016492, respectively. Nodes

labelled at order level (If unclassified at the order level, higher taxonomic

affiliations are provided).

Table S1: Information on selected EMP studies

Table S2: Correlations recovered in marine environments between

microalgae and bacteria

Table S3: Correlations recovered in freshwater environments between

microalgae and bacteria