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AQUATIC MICROBIOMES ON A CHANGING PLANET

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Book of abstracts

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- —**Dolors Vaqué**, Institute of Marine Sciences (ICM-CSIC), Barcelona, Spain

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BOOK OF ABSTRACTS

Keynote Speakers

Diversity and function of freshwater microbial communities

Stefan Bertilsson [1]

[1]*Swedish University of Agricultural Sciences and Science for Life Laboratory, Sweden.*

There is a growing number of studies from around the globe that describe freshwater microbiomes in very great detail, thereby providing ample opportunities for investigating global patterns in microbial diversity, the mechanisms creating such patterns, and its ecological and biogeochemical consequences. In this presentation I will draw information from several such studies to provide my personal birds eye view on the composition and functioning of the interactive and complex microbial communities that populate our inland waters and shed some light on how the most abundant and functionally significant populations are structured along geographical and environmental gradients. I will also highlight selected biogeochemical processes mediated by freshwater microorganisms and discuss linkages to microbial community composition and environmental change.

Thalastasi: The Ocean's Symphony Illuminating the Invisible

Marta Royo-Llonch [1], Antonio Aranda-Ramos [1], Pablo Sánchez [2], Alba G. Corral [3], Santi Vilanova [3], Vanessa Balagué [2]

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Thalastasi is a transdisciplinary project that merges scientific research with artistic expression to highlight the essential role of marine microorganisms in regulating Earth's climate.
In this contribution to SAME, we will present the process behind the creation of Thalastasi, based on the artistic interpretation of published data on the genetics of microbial processes (such as photosynthesis, cloud formation and organic matter degradation) and physico-chemical environmental parameters collected during the Malaspina 2010 oceanographic expedition. This scientific effort is transformed through creative generative algorithms into a digital art piece. The resulting audiovisual compositions recreate the ocean's water column, down to the bathypelagic, embodying the Gaia Theory's concept of Earth as a living meta-organism. Through this innovative lens, the invisible marine world becomes both accessible and emotionally evocative. The project includes an educational resource and presentations at academic forums throughout 2024-2025. The art piece has been shown at Eufònic arts festival and at the Digital After All digital art exhibition. Thalastasi exemplifies new contemporary formats for science transference and fosters deeper public engagement and awareness with ocean ecosystems in the context of climate change.

Multifaceted insights into particle-associated N2 fixation

Mar Benavides [1]

[1]*National Oceanography Centre, United Kingdom.*

Dinitrogen (N2) fixation by diazotrophs provides the largest external source of reactive nitrogen in the ocean. Over the past decade, nifH gene analyses have shown that non-cyanobacterial diazotrophs (NCDs) can dominate diazotroph communities. Some prominent NCDs like Gamma-A are recurrently found in large filter size fractions, suggesting a particle-associated lifestyle. However, nifH gene sequencing based approaches are insufficient to understand how NCDs interact with the particles they presumably inhabit. We investigate NCDs in suspended, slow sinking and fast sinking particles collected with a marine snow catcher in the North Pacific, North Atlantic and Arctic Oceans. Combining qPCR, nifH amplicon sequencing and stable isotope probing we find that NCD groups have different affinities for particle types. Our results provide novel insights into the relationship between NCDs and particles, paving the ground towards elucidating their role in nitrogen cycling in the ocean.

Linking Microbial Community Structure to the Fate of Marine Contaminants

Maria Vila-Costa [1]

[1]*IDAEA-CSIC*

The persistence of synthetic organic contaminants in marine environments poses a growing concern for ecosystem functioning and biogeochemical stability under global change. Microbial communities play a critical role in modulating the fate of these compounds, yet the mechanisms governing their biodegradation and the ecological consequences of pollutant exposure remain insufficiently resolved. I will talk about the bidirectional interactions between marine microorganisms and emerging organic contaminants, using per- and polyfluoroalkyl substances (PFAS) and organophosphate esters (OPEs) as representative chemical classes. By integrating advanced chemical analyses with microbial genomics and physiological assessments, we characterize how microbial taxa with distinct ecological strategies —such as free-living, particle-attached, and host-associated forms— contribute to the transformation of anthropogenic compounds. Simultaneously, we assess how these pollutants influence microbial community composition and activity, potentially restructuring microbial networks and functions. These findings provide new insights into the microbial drivers of contaminant turnover in the ocean and the reciprocal impacts of pollution on microbial ecosystem dynamics, with implications for understanding contaminant persistence and feedbacks to marine biogeochemical cycles in a changing ocean.

Patterns and processes of aquatic microbial communities across space and time

Hugo Sarmento [1]

[1]*Universidade Federal de São Carlos*

Microbes are the engines of the Earth. In aquatic environments, they regulate nutrient cycling, mediate energy flow, and are key to ecological resilience. Despite their key role, the ecological and environmental factors that shape microbial diversity, composition, and function across spatial and temporal dimensions remain at the forefront of research. From freshwater systems to coastal areas and the open ocean, aquatic microbes inhabit dynamic habitats structured by gradients in light, temperature, nutrients, and physical connectivity. Unlike terrestrial ecosystems, aquatic environments are in constant motion, with water masses, currents, and mixing processes continuously redistributing organisms and resources. This inherent fluidity has profound implications for microbial ecology, influencing dispersal, connectivity, and the scale at which environmental selection operates, which makes prediction and modeling challenging. This presentation examines how ecological processes (selection, dispersal, drift and speciation) interact with spatial (horizontal and vertical) and temporal (seasonal and interannual) variability to structure microbial communities. Insights from global surveys highlight consistent biogeographic patterns, including latitudinal diversity gradients and vertical stratification, while long-term time-series emphasize the influence of seasonality, climate variability, and energy flux on microbial dynamics. Drawing from recent findings in both marine and freshwater systems, this talk emphasizes the importance of integrating broad-scale spatial observations with high-resolution temporal data to advance understanding of aquatic microbial ecology. The urgent need to expand monitoring with standardized protocols in underrepresented regions and timeframes will also be discussed, with emphasis on ethical collaboration and capacity building to improve our understanding of how aquatic microbial communities respond to environmental change in a rapidly changing world.

How can microbial traits help understand and predict ecosystem functions?

Elena Litchman [1]

[1]*Michigan State University*

Understanding how microbial communities and ecosystems will function in the future is a question of both fundamental and applied importance. Major ecosystem functions, such as productivity, biogeochemical cycling, carbon sequestration and their resilience to perturbations depend on the performance of diverse microbes that respond differently to environmental conditions. Predicting how microbial community composition drives ecosystem functions requires a mechanistic approach that allows to incorporate biodiversity and can be scaled up. Here I discuss how functional traits can provide such a mechanistic foundation to help tackle fundamental and applied problems. I will provide examples of using traits to answer key questions in microbial ecology and ecosystem functioning. I will show how the diversity in phytoplankton thermal performance curves can help maintain productivity under temperature fluctuations and how comparing traits of harmful algal bloom (HAB)-producing species to other phytoplankton and to environmental conditions may help predict HAB occurrence. Finally, I will outline some challenges and future directions of using trait-based approaches in microbial ecology.

Ordering microbial diversity into ecologically cohesive units

Martin Polz [1]

[1]*University of Vienna*

Microbial diversity is vast and ordering it into units of diverstiy that reflect cohesive ecology remains challenging. Theory predicts that the balance of gene flow and selection is key in determining one of tow evolutionary paths towards creating units of genetic similarity, and of phenotype and ecological function. First, if if recombination is frequent and selection moderate, ecologically adaptive mutations or genes can spread in niche-specific manner within populations independently of their original genomic background (gene-specific sweeps). In fact, these gene-specific sweeps may serve to predict recent adaptations that differentiate populations from their next of kin, an approach we have previously termed "reverse ecology". Second, if the effect of recombination is smaller than selection, genome-wide selective sweeps should occur creating a genomically homogeneous population. We will show that both paths towards creating adaptive popualtions are realized in diverse microbial communities and we will end with a discussion of how such ecological population structure relates to common sequence similarity cutoffs frequently used to delineate species and populations.

The role of behaviour in inter-microbial relationships in the pelagic ocean

Justin Seymour [1]

[1]*University of Technology Sydney*

Rather than solo travellers on the ocean's currents, planktonic marine microbes are embedded within intricate ecological networks, whereby complex inter-species relationships can often be just as important as abiotic environmental drivers in governing microbial community composition and function. Arguably among the most important of these inter-microbial interactions are those involving phytoplankton and bacteria. These relationships can be symbiotic in nature, and often involve reciprocal exchanges of metabolites, resulting in mutual growth enhancement and sometimes ecosystem-wide biogeochemical outcomes. The physical nature of the pelagic realm, along with the specificity of chemical exchanges involved in these interactions, often means that close proximity of partner organisms will be crucial for phytoplankton-bacterial symbioses. Therefore, microbial behaviours including motility and chemotaxis will potentially be key for the establishment and maintenance of relationships. In my talk I will synthesize results derived from laboratory-based experimentation and in situ behavioural assays, which have provided a view into the intricate social lives of planktonic marine microbes. These studies have revealed the importance of specific chemical currencies, unique behavioural strategies and metabolic co-dependencies in shaping the microscale organisation, and potential large-scale influence, of the ocean's microbiome.

Cells to Systems: Tracing Protistan Biodiversity and Activity in Ocean Food Webs

Sarah Hu [1]

[1]*Texas A&M University*

Single-celled microbial eukaryotes play ecologically significant roles in virtually all environments on the planet. In marine food webs, protists are tasked with primary production, the consumption and mortality of other microbes, and recycling of key nutrients. Using methods in meta'omics and assays to estimate microbial carbon transfer and biomass, we present two examples that explore how these single-celled microbes contribute to ecosystem function. In an extremely dynamic, anthropogenically-impacted ecosystem – we investigate how marine heat waves, nutrient runoff, and exposure to oil leakage shape protistan community diversity and structure. In the deep ocean, where a chemosynthetically fueled, biologically rich habitat exists we examine the impact that protists have in the food web and consider what this means for the deep-sea carbon budget. Our findings illustrate several examples for how protists fulfill their critical place in marine ecosystems.

Diversity of giant viruses across ecosystems

Matthias Fischer [1]

[1]*MPI for Marine Microbiology*

Unicellular eukaryotes are host to various types of viruses, including a large and diverse phylum of dsDNA viruses, the Nucleocytoviricota. Commonly known as giant viruses, they rival bacterial genomes in terms of length and coding capacity, typically encoding several hundred proteins. Much about the lifestyle and ecological importance of giant viruses remains unknown, but both metagenomic studies, as well as isolation and structural characterization, have revealed a stunning diversity of genes, shapes, and infection strategies. I will highlight some fascinating aspects of giant viruses from marine, freshwater, and soil ecosystems, offering just a glimpse into their diversity and complexity.

Machine learning in marine microbial ecology: A skeptic's guide

Andrew Steen [1]

[1]*University of Southern California, USA*

Machine learning, and in particular deep learning, has made an impact on nearly every field of science, not least marine microbial ecology. Meanwhile, nucleic acid sequencing technologies, and to a lesser extent protein sequencing technologies, have given us more data than we sometimes know what to do with. It might seem that machine learning approaches are the future of microbial ecology. In this talk, I'll argue that they are – partly – and I'll attempt to separate some of the hype from the reality. I'll review some of the basic types of machine learning models that have been used in microbial ecology. Using examples from my own recent work, I'll discuss some ways various machine learning models can be effective in microbial ecology - and some ways they can be ineffective. Lastly, I'll offer some perspectives on how those of us who, like me, are not trained as computer scientists, can most effectively use machine learning approaches as one tool of many in the toolbox we use to understand how aquatic microorganisms interact with each other and the Earth.

BOOK OF ABSTRACTS

Oral Presentations

Monday, September 29

Microbial functions and biogeochemical cycles

Expanding the view of marine carbon fixation through genome-resolved metagenomics

Anna Mankowski [1], Sander Wuyts [2], Anthony Fullam [1], David Eckey [3], Peer Bork [1]

[1]*EMBL Heidelberg*
[2]*ImmuneWatch*
[3]*Heidelberg University*

Autotrophy is a fundamental biogeochemical process, driving food webs and sequestering atmospheric carbon dioxide. The Calvin-Benson-Bassham (CBB) cycle is the primary carbon fixation pathway in marine ecosystems, dominating photosynthesis in the surface ocean but also contributing to chemosynthesis. However, growing evidence suggests that alternative autotrophic pathways coexist alongside the CBB cycle, especially in environments such as oxygen minimum zones (OMZs), hydrothermal vents and sediments. The global biogeographic distribution of organisms using these pathways remains largely unknown, despite their potential ecological significance. To address this gap, we developed a computational framework to detect seven autotrophic pathways in metagenomes and metagenome assembled genomes (MAGs). Screening >1,000,000 MAGs, we identified >1000 species of putative autotrophs. Among these, >250 species encoded the genetic potential for alternative pathways. By linking each genome to sample metadata, we revealed that these organisms occur in diverse ecosystems, including previously described habitats such as OMZs, hydrothermal vents and sediments . Notably, we also detected organisms putatively capable of alternative carbon fixation in the surface ocean, suggesting their contribution to carbon cycling in photosynthesis-dominated regions. Our findings highlight the ecological relevance of alternative carbon fixation and provide a foundation for further characterisation of autotrophs, their roles in ocean carbon cycling.

Multi-omic insights into chemoautotrophic carbon fixation in deep Antarctic Water masses

Rita Varchetta [1], Donata Canu [1], Angela Garzia [2], Naomi Krauzig [3], Pierpaolo Falco [3], Vincenzo Manna [1], Marco Puglia [1], Elisa Banchi [1], Mauro Celussi [1]

[1]*National Institute of Oceanography and Applied Geophysics—OGS*
[2]*Università La Sapienza, Roma, Italy*
[3]*Università Politecnica delle Marche, Ancona, Italy*

The Ross Sea (Antarctica) hosts the process of formation and export of two important water masses: High Salinity Shelf Water (HSSW) and Ice Shelf Water (ISW). Along the continental slope they mix with Circumpolar Deep Water (CDW) to produce Antarctic Bottom Water (AABW), ultimately affecting the global circulation. In these waters,

chemoautotrophic archaea and bacteria actively participate in CO2 sequestration, playing a key role in the global carbon cycle. To explore potential chemoautotrophic pathways within the prokaryotic communities in the different water masses we used a multi-omic approach, including metabarcoding and metagenomic analysis, by examining the abundance of key genes involved in inorganic carbon fixation and energy-generating metabolic pathways related to the oxidation of nitrogen, sulfur and methane compounds. Our data show that microbial communities in these water masses potentially utilize four autotrophic CO2 assimilation pathways: the Calvin-Benson-Bassham cycle (CBB), the 3-hydroxypropionate cycle (3HP), the dicarboxylate-hydroxybutyrate cycle (DC/4HB) and the reverse tricarboxylic acid (rTCA). Production of new organic carbon is likely driven by ammonia-oxidizing archaea, (e.g. Candidatus Nitrosopumilus) in HSSW and ISW, and by sulfur-oxidizing bacteria (e.g. SAR324 and SUP05) across all water masses.

Microbial incorporation of methionine and leucine in the pelagic ocean

Chie Amano [1], Zihao Zhao [1], Noémie Lebon [1], Sophie Dobianer [1], Thomas Reinthaler [1], Eva Sintes [2], Gerhard Herndl [1]

[1]*University of Vienna*
[2]*IEO-CSIC*

Leucine incorporation is widely used as a proxy for heterotrophic bacterial production in the ocean. Methionine is also essential for protein synthesis. However, methionine incorporation is typically lower than that of leucine. While both substrates are widely used for heterotrophic production of microbial communities, it is not known whether all the heterotrophic microbes are able to take up these substrates. Thus, we investigated methionine and leucine transporter gene distributions and expression levels using both publicly available and in-house metagenomic and metatranscriptomic datasets. In parallel, we measured single-cell uptake of 3H-leucine and 3H-methionine in specific microbial groups with distinct substrate transport systems (ABC vs. TonB transporters) using MICRO-CARD-FISH. Although microbial taxa incorporating methionine and leucine were similar, leucine transporter expression was approximately an order of magnitude higher than methionine, consistent with lower methionine incorporation observed at the single-cell level. This difference suggests widespread de novo synthesis of methionine over reliance on external uptake. Certain taxa (e.g., SAR202) exhibited disproportionately high methionine transporter expression relative to genomic abundance in the mesopelagic, indicating regulated uptake. Our findings confirm leucine's robustness as a production proxy and demonstrate that methionine is a viable complementary tracer when using sensitive detection approaches including an optimized BONCAT protocol.

Distinct communities of particle-associated microbes drive organic carbon degradation in the dark ocean

Marilena Heitger [1], Chie Amano [1], Thomas Reinthaler [1], Maria Papadatou [1], Leo Pokorny [1], Xosé Antón Álvarez Salgado [2], Gerhard J. Herndl [1]

[1]*University of Vienna*
[2]*CSIC Instituto de Investigacións Mariñas (IIM-CSIC), Vigo, Spain*

The biological carbon pump mediates the export of particulate organic carbon from the euphotic zone to the deep ocean, where it provides the base of the food web. Although deep-sea microbial metabolism is generally considered to be largely mediated by macroscopic particles, such as marine snow, the specific contribution of particle-associated microorganisms to the utilisation of bulk organic matter has rarely been quantified. We used in situ pumps to collect particles larger than 3 µm from meso- and bathypelagic waters along a latitudinal transect in the North Atlantic. Prokaryotic abundance, respiration, biomass production, and community composition were determined and compared to the bulk microbial community, which was collected by Niskin flasks. Although particle-associated heterotrophs represented less than 1% of total prokaryotic abundance, they contributed on average 28% to total prokaryotic respiration and 12% to biomass production. Organic carbon turnover times were 40 times shorter in the particulate than in the total organic carbon fraction, highlighting particles as ‘hotspots’ of organic carbon remineralisation. Furthermore, metagenomic analyses revealed clear differences in taxonomy and diversity between the free-living and particle-associated microbial communities. Our results emphasise the significant role of particle-associated prokaryotes in driving organic matter utilisation in the dark ocean.

Lateral inputs disrupt the vertical connectivity of the marine particle microbiome

Paula Sabaté-Fàbregas [1], Viena Puigcorbé [1], Marta Sebastián [1], Ona Deulofeu-Capo [2], Olga Sánchez [2], Laura Alonso-Sáez [3], Josep M Gasol [1]

[1]*Institut de Ciències del Mar (ICM-CSIC)*
[2]*Universitat Autònoma de Barcelona*
[3]*AZTI*

Marine particles in the surface ocean are hotspots for microbial colonization and play a key role in transporting organic matter to the deep sea. As particles sink through the water column, they undergo microbial degradation, reflected in changes in the composition of their associated microbiomes. In this study, we examined the particle-associated and free-living communities of prokaryotes, eukaryotes, and fungi across multiple stations in the Northwestern Mediterranean Sea during different stages of phytoplankton blooms. Vertical particle fluxes were quantified using the 238Uranium:234Thorium disequilibrium method, and depth-resolved microbial communities were assessed through metabarcoding. Stations with the highest particle export fluxes were located within submarine canyons, where lateral inputs introduced new microbial sequences into the water column. These inputs disrupted the expected vertical succession of microbial communities, particularly in prokaryotes and eukaryotes, and to a lesser extent among fungi, which exhibited limited recruitment of

new sequences with depth. Our findings suggest that lateral transport can significantly alter the composition and structure of sinking particle-associated microbiomes, potentially decoupling surface and deep microbial communities. These processes may ultimately influence the efficiency of carbon export to the deep sea and the functioning of the biological carbon pump in coastal-influenced marine systems.

The spatial arrangement of non-cyanobacterial diazotrophs within particles impacts their N2 fixation and respiration activity

Arthur Coët [1], Olivier Grosso [1], Nicolas Brouilly [2], Pierre Ronceray [3], Mar Benavides [4]

[1]*Mediterranean Institute of Oceanography*
[2]*Institut de Biologie du Développement de Marseille*
[3]*Centre interdisciplinaire de nanoscience de Marseille*
[4]*National Oceanography Centre*

Dinitrogen (N_2) fixation is a crucial reactive nitrogen source for marine ecosystems, with non-cyanobacterial diazotrophs (NCDs) increasingly recognized as important contributors across diverse oceanic regions. Marine organic particles are considered an ideal niche for NCDs due to their dual role as low oxygen refuges and carbon availabilityHere, we investigate how the spatial architecture of particle colonization facilitates N2 fixation by NCDs. Using Vibrio diazotrophicus and diatom aggregates as NCDs and particle models, respectively, we combine nitrogenase protein immunolabeling and cell respiration indicators, and stable isotope tracing to map NCDs and their metabolic activity at the microscale. Our results reveal a temporal succession starting with early respiration depleting oxygen in the particle, forming anoxic microniches that allow nitrogenase expression and N_2 fixation. This dynamic reflects microbial niche engineering, where NCDs modify their environment to meet their metabolic needs. Notably, nitrate enrichment altered the colonization patterns, reducing nitrogenase expression despite similar oxygen consumption, indicating complex, resource-driven assembly. These findings suggest that marine particles act as microscale oxygen minimum zones, enabling diazotrophy even in oxygenated waters. By resolving these spatial dynamics, our study provides new understanding of how microscale processes shape oceanic nitrogen cycling.

Dissolved organic matter impacts Arctic nitrogen fixation

Stine Zander Hagen [1], Lisa W von Friesen [2], Amane Fujiwara [3], Urban Wünsch [4], Olivier Grosso [5], Mar Benavides [6], Takuhei Shiozaki [7], Colin A Stedmon [4], Lasse Riemann [1]

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Nitrogen is a key limiting nutrient for primary production in the Arctic Ocean. Hence, knowledge of the sources and sinks of nitrogen is essential for understanding current and future productivity in the region. N_2 fixation, performed by microorganisms called diazotrophs, releases bioavailable ammonium to the ocean by reducing inert dissolved N2 gas. Dissolved organic matter is known to stimulate N_2 fixation in tropical and coastal waters, however, its role in the Arctic Ocean remains unclear. Here, we report N_2 fixation in the upper pelagic layer (0 – 100 m) of the Pacific Arctic Ocean in early autumn, ranging from 0 – 2.34 nmol nitrogen L^{-1} d^{-1}, with the highest rates at a coastal station in the Beaufort Sea. Additionally, we found that dissolved organic matter occasionally stimulated N_2 fixation. The UCYN-A2 (cyanobacterial nitroplast of a haptophyte alga) dominated the nifH gene (biomarker for diazotrophs) reads (47%) and expressed nifH across most locations and depths. Our results suggest that N_2 fixation is a widespread nitrogen source in the Pacific Arctic Ocean, supporting 0.4 to 17% of new in situ primary production in surface waters.

Response of marine microbial communities to iron contained in glacial colloids in the Southern Ocean

Rhea Thoppil [1], Marine Landa [1], Ingrid Obernosterer [1], Stéphane Blain [1]

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Trace element iron (Fe) availability is a major constraint for microbially-mediated processes in the Southern Ocean (SO). Glacial erosion presents a novel source of this element, but whether glacial Fe is bioavailable to marine microbes is poorly known. We carried out on-board incubation experiments with marine microbial communities amended with Fe contained in glacial colloids (20 nm – 200 nm) collected at the outlet of the Cook Ice Cap on Kerguelen Island (SO) during the project MARGO (Matter of Glacial Origin and its Fate in the Ocean). Glacial colloids stimulated phytoplankton and heterotrophic prokaryotic growth as compared to non-amended controls. The abundance and expression of genes related to prokaryotic Fe-uptake, in particular siderophore synthesis and transport, were higher in the colloid-amended treatments as compared to the control, suggesting this process could play a role in the access to glacial Fe. We further identified the metagenome-assembled genomes (MAGs) that harboured and expressed these genes, and explored the link between iron utilization and central carbon metabolism. Our findings provide insights into the potential role of distinct prokaryotic taxa in rendering glacial Fe bioavailable thereby shaping microbial dynamics and carbon cycling in the SO.

Spatial patterns of nitrogen-transforming processes in the Southern Ocean: potential role of Dissimilatory Nitrate Reduction (DNR)

Sharvari Sunil Gadegaonkar [1], Rui Zhang [1], Hélène Planquette [2], Antoine Rigaud [2], Corentin Baudet [2], Stéphane Blain [1], Ingrid Obernosterer [1]

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[2]*CNRS, Université Brest, IRD, Ifremer, Laboratoire des Sciences de l'environnement marin, Technopôle Brest-Iroise, Plouzané, France.*

The nitrogen (N) cycle is crucial for aquatic systems, as it governs the availability of this essential nutrient and thereby supports the productivity of marine ecosystems. While most research in the oxygenated ocean has thus far focused on assimilatory nitrogen fixation and nitrification, the potential role of dissimilatory processes remains poorly investigated. The objectives of the present study were to determine the spatial patterns of N transforming processes, their associations with trace metals that are cofactors, and to identify the involved microbial taxa. We analyzed metagenomes collected along a latitudinal gradient and divers water masses in the Indian sector of the Southern Ocean during the South West Indian GEOTRACERS section cruise. Genes involved in DNR [denitrification and dissimilatory nitrate reduction to ammonium] dominated over those of other N-transforming processes across all water masses, with a notable preference for enzymes requiring copper (Cu) or molybdenum (Mo) over iron (Fe), indicating alternative trace metal adaptation. Metagenome-assembled genomes (MAGs) associated with DNR possessed carbon fixating pathways suggesting mixotrophic strategies. Our observations show the metagenomic potential of DNR as an alternative or additional source of energy for the microbial community in the carbon limited deep ocean.

Seasonal and stable heterotrophic guilds drive Arctic benthic microbiome functioning across polar day and night

Chyrene Moncada [1], Carol Arnosti [2], Jan David Brüwer [1], Dirk de Beer [1], Gunter Wegener [1], Peter Stief [3], Marit R van Erk [4], Jürgen Titschack [5], Rudolf Amann [1], Katrin Knittel [1]

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[4]*Department of Microbiology, Radboud Institute for Biological and Environmental Sciences, Radboud University*
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The remineralization of organic matter by benthic bacteria is an essential process in the marine carbon cycle. In polar regions, strong variation in daylength cause pronounced seasonality in primary productivity, but the responses of sedimentary bacteria to these fluctuations are not well understood. We investigated the seasonal dynamics of benthic

bacterial communities from an Arctic fjord and found a partitioning of the communities into seasonally-responsive and stable guilds. We separately analyzed the fractions of cells in the porewater, and those loosely and firmly attached to sand grains through 16S rRNA gene sequencing, cell counting, rate measurements, and geochemical analyses. The porewater and loosely attached bacterial communities showed a dynamic response in composition and activity, suggesting that they play a central role in bentho-pelagic coupling by responding rapidly to seasonal fluctuations in organic matter availability. In contrast, the majority of the firmly attached cells showed a more buffered response, as reflected, for example in the consistently high cell numbers of Woeseia. This fraction is potentially key to maintaining baseline remineralization processes throughout the year, independent of fresh organic matter input. These findings provide new mechanistic understanding on carbon cycling in Arctic surface sediments that may also apply beyond polar regions.

Aerobic methane production associated with microbial methylphosphonate utilization in the Mediterranean Sea

Judith van der Giessen [1], Juliane Schoetz [1], Jan N. von Arx [1], Benjamín A. Pérez López [1], Fabian Lange [1], Hon Lun Wong [1], Mertcan Esti [1], Wiebke Mohr [1], Gaute Lavik[1], Jana Milucka [1]

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Oxic surface waters of the open ocean are supersaturated with methane. Microbial utilization of methylphosphonate, an organic phosphorus compound, has been shown to be a major source of aerobically produced methane when inorganic phosphorus is scarce. The Mediterranean Sea is strongly depleted in phosphate. However, little is known about in situ methylphosphonate utilization and the associated methane formation in this region. We detected methane concentration maxima in surface waters off Elba (Italy) in October (up to 8.6 and 11.3 nM), and to a lesser extent in June (up to 5.2 nM). Rates of 13C-methane production from 13C-methylphosphonate in the oligotrophic surface waters were highest in June (up to 0.64 nM d^{-1}) and decreased in autumn (up to 0.21 nM d^{-1}). Our molecular analyses revealed that methylphosphonate utilization coincided with a high abundance and transcription of the phnJ gene, a marker gene for phosphonate utilization, by small heterotrophic bacteria (e.g. Enterobacterales, and SAR11). Our combined results show that methylphosphonate utilization by abundant heterotrophic bacteria contributes to the observed methane supersaturation in the oligotrophic Mediterranean Sea over the year. Our findings shed light on aerobic methane formation, which is projected to become more prevalent in future ocean systems due to ocean warming.

Genome-resolved metatranscriptomics reveals population-specific strategies behind rhodopsin expression in an Atlantic Coast-offshore gradient

Danilo Di Leo [1], Sandra Martinez-Garcia [2], Benjamin Pontiller [3], Clara Pérez-Martínez [1], Daniel Lundin [1], José M. Gonzaĺe [4], Emilio Fernández [2], Eva Teira [2], Jarone Pinhassi [1]

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Although photoheterotrophy is a widespread life strategy among marine prokaryotes to support metabolism, its ecological relevance in natural environments remains unclear. Proton-pumping rhodopsins harvest sunlight to generate chemical energy in the form of ATP and are found in around 80% of marine bacterial species. To investigate the mechanisms by which rhodopsin light harvesting influences prokaryote activity, we analyzed 22 metatranscriptomics samples collected in three different seasons in the Atlantic coast-offshore gradient off northwest Spain. Mapping of transcript reads against metagenome-assembled genomes (MAGs) uncovered pronounced differences in metabolic processes of particular populations (the reads mapped to 2,100 MAGs, and 732 MAGs showed active rhodopsin transcription). In offshore waters, rhodopsin expression increased from winter to summer, with dominance of Pelagibacteraceae (up to 60% of rhodopsin transcripts) and decreasing proportions of Archaea. In contrast, in coastal waters, the Archaea increased from 3 to 25% of rhodopsin expression towards summer. Coexpression analysis identified linkages of rhodopsin expression with central metabolism in Pelagibacteraceae and Flavobacteriaceae, flagellar genes for motility in Puniceispirillaceae, and nutrient uptake in Flavobacteriaceae. These observations highlight that taxon-specific differences in how rhodopsin light energy is harnessed sheds new light on how ecological drivers influence microbiome structure and function in coast-offshore gradients.

Membrane transporter for dimethylsulfoniopropionate (DMSP) uptake in marine eukaryotic phytoplankton

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Sulfur is an essential element that marine microalgae acquire through energetically costly, assimilatory reduction of sea-salt sulfate. Incorporation of exogenous reduced sulfur by osmotrophic uptake would confer to non-phagotrophic phytoplankton an

adaptative advantage under energy-fluctuating conditions. We used radioisotope assays with the ubiquitous sulfur compound dimethylsulfoniopropionate (DMSP) to show that uptake occurs in cultured eukaryotic phytoplankton across various phyla. The strains capable of DMSP incorporation all harboured a betaine/choline/carnitine transporter (BCCT) gene. Using luciferase reporter and transcriptional assays in response to DMSP additions to picoalgae, we identified BCCT as the likely DMSP transporter. This was confirmed through the DMSP-uptake recovery of a BCCT-deficient E. coli mutant after recombination with a picoalgal BCCT gene. DMSP uptake and BCCT gene expression rates in ecologically-relevant algae increased with decreasing light, suggesting that many oceanic phytoplankton have developed versatile strategies to use exogenous reduced sulfur to survive energy limitation. Analysis of the TARA Oceans metatranscriptomes revealed that algal BCCT genes were expressed across phylogenetic groups all over the global surface ocean, with pronounced expression in the DMSP-rich polar regions. This work identifies the membrane transporter for DMSP uptake by marine eukaryotic phytoplankton, and highlights its widespread diversity and ecological relevance across the global ocean.

Limited nitrate assimilation ability of Synechococcus in the subarctic HNLC region revealed by metagenomic and NanoSIMS analysis

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Synechococcus is a cosmopolitan picocyanobacterium inhabiting from the tropical to polar regions, contributing significantly to marine primary production. Synechococcus populations are known to gain and lose genes to adapt to diverse environments. However, as most studies have focused on subtropical regions, their ecological adaptations in iron-limited high-latitude High Nutrient Low Chlorophyll (HNLC) areas remain poorly explored. To investigate how Synechococcus adapts to different nutrient conditions, we analyzed metagenomic data and single-cell nitrogen assimilation at nitrogen-depleted subtropical and iron-depleted subarctic stations in the western North Pacific. Metagenome read mapping showed that most Synechococcus populations in the subtropical region possessed genes for nitrate assimilation. This is consistent with previous knowledge that Synechococcus in the subtropical regions primarily relies on nitrate. In contrast, Synechococcus populations in the subarctic region largely lack genes for nitrate assimilation, while genes for reduced nitrogen utilization were retained. Consistently, NanoSIMS analysis in the subarctic regions revealed that picophytoplankton, including Synechococcus, actively assimilated ammonium but exhibited minimal nitrate uptake, likely reflecting their ecological strategy to reduce cellular iron demand. Such metabolic flexibility, particularly the ability to avoid iron-expensive nitrate assimilation, may support the ecological success and broad distribution of Synechococcus.

Unravelling Polyphosphate Metabolism in marine Beggiatoa

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Filamentous sulfide-oxidizing bacteria like *Beggiatoa spp*. have the ability to significantly alter benthic phosphate fluxes due to their polyphosphate storage capacity. While studies have shown the importance of these bacteria for phosphorus availability in fluctuating redox conditions of marine sediments, the genetic regulation of their polyphosphate metabolism is poorly understood. Here we combined physiological experiments with metatranscriptomic sequencing to investigate polyphosphate dynamics in the marine strain *Beggiatoa sp*. 35Flor under phosphorus starvation and subsequent reintroduction. We show that significant polyphosphate depletion occurred only in the fifth generation of starved cultures, suggesting its long-term retention and importance for metabolic functions. After phosphorus addition to starved cultures, phosphate uptake occurred rapidly within the first 10 minutes following the exponential decay model. Although polyphosphate formation was detected already within 5 minutes after phosphate addition, the ppk1 gene, which is typically linked to polyphosphate synthesis, exhibited downregulation during active polyphosphate formation. To explore holistic response, we applied expression-based filtering combined with functional annotation and protein domain prediction to screen for additional genes potentially involved in polyphosphate metabolism. Our results provide insights into the genetic basis of polyphosphate metabolism in *Beggiatoa* and raise questions about the postulated role of ppk1 enzyme in polyphosphate synthesis.

How genome-scale metabolic models and metabolomics help the functional understanding of microbes and viruses at work in salt marsh sediments

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Tidal salt marshes with their peaty soils, halophilic plants and large chemical-physical fluctuations, act as biodiversity and biogeochemistry hotspots. The worldwide loss of salt marshes, caused by erosion, storm surges, rising sea levels, and human activities, calls for multidisciplinary research and new metrics enabling their conservation, restoration and construction. Focusing on one natural and one restored zone in a salt marsh of the Venice Lagoon, we characterised abiotic and biotic components of tidal creek surface sediments, using prokaryotic Genome-scale metabolic models (GEMs), sediment metabolome and virus-encoded genes, to pinpoint key biological processes and ecosystem services. GEM analysis and sediment metabolite profiles outlined the fermentative and aerobic utilisation of multiple carbon sources and a balance between catabolism and anabolism. The predicted virus hosts largely mirrored prokaryotes' taxonomy. Moreover, GEMs and annotated viral genes outlined the influence of viruses on pentose phosphate and amino-sugar pathways, redox metabolism and

lipopolysaccharides biosynthesis. Our results point to the competence of the studied microbial community for degradation of complex organic matter, maintenance of metabolite pools, detoxification of fermentative intermediates and products, and highlight the importance of multidisciplinary approaches for microbiome studies. Study mostly funded by NextGenerationEU-PNRR-M4C2 Investments 1.4 (NBFC CN_00000033-C93C22002810006) and 1.5 (iNEST ECS00000043-C43C22000340006).

Low denitrification rates and high DNRA gene abundance in an oligotrophic stream during suboxic low-flow periods.

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Water scarcity and global warming can intensify anaerobic biogeochemical processes in streams. However, most knowledge about carbon (C) and nitrogen (N) cycling in headwater streams comes from studies under aerobic conditions. In this study, we examined how dissolved oxygen (DO) depletion during the drying phase of an intermittent stream affects N metabolism. As the stream shifted from wet to dry, we periodically measured: (i) potential denitrification and anaerobic respiration rates, (ii) microbial composition, and (iii) N-cycling gene abundance (nitrification, denitrification, DNRA, and anammox). DO dropped from 10 to 2.4 mg O_2 L^{-1} while CO_2 rose from 6.2 to 12.7 mg CO_2 L^{-1}. Under suboxic conditions (800 ng N-CO_2 cm^{-2} h^{-1}), denitrification was low (1000). DNRA gene abundance exceeded that of denitrification (10.0% vs 2.4% relative to 16S rRNA), with a close link to nitrification genes, suggesting DNRA prevalence over denitrification. These findings underscore the role of anaerobic metabolism in N retention and its potential impact on greenhouse gas emissions under low-flow conditions.

Microbes under global change

Driving the adaptation of microbial communities of the thawing permafrost: Mobile genetic elements at play

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With climate change, the thawing of permafrost leads to the expansion of thermokarst ponds and lakes, which are recognized sources of greenhouse gas emissions and can release metallic compounds. Despite the recognition of a high microbial biodiversity and their roles in biogeochemical cycles, mechanisms underlying the trajectories of microbial communities remain poorly understood. Mobile genetic elements such as plasmids, which are known to evolve rapidly, may facilitate microbial adaptation as communities rapidly transition from terrestrial to aquatic habitats. We investigate the role of plasmids in shaping the eco-evolutionary dynamics of microbial communities using a multi-omics approach. Samples were collected along soil-water continua in thermokarst and coalescent ponds of continuous permafrost (Bylot Island, Canada). Preliminary analyses reveal that water plasmidomes encode a higher proportion of genes involved in plasmid conjugation. A multilayer plasmid network based on genetic similarity highlights the connectivity between soils and thermokarst samples, and the emergence of a distinct plasmidome in coalescent ponds. Notably, transitioning plasmids carry genes encoding key functions involved in arsenic resistance or phosphate metabolism. These findings suggest that plasmids play a significant role in the adaptation of permafrost microbial communities, with important implications for predicting their eco-evolutionary trajectories as climate change accelerates permafrost thaw.

When all you have is a microplastic particle… everything looks like a deadly pathogen!

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The study of microbial communities that find refuge or proliferate on microplastic particles in freshwater and marine environments has become a well-established field within microbial ecology. These communities have even earned a dedicated name for over a decade now: the plastisphere. Hundreds of studies have “demonstrated” that plastic particles in aquatic environments can serve as hotspots for the potential proliferation of allochthonous, often pathogenic and antibiotic-resistant bacteria, an assumption largely based on the evident differences between plastisphere communities and the surrounding free-living microbial assemblages. However, the vast majority of these studies neglect the presence of natural organic particles in the water column and the microbial communities associated with them. In this communication, using both experimental data and oceanographic surveys produced by my group and others, I will show that the differences between the plastisphere and the microbial

communities inhabiting the marine snow are minimal and often driven more by the type of plastic or organic particle than by the synthetic nature of the substrate. I will also illustrate how sampling and analysis methods for microplastic particles can introduce substantial biases, issues that are rarely acknowledged in public communication, ultimately leading to a significant overestimation of the problem in the media.

Metagenomic analyses reveal the distribution of antimicrobial resistance genes in bacterioplankton communities of coastal marine aquaculture systems

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The rapid growth of aquaculture as a vital protein source has raised concerns due to intensive practices with extensive antibiotic use increasing antimicrobial resistance (AMR). However, mechanisms of AMR gene transfer among aquatic microbes remain largely underexplored. Therefore, we used genome-centric analyses to investigate distribution and transmission of antibiotic resistance genes (ARGs) in aquaculture while assessing potential health risks to humans. Metagenomes of water samples from coastal aquaculture facilities in Bolinao, Philippines, were sequenced and assembled. The assembled contigs were annotated with deepARG, and mobile genetic elements were identified using geNomad to assess the colocalisation of genes. Our findings indicated that ARGs were widely distributed in bacterioplankton communities of the aquaculture and the surrounding coastal ecosystem. In total, 453 ARG-carrying contigs were identified, encompassing 14 unique ARG classes. The most dominant ARG classes were multidrug, peptide, tetracycline, MLS, and β-lactam, accounting for 84% of all ARGs. Furthermore, more ARGs were present on plasmids than on phages and chromosomes. Primary hosts of ARGs included *Vibrio, Stenotrophomonas, Mycobacterium, Pseudomonas, Acinetobacter*, and *Enterococcus*, which were also the main genera harbouring multiple ARGs. In conclusion, coastal areas subjected to intensive aquaculture favour (pathogenic) bacteria resistant to multiple antibiotics with potential for horizontal gene transfer, greatly increasing human and environmental health risks.

Linking productivity and community size to stochasticity in microbial community assembly

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Biodiversity loss threatens ecosystem functions, making it crucial to understand how species form communities and affect ecosystems. Community assembly is governed by both determinism, interactions between species and their environment, and stochasticity, random events such as drift and priority effects. Stochasticity can play a significant role in shaping community composition, especially under high productivity due to environmental change and in smaller communities caused by habitat destruction. To explore interactive effects of productivity and community size on assembly processes, we conducted a microcosm experiment by diluting freshwater bacterial communities to different sizes and subjecting them to varying nutrient levels. Stochasticity was measured as dispersion in beta-diversity among replicate communities. Under low nutrient conditions, communities were primarily driven by determinism, especially in smaller communities. In contrast, stochasticity had a stronger influence in larger communities exposed to higher productivity. This may be due to enhanced priority effects, where increased productivity and community size promote the growth of a broader range of species, resulting in greater variation among colonizing communities. Our study highlights the importance of stochasticity in microbial community assembly and further studies are needed to unravel the relationship of community size and stochasticity in interaction with other environmental change drivers.

Diatom-bacteria interactions in the context of temperature stress

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In the context of Global Warming, discrete and extreme high-temperature events (Marine Heat Waves, MHW) occur more frequently and intensively. In addition to deadly threats to individual species, increasing temperature can reshape organisms' interactions because of thermal tolerance impairments. This can disrupt the entire community structure and function, leading to large-scale effects. In particular, we are interested in understanding how the host-associated microbiome may impact the thermal tolerance of the phytoplankton host, focusing on the cosmopolitan and bloom-forming diatom Chaetoceros. We first aim to describe the spatial distribution of diatom-bacteria interactions at planetary scale and single-cell resolution. Thus, we are developing a pipeline combining AI-supported photo-activation, single-cell isolation and high-throughput sequencing techniques to reveal true physical interactions from complex environmental samples. We will apply this method on the Tara Ocean ethanol-fixed biobank to compare physical interaction networks involving Chaetoceros in the poles and equator areas. Also interested in the mechanisms and dynamics of these interactions, we are conducting MHW simulations in the lab on axenic and xenic

Chaetoceros strains. With evidence that the microbiome may increase resilience, we seek to decipher mechanisms. To scale-up our understanding, we are planning MHW mesocosm experiments (July 2025) on the natural community.

European Anthropocene: biodiversity shifts driven by human activities revealed through paleogenomics

Raffaele Siano [1], Arthur Monjor [2], Dimitra Ioli Skouroliakou [1], Mathisse Meyneng [1], Guillermo Andres Rangel Piñeros [3], Thomas Haize [4], Joanna Pawłowska [5], Antonio Fernandez-Guerra [6], Paola Bertucci [7], TREC Paleocore project consortium

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Since the onset of significant human activities on Earth —marking the Anthropocene— coastal biodiversity has undergone profound structural and compositional changes. Paleogenomic approaches have made it possible to analyze these changes over centuries, even in heavily disturbed ecosystems. To assess the impact of eutrophication, industrialization, aquaculture, and urbanization in Europe, shifts in prokaryotic and eukaryotic biodiversity have been studied using metabarcoding and metagenomic data derived from sedimentary ancient DNA (sedaDNA). As part of the TREC expedition, sediment cores approximately 1 meter in length were collected from 15 coastal sites across 9 European countries (France, Spain, Italy, Greece, Sweden, Germany, Poland, Estonia, and Finland). Radioisotope dating confirmed that all cores encompass time periods associated with pollution events and/or ecosystemic changes at each site. Barcoding (V4, V9 18S rDNA and V4-V5 16S rDNA) and metagenomic analyses were performed on samples corresponding to periods of human impact, as corroborated by contaminant profiles. Cross-site and cross-country comparisons will test hypotheses regarding biodiversity loss, shared community trajectories in response to specific anthropogenic pressures, and regional temporal dynamics of invasive species. Preliminary results supported by the EU-BIOcean5D project will be presented and discussed within the broader spatial context of the TREC project.

Resistance and resilience of lacustrine microbial communities facing eutrophication

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Microbial communities are key indicators of aquatic ecosystem health due to their rapid response to environmental changes. While they are central to ecosystem functioning and sensitive to disturbances, their resistance and resilience to nutrient enrichment remain understudied in lacustrine systems. In this study, we investigated how microbial assemblages from three Québec lakes with contrasting trophic statuses responded to experimental exposure to eutrophic conditions. Using dialysis bags incubated in a shared eutrophic environment, we assessed differences in community resistance and resilience, identified key microbial taxa and functions associated with these responses, and explored potential microbial indicators of disturbance. Microcosm experiments, combined with amplicon sequencing and metatranscriptomics, showed marked differences in community stability across lakes, with the oligotrophic lake's microbiome exhibiting distinct responses compared to those from more nutrient-rich systems. Notably, Paucibacter emerged as a potential keystone taxon, suggesting a central role in structuring oligotrophic microbial communities under nutrient stress. These findings enhance our understanding of how lake microbiomes respond to global change stressors and highlight the importance of context-dependent microbial dynamics in assessing ecosystem health.

Under-ice methanotrophy may offset Baltic sea ice methane fluxes

Eeva Eronen-Rasimus [1], Igor Pessi [1], Nicolas-Xavier Geilfus [2], Lumi Haraguchi [1], Eric Collins [3], Jenni Hultman [4]

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Sea ice is an active component of Earth's climate and oceanic systems, e.g. altering the energy flow between the atmosphere and sea as well as limiting gas exchange. Recent evidence suggest that Baltic Sea ice could serve as a temporary storage for greenhouse gases (GHGs) such as methane and N2O. Sea-ice brine is a habitat for diverse microscopic organisms including archaea, viruses, protists and bacteria. These bacteria decompose particulate organic matter, remineralize nutrients and convey carbon to the upper trophic levels through microbial loop. However, their specific metabolic functions still are still largely unknown. The aim of this study was to investigate the microbial community composition and activity using metagenomics and metatranscriptomics with special emphasis on assembling potential methanotrophic bacteria from sea ice and under-ice water. The results show that under-ice water had

pmoA transcripts mainly belonging to Gammaproteobacteria, pointing to active methanotrophy in the under-ice water. Our results suggest that sea ice may hinder the methane flux from seawater to atmosphere if the methane trapped under semi-permeable ice is consumed by methanotrophic bacteria.

Evidences of human influences on microbial processing of dissolved organic matter in a Mediterranean coastal environment

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Dissolved organic matter (DOM) and heterotrophic prokaryotes (HP) are key players in the oceanic carbon cycle. There is growing interest for DOM chemical and ecological properties. However, their variability in the heterogeneous coastal environment and their hierarchy of influence on DOM fate are still unclear. Here, in situ observations associated to lab experimentations demonstrated that DOM composition (C, N and P content) and optical properties varied through time and space in relation to freshwater inputs, anthropogenic pressures and primary production. DOM potential to fuel HP growth appeared strongly supported by human contaminations and temperature. Monitoring DOM molecular composition during incubation of microbial communities with DOM from a human-impacted area revealed high HP growth, consumption of a few molecules and production of a large diversity of more oxidize and hydrated compounds. Such same compounds characterized DOM collected in a remote site, along with lower inorganic nitrogen availability. Incubated with microbial community, this remote pool yielded 3-times lower HP growth, consumption of a larger diversity of compounds and production of a far lower diversity of compounds. Community origin and taxonomic diversity did not influence this pattern. While supporting the emerging recalcitrance concept, these results highlight human influence on coastal marine DOM fate.

Interactive effects of warming and zooplankton grazing on a spring community of the Central Baltic Sea

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Climate warming has been suggested to be the main driver of the observed changes in phytoplankton spring bloom biomass and community composition in the Central Baltic Sea. Since the 1990s dinoflagellates dominate the bloom to a large extend. To find out

for dinoflagellate`s potential profiting from increasing SST and related changes in the microbial pelagic food web, we conducted a mesocosm experiment with a natural dinoflagellate-dominated spring plankton community from the Central Baltic Sea under the combined effects of elevated temperature and excluding/including mesozooplankton grazing. Results show that elevated temperature in combination with mesozooplankton abundance led to a faster drop-down of the total dinoflagellate bloom biomass due to warming-enhanced grazing pressure. Contrasting to our expectations, biomass of the mixotrophic ciliate M. rubrum likewise decreased significantly. Analyses further show that mesozooplankton grazing on heterotrophic microzooplankton ciliates occur earlier in the spring bloom than previously observed and is enhanced at elevated temperature, changing the phytoplankton community size structure to a dominance of small-sized pico- and nano- phytoplankton. Our results suggest that under future climate change the phytoplankton spring bloom community composition of the Central Baltic Sea will be further altered due to temperature-induced changes in zooplankton abundances and trophic interactions.

Wetlands in drylands: microbial aerosolization, ecosystems services and global change impacts

Emilio O Casamayor [1], Xavier Triadó-Margarit [1], Joan Caliz [1], Josep Ramoneda [1]

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Microorganisms can be easily transported by winds to very distant places, and total dispersal success has been postulated based on their small size, vast population sizes, long survival, low extinction rate, and astronomic global numbers that may lead to microbial ubiquity. Bioaerosols originated from different terrestrial and marine sources (e.g., deserts, oceans, lakes, forests, agricultural and urban areas) are continuously injected into high atmospheric altitudes reaching the free troposphere, remaining suspended for days or weeks and being long-range transported over continents facilitating connectivity among habitats at multiple spatial scales. However, we have only a vague idea on what are the evolutionary parameters that lead to barriers in some microbial species, but not in others. Microbial aerosolization and the potential for remote colonization of tropospheric airborne microbes may change along different environmental and geographical gradients, and by the presence or not of certain traits in microbial immigrants, and further understanding of the local/regional/global interactions that exacerbate massive mobilization and long-distance dispersal of microorganisms is needed. Wetlands in drylands provide sometimes unseen ecosystem services, are very sensitive to global change effects, and can be used to test hypotheses to evaluate the colonization potential of immigrant airborne taxa, microbial aerosolization by changes in land and water uses, and ecological connections from local to global. In this talk, we will show some of the potentials, limitations and recent results on this topic.

Core Microbiomes and Functional Adaptations of Arctic Lakes in a Changing Climate

Emily Hallett [1], Jérôme Comte [1]

[1]*Institut national de la recherche scientifique*

Arctic lakes are experiencing drastic changes in their structure and functioning in response to climate change, yet their microbial communities and functional roles remain underexplored. Here, we characterized microbial communities in clear-water Arctic lakes using high-throughput 16S rRNA gene sequencing, identified an Arctic core microbiome, and assessed its response to projected browning. Comparisons were made with an analogous temperate lake. Additionally, we isolated two closely related Iodobacter fluviatilis strains from Arctic and temperate lakes to examine their responses to shifts in dissolved organic matter (DOM) quality and quantity. Distinct core assemblages were identified for each region, with Arctic lakes exhibiting higher diversity and only a limited, predominantly freshwater-shared core. Community structure in both climates was shaped by environmental connectivity gradients driven by DOM quality. Incubation experiments revealed that both Iodobacter strains displayed high metabolic versatility and rapid adaptation to various DOM sources, particularly favoring permafrost-derived carbon and elevated DOC. Contrasts with an Arctic Polaromonas sp. highlighted differing metabolic strategies and the complex interplay of inherent and induced microbial functions in response to environmental change. Our findings advance understanding of microbial community structure and function in remote Arctic lakes, emphasizing both community-level patterns and adaptability of key taxa to climate-driven shifts.

Tuesday, September 30
Microbial spatio-temporal distributions

From meta-omics to satellites: can we predict marine microbial community composition from pigment concentrations?

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Meta-omics have revolutionized our understanding of marine microbial communities, offering unprecedented taxonomic and functional resolution. However, these methods provide only discrete snapshots of microbiomes. In contrast, remote sensing retrieves daily global surface ocean color data, which is directly influenced by the pigment composition of microbial communities. This makes ocean color a promising interface between satellite observations and microbial meta-omics. Here, we integrate pigment and meta-omics bi-weekly to monthly data from two coastal time-series in the NW Mediterranean Sea—in the bays of Banyuls (France) and Blanes (Spain)—to test whether microbiome meta-omics can be predicted from photosynthetic pigment concentrations. We constructed four meta-omics datasets (rDNA, psbO, mTAGs, MAGs) covering over 25 photosynthetic groups and dominant microbial taxa, and trained machine learning models to predict these features from pigment profiles in one time-series. We then assessed model performance on the second time-series. Despite low comparability across omics datasets and the microbial communities of the two connected sites, our results reveal that the relative abundance of some specific photosynthetic groups was reliably predicted from pigments. By applying these models to historical satellite-derived pigment estimates, we uncover decadal-scale trends in microbial community composition across the Mediterranean basin.

The contribution of pelagic fungi to ocean biomass

Eva Breyer [1], Constanze Stix [2], Sophie Kilker [2], Benjamin R.K. Roller [2], Fragkiski Panagou [2], Charlotte Doebke [2], Chie Amano [2], Daniel E. M. Saavedra [2], Guillem Coll-Garcia [2], Barbara Mähnert [2], Jordi Dachs [3], Naiara Berrojalbiz [3], Maria Vila-Costa [3], Cristina Sobrino [4], Antonio Fuentes-Lema [4], Franz Berthiller [5], Martin F. Polz [2], Federico Baltar [1]

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Metagenomic analysis has recently unveiled the widespread presence of pelagic fungi in the global ocean, yet their quantitative contribution to carbon stocks remains elusive, hindering their incorporation into biogeochemical models. Here we revealed the biomass of pelagic fungi in the open ocean water column by combining ergosterol extraction, Calcofluor-White staining, CARD-FISH, and microfluidic mass sensor techniques. We compared fungal biomass to the biomass of other more studied microbial groups in the ocean such as archaea and bacteria. Globally, fungi contributed 0.32 Gt C (CI: 0.19-0.46), refining previous uncertainty estimates from two orders of magnitude to less than one. While fungal biomass was lower than that of bacteria, it exceeded that of archaea (Archaea:Fungi:Bacteria biomass ratio of 1:9:44). Collectively, our findings reveal the important contribution of fungi to open ocean biomass and, consequently, the marine carbon cycle, emphasizing the need for their inclusion in biogeochemical models.

Marine bacterial extracellular vesicles - elucidating the functional potential of ubiquitous nanoscale structures at the oceanic scale

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Bacterial extracellular vesicles (EVs) are spherical membranous nanoparticles that are released by cells and are capable of transporting and delivering diverse biological compounds. Experimental investigation of EVs in marine bacteria has been largely limited to laboratory model systems, while their functional potential has not been explored in the ecological contexts of the marine environment. Here we report on the first large-scale oceanographic survey of marine bacterial EVs carried out over more than 5,000 nautical miles of surface waters in the South Pacific. We found that marine BEVs were present across a range of biogeochemical conditions, with an overall abundance comparable to that of bacterial cells. Linking the EV cargoes to the protein and gene content of the bacterial communities that produced them revealed significant differences under specific environmental conditions. We found that in phytoplankton blooms bacterial EVs were enriched in carbohydrate transporters and hydrolytic enzymes, and in nutrient-limited waters the EVs were enriched in iron binding proteins.

Our observations highlight the ubiquity of marine bacterial EVs and suggest that some extracellular functions of marine bacteria may be mediated in part by EVs, with implications for our general understanding of key processes in marine microbial ecology.

Unravelling bloom complexity: Genotype-specific interactions shape Fragilaria crotonensis bloom in freshwater reservoir

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Diatoms are key primary producers in freshwater ecosystems, yet the processes driving their bloom development and collapse remain poorly understood. We tracked a Fragilaria crotonensis bloom along the Římov Reservoir (Czechia), from its initiation in the riverine zone to its decline near the dam. Chlorophyll maxima were monitored throughout the reservoir, and samples were collected twice weekly for microbial enumeration, amplicon sequencing (16S and 18S rRNA genes) of individual colonies, and metagenomic and metatranscriptomic analyses of F. crotonensis-associated size fractions. Single-colony rRNA analyses unexpectedly revealed the presence of two distinct, co-occurring F. crotonensis genotypes, confirmed by nuclear, chloroplast, and mitochondrial rRNA sequences. After the mid-bloom phase, one genotype disappeared due to highly specific infection by chytrid fungi of the genus Zygophlyctis. The remaining genotype was associated with another chytrid (Rhizophydiales spp.), which infected nearly all colonies by the bloom’s end, suggesting that chytrid parasitism was a key driver of bloom collapse. In addition to genotype-specific chytrid infections, we identified several bacterial lineages and metabolic pathways exhibiting temporal succession and specific associations with Fragilaria genotypes. These findings reveal bloom dynamics at an unprecedented level of complexity and highlight the importance of inter-kingdom interactions in freshwater ecosystems.

Seasonality of microbial communities across a surface-depth gradient in the NW Mediterranean Sea

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Marine microbial communities vary across space, depth, and time, yet these dimensions are often considered separately in ecological studies. In the Bay of Banyuls (NW Mediterranean Sea), the seasonality of planktonic microbes has been well documented, using surface data from the coastal SOLA station. In this study, we

expand the temporal perspective by including both the horizontal and vertical dimensions. Over a three-year period, microbial communities have been monitored at multiple depths in two contrasting stations of the Banyuls Microbial Observatory: the long-term coastal site SOLA and the open-ocean station MOLA. Using amplicon sequencing data from the 16S and 18S rRNA genes, our data explores microbial dynamics across the three domains of life. This study extends the understanding of microbial seasonality to deeper layers of the ocean and investigates the link between surface and deep communities throughout the year. By comparing these two sites, we also assess the coastal-offshore connectivity and show how coastal perturbations influence offshore microbial assemblages. Our findings aim to provide new insights into the factors structuring marine microbial communities, with particular attention to the roles of oceanic circulation, upwelling and mixing processes in shaping seasonal community dynamics.

Consequences of functional redundancy for structural stability and species interactions: insights from decadal freshwater time-series

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Biodiversity-function relationships are critical for understanding how long-term environmental change impacts microbial community composition, as well as carbon and nutrient cycling. Functional redundancy, between microorganisms allows for the maintenance of essential biogeochemical and ecological processes under fluctuating or changing environmental conditions. Long-term metagenome time-series allow us to observe seasonal and long-term changes in species and functional diversity. These time-series provide us with opportunities to quantify functional similarity within communities and populations and whether environments with higher functional redundancy in fact resist environmental change. Employing six decadal time-series from four German lakes we demonstrate that lakes with higher levels of functional redundancy, arising due to greater species richness rather than lower functional diversity, experience reduced rates of turnover in their microbial populations. Furthermore, we are able to demonstrate that this higher functional redundancy places a reduced dependency on species interactions, with greater promiscuity between interaction partners. We discuss how these biodiversity-function relationships and species interactions impact both the structural and long-term stability of microbial populations and implications for freshwater ecosystem functioning.

Bacterial blooming dynamics and their impact on ecosystem functioning in a coastal marine system

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The blooms of eukaryotic phototrophs and phototrophic bacteria and their impacts on ecosystem function have been profusely investigated in marine systems. In contrast, blooms of heterotrophic bacteria and their ecological consequences remain largely unexplored, despite growing evidence of transient short-lived increases of fast-growing taxa that might alter ecosystem structure and function. Here we combined a monthly time-series with two high-frequency short samplings (twice daily along 8 days, in Winter and Summer) to explore bacterial bloomers and their dynamics at the Blanes Bay Microbial Observatory in the NW Mediterranean Sea. We investigated the frequency of blooming events, the identity of bacterial bloomers, and their impact on ecosystem function, using a multifaceted approach. Our results show that blooming events occur frequently in the natural environment, even in oligotrophic sites, leading often to marked shifts in community function. This highlights the importance of studying bacterial bloomers and the need for sampling at fine-scale to fully comprehend community dynamics and their influence on biogeochemical cycles.

Biogeographical patterns of eukaryotes communities from bathyal to hadal depths in the north pacific and atlantic trenches.

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Deep-sea ecosystems play key role in the regulation of Earth's climate by storing the organic carbon captured by phototrophic plankton from the atmosphere. However, although plankton communities are now relatively well studied and documented using environmental genomics tools, the taxa reaching the deep-ocean floor (especially hadal trenches, 6000-11000m below sea level), and their spatial distribution at such depths, remains largely unknown. Here, we used environmental DNA from undisturbed surface sediment samples collected from multicores, and amplified the 18S V9 rRNA gene, to investigate eukaryotic communities in 137 samples collected at bathyal, abyssal and hadal depths across three deep-ocean trenches in the North Pacific (Kuril–Kamchatka and Aleutian) and Atlantic Oceans (Puerto Rico). Preliminary results indicate that the planktonic signal (relative abundance of plankton DNA) within sediment samples decrease with increasing depths and that the overall eukaryotic communities composition was significantly shaped by depths. Furthermore, at a larger spatial scale, communities at bathyal and abyssal depths, both within and between trenches, showed less variation than between deepest parts of the trenches. Our results provide key new

insights into the eukaryotic communities of deep-sea sediments and their links with sinking plankton communities across depths.

Ecophysiology and global dispersal of the freshwater SAR11-IIIb clade

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The freshwater SAR11-IIIb genus Allofontibacter (initially described as ‘Ca. Fonsibacter’) within the order Pelagibacterales is recognised for its ubiquitous presence and high abundance in freshwater environments. However, it remains poorly understood due to cultivation limitations, with only one cultured genome published to date. As a result, its genetic diversity, metabolic capabilities and ecological roles remain largely unexplored, with most available data limited to lakes in the Northern Hemisphere. Here, we present seven new isolates representing two novel species, along with 93 high-quality metagenome-assembled genomes (MAGs) derived from a global survey including long-read sequenced samples from five continents. Phylogenomic analysis revealed 16 species forming nine distinct biogeographic clusters, indicating speciation patterns linked to water temperature and latitude. Notably, we observed phylogeographic partitioning, including endemic species restricted to African lakes, quasi-endemic species confined to either the Northern or Southern Hemisphere, and the co-existence of cosmopolitan species alongside regionally constrained lineages. Furthermore, metabolic profiling and growth experiments uncovered species- and strain-specific adaptations for nutrient uptake, along with unique pathways for sulfur metabolism. These findings provide the first global-scale genomic and ecological overview for this lineage, raising key questions about dispersal barriers, priority effects, evolutionary trajectories, and mechanisms of niche adaptation in freshwater SAR11.

Microbial functions and biogeochemical cycles

Organic carbon as electron source in the metabolism of ammonia-oxidizing archaea.

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Ammonia-oxidizing archaea (AOA) are key players in the nitrogen cycle and they are among the most abundant prokaryotes in the ocean. A recently discovered metabolic pathway, nitric oxide dismutation, allows AOA to generate oxygen and nitrous oxide or nitrogen gas upon oxygen depletion, offering a possible explanation for their persistence in oxygen-depleted environments. Yet, the source of electrons fuelling this pathway remains unknown. While AOA fix inorganic carbon into biomass and do not seem to assimilate organic carbon, its role as an electron donor has been unexplored. Here, we tested the capability of AOA to utilize organic carbon as potential electron donor for their metabolism. Using pure cultures incubated with 13C labelled compounds under oxic and oxygen-depleted conditions and isotope ratio mass spectrometry, we observed 46CO2 production from 13C-organic compounds. The oxidation of organic carbon substrates was concurrent with ammonia oxidation and nitric oxide dismutation respectively. These findings provide direct evidence that AOA can oxidize organic carbon in addition to ammonia, demonstrating their metabolic flexibility as both lithotrophs and organotrophs a key insight into a novel trait that likely plays a pivotal role in the ecological success of AOA and reshapes our understanding of their functional potential.

Diving into the seaweed phycosphere: mechanisms of polysaccharide degradation by marine planctomycetota

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Macroalgae are key primary producers in some of the largest and most productive coastal habitats on Earth. A significant fraction of their primary production is released as polysaccharides, in both dissolved and particulate organic carbon forms, which can be exported to the deep ocean and contribute to carbon sequestration. The efficiency of this carbon sequestration process critically depends on the capacity of marine bacteria to degrade algal polysaccharides before they reach the deep ocean. However, the molecular and cellular mechanisms underlying the degradation of complex polysaccharides remain poorly understood. We have found that certain marine Planctomycetota inhabiting the phycosphere of brown algae possess exceptional

capabilities to degrade algal polysaccharides, even some of the most recalcitrants types such as fucoidan. Using Planctomycetota isolates as model organisms, and integrating omics and visualization analyses, we have explored the enzymatic machinery involved in fucoidan hydrolysis and unveiled the coexistence of both selfish and external degradation strategies in these bacteria. Overall, our findings uncover a remarkable diversity of mechanisms for seaweed polysaccharide degradation in marine Planctomycetota and suggest a relevant ecological role of these microorganisms within the macroalgal holobiont.

Digging into the role of microorganisms in Mediterranean Coastal aquifers

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Submarine groundwater discharge (SGD) delivers significant amounts of nutrients and other solutes to coastal oceans, profoundly impacting marine ecosystems. Both groundwater quality and the effects of SGD are shaped by biogeochemical processes occurring at the land-sea interface, many of which are mediated by microorganisms inhabiting the transition zones where freshwater mixes with seawater. However, studies on SGD and coastal groundwater have largely overlooked the microbial component, leading to an incomplete understanding of the functioning of coastal aquifers and their relevance for marine ecosystems. In recent years, we have characterized microbial communities from various coastal aquifers in Spain to shed light into their poorly known diversity, and to better understand their role in regulating SGD and its effects. We show that coastal groundwater communities are extremely diverse and heterogeneous, sharing very few species with connected freshwater, sediment, and seawater microbial communities. They harbor many potentially unknown species and metabolisms, and are dominated by some enigmatic groups such as Patescibacteria and Nanoarchaeota, which might be mobilized to the sea via SGD. Ongoing research targeting microbial functional genes will allow to characterize the functional potential of these communities to elucidate their role in modulating nutrient fluxes to the sea.

Microbial drivers of methane cycling in meromictic as compared to holomictic lakes

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Meromictic lakes, characterized by incomplete mixing, are expected to increase in prevalence due to climate change and anthropogenic activities. Their ability to store elevated concentrations of methane (CH_4) highlights the need for understanding these lakes' role in global carbon cycling. However, there is currently limited understanding of meromictic lakes' potential contribution to global CH_4 levels. This study compared the methane cycling, accumulation, and degassing potentials of boreal meromictic lakes in comparison to holomictic lakes. As expected CH_4 accumulation in the deeper, anoxic water layers of the meromictic lakes had maximum values 15 times higher than what we observed in holomictic lakes. Interestingly, microbial "methane filters" were evident in the meromictic lakes, significantly reducing the CH_4 concentrations towards the oxygenated water masses, thus potentially mitigating these lakes' CH_4 emissions to the atmosphere. We observed differences in the metabolic profiles of the lakes, with a more homogenized metabolic pattern in holomictic lakes compared to the stratified profiles seen in meromictic lakes. Ultimately, these findings provide increased understanding of different lakes' complex roles in global carbon cycling which ultimately may contribute to more accurate predictions of greenhouse gas impacts from freshwater ecosystems.

FizzingSoda: gas fluxes of inland saline-alkaline lakes and the microbial processes determining their carbon balance

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Inland waters are important components of the global carbon cycle and major contributors to terrestrial greenhouse gas (GHG) emissions. Saline-alkaline lakes, which are biogeochemically distinct from freshwater and marine systems and hold globally significant water volumes, remain underrepresented in emission estimates due to a lack of comprehensive gas flux data. The FizzingSoda project investigated diurnal and seasonal greenhouse gas fluxes (CO_2, CH4, N2O) across 12 saline-alkaline lakes in Central and Eastern Europe, integrating direct emission measurements, water chemistry, metagenomics and metatranscriptomics. In the studied lakes, CH_4 fluxes

frequently exceeded the average CO_2 fluxes with mean emissions of 540 mg C/m2/day and peak values reaching 19400 mg C/m2/day based on >350 individual measurements. CO_2 fluxes ranged from sinks to substantial emitters (-181 mg C/m2/day1 to 3883 mg C/m2/day). Meta-omic analyses showed that methylotrophic methanogenesis was the dominant methane-producing pathway throughout the year, while the expression of denitrification genes varied seasonally and spatially. Despite fluctuating environmental conditions, microbial gene expression profiles linked to GHG turnover exhibited an overall low diel variability, suggesting persistent microbial activity. The findings highlight the importance of saline-alkaline lakes as dynamic hotspots of carbon processing and their potential impact on terrestrial greenhouse gas budgets.

Hypolimnion-specific bacteria in a monomictic lake modulate their gene expression to survive winter holomixis

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The CL500-11 lineage (phylum Chloroflexota) is a ubiquitous and dominant group of bacterioplankton inhabiting the oxygenated hypolimnion of deep freshwater lakes worldwide. In monomictic lakes, their dominance ends with the collapse of the hypolimnion by the winter holomixis, and their abundance increases again following re-stratification in the subsequent spring. This annual population cycle raises the question of how CL500-11 cope with the abrupt and seasonal disappearance of their preferred habitat (i.e., the hypolimnion) and persist through the winter. To address this issue, we investigated the seasonal gene expression dynamics of CL500-11 using monthly metatranscriptomic data collected from monomictic Lake Biwa. The results revealed that their transcriptomic profile remained stable in the hypolimnion during the stratified period and shifted significantly with the onset of winter holomixis. Intriguingly, genes involved in oxidative phosphorylation, chromosomal replication, sigma factors, ribosomal protein, and rhodopsin were upregulated after the mixing. These findings suggest that CL500-11 do not simply endure the habitat loss during winter holomixis, but instead actively exploit the increased availability of oxygen and light to sustain growth and avoid population collapse. This rapid and active seasonal metabolic modulation likely represents a key mechanism underlying the dominance of CL500-11 in global deep freshwater lakes with varying mixing regimes.

Connectivity-driven microbial assembly mediate organic matter degradation and methane cycle in high-arctic transitioning permafrost

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As continuous permafrost thaws, thermokarst ponds form and act as hotspots for greenhouse gas emissions. Their numbers are increasing in some areas across the circumpolar region, drawing attention to the ecological functioning of these small but significant aquatic systems. They are key interfaces between crysoils and waters, yet the microbial processes governing their ecological function remain poorly understood. We investigated microbial community assembly and functional potential from three permafrost cores, three thermokarst, and three non-thermokarst soil–water continua (Bylot Island, Canada) using 16S rRNA gene amplicon sequencing and gene and genome-resolved metagenomics. Thermokarst ponds showed soil microbial mass effects, with characteristic genomes encoding pathways for degrading terrestrial aromatic compounds. Methane cycling activity was concentrated in littoral submerged peat soils, but thermokarst ponds lacked functional stratification, limiting anaerobic methane oxidation. In contrast, non-thermokarst ponds were shaped by more selective processes, with characteristic genomes geared toward processing plant-derived organic matter, their sediments were hotspot for coupled carbon and nitrogen cycles. Our findings suggest that ongoing permafrost thaw and rewetting could drive substantial methane emissions from thermokarst ponds littoral before methanotrophic communities are established. Understanding microbial community assembly and functions in these environments is crucial to predicting their role in future greenhouse gas dynamics.

Greenhouse gas production kinetics and metabolic traits in thawing permafrost peat

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While numerous drivers of greenhouse gas emission have been suggested to drive variabilities across thermokarst landscapes, experimental validations are still widely lacking. Here, I will present results from several experimental studies capturing drivers of greenhouse gas production across thawing permafrost landscapes like redox, organic matter and microbial source community. Our findings reveal clear responses of the greenhouse gases to redox conditions across the landscape. CO_2 production

showed a drastic decline when O_2 dropped below 100 μM; specifically, under anoxic conditions, CO_2 yield and maximum net production rates decreased by around 90 % compared to oxic conditions. CH_4 levels remained consistent across O_2 gradients, until at least 6 months upon thawing. Functional groups like N_2O reducers, fermenters, denitrifiers, and sulfur reducers increased under anoxic conditions, and reduced CO_2 production rates and yields under anoxic conditions were reflected by genomic changes towards reduced central metabolic efficiency. Our data thus points towards critical greenhouse gas emission thresholds under suboxic conditions across thawing landscapes. Moreover, gas dynamics seem to be primarily influenced by permafrost conditions rather than source community colonization. Our experiments provide increased knowledge on the variable greenhouse gas emissions across thermokarst landscapes influenced by variable redox conditions and permafrost's organic matter legacy.

Intracellular calcite formation linked to photosynthesis reduces carbon dioxide emissions and sequesters carbon in hardwater ecosystems

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In hardwater ecosystems, most of the available inorganic carbon for photosynthesis is in the form of HCO^{3-}. Some species of bacteria and microalgae have developed carbon-concentrating mechanisms (CCMs) that increase the inorganic carbon inside the carboxysomes, where carbonic anhydrase converts the HCO^{3-} into CO_2. Moreover, other species also couple photosynthesis with calcite formation, removing two bicarbonates: one for the calcite formation (CaCO3 + H+) that eventually can precipitate sequestering carbon and the other one for the photosynthesis converting this second HCO^{3-} into organic matter. How this coupling between calcite formation and photosynthesis can affect CO_2 emissions is still a controversial process. Here, we examine for one year the links between calcite formation during photosynthesis and CO_2 emissions in a eutrophic and hardwater reservoir of Southeastern Spain. We observed that during the most productive period (high photosynthesis), concomitant with the proliferation of cyanobacteria in the summertime, there is a substantial reduction in the alkalinity (HCO^{3-} concentration), calcium, and CO_2 fluxes. This decline in HCO^{3-} concentration and calcium to form calcite during the cyanobacteria bloom reduced CO_2 fluxes during the summertime and contributed significantly to carbon sequestration. Therefore, this process should be included in the carbon budgets of hardwater ecosystems.

Microbial traits and their roles in community function

Waking the sleeping chytrid parasite beauties

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Chytrids are zoosporic fungi and widespread parasites of aquatic primary producers. Their infections can reach epidemic levels, strongly regulating host population dynamics and community structure. Many chytrid species produce resting spores that enable persistence during host absence or unfavourable environmental conditions. These spores may be the key to initiating new infections cycles, especially during algal blooms. Yet, the environmental cues triggering germination remain poorly understood. To investigate the effects of temperature, light, and host presence in germination dynamics, we conducted a microcosm experiment using the model system Staurastrum sp. – Staurastromyces oculus. Resting spores were stored for 3 months at two temperatures (4°C, 16°C) and two light regimes (darkness or light). Subsequently, spores were exposed to combinations of temperature, light, and host presence. We monitored germination and new infections over 14 days using automated fluorescence microscopy. Our preliminary results indicated that temperature increase following 4°C storage is a strong germination cue, while light had no clear effect. In contrast, resting spores stored at 16°C did not exhibit the classical germination phenotype, yet infections occurred, suggesting undetected low-level germination or sustained infection activity. These findings suggest multiple germination pathways in chytrid resting spores, primarily involving temperature shifts and potentially host-related cues.

Metagenomic and lipidomic analysis of a marine subsurface biofilm involved in the anaerobic oxidation of methane

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Deep marine sediments produce large amounts of methane, but most of this gas is consumed by anaerobic oxidation of methane (AOM) coupled to sulfate reduction. AOM is mediated in a syntrophic interaction of anaerobic methane-oxidizing archaea (ANME) and sulfate-reducing bacteria. Sedimentary AOM biofilms have been rarely described, but recent publications have suggested a potential role as methane sinks. In this study, we investigated the microbial community of two subsurface biofilm samples collected from the Ginsburg mud volcano (Gulf of Cadiz), in a deformation influenced

by upward fluid flow. From metagenomic and lipidomic analysis, we detected a microbial community dominated by archaea. Actually, 63% of the reads mapped to ANME-1b organisms that have been detected in biofilm slimes. Our analysis revealed the presence of two genes encoding the subunit alpha of the methyl coenzyme M reductase, the key enzyme of methane oxidation, which usually appear as single copy. This duplication might be connected to a horizontal gene transfer event, which might be connected to the biofilm nature. The rest of the community included two sulfate-reducing bacteria, which would act as potential partner bacteria, and four microorganisms with a potential heterotrophic lifestyle, likely growing on AOM cell exudates or necromass.

Spirochetes in marine invertebrates: adversaries or allies?

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Spirochetes are often associated with animals; the most well-studied clades are pathogens that cause a variety of mammalian diseases. Very little is known about spirochetes associated with marine hosts and whether these are harmful or beneficial. We recently discovered a clade of spirochetes that is regularly present in the microbiome of gutless marine worms (oligochaetes). The dominant members of these worms' microbiomes are sulfur-oxidizing and sulfate-reducing bacteria that provide nutrition to the host and recycle fermentative products. To understand the role the spirochetes play in these symbioses, we analyzed metagenomes and optimized a method to generate paired metaproteomes and metabolomes from single host animals. Our analyses revealed that the spirochetes ferment carbohydrates (including disaccharides) to acetate and H2. These products are used by the sulfur-oxidizing and sulfate-reducing symbionts, thus contributing to the overall efficiency of the symbiosis. With this export of carbon and energy to neighboring bacteria and the lack of evidence for harmful effects to their hosts, we conclude that these spirochetes fill a mutualistic role in gutless marine worms. Exploring the diversity of mutualistic associations between spirochetes and marine hosts will expand our understanding of these ubiquitous bacteria and how they interact with their partners, beyond pathogenicity.

Niche Partitioning of Marine Microbiota in Deep-sea Organic Matter Cycling

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The ocean harbors a vast pool of organic matter, much of which is produced and transformed by microbial communities. Proteins, as both structural and functional biomolecules, offer insight into microbial activity and biogeochemical cycling. We used a multi-omics approach, including metagenomics, metatranscriptomics, and metaproteomics, to analyze microbial protein expression across size fractions and depths in the Pacific, Atlantic, and Southern Oceans, with a focus on the deep ocean (>200 m). We found that zooplankton proteins contribute more to the deep-sea metaproteome than algal proteins, indicating significant inputs from higher trophic levels. Gammaproteobacteria were highly active, contributing up to 30% of bacterial proteins and 80% of the dissolved proteins, potentially due to tight virus-host interactions. Despite expectations that enzymes would remain cell-associated in dilute deep-sea conditions, extracellular enzymes like peptidases and CAZymes were mostly found dissolved. These enzymes increased in abundance and diversity with depth, suggesting that particle-associated microbes release them into diffusion-limited microenvironments. Transporter proteins showed size- and taxon-specific expression patterns, with free-living taxa using ABC transporters for organic nitrogen and particle-associated taxa using TonB-dependent transporters for carbon-rich substrates. These findings reveal a complex, depth-stratified network of microbial metabolism that drives the cycling of organic matter in the deep ocean.

Foraminifera-Microbiome co-occurrence predictions using multi-omics link ecosystem function to diversity in deep-sea mining areas

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Deep-sea mining will profoundly disturb benthic ecosystems, including microbial functions and interactions with faunal communities. Meanwhile, multiomic methods matured to yield detailed taxonomic and genomic information that for environmental DNA samples from the global deep-sea benthos can help understand compositional changes and the ecology of prokaryotic and eukaryotic communities. Meiofauna and protists thriving in metal-rich, deep-sea sediment are particularly elusive, and whether their diversity is maintained by that, and specific functions of prokaryotic partners, is only documented for a few, emblematic host model species. Here, we integrate metabarcoding and metagenomics for a global set of abyssal, deep-sea sediment samples covering an area dedicated for mineral mining, the Eastern Clarion-Clipperton Zone (CCZ), and predict co-occurrences of Metazoa and Foraminifera with microbes. We identify functions and reconstruct genomes, and measure their evolutionary significance in phylogenies, with focus on metal-dependent metabolisms enriched in

the CCZ. We show that the abundance of microbes performing extracellular electron transfer and metal harvesting functions determine eukaryotic diversity, in the CCZ only. We propose that compelling, prokaryotic-eukaryotic co-occurrence predictions substantiated by multiomics with underlying eco-evolutionary mechanisms are considered to prioritize the probing for new ecological models, and saveguard undocumented, yet key interactions in threatened deep-sea benthic ecosystems.

A taxonomy-free approach for diatom bioindication using three gene markers

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Diatoms are key bioindicators for assessing the quality and health of water ecosystems. However, traditional microscopic analysis poses challenges due to misidentifications, resulting in economic losses. DNA metabarcoding provides a rapid and objective characterisation of numerous samples to assess the water quality of rivers and lakes. Yet, its full potential is limited because many DNA sequences cannot be assigned to species due to incomplete reference databases. We propose a taxonomy-free diatom-based approach to overcome this limitation and extract ecological information directly from sequence data. Among the various diatom markers, rbcL is widely used in freshwater diatoms, 18S in marine samples, and COI shows great promise due to its high resolution and ability to detect cryptic and intra-species diversity. Our approach uses a taxonomy-free, Machine Learning workflow, based on Random Forest classifiers, to identify which sequences from multiple metabarcoding markers (COI, 18S, and rbcL) are ecologically informative. This workflow facilitates the characterisation of indicative values not only for diatom operational taxonomic units but also for other ecologically informative microeukaryotes. It enables the reconstruction of their co-occurrence networks and expands the utility of diatom indicators across diverse ecosystems, from peat bogs and littoral seawater to marine sediments.

Solid vs. liquid: microbial community effects on enterovirus persistence in estuarine ecosystems

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Microbial community composition plays an important role in modulating the stability of human pathogens in aquatic ecosystems. Waterborne pathogens can remain infectious in the environment for extended periods, impacting water quality and posing public health and economic risks. In this study, we investigate how indigenous microbial communities affect Enterovirus persistence in estuarine water. Estuaries support economically valuable activities such as oyster farming, where viral stability directly

impacts food safety. We performed batch inactivation experiments by spiking estuarine water samples with Enteroviruses under varying biotic and abiotic conditions. Bacterial communities were characterized before and after the experiments using full-length 16S rRNA gene sequencing (Oxford Nanopore). Viral stability and bacterial communities were assessed separately in the solid and liquid phases of water samples. We observed distinct bacterial community compositions between these phases. Enteroviruses remained more stable when adsorbed onto solid particles, while viral inactivation occurred more rapidly in the liquid phase. These results suggest that biofilm-associated microorganisms play a limited role in viral inactivation, whereas free-living communities contribute more strongly to viral decay. Our findings highlight the need to consider solid particle concentration and its associated microbial communities when assessing the environmental stability of waterborne pathogens, particularly in coastal areas.

Manipulation of the seed microbiome in Zostera marina affects germination rates

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In many ecosystems, microbial communities significantly affect plant growth, serving as key mechanism for adaptation to global change. Seagrasses are the only marine macrophytes capable of sexual reproduction through flowers and seeds, like terrestrial angiosperms. In terrestrial settings the microbiota of seeds affects germination as well as the health of seedlings through various beneficial functions. However, this is relatively unknown for seagrass seeds which often display low germination rates. Here, we investigated the influence of plant growth promoting bacteria on the seeds of the temperate seagrass *Zostera marina*. We isolated microorganisms from roots, leaves and seeds of *Z. marina* and performed assays for plant growth promoting traits. Based on this we selected 10 strains and incubated them with natural and disinfected *Z. marina* seeds from the Western Baltic Sea. Treated seeds were incubated in seawater in 96 well plates as well as in sediment in aquaria and germination rates were documented. The results indicate that seed microbiota can both positively and negatively influence the germination rate of seagrass seeds. Our results suggest that manipulating the seed microbiome by adding plant growth promoting microbes can enhance germination rates and thereby increase restoration success.

Phytoplankton and particle attachment drive seasonal abundance and photoheterotrophy of aerobic anoxygenic phototrophs

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Aerobic anoxygenic photoheterotrophic (AAP) bacteria are a functional group characterized by their relatively large size, high metabolic activity, and rapid growth, which can be partially attributed to their ability to harvest extra energy from light via bacteriochlorophyll-a-containing type II reaction centers. Despite their widespread occurrence and contribution (1–23% of aquatic bacterial communities), their ecological roles remain insufficiently understood. To better understand their role in the carbon cycle and microbial food webs, we conducted three complementary field studies: (1) a two-year monthly sampling in a freshwater lake, (2) three seasonal campaigns in the Adriatic Sea, and (3) a three-year biweekly sampling in a freshwater lake. We found that light-derived energy enables AAP bacteria to reduce microbial respiration while enhancing carbon uptake. In coastal marine waters, they were notably more abundant in the particle-attached fraction during the most productive seasons. In freshwater, their abundance followed a recurrent seasonal pattern, peaking during spring phytoplankton blooms. These results suggest that AAP bacteria recycle and use both dissolved and particulate phytoplankton-derived organic carbon. Their ability to thrive on carbon-rich particles while minimizing respiratory carbon loss highlights their potential to channel organic matter efficiently through microbial food webs toward higher trophic levels.

Wednesday, October 1

Microbial diversity across evolutionary scales

Niche-driven specialization of Akkermansiaceae across marine and gut ecosystems

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Though widespread, the phylum Verrucomicrobiota remains understudied due to its low abundance and challenges in cultivation. Within it, the family Akkermansiaceae inhabits diverse ecological niches, with gut-associated species specializing in mucin degradation and aquatic species targeting complex sulfated polysaccharides. While gut Akkermansia species are well-characterized for their role in mucin breakdown, aquatic Akkermansiaceae remain poorly understood despite their possible role in marine carbon cycling. Here, we focused on elucidating substrate transport mechanisms in environmental Akkermansiaceae, employing structural predictions to understand how these mechanisms adapted to different aquatic niches within the Akkermansiaceae. We identified conserved substrate transport mechanisms across aquatic and gut-associated Akkermansiaceae using comparative genomics and protein structure predictions. In particular, we found proteins from the recently described mucin utilisation loci of gut Akkermansia conserved within environmental genomes, specifically the MUL2A protein thought to aid mucin acquisition. The high degree of structural similarity between our predictions and available experimental structures suggests evolutionary conservation of transport mechanisms despite environmental differences (predicted protein structures with pLDDT >70% from 233 aquatic Akkermansiaceae genomes had RMSD.

Marine and terrestrial Trichoderma reesei biodiversity: adaptive traits unlocking biotechnological potential

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Trichoderma reesei is a filamentous fungus widely used in industrial biotechnology due to its capacity to secrete high levels of cellulolytic enzymes. However, industrial strains are almost exclusively derived from a single terrestrial isolate, QM6a, leaving much of

T. reesei's natural diversity unexplored. In this study, we explore the genetic and phenotypic diversity of eight natural strains globally distributed environments, including one rare isolate from marine habitat, to investigate ecological adaptations with biotechnological implications. While genome content appeared largely conserved across strains, phenotypic assays revealed substantial differences between marine and terrestrial strains. Remarkably, the marine strain exhibited exceptional salinity tolerance and distinct growth dynamics under salt stress, suggesting distinct mechanisms of environmental adaptation, although all strains remained viable under marine condition. These findings underscore the overlooked potential of marine fungi as reservoirs of adaptive traits valuable for biotechnology. The marine *T. reesei* isolate represents a promising candidate for developing more resilient fungal platforms suited to challenging industrial conditions. By linking ecological origin to phenotypic performance, our study highlights the evolutionary plasticity of marine fungi and their relevance in the context of a changing planet and sustainable bioprocess design.

The hidden genetic reservoir: structural variants as drivers of marine microbial and viral microdiversity.

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Intraspecific genetic diversity is essential for understanding microbial evolution, adaptation, and ecosystem stability. Traditional short-read metagenomics often overlooks this diversity—particularly structural variants (SVs)—due to assembly challenges in complex communities. Using third-generation metagenomics, we examined the eco-evolutionary roles of SVs in microbial and viral populations across distinct ecological niches within the photic zone of the marine water column. Insertions and deletions were the most common SVs, found in both core and flexible genomes, often within genomic islands. In cellular microbes, especially streamlined taxa like *Pelagibacter* and *Nitrosopumilus*, insertions exceeded 2 Kb. Viral SVs were smaller (~430 bp) and more size consistent. Functionally, SVs were enriched in genes for nutrient uptake, amino acid metabolism, and regulatory elements, including non-coding RNAs. These often-spanned full genes or operons, promoting lineage diversification, such as rhodopsin-bearing microbes in surface waters. In viruses, SVs enhanced host range and metabolic control. Stable populations of cyanophages and pelagiphages used SVs as a major diversification source. Some pelagimyophages carried a functional pstS gene, improving phosphate uptake and replication in nutrient-poor waters. Our findings underscore the value of long-read sequencing in revealing hidden genomic variability, offering new insights into microbial ecology and marine ecosystem resilience.

Shared microbial genomes in distant oceans reveal signatures of seasonal and ENSO population selection

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The response of the ocean microbiome to global change remains poorly understood. Analyzing long-term genomic variation in microbial populations exposed to distinct climatic regimes may provide insights into their responses to future environmental conditions. We analyzed coastal marine microbiomes from two distant locations: the Blanes Bay Microbial Observatory (Catalonia, Spain) and the Microbes in the Coastal Region of Orange County, Newport Beach (California, USA). Over 15 and 11 years, respectively, metagenomic sampling conducted at least monthly yielded 1,535 Metagenome-Assembled Genomes (MAGs) in Blanes and 1,068 MAGs plus 187 Single-Amplified Genomes (SAGs) in Newport, from short and long reads. Dereplication at 95% ANI identified 615 shared genomes, while dereplication at 99% revealed 16 genomes present at the strain level in both sites. Focusing on a shared Prochlorococcus genome featuring >96% ANI, we performed variant calling and tracked mutations over time. In both locations, recurrent mutations and pN/pS ratios exhibited pronounced seasonal patterns. We detected a potential imprint of the El Niño Southern Oscillation (ENSO) in the Newport time-series: higher pN/pS corresponded with El Niño events. These results suggest that, beyond seasonality, long-term climatic oscillations may shape microbial populations, with implications for understanding how the ocean microbiome responds to long-term environmental change.

Composition of bacterial communities in sediment cores of Lago Grande di Monticchio over 30,000 years

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Ancient sedimentary DNA provides a powerful archive for reconstructing microbial community dynamics and ecosystem processes over millennial timescales. In this study, we analyzed 163 stratigraphic samples from a 14-meter core retrieved from Lago Grande di Monticchio (southern Italy), covering ~30,000 years of depositional history. High-resolution 16S rDNA amplicon sequencing, yielding over 5 million high-quality reads and 88,514 amplicon sequence variants, was used. Alphadiversity showed a non-linear decline with depth/age, peaking in mid-Holocene sediments (~1.4–1.6 ka median age), and correlated significantly with sedimentary chlorophyll derivatives and loss-on-ignition. Betadiversity patterns revealed ten distinct community zones reflecting shifts in lake trophic state, redox gradients, and climate. Community composition transitioned from Chloroflexota- and Atribacterota-dominated assemblages in younger sediments to Firmicutes and Acidobacteriota prevalence in mid-Holocene, with deep glacial samples (>20 ka) enriched in Actinobacteriota. Functional prediction

suggests temporal succession of metabolic guilds, including fermenters, sulfate reducers, and phototrophs, with evidence of stratified anoxic niches and methanogenic pathways in deeper sediments. These findings underscore the resilience and transformation of microbial ecosystems in response to long-term environmental forcing and demonstrate the utility of ancient sedimentary DNA for probing biogeochemical histories in closed volcanic lake systems.

Microbiome richness varies among phytoplankton species of different sizes

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One of the general laws of ecology is the island-species-area relationship: larger islands harbor more species than smaller ones. In this study, we isolated 13 phytoplankton species of different sizes from Port Hacking, Australia, and analyzed the richness of their associated microbiomes. Indeed, we found higher microbiome richness associated with larger phytoplankton species, confirming the ubiquity of the island-species-area relationship. However, the slope of this relationship was lower than that observed in other habitats involving aquatic bacteria. To investigate this further, we applied a model simulating phytoplankton-bacteria interactions, and identified several mechanisms contributing to the lower slope: i) reduced release of DOM from the phycosphere of larger phytoplankton cells, caused by more bacteria taking up more DOM in the phycosphere, which decreases encounter rates of chemotactic bacteria; ii) a lower probability of "naked" large phytoplankton cells (i.e., cells without attached bacteria) after cell division, i.e. less space and substrate for new attachments; and iii) lower abundances of larger phytoplankton species compared to smaller ones, resulting in an overall smaller encounterable population area. Finally, we discuss factors that may have influenced our findings, such as differences between single-cell and population-level microbiomes, and place the observed patterns within a broader theoretical context.

Global DNA signatures of temperature and nutrient limitation in prokaryotes

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Microorganisms adapt to their environment through changes in both their genes and overall genome composition. However, identifying universal principles of genomic adaptation remains challenging because different environments present multiple, overlapping selective pressures. To overcome this challenge, we analyzed DNA composition patterns across diverse environments using machine learning. By examining tetranucleotide frequencies from 1,112 marine and soil metagenomic

samples, we discovered that environmental temperature can be accurately predicted from DNA composition alone (R2=0.82). This temperature signal remained robust even when analyzing individual bacterial phyla and classes, suggesting a fundamental adaptive response. To examine this adaptation mechanism, we analyzed GC content relationships with temperature and found opposing relationships in different environments: positive correlations in soil but negative correlations in marine samples. We show that this inverse relationship in marine environments is driven by nutrient availability, as GC content increases with nutrient levels while nutrients decrease with temperature in marine ecosystems. Despite these contrasting GC patterns, we identified specific tetranucleotides composed of equal numbers of GC and AT bases (50% GC content) that showed consistent temperature correlations across all environments. These findings reveal a complex interplay between temperature adaptation and nutrient limitation in shaping microbial genomes.

Microbial spatio-temporal distributions 2

Fungal parasitism during a phytoplankton spring bloom along a Baltic Sea salinity gradient

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Parasites of phytoplankton are an integral part of aquatic ecosystems. Chytrid fungi are known to cause large epidemics during diatom spring blooms in freshwater lakes. However, they remain largely unexplored in the Baltic Sea, one of the largest brackish water bodies with a unique horizontal salinity gradient. To address this knowledge gap, we aimed to (i) characterize the diversity and abundance of parasitic chytrids and (ii) identify potential drivers of their occurrence and diversity during a spring bloom along a north-south salinity gradient in the Baltic Sea. Samples were collected during five cruises along a salinity gradient with salinities 5 to 15 (59.94°N, 25.01°E to 54.13°N, 11.28°E). We applied high-throughput quantification and sequencing methods, as well as cultivation and single-cell sequencing of individual host-parasite associations, to characterize the spatiotemporal distribution patterns of chytrid parasites and their hosts. We identified five novel chytrid species infecting cold water diatoms (Thalassiosira spp., Pauliella taeniata and Guinardia delicatula). The occurrence and relative abundance of parasitic chytrids varied temporally and spatially and were linked to host distribution and phytoplankton bloom development. Both salinity and host community composition significantly explained chytrid parasite community composition, underscoring salinity as an important driver of host-parasite associations in the Baltic Sea.

Niche-specific distribution of freshwater Methylopumilus spp. across the European continent

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The genus Methylopumilus consists of ubiquitous methylotrophs that are abundant in freshwater systems. Three currently described species seem to occupy distinct ecological niches. We identified eleven additional species (total = 14) of Methylopumilus by including complete genomes of new cultures and high-quality metagenome-assembled genomes (MAGs). We analysed samples from 77 European lakes (n = 458) taken at different seasons to assess the geographical and seasonal

distribution of Methylopumilus using four different methods for abundance estimation. Two described species could be differentiated based on lake trophic states. Further, we could pinpoint niche-specific adaptations of four additional species of Methylopumilus, which we propose as new taxa. Two of them (*M. alpinus* and M. profundus) occurred mainly in the deep zone of lakes but showed different distributions based on latitude and depth. Two more taxa (*M. autumnalis* and *M. hivernalis*) were associated with specific seasons in a 5-year time series of a eutrophic reservoir. We were able to connect distribution patterns to specific differences in the flexible genome of Methylopumilus that facilitate adaptations to cold temperatures among others, and identified variations in a phosphate-selective porin. The widespread occurrence of the genus in freshwater ecosystem might be explained by these niche-driven genomic differentiations.

Microbial fingerprinting of marine water masses in an Antarctic and hydrographically complex area

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Microorganisms are ubiquitous components of marine ecosystems, yet the role of water masses in their distribution remains underexplored, particularly in remote and extreme environments such as Antarctica. This work studies the microbial communities in the Gerlache-Bismarck Strait, an area with complex hydrography. In this area, we measured microbial abundances and diversity (both prokaryotic and eukaryotic), and we recorded multiple oceanographic and biogeochemical variables. Samples covered from 0 to 400m depth, and we included 3 size fractions: pico- (0.2-3.0 µm), nano- (3.0-20 µm) and microparticles (20-200 µm). The results revealed that the water mass was the main driver determining the assembly of microbial communities and was more relevant than location, depth, size fraction, and the measured environmental variables. Water masses detected in this area included AASW, GMW, TBW (in surface waters), TWW, and CDW (in intermediate and deep waters). To examine in detail the links between water masses and microbes, we defined the microbial fingerprint of water masses, which includes different descriptors, such as the core microbiome and tracer microbes. Each water mass had a characteristic microbial fingerprint, and the fingerprint revealed ecological roles of the water masses linked to biogeochemical cycles (e.g., the ammonia-oxidizing archaea *Nitrosopumilaceae* in the CDW) and food webs (e.g., the phototroph *Chrysochromulina simplex* in the GMW). This study provides direct evidence of the strong links between microbes and water masses, revealing that microbial communities found in seawater are shaped not only by direct local conditions but also by their transport through water masses. We also developed the concept of “microbial fingerprinting of water masses”, which may be explored and applied elsewhere.

Seasonal dynamics of rhodopsin phototrophy reveal overlapping distributions with phytoplankton blooms in productive marine systems

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Rhodopsin phototrophy is the single most abundant light-dependent metabolism in the surface ocean, potentially modulating the biogeochemical cycles. Previous studies suggest that rhodopsin-containing bacterioplankton are adapted to use light in the most severely nutrient-depleted environments. However, rhodopsin genes are also found among multiple copiotrophic bacteria, suggesting that this type of phototrophy could also be relevant in productive environments. To explore this hypothesis, we studied the seasonal rhodopsin dynamics in the upwelling system of the Southern California Bight. In contrast to oligotrophic waters, rhodopsin levels in this environment peaked during the most productive season, the Spring phytoplankton bloom, when chlorophyll concentrations were also the highest. We found that heterotrophic bacterial abundance was significantly correlated to rhodopsin concentrations, allowing us to build linear models to predict rhodopsin distributions in a productive environment. Moreover, rhodopsin genes within the order Flavobacteriales–a microbial group typically associated with phytoplankton–dominated the rhodopsin gene pool when the highest rhodopsin levels were observed, suggesting a significant flavobacterial contribution to the rhodopsin distributions in this productive environment. Our findings provide clear evidence of the importance of microbial rhodopsin light-capture beyond oligotrophic marine systems.

Niche-partitioning of AEGEAN-169, an abundant and ubiquitous marine bacteria in the global ocean

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AEGEAN-169 (also known as HIMB59) is a globally abundant but poorly characterized group of free-living marine bacteria. Here, we investigated their distribution and ecological diversity across the global ocean. Using 16S rRNA metabarcoding data from 1,987 samples collected during the Tara Oceans, Malaspina, and Hotmix expeditions, along with a 22-year coastal time-series (BBMO) from the NW Mediterranean, we analyzed spatial and temporal patterns of AEGEAN-169 relative abundance. The group was consistently detected in surface waters, with higher relative abundance in tropical and subtropical regions compared to cold polar or deep waters, and in summer/autumn compared to winter/spring. AEGEAN-169 alpha-diversity negatively correlated with relative abundance in the BBMO time-series, suggesting seasonal dominance patterns during warmer months. Amplicon sequence variants within AEGEAN-169 exhibited distinct temperature preferences, consistent with niche partitioning. Furthermore, we assembled four new single-amplified genomes (SAGs) from Tara Oceans and recovered fifteen metagenome-assembled genomes (MAGs) of medium to high quality (>70% completeness.

Changes in landscape connectivity and impacts on microbial diversity and freshwater ecosystem functioning

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Most of the lakes on Earth are located in Arctic and sub-Arctic regions. Rapid climate warming in the Arctic is promoting both lake expansion and shrinkage depending on the location, altering connectivity among aquatic ecosystems. Lakes support global biodiversity, contribute to climate regulation and serve as crucial resources for northern communities. Changes in connectivity will affect the movement of microorganisms across the landscape, with largely unknown implications for the functioning of these sentinel ecosystems. We focus on two continuous permafrost regions in Canada: Bylot Island, Nunavut, where the number of lakes is increasing, and Ts'udé Nilįné Tuyeta protected area, Northwest Territories, where some lakes are being drained. We are 1) Assessing the taxonomic (Nanopore long-read 16S, metagenomics) and functional diversity (Biolog Ecoplates and nitrogen, phosphorus and sulfur utilization assays) of bacterial communities along local connectivity gradients; 2) Comparing bacterial diversity and ecosystem functions at the two sites with contrasting regional

connectivity; and 3) Examining the mechanisms controlling ecosystem functioning. Preliminary results from Bylot Island indicated that bacterial community beta-diversity correlated with functional dissimilarity, but not with landscape position. Analyses are ongoing to determine whether similar patterns occur in a reduced connectivity context.

Ecogenomics and functional biogeography of the Roseobacter group in the global oceans

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The Roseobacter group is a major component of prokaryotic communities in the global oceans. Assessments in the pelagic of the global oceans indicated an unveiled diversity of this group but detailed studies of the diversity and biogeography of the entire group are still missing. Hence, we aimed at such an assessment on the basis of 610 MAGs and 43 SAGs recruited from all major ocean basins. The phylogenetic analysis delineated six clusters, two of which were completely novel: These clusters account for ~70% of all Roseobacter MAGs and SAGs in the epipelagic. Four clusters are among the most deeply branching lineages of the Roseobacter group. These and several sublineages of the two other clusters exhibit features of genome streamlining and exhibit sound differences in their functional features, also compared to other lineages of the Roseobacter group. Proteorhodopsin is encoded in most species of four clusters and in a few species of another one. Biogeographic assessments showed that four clusters constitute the Roseobacter group in the temperate to polar regions to great extent. The two other clusters dominate in the tropics and subtropics and thus in regions not known before to be important regions for this group of marine bacteria.

The long-term view: inter- and intra-annual plankton diversity patterns in the Western English Channel from a 20-year metabarcoding dataset.

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Plankton underpin coastal productivity and ecosystem services. Here we examine long-term inter and intra-annular diversity patterns in coastal plankton at the Western Channel Observatory Station L4 through a twenty-year dataset of environmental DNA samples of near weekly resolution. Metabarcode sequencing of eukaryotic communities was conducted on 587 samples via 18S rRNA gene amplification (V9 region). Amplicon sequences were processed using the DADA2 pipeline, and individual biological sequences (i.e. Amplicon Sequence Variants: ASVs) were classified against

the Protist Ribosomal Reference (PR2) database, with further interrogation of abundant, poorly classified ASVs via the NCBI database. Intra-annually, we observed that ASV diversity was highest during the winter months. Over summer, diatoms (Bacillariaceae) were more abundant, while Phaeocystis abundance declined. Inter-annually, winter and spring communities were more similar across years compared to summer communities. Environmental drivers are not yet ascertained, but we predict that summer stratification is important. Over the 20 years, we observed three major shifts to phases of low diversity, which may be linked to regional weather patterns. The timescale of these patterns shows the importance of long-term eDNA datasets for determination of diversity 'baselines' and multi-year anomalies, which may be used for developing indicators of ecosystem health.

Unique microbial communities at Arctic cold seep dark oxic-anoxic interfaces: insights into seasonal and spatial dynamics

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Methane fuels methanotrophic microorganisms, initiating energy flow into light-deprived ecosystems. In the Arctic, terrestrial methane seeps develop where groundwater from below the permafrost surfaces. At Lagoon Pingo, a methane-saturated Arctic seepage, a sulfur-oxidizing microbial community fueled by anaerobic methane oxidation was recently discovered entrapped within the ice. Our study investigates whether (1) this microbial community is unique to the winter ice-water interface, and depends on ice growth and environmental factors, and (2) ice cores preserve biochemical and genetic signals of this microbial activity. We applied shotgun metagenomics to summer and winter samples from Lagoon Pingo and performed 16S rRNA gene sequencing on winter time-series ice cores. Metagenomic analyses revealed a chemosynthetic gene pool unique to recently formed ice at the core bottom, absent from both summer oxygenated and winter anoxic waters. Time-series data identified two distinct ice-associated microbial communities: (1) anaerobic methanotrophs and sulfate-reducing bacteria resembling water communities and (2) sulfur-oxidizers and aerobic methanotrophs absent from water. The sporadic distribution of the latter within ice cores suggests alternating capture of waterborne or interface-specific communities by the ice, likely seeded by overwintering sediments. Our findings highlight the ecological significance –and vulnerability– of under-ice microbial processes as polar seasonality faces increasing disruption.

A genomic atlas of 11,938 metagenome-assembled genomes highlights the diversity and originality of the Southern Ocean

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The Southern Ocean (SO) hosts some of the most productive and unique marine biomes. The high susceptibility to climate change of the SO calls for a reference atlas of its largely uncharacterized planktonic ecosystems and their genomic content. We present findings from the Antarctic Circumnavigation Expedition, the largest metagenomics sampling effort to date in the SO. To characterize the SO microbial diversity and function, we analyzed 218 metagenomes sampled from 30 stations during the 2016-2017 austral summer, at depths ranging from 4 to 3460 meters. We reconstructed 11,938 prokaryotic MAGs representing 1,478 distinct genomospecies. The evaluation of their content against recent marine prokaryote reports highlighted the SO unicity and functional novelty: we identified 661 potentially new genomospecies, which exhibits comparatively higher content of novel genes. We identified SO water masses as the main driver shaping microbial communities distribution, and discovered that several metabolic pathways were enriched across microorganisms endemic from the SO. This wealth of new genomic diversity from a circum-Antarctic track constitutes an important contribution to advance our knowledge on marine microbial life and can serve as a reference to evaluate polar-specific adaptations and future climate related biodiversity changes in this highly sensitive region.

Thursday, October 2

Microbial interactions and communication

Fish microbiome: a driver for aquaculture sustainability and welfare through holobiont insights

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The study of microbiomes in aquaculture, as in many other fields, represents a crucial point for productive progression. Microorganisms are in fact the cornerstone of the synergistic system which puts the host and the environment in close communication, generally defined as holobiont. However, the complex nature and variability of microbiomes make interpreting the hierarchy and functional interactions within this network a real challenge. Accordingly, our research aims to disclose the inner mechanisms that regulate the cross-talk within the holobiont, evaluating the different dynamics of the microbes' populations associated with water, skin and intestine of farmed fish. For this purpose, considering multi-omics, computational and modelling analysis, we analyzed how different extrinsic and intrinsic variables, such as diet, temperature, oxygen and host genetics modulate the microbiomes responses both from taxonomic, functional and temporal perspectives. Our results obtained so far demonstrate that the definition of a healthy core microbial profile, together with the identification of specific prokaryotic biomarkers, constitute a proxy for welfare and productivity evaluation in the sector. Furthermore, revealing the factors that shape microbiomes across generations, our findings can also be exploited for the future selective breeding programs and more sustainable tailor-made strategies for the development of aquaculture.

Uncovering the metabolic and biosynthetic potential of the microalgae microbiome

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Microalgae are a promising source of sustainable energy and nutrition, due to their photosynthetic efficiency and production of valuable compounds. However, large-scale microalgae cultivation faces technical challenges. Diverse microbial communities influence the performance of large-scale microalgae production, yet their specific

effects remain poorly understood. Microbes interact with microalgae in beneficial and detrimental ways, influencing the microalgal growth or modulating their microbiome by producing secondary metabolites. Here, we explore the metabolic and biosynthetic potential of the microbiome of *Desmodesmus armatus* (green microalgae) cultivated in two large-scale reactors during 8 months. We aimed to identify timepoints where specific microbial genes, metabolic functions, or pathways were over- or under-represented, detecting temporal trends, and exploring associations with production conditions. We focused on gene clusters in Metagenome-Assembled Genomes (MAGs) associated with synthesizing bioactive compounds. Results suggest temporal stability in core metabolisms, despite substantial taxonomic turnover. However, specific secondary pathways and functions exhibited marked changes. Multiple biosynthetic gene clusters (BGCs) were detected across >4,000 bacterial MAGs, with differences between reactors. These BGCs include pathways potentially involved in synthesizing antimicrobial, antifungal, or cytotoxic compounds. Functional profiling provides a deeper understanding of the metabolic potential encoded in the microalgae microbiome and may contribute to optimizing large-scale cultivation.

Seed and sediment microbiomes influence the germination of seagrass seeds

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Seagrasses are the only flowering plants in the marine environment, forming vast meadows which fulfil important ecosystem services. Despite clonal growth, the recruitment of seagrasses from seeds is essential for maintaining genetic diversity and facilitating dispersal. While the seed microbiome of terrestrial plants has been shown to influence germination and seedling survival, the microbiome of seagrass seeds has received limited attention. We investigated the microbiome of eelgrass (*Zostera marina*) seeds along the salinity gradient of the German Baltic Sea to establish a community baseline by 16S and 18S rRNA gene amplicon sequencing. To assess the influence of the microbiome on germination and seedling development we then manipulated the microbiome of *Z. marina* seeds and sediments. Our analysis revealed that *Z. marina* seeds carry distinct microbial communities with several taxa shared across the different sampling locations. Further we found three times higher germination rates for seeds with intact microbiomes incubated in sterilized sediments compared to sterilized seeds incubated in native sediments. We conclude that seagrass seeds have a microbiome distinct from other plant parts, featuring several potentially plant growth-promoting taxa. This microbiome is likely critical in germination and establishment of healthy seedlings, a prerequisite for recovery and restoration of seagrass ecosystems.

Compensatory dynamics driven by species interactions revealed in the aquatic microbiome

Carmen García-Comas [1], Chun-wei Chang [2], Judith Traver-Azuara [1], Caterina R. Giner[1], Ramiro Logares [1], Chih-hao Hsieh [2], Pedro Cermeño [1]

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The aquatic realm harbors a diverse and complex community of microbes that interact in non-linear ways, with context-dependent episodic interactions difficult to capture with traditional network analyses. To account for non-linearity, we applied empirical dynamic modeling (EDM) to infer temporal interactions among approximately 200 commonly observed species in a microalgae biomass production raceway. To be able to grasp the episodic interactions, we sampled the microbial community three times per week during eight months in two consecutive years. Comparing the species' relative abundances in the time series with the balance of positive versus negative interaction strengths they receive, a pattern of compensatory dynamics driven by species interactions emerges. That is, most of the time in a community species are rare and tend to receive greater relative strength of positive interactions, while in the few occasions in which a species dominates (e.g., relative abundance>0.5), this receives greater relative negative interaction strength. In line with ecological theory, this reflects negative frequency dependence, a stabilizing mechanism that supports biodiversity and ecosystem functioning. In this presentation we will further delve into the interactions involved in the dynamics of those species that occasionally dominate, offering insights into the mechanisms underlying this intricate 'dance of ecological interactions'.

Single-cell C and N assimilation in a diazotrophic cyanobacterium – implications for cell specialization and transport

Meri Eichner [1], Rebeca Lopez Adams [1], Anxhela Hania [1], Anežka Mišková [1], Michiel Kienhuis [2], Kasia Piwosz [3], Lubos Polerecky [2], Ondrej Prasil [1]

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To protect the O_2-sensitive nitrogenase enzyme, N_2-fixing cyanobacteria separate N_2 fixation form photosynthesis either in space or in time. In the filamentous *Trichodesmium*, conflicting results and technical limitations have so-far prevented a consensus on the separation mechanism. Here, we combined traditional staining with advanced single-cell methods specifically adapted for capturing the dynamics of gene expression, fixation and transport of both carbon (C) and nitrogen (N) simultaneously. Using mRNA CARD-FISH, we found that about half of the cells expressed nitrogenase at a given time, mostly in stretches of few cells within a filament where nifH and psbA expression were often anticorrelated. Short stable isotope incubations coupled with nanoSIMS allowed us to reveal a significant fraction of cells that fixed only N but not C. NanoSIMS also showed that within 30 min, most cells within a filament receive N most likely by rapid intercellular transport. Our results also indicate that filaments may switch between fixation, transport and storage phases over time. These findings highlight key

mechanisms that allow for spatial separation of C and N fixation in *Trichodesmium*, providing new insights into the physiological characteristics that allow this globally important genus to thrive.

Vitamin biosynthesis drives seasonal microbial interactions and community structure in the Mediterranean Sea

Nestor Arandia-Gorostidi [1], Lidia Montiel [1], Vanessa Balagué [1], Ramon Massana [1], Josep M. Gasol [1], Caterina R. Giner [1], Rafel Simó [1], Ramiro Logares [1]

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Microbial interactions are central to ocean biogeochemistry and ecosystem functioning, yet remain poorly understood due to methodological challenges of in situ observations. In silico approaches, such as ecological network analysis of metagenomic data, provide powerful tools for inferring microbial interactions and their underlying metabolic mechanisms. Here, we analyzed a ten-year metagenomic time-series from the Blanes Bay Microbial Observatory (NW Mediterranean) to investigate the main metabolic pathways shaping microbial associations and seasonal dynamics. Gene co-occurrence and metagenome-assembled genome (MAG) analyses revealed that vitamin-related metabolism accounted for the highest number of associations, with cobalamin (B_{12}) and thiamine (B_1) biosynthesis playing a central role in network structure. Most associations occurred between partial synthesizers, indicating that vitamin precursor cross-feeding might be relevant in modulating seasonal microbial interactions, and hence, microbial community composition. We observed distinct seasonal patterns, with cobalamin prototrophs peaking in summer, while partial synthesizers dominated in winter. Thiamine followed the opposite trend, with complete synthesizers more abundant in winter. We also detected strong associations between prokaryotic vitamin producers and small eukaryotes, highlighting inter-domain metabolic dependencies. These findings underscore the importance of vitamin biosynthesis in structuring microbial communities and demonstrate the value of gene-based network approaches for revealing functional interactions in marine ecosystems.

The role of jellyfish microbiome in the decay of its gelatinous host

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Blooms of gelatinous zooplankton, or ‘jellyfish’, are increasingly reported worldwide, often collapsing abruptly and disrupting ecosystems, though underlying mechanisms remain unclear. We previously showed that such blooms trigger consistent bacterial

responses, with Pseudoalteromonadaceae and Vibrionaceae driving remineralization. Notably, these lineages were also reported as part of microbiome of living jellyfish. We hypothesized that aging or stress alters host tissue biochemistry, destabilizing the microbiome, weakening defenses, increasing susceptibility to pathogens and shifts to harmful symbionts. To investigate whether bacteria involved in jellyfish decay also reside in the living host, we analyzed the microbiome of the invasive ctenophore Mnemiopsis leidyi throughout its bloom in the northern Adriatic Sea. We sampled bi-monthly across multiple locations and assessed jellyfish mucus microbiota via 16S rRNA sequencing and measured single-cell metabolic activity using FISH and HPG. Bacterial abundance in mucus was 5.5 times higher than in ambient seawater, with ~50% metabolically active. Pseudoalteromonadaceae and Vibrionaceae made up 20% and 40% of the community, respectively, with the majority being metabolic active (~75% and ~60%). Thus, key bacterial taxa linked to jellyfish decay are already present during the host's life, offering new insights into host–microbiome dynamics in gelatinous zooplankton.

Multi-omics driven design of marine microbial consortia for biotechnological applications

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The marine microbiome is a key driver of the blue economy, making its study essential to discover, protect, and use ocean resources sustainably. It consists of microbial communities that interact with one another, often competing, but also cooperating, enabling functions beyond individual capabilities and opening avenues for biotechnological applications. To harness this potential, we propose a computational pipeline for designing microbial consortia tailored to specific applications. As a showcase, we focus on pollutant degradation, demonstrating how microbial interactions can be leveraged to establish functional communities. The pipeline consists of two major steps: (1) the characterization of the metabolic capacity of strains, and (2) the inference of pairwise interactions. We began by collecting 23,000 metagenome-assembled genomes (MAGs) and 6,000 prokaryotic and eukaryotic genomes from multiple databases. Each MAG and genome are characterized by the construction of genome-scale metabolic models and the retrieval of their KEGG Orthologs. Candidate species are identified based on the presence of KEGG Orthologs related to pollutant degradation pathways, either by completing or complementing such pathways through metabolite cross-feeding. Next, to infer interacting genome pairs, we propagate the information from a co-abundance network previously built on 234 TARA Oceans metagenomes. We link inferred interactions between TARA MAGs to isolate genomes by evaluating their similarity using Tanimoto and Hamming distances in the metabolic space. Finally, we assess the likelihood of pairwise genome interactions using a graph neural network for edge predictions and community metabolic modeling for exchanged metabolite predictions, which allows us to prioritize species selection for subsequent

mono- and co-cultivation experiments. In conclusion, the computational pipeline provides a framework to infer interacting microbial communities for targeted applications using multi-omics data.

Exploring nutrient-driven microbial interactions through co-culture experiments

Teresa Peil [1], Luuk van Wieren [1], Carina Bunse [1]

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Heterotrophic marine microorganisms play a significant role in the global carbon cycle by transforming organic carbon and consequently influencing long-term carbon storage in the ocean. It is predicted that nutrient stocks and stoichiometry will change with anthropogenic activities. However, the specific responses of marine heterotrophic microbial communities and their interactions to changing nutrient stoichiometry remain unclear, thereby limiting our understanding of their role in ocean carbon cycling under global change. With this study, we aim to demonstrate the influence of nutrient stoichiometry on microbial community composition and the transformation of dissolved organic matter. Using high-throughput laboratory techniques, we are looking at microbial monocultures as well as synthetic communities with the objective of elucidating microbial interactions, physiological responses and growth patterns under varying stoichiometric drivers. For that, we have isolated heterotrophic marine model microorganisms from the Swedish West Coast. We have scrutinized them for their interaction behavior towards each other as well as natural communities to understand metabolic exchange patterns within marine heterotrophic microbes. Our results highlight species-specific metabolic responses, demonstrating how community assembly patterns affect organic carbon processing.

Long-term dynamics of microbial metabolic interactions in the ocean

Clàudia González-Valdivia [1], Lidia Montiel [1], Francisco Latorre [1], Vanessa Balagué [1], Caterina R. Giner [1], Josep M. Gasol [1], Nestor Arandia-Gorostidi [1], Ramiro Logares [1]

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Microbial interactions underpin marine food webs and sustain biogeochemical cycles, yet they remain poorly understood. These interactions are not static and often fluctuate in response to environmental heterogeneity. Unraveling the dynamics of microbial interactions, such as temporal variations in cross-feeding and competition, is essential for a deeper understanding of marine ecosystem functioning. Here, we integrate metagenome-assembled genomes (MAGs) with genome-scale metabolic models (GEMs) and co-occurrence networks to reconstruct patterns of potential microbial interactions along a 12-year coastal marine time series at the Blanes Bay Microbial Observatory (Blanes, Spain) in the North-Western Mediterranean Sea. This integrative approach allows us to examine predicted interaction trends over time, including shifts between competitive and cooperative assemblages, as well as variations in the types of metabolites exchanged within the microbial network. Our study offers new insights

into long-term metabolic interactions within marine microbial communities, which are central to comprehend ocean ecosystem functioning and the self-organization of these communities in heterogeneous conditions.

Exploring the unseen: chytrid fungi in Arctic and Antarctic microphytobenthic communities

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Fungal parasites have been recognized as critical and abundant components of every ecosystem. They have the potential to regulate host populations, mediate interspecific competition between hosts and other species, and thus affect biodiversity and food web structure. However, parasite diversity is still poorly known, particularly for fungal parasites in aquatic ecosystems, and only rough estimates exist on total species diversity and abundance. A few recent studies in polar regions indicate that parasitic species, in particular of the early fungal lineages, are highly abundant in both Antarctic and Arctic aquatic ecosystems, yet their diversity and ecological roles are still poorly understood. Therefore, we aimed to address these gaps and investigate the relevance of fungal parasites for phytoplankton and microphytobenthic communities, forming the basis of aquatic food webs and are key components for trophic interactions in polar ecosystems. We show that fungal parasitic taxa are present in these habitats in high abundances, whereby their correlations with benthic diatoms indicate potential parasitic interactions. Moreover, we show that temperature and salinity are major drivers of fungal diversity, community composition, and interactions with potential hosts. We emphasize the need for further research, considering the effects of increased water temperatures and salinity following climate warming.

Macroalgal biofilm harbours a wide diversity of parasitic protists with distinct temporal dynamics

Albert Reñé [1], Elisabet Alacid [2], Natàlia Timoneda [1], Esther Garcés [1]

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Marine macroalgae surfaces create a nutrient-rich environment that promotes the formation of epiphyte biofilms. Biofilms are complex systems that facilitate ecological interactions within the community. This study describes the diversity and temporal dynamics of microeukaryotic community in the biofilm of Mediterranean macroalgae during summer, focusing on parasitic groups. Protist diversity was assessed using metabarcoding sequencing of the V4 region of the 18S rDNA gene. The macroalgal biofilm exhibited dynamic shifts in the microeukaryotic community structure associated to three phases of biofilm formation. Each phase was characterised by the dominance of specific eukaryotic groups with succession between them. Our study revealed a high diversity of parasites from different lineages infecting a wide variety of hosts, including the basibiont, colonizers within the biofilm, nearby marine hosts and terrestrial organisms. The highest diversity and abundance of parasites were found in the mature phase of the biofilm, where the complexity and stability of the system seem to favour parasitism. The parasite assemblage was dominated by Apicomplexa, mainly corresponding to unknown diversity. Parasitism potentially affects the dynamics of these communities and facilitate ecological interactions between the biofilm and surrounding organisms, suggesting that parasitism play a key, but unexplored role in shaping complex marine biofilms networks.

Protists and their roles in aquatic microbiomes

Resolving microbial food web structure and dynamics at unprecedented taxonomic resolution indicates unexpected importance of omnivores as bacterial grazers.

Sohrab Khan [1], Kasia Piwosz [1], Jared V Lacaran [1], Anetta Ameryk [1], Krzysztof Rychert[2], Lidia Nawrocka [1], Vojtech Kasalicky [3], Indranil Mukherjee [3], Tanja Shabarova [3], Usman Asghar [3], Karel Simek [3]

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The diversity of pelagic protistan predators is well recognised, but the trophic roles of ecologically relevant groups remain little known. Moreover, it is unknown to what extent their community is bottom-up or top-down controlled. Here, we combined size-fractionated experiments with high-frequency environmental observations to disentangle the trophic roles of protistan predators in the coastal waters of the Baltic Sea, with a focus on heterotrophic nanoflagellates (HNF). Trophic cascades upon size fractionation indicated strong top-down control on HNF by predatory ciliates. Adding bacterial prey induced rapid growth of HNF but did not affect their community composition. Interestingly, omnivorous kathablepharids and predatory MAST-2 dominated protistan communities, while bacterivores, such as Cry-1 cryptophytes or MAST-1, were less abundant. The high-frequency sampling campaign confirmed the importance of kathablepharids and MAST-2 in the environment, and their dynamics suggest a trophic relationship between them. Moreover, ciliates were confirmed to be a key group exerting top-down control on HNF. Combining the experiments and high-frequency sampling allowed us to resolve trophic interactions between HNF and microzooplankton at unprecedented taxonomic resolution. Moreover, our results indicate the potential importance of omnivorous kathablepharids as bacterivores in the coastal water of the Baltic Sea, compared to typical bacterivorous groups.

Heritable diel energy reserves enhance diatom growth

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Diatoms are important drivers of marine primary production and biogeochemistry. Unlike most other photosynthetic organisms, diatoms can divide asynchronously, with growth and divisions occurring day and night. However, the mechanisms and rates of asynchronous division have remained elusive. Here, using microfluidics-based time-resolved cell tracking, we measure the growth dynamics of individual cells of the diatom Thalassiosira pseudonana and show that cells growing mostly during the dark phase achieve rapid generation times (8 hours), dividing as fast as cells growing fully in

light. We found that this remarkable ability of rapid growth in the dark is a consequence of the light history of both the cell and its parent cell, as light history controls the amount of photosynthetic energy the cell has stored in the form of the polysaccharide chrysolaminarin when entering the night. Furthermore, a mathematical model of this mechanism yields a 14% increase in the daily asynchronous population growth rate compared to growth without diel energy reserves, resulting in a 17-fold increase in cell abundance over a typical 10-day diatom bloom. Our results demonstrate that heritable diel energy reserves can thus be a major contributor to the rapid growth of diatom populations in the ocean.

From Bacteria to eukaryotes and nucleomorphs: the prominent evolutionary journey of the DNA polymerase III

Thibault ANTOINE [1]

[1]*Genoscope*

Cryptophytes have acquired a plastid from red algae (secondary endosymbiosis) and a remnant of the engulfed red algae nucleus, the nucleomorph, persists in multiple lineages. Here, we completed the first genome-resolved metagenomic survey dedicated to nucleomorphs using the Tara Oceans ‘omics legacy. We successfully resolved the triumvirate environmental genomes (nuclear, nucleomorph and plastid) of the genus Teleaulax, which is abundant and widespread in the sunlit oceans. Unexpectedly, Teleaulax nucleomorphs encode the first DNA polymerase III (DNApol-III) genes documented among eukaryotes. Phylogenetic analyses firmly connect the Teleaulax DNApol-III to Cyanobacteria, favoring a primary endosymbiosis origin for this prominent gene. This scenario implies that some red algae have maintained this gene at least up to the point of the Cryptista-related secondary endosymbiosis event. By leveraging eukaryotic genomic databases, we found that a few red and green algae also contain this DNApol-III, demonstrating its occurrence in distantly related eukaryotes and supporting a primary endosymbiosis origin. Finally, we suggest that DNApol-III plays a critical ecological role. Indeed, the plastid and nucleomorph of Teleaulax are sequestrated together by the ciliate Mesodinium over relatively long periods of time. We hypothesize that DNApol-III is used for plastid genome replication in both Teleaulax and Mesodinium.

Phagocytosis-mediating genes in cultivated species of heterotrophic flagellates

Aleix Obiol [1], Irene Forn [1], Elisabet Sà [1], Ramon Massana [1]

[1]*ICM-CSIC*

Microbial organisms contain the vast majority of genetic and functional diversity within eukaryotes and populate all ecosystems on Earth. Through their activity, microbial eukaryotes play key roles in global biogeochemical cycles as primary producers, grazers, parasites and decomposers. Among them, marine heterotrophic flagellates (HFs) are tiny bacterivores responsible for channeling carbon to higher trophic levels, nutrient recycling and controlling bacterial abundances. The study of this functional

group has long been hindered by the absence of cultured species and robust reference genomes. As a result, phagocytosis, an ancient eukaryotic process whereby HFs engulf and digest bacteria, has been inadequately characterized as a microbial feeding mechanism. In this study, we analyzed transcriptomic data from three species of heterotrophic flagellates to advance our understanding of the genes involved in phagocytosis. We first built high-quality genomes based on long-read sequencing for the studied species and then performed incubations to obtain RNA-Seq data from different stages of cell growth. We used the newly-generated genomes to perform comparative genomics between the different species and process the obtained transcriptomes. Our results revealed distinct patterns of overexpression for certain orthologous groups annotated with a phagocytosis-related function during the exponential phase of cell growth. Some of these groups were shared and actively expressed in the three species, which supports the idea that phagocytosis is a conserved mechanism in eukaryotes and that describing its genetic toolkit is something feasible in the following years.

Monitoring sexual reproduction in diatoms: a global perspective on aquatic microbiomes

Lucia Campese [1], Gust Bilcke [2], Rossella Annunziata [1], Luz Amadei Martínez [2], Camilla Borgonuovo [1], Nadine Rijsdijk [2], Peter Chaerle [2], Koen Van den Berge [3], Daniele Iudicone [1], Marina Montresor [1], Maria Immacolata Ferrante [4], Klaas Vandepoele [2], Wim Vyverman [2]

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Sexual reproduction is a fundamental yet underexplored process in the life cycle of eukaryotes, particularly in aquatic microorganisms like diatoms, which play a crucial role in marine and freshwater ecosystems. Despite its ecological importance, sexual reproduction is rarely observed in natural diatom populations. This study presents a method for in situ monitoring of diatom sexual reproduction, developed through controlled sex transcriptome experiments on four diatom species. A panel of conserved marker genes was validated for specificity and sensitivity using metatranscriptomic data from an estuarine community experiencing massive sexual reproduction in response to salinity changes. The analysis, combined with Metagenome-Assembled Genomes (MAGs) from Tara Oceans, revealed widespread and coordinated expression of these markers across nine diatom genera, including both bloom-forming and rare species. Additionally, automated imaging confirmed the presence of sexual cell stages. These findings challenge the prevailing view that diatom sexual reproduction is rare, suggesting it is a common and coordinated process in the global ocean. This marker panel offers a new tool for monitoring sexual reproduction in natural diatom populations, advancing our understanding of the environmental and evolutionary factors that govern this essential process.

Cultivation, genomics and giant viruses of a ubiquitous freshwater heterotrophic cryptophyte

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Heterotrophic nanoflagellates are the chief agents of bacterivory in the aquatic microbial loop but remain underrepresented in culture collections and in genomic databases. Using a genome-streamlined, ultrasmall and abundant microbe as a prey and CARD-FISH screening, we isolated and characterized Tyrannomonas regina, one of the most dominant ubiquitous heterotrophic cryptophytes in freshwaters and is the first cultured representative of the widespread and abundant freshwater CRY1 lineage. Tyrannomonas has the smallest genome of any cryptophyte sequenced thus far. The compact genome (ca. 69 Mb) revealed no traces of loss of an ancestral photosynthetic lifestyle, consistent with its phylogenomic placement prior to the acquisition of the red-algal symbiont in photosynthetic cryptophytes. Moreover, its genome is substantially depleted in repeat content and endogenous viral elements in comparison to its photosynthetic counterparts. Genomes of two giant viruses, Tyrannovirus reginensis GV1 and GV2 were also recovered from the same culture and are the only representatives of a genus described solely by metagenome-recovered giant viral genomes. Collectively these findings provide insights into genomic ancestry and evolution, widespread ecological impact and interactions of an elusive protist lineage and illustrate the advantages of culture-centric approaches towards unfolding complex tapestries of life in the microbial world.

New parasitic oomycete: A potential driver of the spring diatom bloom termination in Sagami Bay, Japan

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Marine zoosporic fungi remain understudied despite growing evidence of their potential influence on ocean productivity, primarily due to the limited availability of cultured isolates. In this study, we report a novel species of marine diatom-infecting fungus from Sagami Bay (Japan), which has been successfully isolated and maintained in culture. This fungal parasite exhibited a high prevalence of infection on the same host during spring diatom blooms for three consecutive years, suggesting ecological resilience and a potential role in bloom dynamics. The objectives of this study were to (i) determine the organism's phylogenetic placement, (ii) characterise its full life cycle, (iii) assess its host specificity under laboratory conditions, and (iv) evaluate its seasonal occurrence.

Phylogenetic analysis of the SSU rDNA placed the isolate within the genus Miracula, and its life cycle was consistent with Oomycetes' characteristics. Laboratory experiments revealed its capacity to infect additional diatom species. Notably, reinfections were observed in healthy host cultures after inoculation with material from a two-month-old inactive culture, suggesting the presence of a dormant stage capable of reactivation when conditions become favourable. These findings expand our understanding of the diversity, ecological roles, and survival strategies of marine fungal parasites, with direct implications for marine biogeochemical cycles.

Functional Diversity and Metabolic Strategies of Microeukaryotes from the Epipelagic to the Bathypelagic Zone of the Tropical and Subtropical Ocean

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Microeukaryotes are vital to marine ecosystems, influencing food webs, fisheries, and the ocean's carbon cycle. Yet, limited genomic data from deep-sea environments restricts our understanding of their metabolic capabilities and ecological roles across depth gradients, complicating predictions of climate change impacts. In this study, we analyzed 116 metagenomic samples from 11 vertical profiles collected during the Malaspina expedition, spanning epipelagic to bathypelagic zones. We focused on the metabolic potential of pico- and nano-microeukaryotes in tropical and subtropical oceans. Using a new bioinformatics workflow, we extracted eukaryotic genes, curated taxonomy, and reduced contamination. Our curated gene catalog includes 7.7 million eukaryotic open reading frames (ORFs), with Dinophyceae genes dominating across different depths. Taxonomic comparisons between the gene catalog and ribosomal RNA (mTags) showed 42% and 53% agreement for pico- and nano-microeukaryotes, respectively. Gene-based taxonomic richness correlated with ORF and KEGG KO counts across depths. Differential abundance analyses showed depth-specific metabolic strategies: photosynthesis in surface waters and nitrogen, sulfur, and carbon metabolism deeper down. Low-variation KO functions showed diverse taxa contributing similarly, indicating metabolic redundancy across depths. Overall, these results underscore the functional adaptability of microeukaryotes, suggesting they may possess resilience to environmental shifts across oceanic depth layers.

Disentangling a novel protistan lineage: diversity and trophic strategies of picozoa in the Mediterranean Sea

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A global repository of metabarcoding datasets has revealed a dominant group of protists, known as *Picozoa*, to be a significant component of marine microbial planktonic communities, with a substantial biogeographic distribution spanning both polar and non-polar regions. However, key aspects of their biodiversity and ecological function remain largely unresolved. The present study aims to investigate the phylogenetic structure of *Picozoa*, as well as their trophic strategies, with a particular focus on populations from the Mediterranean region. *Picozoa*-specific probes were developed based on environmental 18S rDNA sequences from independent global datasets, and applied in a seasonal sampling at the Mediterranean region. Within this framework, the PIC-1 clade exhibited the greatest cell abundance, followed by PIC-2 and PIC-3. To assess trophic behavior, grazing experiments were conducted by applying fluorescence in situ hybridization (FISH) to natural assemblages to evaluate ingestion patterns in each clade. Variables such as ingestion time (1–24 hours), prey type (microalgae, bacteria, and picocyanobacteria), and diel variation (day vs. night) were considered. Our data will reveal if *Picozoa* are phagotrophic and whether they can exhibit specific feeding strategies.

Use it or lose it: Maintaining adaptive phenotypic defence against a protistan predator in a freshwater bacterium

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Theoretical analyses of eco-evolutionary predator-prey dynamics suggest that constant environments should select for the loss of phenotypic plasticity, whereas adaptive formation of defence phenotypes should be preserved at fluctuating environmental conditions. We experimentally tested these predictions with a freshwater bacterial isolate that overproduces grazing-protected aggregates upon a chemical cue from a bacterivorous flagellate. This defence trait was either constitutively enhanced or reduced in isolates that were grown for 20 cycles with (P+) or without (P-) predators, and the evolved strains lost the ability to increase aggregate formation in supernatants of predator-prey co-cultures. However, if predators were only present during every second growth cycle, phenotypic plasticity was preserved and the size of the aggregated bacterial subpopulation in evolved strains was indistinguishable from the ancestor. 90% of the P+ population lost a 156 kbp fragment on their chromosome, and all populations under (constant or alternating) predation pressure had a mutated glycosyltransferase gene that might play a role in the formation of the grazing protected phenotype. Our results confirm that predation may affect genome architecture, and we provide experimental evidence for an evolutionary mechanism that was proposed to select for either fixed trait variability or phenotypic plasticity.

Describing the diversity and global distribution of plastid-bearing coral symbionts re-using published microbiome data

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Corals form intricate symbiotic relationships with a diverse microbial community that influences their physiology, evolution, and ecological function. In microbiome analyses, sequences of plastid origin are often discarded as noise, yet they carry valuable ecological information and should be retained and analyzed. In this study, we performed a meta-analysis of publicly available 16S rRNA gene datasets to examine the diversity and global distribution of plastid-bearing microeukaryotic symbionts in corals. We reprocessed 3,735 samples from 48 studies, generating a unified dataset of 17,732 Amplicon Sequence Variants (ASVs) and over 13 million reads. *Coccidiomorphea* (Apicomplexa) were highly abundant across all coral types, health states and locations, confirming that they are the second most widespread and abundant eukaryotic symbiont after the *Symbiodiniaceae*. Phylogenetic placement identified most apicomplexan reads as *Corallicolidae*. *Bacillariophyceae* (diatoms) were also common though they may represent epibionts or ingested prey. Healthy and bleached coral eukaryomes exhibited substantial overlap, whereas diseased corals harbored distinct communities, highlighting the reversibility of bleaching and the severity of disease. The most abundant ASVs exhibited strong host specificity, a pattern supported across the dataset by ASV profiles. This large-scale analysis provides a comprehensive overview of plastid-bearing coral symbionts and illustrates the value of reanalyzing existing microbiome data.

Predator-prey interactions in aquatic microbial communities under different resource fluctuation regimes

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Heterotrophic and mixotrophic protists, as key microbial predators, play a crucial role in structuring bacterial communities in aquatic ecosystems. Yet, the extent and drivers of their interactions with bacterial communities remain unclear. To examine how resource fluctuations affect the top-down control of mixotrophic and heterotrophic protists on bacterial communities, we conducted a controlled microcosm experiment with natural freshwater plankton communities. Communities were exposed to alternating resource enrichment via soil extract addition for six days, followed by resource depletion via dilution for six days, and vice versa. Microbial responses were monitored using 16S and 18S rRNA gene sequencing, flow cytometry, fluorescence microscopy, and grazing assays. Regardless of the addition order, enrichment increased mixotrophic and heterotrophic protist abundances, while depletion increased grazing rates, indicating rapid protist responses to both conditions. However, when enrichment followed dilution, the bacterial communities more closely resembled the control treatment. In contrast, when enrichment occurred first, bacterial abundances stabilized and diverged more

from the control. These results emphasize that the history of resource fluctuation can modulate the impact of different protist feeding modes on bacterial community composition. In the next phase of this study, we will compare changes in protist communities estimated through sequencing.

Friday, October 3

Viral diversity and ecology

Giant viruses and virophages: unveiling their presence in marine protist cells

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Marine protists are key to ocean biogeochemistry and host complex viral communities, including giant Nucleocytoplasmic Large DNA Viruses (NCLDVs) and their virophage parasites. These infect diverse eukaryotes, shaping host biology and contributing to nutrient cycling through lysis. Research on eukaryotic viruses has traditionally relied on host culturing, but most microbial and viral diversity remains uncultivable, limiting culture-based approaches. In parallel, while metagenomics has been key in uncovering and enlarging the diversity of eukaryotic viruses, it lacks the ability to link these viruses to their specific hosts. To overcome this, we applied Single Amplified Genomes (SAGs) from ~400 marine protist cells in Blanes Bay, providing a direct, lineage-resolved view of virus–host interactions. Our analysis revealed widespread interactions of NCLDV-like DNA across various protist lineages, indicating a significant and often overlooked presence of these large viruses within host genomes. Furthermore, we identified endogenous virophage sequences in several protist SAGs, suggesting a potential for host-mediated defense against giant virus infections. Our findings demonstrate that single-cell genomics can uncover hidden virus–host interactions in uncultured marine protists. These insights highlight the ecological significance of giant viruses and virophages in shaping marine microbial community structure and dynamics.

Polinton-like viruses associated with a genome-streamlined heterotrophic cryptophyte

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Polinton-like viruses (PLVs) are diverse eukaryotic DNA elements (14–40 kbp) that often expand within protist genomes through repeated insertions. Emerging evidence suggests they act as antiviral agents, limiting giant virus (*Nucleocytoviricota*)

proliferation and modulating host–virus interactions. While numerours PLVs have been identified, most are derived from metagenomic data, leaving host associations unresolved. In previous work, we uncovered over a thousand complete PLVs in large, repeat-rich genomes of photosynthetic cryptophytes, revealing lineage-specific expansions and enabling host-virus linkage inference in environmental datasets. However, the extent of PLV diversification in small, heterotrophic cryptophytes remained unknown. Here, we used long-read sequencing to assemble the genome of *Tyrannomonas regina*, a newly cultured, ultrasmall, and abundant heterotrophic cryptophyte. We identified 13 endogenous PLVs, each encoding hallmark features such as major capsid proteins, protein-primed DNA polymerases, and integrases. Most elements are flanked by terminal inverted repeats, while two possess terminal direct repeats and encode tyrosine recombinases, indicating alternative integration mechanisms. All PLVs share a conserved sialidase-like domain, suggesting a role in host specificity. Additionally, two giant virus genomes were recovered from the same culture. These findings highlight the value of culture-based approaches for unravelling viral diversity and host-virus coevolution in aquatic microbial systems.

Biogeography of giant viruses across global deep freshwater lakes

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Previous studies have collectively illustrated that microbial communities generally follow distance-decay patterns, where genetic similarity decreases as geographic distance increases, suggesting strong local diversification over long-distance dispersal. However, recent observations of giant viruses in marine systems suggest that nearly identical genomes may be globally distributed regardless of distance, raising questions about whether viruses follow the same ecological rules as cellular microbes. To address this, we investigated the global biogeography of giant viruses in deep freshwater lakes, which serve as isolated and stable environments ideal for weighing the impact of dispersal and local diversification on shaping the biogeographic pattern. We reconstructed thousands of giant virus genomes from over 200 metagenomic datasets collected across deep lakes on five continents. These metagenome-assembled genomes represent extensive viral diversity, including a large number of previously unknown giant virus species. This dataset also enabled systematic investigations of giant virus diversity and distribution at a global scale, leading to the identification of cosmopolitan species found across distant lakes on different continents. This study provides a foundation for understanding the biogeographic structure of giant viruses, and contributes to broader questions about the evolutionary and ecological drivers shaping viral communities in freshwater systems.

Synergy in bacterial-viral coinfection expedites algal bloom demise

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Algal blooms are considered hotspots for diverse microbial interaction ranging from symbiosis to pathogenicity. These microscale interactions can control algal cell fate and have a profound impact on bloom demise. Viruses are thought to recycle more than one-quarter of oceanic photosynthetically fixed organic carbon via a fundamental ecosystem process termed the viral shunt. However, while algal blooms typically decline in a synchronized manner, recent single-cell analysis revealed that only 10-30 % of cells showed active viral infection, suggesting additional mortality factors may act in concert. Here, we revealed that bacteria significantly enhance virus-mediated algal mortality, using a microbial community composed of isolates from natural coccolithophore blooms. We quantified this tripartite interaction using two novel metrics that described bacterial lifestyles during co-infection: the Synergy Index (SI) measuring killing interaction strength, and the Benefit Index (BI) assessing bacterial fitness. These measurements identified Alteromonas as a key driver of this tripartite killing mechanism. Members of this genus exhibited high chemoattraction towards infected algal cells which led to their enrichment in particles derived from bloom demise. We propose that bacterial-viral synergy in pathogenicity is a previously unrecognized mortality agent controlling algal bloom demise, consequently modulating the balance between carbon recycling and export.

Bacterial-viral interactions in atmospheric cloudwater

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Clouds serve as an atmospheric refuge for various microbes. However, the extent of bacterial adaptation to the cloud environment and interactions with viruses remain a knowledge gap. Here, six cloudwater collectors were used to sample clouds on Mount Verde (744 m), located on São Vicente Island in the tropical Atlantic Ocean. Cloudwater samples were analyzed using metagenomic sequencing and bacterial isolation, revealing Gram-positive species such as *Agrococcus sp., Bacillus spizizenii, and Deinococcus sp*., as well as Gram-negative species like *Paracoccus marcusii* and *Sphingomonas sp*. Some bacterial genomes encoded for carbon monoxide dehydrogenase (CoxL), likely involved in atmospheric CO metabolism. The genomes

contained 24 prophages, including inducible ones, and diverse antiviral defense systems. The strain *Sphingomonas sp*. MPC37 had the highest number of defense systems (12) including CRISPR immunity targeting viral operational taxonomic units (vOTUs) demonstrating bacterial-viral interactions in cloudwater. Metagenomic sequencing detected 458 vOTUs, with *Sphingomonas spp*. (75 vOTUs), *Deinococcus spp*. (15), *Novosphingobium spp*. (14), and *Methylobacterium spp*. (13) as the main predicted bacterial hosts. Marine-origin cloudwater had the highest counts of both unique and total vOTUs. Our findings underscore the dynamic nature of clouds as microbial and viral ecosystems, where complex survival strategies and interactions shape the airborne microbiome.

Cyanophage plastocyanin supercharges photosynthetic electron transport during infection

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Picocyanobacteria are globally important players in Earth's carbon cycle, whilst the cyanophages that infect them are not only key mortality agents but also trigger significant metabolic shifts during host infection, including inhibition of host CO_2 fixation. Phage genomes contain a number of host-like auxiliary metabolic genes (AMGs) related to metabolic process such as photosynthesis and nutrient depletion. However, historical AMG work has prioritised bioinformatic analysis, with minimal physiological characterisation. Here, using genetics and a Joliot-type spectrophotometer to capture sub-microsecond photosynthetic electron transfer reactions, we investigated the function of cyanophage encoded plastocyanin. Infection of a marine Synechococcus host with a plastocyanin containing cyanophage significantly increased linear and cyclic electron transport, but such an increase in transport was impeded under anoxic conditions suggesting an alternate O_2 dependent electron. By expressing cyanophage plastocyanin in a heterologous Synechococcus, we demonstrate that cyanophage plastocyanin preferentially reduces the O_2 dependent Cytochrome c Oxidase (COX) over photosystem I (PSI). This redirected electron flow to COX reduces electron donor pressure, substantially increasing overall electron transport to maximise ATP or NADPH production during infection and exquisitely highlights how cyanophage hijack the photosynthetic process by exploiting the host's co-localization of photosynthetic and respiratory chain components.

Rapid evolution of marine bacteria mediated by phages

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Phages shape bacterial communities through predation and by facilitating horizontal gene transfer, but their dynamics with their hosts in the environment are not well understood. Here, we studied phage-host interactions in a coastal marine time series using community metagenomics and a model system with high-throughput isolation. As a general feature, short host blooms are followed by equally short phage blooms across phylogenetically diverse bacteria. Metagenomic analysis showed a diverse set of phages, suggesting that a phage cocktail rather than single phages limit bacterial blooms. To understand such dynamics mechanistically, we characterized a bloom of *Vibrio cyclitrophicus* and its phages. Using a cross-infection assay involving 1,753 *V. cyclitrophicus* isolates and 76 diverse phages, we tracked changes in host susceptibility. Susceptibility increased to 52% before the bloom, decreased to 9% at its end, and recovered to 28% five days later, highlighting receptor-based resistance and suggesting rapid, phage-driven host evolution. Additionally, phage replication was assessed, and we observed that more bacteria allowed phage attachment than replication , indicating that also internal defenses do play a role. Overall, our findings show that bacterial populations rapidly shift receptor profiles and internal defenses during blooms, underscoring the dynamic nature of phage-host interactions in the wild.

The origins and evolution of phages infecting freshwater picocyanobacteria: A conundrum spanning the salinity divide

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Oxygenic photosynthesis performed by picocyanobacteria is a fundamental biological process contributing to approximately 25% of the ocean net primary production. Here, viruses (cyanophages) infecting these globally distributed phototrophs are responsible for releasing ca. 20% of globally fixed carbon into the ecosystem. Marine picocyanobacteria/cyanophages have been studied for decades, whilst the dearth of data of freshwater representatives has only recently been addressed. We show that freshwater cyanophage genomes are distinct from their marine counterparts with isoelectric point acid shift and prevalence for negatively charged amino acids. Additioanlly, freshwater cyanophages show a distinct pattern of genes inolved with metabolism and lack key genes common in marine counterparts. Coverage in metagenomic datasets unequivocaly discards the presence of marine phages in

freshwater lakes and freshwaters on oceanic regimes. Therefore, salinity dictates a phage diversification where transitions between marine and freshwater systems would require extensive changes in the proteomes. Yet despite this, phylogenetic analsysis shows freshwater cyanophages have emerged more recently and multiple times from their marine counterparts. This is at odds with the phylogenetic relationships seen in their cognate hosts, where marine picoyanobacteria have evolved once from fhreshwater ancestors. This has important implications for horizontal gene transfer across the salinity divide.

Hi-C-inferred virus-host networks: coinfection, generalist and specialist phages, and viral metabolic functions in freshwater ecosystems

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Viruses are central players in the ecological and evolutionary dynamics of aquatic ecosystems, yet identifying their microbial hosts remains challenging, especially for environmental viruses known solely from their genomic sequences obtained through culture-independent approaches. In this study, we applied Hi-C technology to detect active virus–prokaryote interactions in the freshwater Amadorio reservoir (Alicante, Spain). From metagenomic data, we reconstructed over 1,000 bacterial genomes (HAGs), mainly affiliated with genera such as Limnohabitans, *Candidatus* Planktophila, and Fonsibacter, as well as more than 33,000 viral genomes (vHAGs), primarily from the class Caudoviricetes. Importantly, we identified over 100 active phage–host associations. Interaction networks revealed broad-host-range phages, multiple infections per host, and coinfection events. Active associations were more common among abundant bacterial taxa, and generalist phages exhibited higher relative abundance. Novel phages were identified infecting genera from which no viruses had previously been reported, including *Polynucleobacter* and *Candidatus Nanopelagicus*. Nearly half of the associated phages encoded auxiliary metabolic genes (AMGs), indicating functional impacts on host metabolism. Overall, this study underscores the power of Hi-C to advance genome-resolved metagenomics and, critically, to reveal active virus–host interactions within complex freshwater microbial ecosystems.

New tools & big data in microbial ecology

The Ocean Microbiomics Database (OMDB): A Web Repository for the Genome-Resolved Exploration of the Global Ocean Microbiome

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The ocean, Earth's largest interconnected ecosystem, harbors diverse microbial communities shaped by spatiotemporal variation, environmental conditions, and species interactions. High-throughput sequencing has revolutionized our ability to study this microbiome, uncovering taxa, proteins, and bioactive compounds. However, data remains fragmented across isolated studies, with inconsistent methods and metadata that hinder global comparisons, limit reuse, and impede cumulative discovery—complicating global contextualization and increasing the risk of redundant findings. Here, we present the Ocean Microbiomics Database (OMDB), a repository for the systematic exploration of marine microbiomes available at https://omdb.microbiomics.io. OMDB contains 12,260 metagenomes processed with a standardized co-abundance binning method, reconstructing 274,282 genomes. These are grouped into 32,022 prokaryotic species-level clusters and include gene annotations and metadata like geographic location, depth, and temperature. Crucially, this resource opens opportunities to model how abiotic factors, such as temperature, influence microbial composition and function across global gradients. Its vast >500M protein sequences catalog can be queried online in seconds, facilitating the discovery and classification of novel functions, including biotechnologically and biomedically important molecules like biosynthetic gene clusters and often-overlooked antimicrobial peptides. Together, the OMDB enables genome-resolved, globally contextualized analyses of the ocean microbiome and provides a foundation for predictive, trait-based models of marine microbial ecology.

Protein-based functional signatures reveal global patterns of ocean microbial metabolism

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Marine microbes drive ocean ecosystem processes and global biogeochemical cycles. While prior studies have explored their taxonomic and functional biogeography, the potential metabolisms underpinning biogeographical patterns are still poorly understood. To address this, we analyzed 1,379 marine metagenomes using a sensitive clustering strategy that groups protein sequences into Operational Protein Units (OPUs), capturing both known and uncharacterized metabolic functions. This high-resolution profiling approach allowed us to move beyond traditional gene annotations and tap into the so-called “functional dark matter” of microbial life. From this comprehensive OPU-dataset, we used machine learning to identify Ocean Microbiome Signatures (OMSs)—distinct functional assemblages that reflect the metabolic repertoire and ecological roles of marine microbial communities. We identified ten OMSs that collectively capture key metabolic capacities, each exhibiting specific spatial patterns and functional traits. For instance, some (OMS1, OMS3) are enriched in carbon fixation and energy metabolism, supporting productivity in sunlit, nutrient-poor waters. Others (OMS2) consist of resilient heterotrophs adapted to polar environments. Still others (OMS8, OMS10) specialize in stress resistance and nutrient transformation. By integrating both known and novel protein functions, our framework reveals new dimensions of the ocean microbiome's functional ecology, offering deeper insights into how microbial communities shape global biogeochemical processes.

Pangenome-resolved metaproteomics sheds light on population-specific ecophysiology in freshwater microbiomes

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State-of-the-art data-independent acquisition (DIA) metaproteomics enables high-throughput in situ profiling of environmental proteomes, but its sensitivity is undermined by reference databases that subsume strain-specific variation into umbrella MAG constructs. Leveraging cutting-edge high-throughput SAG-gel (single amplified genome) and long-read sequencing, we implemented a strain-aware framework that

combines single-cell genomics, hybrid metagenomics and DIA to profile, at strain-level resolution, the microbiomes of two geographically disparate lakes—Lake Zürich (Switzerland) and Lake Biwa (Japan). We recovered 2’839 SAGs and 418 MAGs from paired epilimnion and hypolimnion samples. By selecting 14 abundant, ecologically coherent species-level populations, we generated a non-redundant pangenome-resolved protein catalogue of 108‘402 entries, retaining core genes and encompassing accessory variation. Searching DIA spectra against this bespoke library recovered 33’786 unique peptides mapping to 6’826 proteins (1% false-discovery rate, ≥2 peptides/protein). Notably, many of the most abundant proteins were encoded exclusively by accessory loci present only in SAGs, revealing population-specific expression signals invisible to conventional MAG-centered searches. By integrating strain-resolved genome content with in situ protein expression, our framework closes a critical gap between sequence potential and realized function. It establishes a new benchmark for genome-informed metaproteomics and a scalable blueprint for revealing how microbial populations deploy their genomic potential in freshwater ecosystems.

Species-resolved metaproteomics sheds light on bacterial ecophysiology

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Genome reduction has been framed as an evolutionary strategy for bacteria adapting to oligotrophic aquatic habitats—enabling ecological specialization yet constraining functional breadth. While compelling, this hypothesis remains largely theoretical and genomically inferred, with limited empirical evidence of how streamlined genomes influence microbial life-strategies. To address this gap, we combined time-resolved metagenomics and metaproteomics to link genome content to realized ecophysiology. We conducted a high-resolution temporal survey of Lake Zurich across 37 dates in spring 2021. By combining Nanopore and Illumina sequencing, we reconstructed 3,000+ high-quality metagenome-assembled genomes, dereplicated into 495 species. This formed the basis for an ecologically resolved custom protein database. Integrated with deep data-independent-acquisition proteomics, this framework enabled unprecedented species-level resolution of microbial protein expression, identifying more than 17,500 high-confidence proteins—nearly 15,000 assigned unambiguously to individual taxa. Our results show that genome-reduced freshwater bacteria overinvested in transporter proteins, maintaining high-level expression irrespective of environmental change. This decoupling from external cues reflects physiological bet-hedging, enabling streamlined microbes to remain broadly responsive despite limited regulatory capacity. By linking genome architecture to dynamic time-resolved protein expression, our study provides direct evidence that genome reduction not only constrains functional versatility but also reshapes microbial life-history strategies in oligotrophic aquatic ecosystems.

Predicting complex microbial traits using genomics and machine learning

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Uncultured bacteria dominate and perform important functions in many ecosystems. However, our inability to bring them into culture limits the characterization of their functional traits and therefore our ability to understand their ecological and evolutionary associations. In this talk, I present a way forward where the integration of genomic information and machine learning tools allows the inference of the environmental preferences of thousands of bacterial taxa. By identifying the pH preferences of bacterial taxa from their distributions along pH gradients in both soils and freshwater systems, we were able to construct a large database of genomes with corresponding pH preference information. This allowed us to identify a set of genes consistently associated with preference for acidic or alkaline conditions across bacterial groups. Information on the presence/absence of these genes was then used to train a machine learning model that predicts the pH preference of any given bacterial genome with a mean average error (MAE) of 0.63. This work highlights the value of integrating modern tools to expand our understanding of complex microbial traits. These methods can help improve the cultivation of recalcitrant taxa, the understanding of microbial responses to environmental change, and microbial species distribution modelling.

A novel artificial intelligence tool for identification of extracellular proteins doubles current estimate of the marine secretome

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Microbes are the engines driving the elemental cycles. In order to interact with their environment and the community, microbes secrete proteins into the environment (known collectively as the secretome), where they remain active for prolonged periods of time. Despite the environmental relevance of microbes, our knowledge of the marine secretome remains limited due to a lack of effective in silico methods for the study of secreted proteins. An alternative approach to characterise the secretome is to combine modern machine learning tools with the evolutionary adaptation changes of the proteome to the marine environment. In this study, we identify and describe adaptations of marine extracellular proteins, which vary between phyla, resulting in differences in ATP costs, amino acid composition and nitrogen and sulphur content. We develop 'Ayu', a machine prediction tool that does not employ homology-based predictors and achieves better and quicker perfor- mance than current state-of-the-art software. When applied to oceanic sam- ples (Tara Oceans dataset), our method was able to recover more than double the proteins compared to the most widely used method to identify secreted proteins. The application of this tool to open ocean samples allows better characterisation of the composition of the marine secretome.

Assessing global microbial performance

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Understanding the ecological processes structuring microbial communities demands disentangling drivers of diversity and coexistence at a global scale. We applied a ranking index adapted from competitive gaming (Elo-rating) to assess bacterial performance across multiple biomes. Our analysis included >4,000 samples from the Earth Microbiome Project dataset comprising both free-living (aquatic, sediments, soils) and host-associated microbiomes (plants and animals-related). The Elo-rating is a metacommunity index that quantifies individual bacterial performance by integrating both relative abundance and occurrence across samples. The ratings exhibited a left-skewed distribution, with sediments, soil and rhizosphere biomes showing greater variability than animal-related ones. This pattern suggests that some biomes harbour few disproportionally strong competitors within their respective metacommunity. We subsequently ranked genera using per-biome Elo-ratings, identifying the most successful ones by their presence in the top decile. These winners were predominantly generalist taxa and common nosocomial pathogens, such as Pseudomonas and Acinetobacter, followed by opportunistic groups like Sphingomonas and Bacillus. Our approach offers a scalable framework for comparing competitive hierarchies across diverse ecosystems, shedding light on the global patterns of microbial success.

Leveraging metagenomics and metatranscriptomics to gain novel insights in the ecological dynamics of microeukaryotic plankton communities and their contribution to biogeochemical cycles

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Seasonal fluctuations profoundly affect marine microeukaryotic plankton composition and metabolism as well as biotic interactions. But accurately tracking these changes has been a long-standing challenge. High-throughput sequencing of environmental samples has dramatically improved our understanding of the molecular activities of complex microbial communities in their natural environments. For instance, by enabling taxonomic profiling and differential gene expression analysis, microbiome studies have revealed intriguing associations between community structure and ecosystem functions. In this study, we present seasonal (monthly samples) and diel (hourly samples) metatranscriptomic data sets from the North Sea. Our data illuminate the contributions of microeukaryotic taxa to biomass production and nutrient and carbon cycling at different times of the year/day and allow delineation of their ecological niches. We characterized the metabolic signatures of different seasonal and diel phases in detail, thereby revealing for example the metabolic versatility of dinoflagellates and the growth and energy metabolism of colonial Phaeocystis globosa during its blooms. While we demonstrate that sequence data analysis is an invaluable

tool to understand the ecology of microeukaryotic planktonic communities, its effectiveness in characterizing microbial ecosystem functioning at the systems level has been limited by the quality and scope of reference sequence databases. We therefore applied state of the art bioinformatics tools to leverage publicly available genome/gene sequences for planktonic organisms to build a customized protein sequence database. Based on this, our goal is to conduct a systems-level interrogation of environmental samples, which can effectively augment the insights obtained through traditional gene-centric analysis. By further expanding on the taxonomic and functional complexity of our database we improve our ability to map the molecular traits that drive changes in the composition and functioning of marine planktonic networks through space and time.

BOOK OF ABSTRACTS

Flow Cytometry Workshop

Flow Cytometry Workshop

Pushing the boundaries of flow cytometry: illuminating the active virosphere with BONCAT and single virus genomic sequencing tecnologies

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Viruses are the most abundant biological entities in nature and reshape every cell in ecosystems through cell lysis and other viral processes influencing the overall function of microbial communities. However, most viruses and their hosts are extremely difficult to culture in the laboratory. Therefore, identifying and quantifying the specific viruses producing cell lysis in a biological sample remains a priority in virology and presents significant technical challenges. Here, we introduce a click-chemistry method to fluorescently label, sort, and sequence the genomes of newly produced uncultured viral particles released from transcriptionally active host microbial cells. This approach, called viral BONCAT-FACS, combines biorthogonal non-canonical amino acid tagging (BONCAT) with single-virus sorting followed by advanced flow cytometry sorting, and sequencing of sorted active viral particles. This study, using a marine natural sample, illustrates the effectiveness of this novel technology for uncovering genome-resolved uncultured viral-host dynamics with potential application in other fields such as Human Microbiome and Virome. By providing a direct approach for tracking active viral infections in biological samples, this method enhances our ability to investigate behavior and interactions of these nanoscale predators, expanding our understanding of their role in nature.

Sketching the invisible: high frequency sampling and computational cytometry of marine microbes

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A reliable depiction of microbial communities is essential for marine ecology and, as in visual arts, the aim is to produce a good sketch of the environment, fast yet informative. This requires balancing resolution and speed, capturing detail while keeping the

process relatively quick and affordable. In this context, flow cytometry has long been the preferred technique, widely used to estimate microbial abundance, size, and biomass. These estimates are traditionally based on Niskin bottle sampling, and a natural improvement, as proposed here, is to increase the sampling frequency through automation. As in nucleic acid sequencing, the large volume of data generated demands suitable bioinformatic tools for processing. These approaches are commonly referred to as computational cytometry. With this methodology, it is possible not only to cluster populations in an objective and reproducible way but also to calculate diversity indices directly from the cytograms. When combined with high frequency sampling, this approach enables the simultaneous acquisition of data on microbial abundance, biomass, and diversity, providing the most comprehensive sketch of microbial communities achieved so far.

Links between the morphological and rank abundance structures of bacterioplankton communities and the optical and molecular structure of dissolved organic matter across Canadian lakes.

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Bacterioplankton and dissolved organic matter (DOM) are fundamental components in aquatic ecosystems and unravelling how they relate along major environmental gradients is a central goal to our understanding of ecosystem function. Here we explore the potential links between two aspects of bacterioplankton structure, i.e. rank abundance structure, derived from 16SrRNA sequencing approach, and the morphological structure, derived from a cytometric approach, and the optical and molecular structure of DOM. These links were explored across 393 lakes in the context of the pan-Canadian LakePulse project, the first standardized national lake survey that cover wide environmental, climatic and human impact gradients. Lakes dominated by terrestrially derived DOM and associated optical and molecular components have bacterioplankton communities with flatter rank abundance curves (i.e. less marked dominance) and with simpler morphological structures (i.e. oblate shape with low-DNA structure and single cytometric population). The opposite pattern was found in lakes with a larger fraction of autochthonous derived DOM and its associated DOM components, where bacterioplankton communities have steeper rank abundances curves (i.e. more marked taxa dominance) with more complex morphological structures (i.e. more diffuse, prolate shapes with distinct cytometric populations). Overall, key aspects of bacterioplankton and DOM structure appear to covary along broad environmental gradients.

Enhancing Biodiversity Studies with Image-Based Flow Cytometry

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Flow cytometry is currently undergoing a major transformation. Following the transition to spectral cytometry, the field is now integrating imaging capabilities. While imaging is not new to flow cytometry, having been available for years in high-resolution platforms such as ImageStream (now from Cytek Biosciences), Attune CytPix (Thermo Fisher), and COPAS Vision (Union Biometrica), the latest advancement involves incorporating imaging modules into cell sorters. An example of this is the CellView system integrated into the FACSDiscover S8 from Becton Dickinson. This technology enables not only the visualization of analysed events but also the verification of sorted populations. In this presentation, we will highlight several examples where image-based cytometry, when integrated into a cell sorter, has demonstrated clear advantages. Notably, it outperforms other image-based sorting systems due to its high-speed cell sorting capabilities, a critical feature for identifying and isolating rare organisms. Additionally, it provides substantial added value by enabling the correlation of morphological image data with single-cell genomic analysis in biodiversity studies. Until now, it was not possible to integrate morphological characteristics with genomic data, an important limitation, especially in studies focused on taxonomy in single-cell research.

BOOK OF ABSTRACTS

Posters

1

Nitrogen cycling in Hungarian soda lakes as revealed by cultivation and metagenomics

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Soda lakes have multiple extreme physico-chemical properties such as alkaline pH, high turbidity and carbonate-dominated ionic composition. Most of these lakes have remarkable nutrient loads from various sources, e.g. feces of migratory waterbirds, groundwater inflow and decaying macrophytes. However, our understanding of the microbial nitrogen cycling in these unique aquatic habitats is still limited. This study combined bacterial growth tests using ammonium salts with the analysis of metagenomic data from water samples of three Hungarian soda lakes. The growth of 56 bacterial strains was tested using various concentrations of ammonium chloride, ammonium sulfate and ammonium bicarbonate. Additionally, the NCyc database was used for metagenomic profiling of nitrogen cycling genes of 253 bins obtained from 42 metagenomes. The isolates exhibited a preference for ammonium chloride compared with the other tested compounds. Metagenomic results showed that organic nitrogen transformation processes were the most abundant among nitrogen cycle processes, while dissimilatory nitrate reduction and denitrification-related genes were also present. Our results suggested that some of the processes in the nitrogen cycle are repressed in these environments, while the pH-dependent increased release of ammonia and the rapidly changing water temperature due to the shallowness of these sites could remarkably affect the biological processes and nitrogen turnover.

2

Prevalence and activity of non-cyanobacterial diazotrophs from the deep ocean

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Biological nitrogen fixation is a crucial process that sustains oceanic productivity. Although cyanobacterial diazotrophs have traditionally been considered the main contributors, non-cyanobacterial diazotrophs may have a bigger and more widespread role than previously recognized. Here, we investigated the distribution and activity of three non-cyanobacterial diazotrophs recovered as metagenome-assembled genomes from the bathypelagic ocean: *Novosphingobium sp*. (MAG-0177), *Ketobacter sp*. (MAG-0081), and *Salipiger sp*. (MAG-0509). Fragment recruitment analysis across 58 metagenomes and 51 metatranscriptomes from the Malaspina Expedition revealed the presence of all three MAGs, although their biogeography and activity differed. Ketobacter was mostly restricted to the Pacific Ocean, associated with the free-living

fraction, and showed the only detectable nifH transcripts. Salipiger was linked to the particle-attached fraction in a few stations of the Pacific or North Atlantic Ocean. However, Novosphingobium differed significantly based on its presence (metaG) or activity (metaT). Additionally, two Novosphingobium strains from bathypelagic samples were isolated and sequenced. Both isolates belonged to the same species as MAG-0177 (ANI> 99%), and consequently also harboured the nifH operon. These results, along with future experimental validation of nitrogen fixation within Novosphingobium isolates, will provide new insights on the ecological relevance of non-cyanobacterial diazotrophs in the deep global ocean.

3

Metagenomic insights into the temporal dynamics of prokaryotic functions during environmental disturbances in an eutrophicated coastal lagoon

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Eutrophication is a major driver of degradation in coastal ecosystems, as illustrated by the Mar Menor lagoon (SE Spain). In 2016, this ecosystem crossed a tipping point that disrupted its ecological balance and severely impacted its biological communities. Since then, recurrent deoxygenation episodes linked to phytoplankton blooms have been detected. Moreover, metabarcoding data have shown rapid shifts in the structure of prokaryotic communities in response to these environmental disturbances. We applied metagenomics to assess the functional potential of these microbial communities. Fourteen metagenomes, collected seasonally over three-years (2019-2022), were analyzed to build a functionally annotated gene catalog (~4 million genes) and track their relative abundances over time. We screened this catalog to identify temporal patterns in biogeochemically relevant functions and the taxa responsible for them. Notably, the abundance of genes involved in anaerobic processes such as denitrification (nirK) or dissimilatory sulfate reduction (aprA/B) varied markedly between deoxygenation events (4-fold increase), suggesting that these episodes may activate microbial processes associated with distinct biogeochemical pathways. Metagenomics provided insights into the role of prokaryotes in nutrient cycling within this coastal ecosystem, which is key to understand the changes in ecosystem functioning produced by eutrophication.

4

Impact of phytoplankton blooms on marine VOCs emission

Cecilia Costas-Selas [1], Eve Galen [1], Suman Son [1], Ane Helberg2 [1], Simon Rohard [1], Klara Li Termansen [2], Riikka Rinnan [2], Lasse Riemann [2]

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Volatile organic compounds (VOCs) are diverse bioactive molecules of low molecular weight and high vapor pressure. VOCs are produced by biotic and abiotic processes and are important for atmospheric chemistry as main biogenic precursors of secondary organic aerosols. In marine environments, the phytoplankton are the main source of marine VOCs, which appear to be an important source of organic compounds fueling bacterioplankton growth. Increased eutrophication and rising temperatures will likely increase intensity of coastal phytoplankton blooms in the coming decades with the potential cascading consequence of elevated VOC production rates and emissions. To assess VOC production by different phytoplankton blooms, we conducted a mesocosm experiment where VOC production by natural, unaltered microbial communities were compared to that by phytoplankton blooms induced by nutrient amendment. VOCs were measured by combining a purge-and-trap system with a high-sensitivity proton-transfer reaction time-of-flight mass spectrometer (PTR-TOF-MS). We observed that the concentrations and compositions of the VOCs changed over the course of the phytoplankton bloom, with, for example, high concentrations of dimethylsulfide (DMS) at the peak of the bloom, and a subsequent decline in DMS concentration. Conversely, highest concentrations of methanethiol (MeSH) were found with the unaltered microbial community. These results highlight the importance of phytoplankton blooms for the VOC composition and production in surface seawater.

5

Diverse mercury-methylating microbial communities inhabit the anoxic waters of the Cariaco Basin

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Microorganisms that possess the hgcAB gene pair in their genomes can methylate inorganic mercury (HgII) in anoxic conditions, transforming it into its neurotoxic form, methylmercury (MeHg). The distribution and lifestyle of these hgc-containing microorganisms, and the surrounding redox conditions, likely influence where and to what extent this toxic compound is produced in the global ocean, particularly in low-oxygen regions. In this study, we investigated the potential for MeHg formation along the redoxcline of the Cariaco Basin, Venezuela, by using the abundance and gene expression of hgc+ microorganisms as a proxy. A total of 48 metagenomes and 48

metatranscriptomes from the particle-attached and free-living prokaryotic fractions of a vertical profile of the Cariaco Basin were obtained during upwelling and non-upwelling periods. In agreement with previous studies, our results showed greater hgc+ gene diversity in particles, likely being an important hotspot for MeHg formation. Interestingly, free-living prokaryotes, mainly Desulfobacterota, seemed to also play a significant role for MeHg formation in euxinic layers during upwelling events. Furthermore, these productivity events enhanced hgcA+ expression along the water column. These findings suggest that the ongoing ocean deoxygenation due to global change could significantly increase MeHg formation potential in oxygen-deficient regions, particularly under expanding euxinic conditions.

6

Volatile Emissions from Estuarine Bacteria

Eve Galen [1], Kajsa Roslund [1], Riikka Rinnan [1], Lasse Riemann [1]

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Microbial volatile organic compounds (mVOCs) are low molecular weight, high vapor pressure molecules that readily diffuse through air and water, enabling chemical signaling over long distances and at low concentrations. The production and cycling of mVOCs may be linked with microbial community composition, yet few studies have examined bacterial mVOC emissions, and almost none with marine bacteria. As mVOCs are, in part, products of primary and secondary metabolism—processes that are generally conserved at the phylum level—mVOCs emissions could be associated with microbial phylogeny. Therefore, we investigated mVOC emissions from 16 bacterial strains cultivated from Baltic Sea surface water, representing Alphaproteobacteria, Gammaproteobacteria, Betaproteobacteria, Bacteroidota, and Actinomycetes. Strains were grown in minimal medium and headspace mVOCs were analyzed using PTR-ToF-MS. A total of 286 unique mass charges were identified, but no correlation was observed between phylogeny and mVOC blend. While Sphingomonas exhibited the highest overall emissions, rates did not align with phylogeny. Acetone was the most abundantly emitted mVOC, predominating in the majority of strains, with acetaldehyde and ethanol dominating in a few strains. Potential biomarkers for eight strains were identified. Overall, bacterial mVOC blends tend to be dominated by the same few compounds, with less abundant compounds driving differences between strains.

7

Discoveries with Roseobacteraceae: Bacterial Models for Ocean Heterotrophy

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The molecular revolution of the 1990s brought insights into the tremendous breadth of evolutionary diversity harbored within the bacterial and archaeal domains of life, enabling scientists to peer into the proverbial microbial black box. Many of these early molecular efforts focused on microbes in marine surface waters, given their global relevance and ease of extraction from seawater via filtration. From molecular surveys of marine microbial communities there emerged a limited number of taxa with marked numerical dominance and distribution across ocean realms. One of these lineages is the now well-studied Roseobacteracae family. Three decades of studying roseobacter members, many of which are amenable to both laboratory culture and genetic manipulation, have led to discoveries in how microbial heterotrophs process diverse marine organic matter, drive biogeochemical cycles, and interact with primary producers.

8

Microbial adaptation and metabolic plasticity shape biogeochemical cycles in the Red Sea brine pool

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The Red Sea brine pools (BP) are among the most extreme and geochemically isolated marine environments on Earth, serving as a unique system for exploring microbial adaptation and evolution. In this study, we analyzed micobial metagenomes from both the free-living (FL) and particle-associated (PA) fraction in a BP and compared them with the microbial community in the adjacent ambient deep water (ADW). Community composition was dominated by Campylobacterota across two BP fractions, while Cloacimonadota, Desulfobacterota, and Planctomycetota were exclusively enriched in the PA fraction in the BP, leading to a high diversity in the PA fraction. In contrast, Pseudomonadota were more prevalent in ADW and the FL fraction in the BP, highlighting habitat-specific selection. The PA metagenome in the BP was enriched in genes related to DNA replication, stress response, anaerobic carbon fixation, and high-affinity transport systems, suggesting enhanced genomic plasticity and metabolic versatility of BP microbiota. Metagenomic assembled genomes (MAGs) affiliated with Campylobacterota, Cloacimonadota, Desulfobacterota, and Planctomycetota recovered from the BP metagenome encoded genes involved in carbon fixation, denitrification, and sulfur oxidation/respiration, underscoring their key roles in brine pool biogeochemistry. These MAGs also belonged to previously

uncharacterized lineages absent from the publically available MAG collections, reflecting evolutionary divergence shaped by environmental filtering.

9

Ecogenomics of the first isolates of the freshwater Actinomycetota order S36-B12

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Actinomycetota are among the most abundant phyla in freshwater lakes, yet several of its lineages remain uncultured. One such lineage, S36-B12 (also known as PeM15 in 16S rRNA databases) was recently classified as a distinct order. Here, we present comparative genome analyses based on the first 38 isolates of the order obtained via dilution-to extinction cultivation. Phylogenomic and average nucleotide identity analyses revealed that the isolates represent two families (S36-B12 and UBA5976), five genera (UBA100649, CAIXFB01, UBA4952, UBA5976, ATZT02) and 17 species. The two families exhibit different degrees of genome streamlining reflected in genome sizes (1.4-3.3 Mbp), GC content (38-67%), median intergenic spacers (14-29 bp) and coding densities (93.1-95.2%). Genome analyses indicate diverse nutrient acquisition strategies including a wide repertoire of carbohydrate-active enzymes, peptidases and in some isolates bacterial microcompartments. All isolates encoded actino- and heliorhodopsins supporting a photoheterotrophic lifestyle and some are likely able to fix carbon via the CBB cycle. Several strains encode aerobic carbon monoxide dehydrogenases and hydrogenases indicating at the potential to supplement energy by trace gases oxidation. These results highlight the ecological and metabolic diversity of the S36-B12 order and establish a foundation for future physiological studies of this previously uncultured lineage.

10

Effects of declining sulfur deposition on methane cycling in lakes

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Atmospheric sulfur deposition has declined significantly due to reduced industrial pollution, leading to measurable changes in the freshwater chemistry in Sweden and other parts of the northern hemisphere. Despite long-term declines in sulfate in Swedish lakes, the impacts on methane dynamics remain unclear beyond that methanogenesis and sulfate reduction are known competing microbial processes in lake sediments, and that a decrease in sulfate availability may promote methane production – amplifying greenhouse gas emissions from lakes. I conducted a field study across 21 lakes in Sweden, investigating how a natural gradient of sulfate levels influences methane dynamics. Depth profiles were sampled during late summer during periods of stable thermal stratification. Samples were analyzed for methane, carbon dioxide, nutrients and ionic composition (including sulfate) while microbial communities were assessed through DNA-based assays. Sulfate concentrations ranged from 1-80 mg/L and methane from 7-3464 ppm across lakes. Preliminary multivariate analyses revealed no clear relationship between sulfate and methane concentrations, suggesting methane oxidation may buffer methane accumulation. Ongoing molecular analyses, including 16S rRNA gene sequencing and quantitative PCR, will describe microbial communities involved in methane production and oxidation, improving our understanding of how long-term sulfur deposition declines may influence methane emissions from lakes.

11

Diverse light-utilization strategies of cyanobacteria with microbial rhodopsins

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Most biological processes on Earth are driven by the energy supplied from sunlight. In the aquatic environment, this sunlight energy is primarily converted into chemical energy through photosynthesis by microorganisms such as cyanobacteria. The

accumulation of genomic sequence information has revealed the widespread prevalence of microbial rhodopsins, which are also commonly found in cyanobacteria. It has been suggested that this dual system enables efficient capture of sunlight energy by using complementary absorption wavelengths. However, the diversity of cyanobacterial rhodopsins remains poorly understood. In this study, we employed a metagenomic mining approach, which led to the identification of a novel rhodopsin clade unique to cyanobacteria: cyanorhodopsin (CyR) and cyanorhodopsin-II (CyR-II). Heterologous expression analysis in Escherichia coli revealed that both CyRs and CyR-IIs function as light-driven outward H+ pumps. The CyR-IIs are further divided into two subclades based on their specific absorption wavelength, which are attributed to light changes in the side chain structure near the retinal chromophore. The evolutionary trajectory of cyanobacterial rhodopsins suggests that both their function and light-absorbing range have been adapted to diverse habitats with variable light and environmental conditions. Collectively, these findings illuminate the significance of rhodopsins in the evolution and environmental adaptation of cyanobacteria.

12

NAD dependency in freshwater 'Ca. Nanopelagicaceae' strains revealed by genomic and culture-based analyses

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Nicotinamide adenine dinucleotide is a universally conserved redox cofactor, and its external acquisition is considered uncommon among free-living bacterial taxa. In this study, two freshwater strains belonging to the 'Candidatus Nanopelagicaceae', an abundant group of free-living freshwater bacteria, were investigated to assess their physiological reliance on NAD. Phylogenetic analysis assigned the two strains, IMCC27789 and IMCC27656, to the 'Ca. Planktophila' (acI-A6 and A7 tribes). Genomic analysis indicated that both strains are deficient in key genes required for the complete de novo biosynthetic pathway. This inference was supported by culture-based experiments, which demonstrated that growth occurred only when NAD was directly supplemented, whereas no growth was observed in the presence of niacin, a known precursor for salvage pathway synthesis. These results suggest that the salvage pathway is either incomplete or non-functional in the examined strains. The persistence of such auxotrophic organisms in natural environments may depend on ecological interactions with cofactor-producing microorganisms. Supplementation of culture media with both NAD and niacin may facilitate the isolation and cultivation of a broader range of free-living freshwater bacteria. The findings highlight the importance of combining genomic information with experimental validation to elucidate metabolic dependencies in previously uncultured freshwater bacterial lineages.

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Microbial methylmercury formation in coastal waters affected by submarine groundwater discharge

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Coastal aquifers are sensitive land-ocean transitional zones receiving nutrient-rich water due mainly to fertilizing practices that may increase microbial activity and reduce oxygen availability. Groundwaters reaching coastal waters (ocean/lagoons) through submarine groundwater discharge (SGD) harbor diverse microbial communities that may play a crucial role in biogeochemical cycles including metal transformations such as mercury (Hg). Yet, the factors determining the microbial Hg transformations in coastal waters affected by SGD has been overlooked. Hence, this work aims at defining the role of nutrient-enriched SGD in microbial Hg cycling from the Mar Menor lagoon (Spain), the largest in the western Mediterranean. Potential Hg methylators (i.e., identified through 16S rRNA gene metabarcoding) were diverse, more abundant in the porewaters from the aquifer and mainly composed by Geobacter (average 18% potential Hg methylators), Bacteroides (13.5%) and Nitrospira (11.2%). Using enriched Hg stable isotopes, microbial Hg methylation was detected in brackish and saline porewaters from the lagoon shoreline, where iron-reducing Geobacter dominated the Hg methylator community. Metagenomic analyses are expected to provide deeper information on phylogeny of the Hg methylation genetic markers hgcA/B genes and the associated main microbial metabolisms. These novel findings are key to understand further Hg bioaccumulation in coastal trophic webs.

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Spatial heterogeneity of dark carbon fixation by prokaryotes and its implications for the biological carbon pump in the mesopelagic zone

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Dark Carbon Fixation (DCF), the conversion of inorganic carbon into biomass using chemical energy, remains poorly understood despite its growing recognition as a key process in supplying organic carbon to the mesopelagic zone and addressing the deep ocean's carbon imbalance. Through a multidisciplinary approach across meso- to sub-mesoscale hydrodynamic features (eddies and fronts), and leveraging data collected during an extensive field study in the North Atlantic Ocean (the APERO cruise from June–July 2023), this study fills key knowledge gaps about organic carbon cycling. For the first time, we quantify the respective contributions of DCF and prokaryotic heterotrophic production (PHP) to the mesopelagic carbon budget, analyzing both free-living and sinking particle-attached prokaryotic communities. Using in situ DCF and PHP measurements, bulk gene-quantification, and nanoSIMS at single-cell resolution, our results revealed strong mesoscale-driven variability of prokaryotic activities over a relatively small area (450 km²). Heterotrophs contributed more to DCF on particles than in the free-living fraction, and conversely for chemoautotrophs. Remarkably, DCF from free-living prokaryotes can supply up to 50% of the total organic carbon available in the mesopelagic zone. These results will be complemented by preliminary metagenomic analyses that promise to deepen these insights, opening new perspectives on mesopelagic carbon pathways.

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Biofilms on plastic, metabolically fantastic: functional dynamics of the microbial plastisphere

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Plastic pollution in marine ecosystems has become an environmental issue of global concern. Plastic items ending up in the ocean serve as a new habitat, named “plastisphere”, harbouring complex microbial communities. While much of the plastisphere-related research has focused on the environmental drivers shaping its structure, its biogeochemical role has been generally overlooked. To assess the microbial plastisphere’s metabolic potential, 1×0.5cm plastic pieces made of five polymers (HDPE, LDPE, PET, PP, PS) were incubated under in situ conditions in two Mediterranean sites (Gulf of Trieste and Gulf of Naples), in winter and summer. The 60-day-long experiments were sampled at four time points to investigate prokaryotic biofilm formation and its metabolic potential through confocal imagery and omic techniques, employing specific stains to detect metabolic activity. Biofilm-specific exoenzymatic rates were assessed through fluorogenic substrate assays. Biofilm developing on HDPE, LDPE and PP showed faster exoenzymatic rates and higher prokaryotic abundances than PET and PS. Generally, glycolytic activity peaked after 15-30 days, whereas protein degradation increased only at the last time points. After long-term observations, biofilm forming on 1cm2 of plastic displayed organic matter degradation rates comparable to those measured in 1L of seawater, highlighting the non-negligible biogeochemical role of the microbial plastisphere.

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Multifunctionally diverse alkaline phosphatases of Alteromonas drive the phosphorus cycle in the ocean

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Phosphorus is a critically limiting nutrient in marine ecosystems, with alkaline phosphatases (APases) playing a vital role in liberating phosphate from organic compounds. However, the dominant taxa and APase families driving the marine phosphorus cycle, particularly in the deep ocean, remain poorly understood. Equally enigmatic remains the (multi)functional diversity and mechanisms of action of different

APases. To address these gaps, we combined global multi-omic analyses, biochemical studies of purified recombinant proteins, and laboratory experiments with proteomics and enzymatic rate measurements. Multi-omics consistently identified Alteromonas as a primary contributor to APase expression and production, with PhoA as the dominant APase family, particularly in the deep ocean. Furthermore, all four major APase families (PhoA, PhoD, PhoX, PafA) exhibited multifunctionality, revealing distinct substrate preferences and regulatory mechanisms. Ultimately, this study expands the mechanistic understanding of the marine phosphorus cycle, while revealing the significance of enzyme multifunctionality in elemental cycles.

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How the sulfated algal polysaccharide fucoidan alters microbial activities in marine ecosystems

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Annually fixing ~50 gigatons of inorganic carbon via photosynthesis, algae invest up to 80% of their organic carbon into glycans. Some of these glycans are highly complex and accumulate in the ocean because they are difficult to break down. Fucoidan is such a complex highly sulfated glycan that is produced and released by marine algae, particularly brown algae and diatoms. The global abundance of these producers and the recalcitrance of fucoidan make it a frequent compound that marine microbes are subjected to. In this study, we investigate how the omnipresence of this compound affects microbial life in the ocean. As a proxy of activity, we measure respiration rates and cell densities of microbial communities in response to different fucoidan concentrations. We first performed these measurements using E. coli monocultures, followed by a coastal microbial community, and finally with an open ocean microbial community. Across all experiments, we observe a consistent pattern: The bulk respiration rate increases significantly along with the cell numbers, whereas the respiration rates per cell are significantly reduced in microbes subjected to fucoidan. These results highlight a neglected influence of complex polysaccharides like fucoidan on microbial communities, which may also impact biogeochemical cycles on a larger scale.

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Seasonal dynamics and potential interactions of free-living bacterioplankton during phytoplankton blooms at Helgoland Roads

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Marine bacterioplankton play a crucial role in global carbon cycling by decomposing microalgal biomass generated during phytoplankton blooms. This study aims to investigate the seasonal dynamics and ecological interactions of bacterioplankton at Helgoland Roads, over a full year (2022–23). Metagenome and metatranscriptome analyses were performed on 44 and 36 samples, respectively. Preliminary analysis generated 855 representative non-redundant metagenome-assembled genomes. Analysis of abundant and highly expressed metagenome-assembled genomes revealed dynamic shifts in community composition, gene expression, and activity patterns that closely correlated with chlorophyll a concentrations. These patterns suggest distinct seasonal responses among bacterial clades (temporal niche partitioning). i.e., adaptations to changing conditions and ecological roles associated with spring, summer, and autumn phytoplankton blooms. Key active groups included members of Bacteroidia (e.g., Aurantivirga), Alphaproteobacteria (e.g., *Pelagibacter*) and *Gammaproteobacteria* (e.g., SAR92). We further hypothesize that increased bacterial cell densities occurring during summer blooms enhance both cooperative and competitive interactions among bacteria and with algae. These interactions, likely mediated by secretion systems and biosynthetic gene clusters, may lead to higher bacterial mortality rates, potentially influencing the balance between organic matter remineralization and carbon sequestration. Our findings contribute to a deeper understanding of microbial functionality and ecological relationships in dynamic coastal marine ecosystems.

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Dissolved organic matter and prokaryote interplay across gradients in the Nares strait: first results of the Refuge-Arctic expedition

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The Arctic Ocean, a key component of Earth's climate, is warming at unprecedented rates. Over the last years, sea ice extent has declined by 10-15% per decade, and multiyear ice has decreased by 70%. The consequences of these changes on ecosystems and biogeochemical cycles remain uncertain, especially in the "Lasting Ice Area", located north of Canada and Greenland, that hosts unique endemic sea ice-dependent ecosystems. The Refuge-Arctic expedition visited this region from August to October 2024 onboard the Canadian icebreaker 'Amundsen', during which we aimed to characterize microbes (prokaryotic production and diversity), nutrients, and dissolved organic matter (DOM as organic carbon, fluorescent DOM and amino acids) across latitudinal (North to South Nares strait and down to the Baffin Bay), vertical (surface to near-bottom waters), glacier-to-ocean, and ice-to-sea gradients. Our overarching goal is to unravel the role of the different DOM sources and temperature as drivers of prokaryotic communities and their role on carbon cycling and export in this vulnerable and unexplored area. First results show increasing gradients in prokaryotic heterotrophic production (3H-leu incubations) from North to South and a positive correlation to labile DOM. These differences in production and substrate quality is reflected by changes in community diversity and composition.

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Shaping deep Atlantic carbon and nitrogen flux: The role of particle-associated microbial processes

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Marine snow aggregates are pivotal to the biological carbon pump, mediating the vertical transport of particulate organic carbon and influencing oceanic carbon sequestration. Microbial transformation of these aggregates modulates the balance between carbon export and remineralization. We studied the functional and phylogenetic composition of particle-associated and free-living microbes in the North Atlantic using integrated 'omics'. Size-fractionated samples (0.2 µm, 1 µm, 3 µm) were collected from 14 stations (475-3800m) using Niskin flasks. Genes for carbon fixation (RuBisCO, PEPcase) and degradation (Enoyl-CoA Reductase, Thiolase) were widespread, while nitrogen metabolism genes indicated organic nitrogen uptake (ABC_tran) and degradation (Pro_CA, Aldedh). Methane-related genes (Fer2,

metalloenzyme) were enriched in particles, suggesting niches supporting methanogenesis and methane oxidation. Taxonomic profiling of 21 metagenomes revealed microbial communities differing with depth and size fraction. Large aggregates (>3µm) were enriched in Myxococcota, Bacteroidota, Verrucomicrobiota, Nitrosphaerota, and Chloroflexota. Functional partitioning across size classes highlights the role of microbes in carbon sequestration and greenhouse gas regulation. Undestanding these processes is crucial for predicting microbial responses to changing ocean conditions and their impact on global carbon and nitrogen budgets.

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Magnetotactic Bacteria in Baltic Sea redoxclines: Distribution Patterns and Ecological Significance

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Magnetotactic bacteria (MTB) are a polyphyletic group of microorganisms that align and navigate along geomagnetic field lines. Previous studies indicate that MTB contribute significantly to nutrient cycling in chemically stratified aquatic systems. For instance, large Magnetococcus species, approximately 5 µm in diameter, have been identified as key contributors to phosphorus cycling due to their ability to accumulate polyphosphate. To explore MTB diversity and ecological roles in the Baltic Sea, high-resolution sampling at meter scale was conducted during the R/V Meteor cruise M200 at the redoxclines of the Gotland, Fårö, and Landsort basins. Water column profiles were obtained using CTD and pump-CTD systems, and samples were collected for full-length 16S rRNA gene sequencing to characterize bacterial communities and assess MTB distribution and abundance. In total, 152 unique sequences affiliated with known MTB taxa were recovered, including two potentially novel bacterial families and twelve candidate genera. Sequences related to Magnetococcus were detected across multiple depths, suggesting their widespread presence. Furthermore, scanning electron microscopy coupled with energy-dispersive X-ray spectroscopy (SEM-EDX) analysis of Baltic Sea samples confirmed magnetotactic cocci containing polyphosphate inclusions. This study broadens our understanding of MTB diversity and distribution in Baltic Sea redoxclines and provides insight into their ecological relevance.

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Ecological drivers of particle degradation in waters of varying levels of productivity

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Marine particles play a fundamental role in ocean biogeochemical dynamics, influencing the magnitude and efficiency of the biological carbon pump (BCP). During their sinking, they undergo microbial colonization and gradual changes in particle composition. As the microbiome develops, it actively degrades particulate organic matter (POM), driving its transformation and the release of dissolved organic matter (DOM). The extent of remineralization depends on particle composition, the associated microbiota, and environmental conditions such as nutrient availability, among other factors. In this study, we investigate the ecological drivers of particle degradation by incubating particles (collected at 200 m) with free-living microbial communities from either mesopelagic (200 m) or bathypelagic (1000 m) waters in two contrasting regions of the Atlantic Ocean: the oligotrophic Subtropical North Atlantic and productive waters from the equatorial and the South Atlantic upwelling systems. We aim to determine whether particle solubilization is shaped more by particle origin, microbial context, or regional biogeochemical conditions. Our approach combines Bioorthogonal Non Canonical Amino acid Tagging (BONCAT) to identify metabolically active cells involved in POM degradation, with the optical characterization of DOM. This will shed light into the microbial mediation of carbon fate and the production of labile and recalcitrant DOM from sinking particles.

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Mechanistic modeling of nitrogen fixation costs and benefits in Nostocales: from laboratory calibration to ecosystem application

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Nitrogen-fixing cyanobacteria of the order Nostocales, such as Dolichospermum and Aphanizomenon, can thrive in nitrogen-limited lakes through heterocyst-mediated N_2 fixation. However, the ecological cost-benefit balance of this trait across nitrogen gradients remains unresolved. We calibrated a dynamic, mechanistic agent-based model (ABM), originally developed for Dolichospermum*, using laboratory data for Aphanizomenon to investigate interspecific differences in nitrogen fixation strategies. The model was calibrated against observations, including growth rate, cell concentrations, nitrogen and phosphorus uptake rate, heterocyst fraction, nitrogen fixation rate and nitrogenase transcripts. The model reproduces species-specific

responses to nitrogen limitation and light conditions. Preliminary results show that Aphanizomenon exhibits distinct adaptive responses under variable light and nutrient regimes, consistent with experimental observations. The calibrated ABM will be integrated into a coupled biogeochemical model of Lake Müggelsee (Berlin, Germany) to examine the ecological implications of diazotrophy under decreasing nutrient levels and variability observed in this system. This integrative approach aims to expand the knowledge of cyanobacterial ecology and support nutrient management strategies in eutrophic lakes. *Hellweger, Ferdi L., et al. "Dynamic, mechanistic, molecular-level modelling of cyanobacteria: Anabaena and nitrogen interaction." Environmental Microbiology 18.8 (2016): 2721-2731.

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Combined effect of emerging concern contaminants and solar radiation on natural marine microorganisms

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Contaminants of emerging concern (CECs) are chemicals that are not currently regulated, however they are increasingly detected in aquatic ecosystems and reports on their potential toxicity to marine biota are accumulating. CECs include pharmaceuticals and personal care products, such as UV filters contained in solar sunscreens, industrial and agricultural chemicals (e.g. pesticides), or microfibers. However, our knowledge on the response of natural microbial communities to the combined effect of CECs and UV radiation exposure remains limited. Natural microbial and prokaryotic communities were amended with organic and inorganic UV filters and incubated in the dark or under natural solar radiation in late spring/summer. Organic and inorganic UV filters caused increased microbial respiration, phosphatase and aminopeptidase activity rates compared to unamended communities, whereas organic UV filters boosted prokaryotic communities' respiration. Exposure to natural solar radiation inhibited respiration and enzymatic activity rates in all treatments. Taken together, our results indicate that UV filters effect on the metabolism of natural microbial communities is modulated by environmental factors, such as UV exposure, and emphasize the need to consider multiple-stressor scenarios to better forecast ecosystem responses to disturbances.

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Life under pressure in prokaryotes and the impact on the biological carbon pump

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The deep ocean, one of Earth's largest and least explored biomes, spans depths with extreme conditions, including high hydrostatic pressure, low temperatures, and minimal organic carbon. These characteristics significantly influence microbial life, requiring unique adaptations for survival and metabolic function. Prokaryotes thriving in high-pressure environments are known as piezophiles (Tamburini et al. 2013 and references therein). Those deep-sea micro-organisms adapt to high-pressure environments through cellular and genetic changes, constituting ‘bathytypes’ specific to ocean depths (Oger and Jebbar 2010; Peoples and Bartlett 2017). Due to technical challenges in sampling under in situ conditions, much of the data about deep-sea microbes has historically been gathered under decompressed conditions. This decompression can result in significant changes in community composition (La Cono et al. 2009; Edgcomb et al. 2016; Garel et al. 2019) and activity (Tamburini et al. 2013; Garel et al. 2019), which generally leads to underestimated assessments of their ecological roles. Innovations, such as pressure-retaining samplers (Peoples and Bartlett 2017; Garel et al. 2019) or in situ microbial incubator (Amano et al. 2022a), now allow for the collection or incubation of samples at in situ pressures, preserving the natural state of these microorganisms for more accurate study. Deep-sea microbes play a critical role in the global carbon cycle, particularly through their participation in the biological carbon pump, which involves the decomposition and mineralization of organic material as it sinks through the water column (Arıstegui et al. 2009). Research suggests that deep-sea microbes are dynamically responsive to organic matter input, with a substantial portion of global heterotrophic production occurring below the photic zone. According to Aristegui et al. (2009) the integrated prokaryotic heterotrophic production (PHP) in the dark ocean represents around 50% of the total water column. Understanding the pressure effect on prokaryotic activities is crucial to better estimate their role in the remineralization in the dark ocean. However, to date, the effect of decompression is controversial, depending on the methodology used and the authors (Amano et al. 2022b versus Tamburini et al. 2013 and reference therein). Continued advancements in sampling technology and high-throughput sequencing will likely reveal more about these resilient organisms' ecological roles and physiological capacities, enhancing our comprehension of the deep ocean's contributions to Earth's biogeochemical processes. Over the last few years, pressure-retaining samplers were deployed during different oceanographic cruises in North Atlantic Ocean and in the Mediterranean Sea, allowing the constitution of a unique dataset of PHP rates (using 3H-Leucine) and MICROFISH, under in situ conditions. Among this cruise, a specific one dedicated to the study of deep convection and re-stratification phenomena brings new insights in the biological and physical coupling of this study area, and the necessity to well understand this context when talking about organisms adapted to pressure or not. Pressure effects on other activities (dark dissolved inorganic carbon fixation, ectoenzymatic activities, and high molecular organic compounds' degradation) will also be presented. Finally, we will also show how surface prokaryotes attached to gravitationally sinking particles cope with the increase in pressure with depth.

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Microbial succession dynamics of rhodopsin phototrophs in a productive coastal system

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Almost all members of the marine microbial plankton have evolved mechanisms to use light as an energy source. Besides photosynthesis, proteorhodopsins (PR) are transmembrane proteins that capture light to generate biochemical energy, supporting the growth and survival of a wide range of marine microbes. Retinal quantification, used as a proxy for this photosystem, has shown that in oligotrophic environments, the highest PR concentrations occur where chlorophyll-a and inorganic nutrients are low. However, a recent study in the productive region of the Southern California Basin found that PR levels peaked alongside high chlorophyll concentrations during the spring algal bloom. Furthermore, those distributions could be attributed to bacterioplankton abundance. To understand these dynamics, we investigated the ecological relationships among nutrient availability, phytoplankton growth, PR-containing microorganisms, and microbial community structure during bloom development. A 24-day mesocosm incubation experiment was conducted using seawater from the Southern California Basin enriched with inorganic nutrients to induce phytoplankton growth. Chlorophyll levels peaked after day 8 of incubation, followed by a dramatic increase in PR and bacterial abundance. Flavobacteriales dominated the post-bloom bacterioplankton community, suggesting that these known photoheterotrophs optimize the uptake and utilization of freshly-produced organic matter using proteorhodopsin light-harvesting.

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Glycan degradation of free-living and particle-attached bacteria during a spring phytoplankton bloom

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Marine phytoplankton account for about 50% of the global photosynthetic carbon fixation, most prominently during massive phytoplankton blooms. These blooms release substantial amounts of polysaccharides, a significant portion of which undergo rapid remineralization by heterotrophic bacteria. We investigated the diversities, activities, and functional potentials of polysaccharide-degrading bacteria across different size fractions during a diverse spring phytoplankton bloom at Helgoland Roads (southern North Sea) at high temporal resolution with microscopic, physicochemical, biodiversity, metagenome, and metaproteome approaches. Particle-attached bacterial communities exhibited a notably greater diversity compared to free-living bacterial communities. Polaribacter, the NS9 and NS5 marine groups, Algibacter, Cd. Prosiliicoccus and Tenacibaculum dominated Flavobacteriia in particles (3-10 µm and >10 µm). A total of 305 species-level metagenome-assembled genomes were obtained, with 43 belonging to Flavobacteriaceae. Particle-attached metagenome-assembled genomes, on average, featured larger metagenome-assembled genomes with higher proportions of polysaccharide utilization loci. These loci were predicted to target a broad spectrum of polysaccharide substrates, from readily soluble, simple-structured storage polysaccharides (e.g., laminarin, α-glucans) to less soluble, complex structural or secreted polysaccharides (e.g., xylans, cellulose, pectins). Our findings suggest that numerous active and abundant flavobacterial clades played a pivotal gatekeeping role in the solubilization and subsequent degradation of various essential classes of algal glycans.

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Oceanic eddies impact the functioning and biogeochemical role of marine microbes

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Mesoscale eddies are widespread oceanographic phenomena that trap, transport and mix water masses for a determinate period of time. Due to their unique characteristics, they have great potential to affect the marine microbial community composition, and therefore, the biogeochemical processes they catalyse. Although the perturbations of microbial community inside of eddies have been previously reported, we are still in the dark as for how these changes affect their metabolic capabilities and thus their biogeochemical role. Here, we combine omics analysis (metagenome and metatranscriptome) with physicochemical parameters and metabolic rates derived from various (cyclonic and anticyclonic) eddies sampled in the Canary Eddy Corridor in the summer of 2022 in order to answer this question. We found significant differences in metabolic rates, carbon fluxes and omic-based metabolic capability between eddy categories. These results reveal the pivotal role of eddies—and biophysical coupling processes more broadly—in shaping marine biogeochemical dynamics, while also providing a mechanistic understanding of these phenomena.

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Pyrenean lakes: microbial ecological insight through long-read sequencing

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The PyriSentinel team investigates microbial biodiversity across ~300 high-altitude lakes in the Pyrenees. These lakes are ecologically unique, shaped by geographic isolation, nutrient scarcity, high UV radiation, and pronounced climatic variability. To explore the microbial ecology of these environments, we conducted a comparative study of two long-read sequencing platforms: Oxford Nanopore Technologies (ONT) and Pacific Biosciences (PacBio). Focusing on the recovery of full-length marker genes, we assessed each platform's ability to resolve taxonomic diversity. This comparison highlights the complementary strengths of both technologies and informs future strategies for microbial monitoring in remote environments. Ultimately, our results contribute to understanding microbial diversity in high-mountain freshwater ecosystems and underscore the role of long-read sequencing in tracking ecological change in sentinel environments.

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Large-scale data reuse enables global distribution and predictive modeling of Vibrio vulnificus

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The amount of publicly available next generation sequencing data has reached Petabyte-scale dimensions providing a vast, but so far only marginally exploited resource for data mining and reuse. Here, we mined the European Nucleotide Archive, screening ~70000 coastal amplicon sequencing data sets, to model the global distribution of Vibrio vulnificus, a potentially pathogenic bacterium that poses a growing public health threat worldwide due to climate change. Together with publicly available satellite data, two random forest models were trained to predict relative Vibrio vulnificus abundances using either remotely sensed environmental variables or the prokaryotic community. These models indicated that, besides temperature and salinity, both chlorophyll-a and surface currents, as well as non-vulnificus Vibrio spp. and Pseudoaltermonas spp. likely played a role as environmental and bacterial predictors of Vibrio vulnificus, respectively. Collectively, our results emphasize the role of decaying phytoplankton blooms in stimulating relative Vibrio vulnificus abundances. These factors should therefore be integrated into predictive modelling to estimate future risks, considering that climate change-induced modifications of phytoplankton dynamics and currents may favor a poleward expansion of Vibrio vulnificus. We further show-case the added value for science and society that may accrue from the large-scale reuse of public data resources.

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Better together – A new filtration device to support a holistic monitoring strategy in the German North and Baltic Sea

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The North and Baltic Seas are heavily impacted by human activities, threatening both biodiversity and economic value. As part of the CREATE I and II projects (Concepts for Reducing the Effects of Anthropogenic Pressures and Uses on Marine Ecosystems and Biodiversity), we integrated DNA-based microbial monitoring into long-term observation strategies within Germany's exclusive economic zone over the past three years. A key advancement was the development of a new filtration system, which standardized and simplified sampling, minimized contamination risk, saved time, and enabled even non-experts to collect samples. In partnership with research institutions and universities (e.g., AWI, IOW, Geomar, HIFMB, ICBM, University of Greifswald), we have collected over 3,000 microbial samples, forming a growing microbial bio-archive. This centralized repository preserves microbial diversity data and supports the

detection of large-scale spatial and temporal patterns. It also enables the identification of microbial indicators that can serve as early warning signs of environmental stress. Integrating this bio-archive into existing monitoring enhances the value of current efforts, offers a more comprehensive ecosystem perspective, and provides a solid foundation for future marine research and informed environmental management.

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Metagenomic Insights into Microbial Diversity and Metabolism in Hypersaline Sinkholes of the Dead Sea

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The continuous desiccation of the Dead Sea has led to the formation of thousands of sinkholes along its shoreline. The physical characteristics of sinkholes vary significantly, creating dynamic and unique aquatic habitats. To investigate these sinkholes, we conducted seasonal sampling of 11 sinkholes from 2021 to 2023, combining chemical and metagenomic analyses. We recovered 888 metagenome-assembled genomes spanning 22 bacterial and 9 archaeal phyla, and identified 2620 known and 285 novel species through community composition analysis. Total dissolved salts, pH, and $\delta^{18}O$ were significantly correlated with microbial community composition ($p < 0.05$, mantel test), with pool identity identified as the primary driver of microbial variation ($R^2 = 0.49$, PERMANOVA). The sodium is positively correlated with both Shannon and Simpson indices ($FDR < 0.05$) but not with richness, suggesting it influences microbial community evenness rather than species count. In addition, metabolic analysis of the species exceeding 10% relative abundance reveals the prevalence of aerobic and chemolithotrophic metabolisms, suggesting that energy acquisition from inorganic compounds is a key strategy in these hypersaline sinkholes. Our findings expand the current understanding of microbial life in extreme environments, and provide a comprehensive view of how environmental variability structures microbial ecosystems in hypersaline sinkholes.

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FISHing for marine protists in the era of big data

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High-throughput environmental DNA sequencing has revealed a vast uncharted microbial diversity. Most of such diversity has withstood culturing thus far and can only be studied through culture-independent approaches. With thousands to millions of DNA sequences, new experimental possibilities are opening for targeted environmental lineages. However, designing taxon-specific oligonucleotides remains a bottleneck in molecular studies relying on primers for Polymerase Chain Reaction (PCR) or probes for Fluorescence in situ Hybridization (FISH). No software currently exists to design specific oligonucleotides using only a custom set of target sequences, and studying the biology, or even the morphology of many microbial lineages remains challenging. We present oligoN-design, a simple, versatile Python tool to ease the design of specific oligonucleotides. OligoN-design is suited for high-throughput sequencing data and freely available from Bioconda. It requires only two fasta files as input (containing target and non-target taxa respectively), and does not rely on phylogenetic trees nor alignments. Designed taxa-specific probes can then be applied in FISH alongside cytological fluorescent markers to study the 3D morphology and life cycles of specific protist lineages. Altogether, this approach links available environmental sequences to their organismal morphology and biology.

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FunAqua: a global DNA-based database of aquatic fungal biodiversity in water and sediments

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Aquatic fungi contribute to the functioning of aquatic ecosystems as decomposers, parasites and pathogens. Despite their importance, they remain under-researched compared to other aquatic microorganisms. To address this, the University of Tartu and the Estonian University of Life Sciences launched the collaborative FunAqua project, aiming to describe factors shaping aquatic fungal biodiversity. The project's first objective is to build a global fungal metabarcoding database to support biodiversity studies of aquatic fungi. We present the dataset derived from 1,080 sediment and 1,299 filtered water samples collected between 2018 and 2024 by voluntary contributors. The sampling points were spread across 87 different countries, and covered 10 aquatic biome types. DNA from the sediment and filtered water samples was extracted, followed by PCR amplification of the internal transcribed spacers region of the rRNA operon. Sequencing was performed at Oslo University using the PacBio Sequel platform. Overall, 137,887 high-quality eukaryotic OTUs were produced, from which 50,175 (36% of OTUs) were fungal. From sediment (n = 1060) and water filter (n = 1212) samples, 34,170 and 26,296 unique fungal OTUs were produced, respectively. This dataset will be openly published to support future research into aquatic fungal diversity.

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Facilitating molecular detection of zoosporic parasites of aquatic primary producers with ParAquaSeq

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[13]*University of Zagreb*
[14]*Université Savoie Mont Blanc, INRAE*
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Zoosporic parasites are highly diverse and significantly impact host populations in natural ecosystems and industrial-scale algae production. Despite a surge in metabarcoding amplicon sequencing and a near-exponential increase in rRNA and ITS sequences for fungal and fungal-like organisms, the annotation of functional traits derived from molecular sequences remains limited. This limitation hampers detection and ecological interpretation of these parasites in molecular datasets. We present PARasite AQUAtic SEQuences (ParAquaSeq), a FAIR database generated within the ParAqua COST Action. ParAquaSeq compiles ribosomal sequences from confirmed or putative zoosporic parasites of aquatic primary producers (microalgae, cyanobacteria, macroalgae, and aquatic vascular plants) by querying public repositories and manually curating over 12,000 sequences. Each entry includes metadata, such as host group, culture availability, location of isolation, and habitat. ParAquaSeq includes 1131 curated sequences from 13 taxonomic groups, with Chytridiomycota and Syndiniophyceae most represented. Dinoflagellates and diatoms are the most common host (each 25%), followed by aquatic plants (15%) and macroalgae (8%). Sequences span marine (46%), freshwater (41%), and industrial (2%) environments. ParAquaSeq, available at https://github.com/ ParAqua-COST/ParAquaSeq_Repository, fills a critical gap supporting research and industrial applications. It also highlights the lack of knowledge in underrepresented taxa and environments, underscoring the need for expanded sampling and research.

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Comparing methods of phytoplankton quantification

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While standard light microscopy, imaging and flow cytometry are commonly used techniques in marine microbial studies, comparisons between these methodologies remain limited. The pulse-shape recording flow cytometry provides distinction of functional groups whereas imaging flow cytometry combines the high throughput of flow cytometry with the imaging capabilities of microscopy, offering a significant potential to enhance the monitoring of marine microbial populations. The main aim of this study is to assess outputs of different phytoplankton quantification methods: the pulse-shape recording CytoSense (CytoBuoy), the Imaging FlowCytobot (IFCB, McLane Labs) and light microscopy. This study utilizes marine samples of microbial autotrophic prokaryotes and eukaryotes collected at the Utö Atmospheric and Marine Research Station located in the Baltic Sea. To explore the power of single-cell based techniques, we compare biovolumes estimated by different methods for cultured organisms analysed with CytoSense and IFCB. Preliminary results on the abundance and size distribution of natural phytoplankton communities yielded by flow cytometry, imaging and light microscopy highlights the overall comparability of cell abundances by different methods for organisms ~5–80µm and demonstrates the importance of combining different methods for observing phytoplankton community structure. Small cells (<5µm) were only recorded by the CytoSense, which provided an ataxonomic classification of the community.

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Influence of temperature on cyanobacteria-cyanophage interactions

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Temperature plays a crucial role in shaping virus-host interactions in the oceans. Elucidating how temperature impacts their dynamics is of prime importance. The relationships between Synechococcus, a major primary producer, and its cyanophages have been studied for years, but their responses to temperature have not been investigated. Here we report on the interplay between cyanobacteria and their cyanophages as a result of changes in this environmental variable. We observed distinct changes in infection patterns, with several Synechococcus-phage systems displaying shifts in resistance/sensitivity when grown at different temperatures. Infection dynamics showed that the viral cycle of a cyanophage was impaired at low temperature, where phage particles could adsorb to the cell surface but viral production was prevented. Conversely, the cyanophage exhibited a full lytic cycle and lysis occurred at higher temperature. We also assessed the impact of temperature on viral life traits of a generalist cyanophage on two Synechococcus hosts. Low temperatures

lengthened lytic cycle on both hosts, while burst size and virulence were affected by temperature in only one of the hosts, highlighting that temperature-driven changes are interaction-dependent. Our findings suggest that temperature can considerably affect the outcome of cyanobacteria mortality, and therefore have implications for biogeochemistry in the environment.

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First genomic insights into phages infecting the freshwater genus Zwartia (GKS98; Gammaproteobacteria)

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Prokaryotic viruses (phages) are key regulators of microbial community structure and nutrient cycling in aquatic ecosystems. However, phage–host interactions in freshwater environments remain poorly characterized, largely due to the lack of cultured representatives for abundant bacterial groups. While recent cultivation-independent methods (e.g., CRISPR-spacer linking) enable host prediction for ~20% of viral sequences, host-based phage isolation remains the most reliable approach for elucidating infection dynamics and host specificity. Here we used a newly cultured representative of the abundant freshwater bacterial genus Zwartia (a.k.a. GKS98 or BetIII, Burkholderiales, Gammaproteobacteria) from the Římov Reservoir (Czech Republic) to isolate its phages. Phylogenetic analysis and average nucleotide identity (ANI) of six obtained lytic phages clustered them into two groups with genome sizes of 30 and 39 kb, showing structural synteny within and evidence of group rearrangements between these clades. Genome annotations identified putative auxiliary metabolic genes (AMGs), including N-acetyltransferases and carboxypeptidases, potentially modulating host metabolism. Long-term temporal dynamics patterns in the reservoir will be shown based on metagenome fragment recruitment. This study presents the first isolated phages infecting the globally abundant genus Zwartia, offering insights into this underexplored lineage and building our knowledge of freshwater viral diversity and phage–host ecology.

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Exploratory hunt for viruses uncovers diverse phages in alpine streams: The Alpine Lotic Phage (ALP) collection

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Microbial biofilms colonizing streambeds critically drive ecological and biogeochemical processes in streams globally. While their significance is increasingly recognized, the role of bacteriophages (phages) – viruses that infect bacteria – remains underexplored. Here, we introduce the Alpine Lotic Phage (ALP) collection; a pioneering initiative to isolate and characterize phages native to alpine streams. We collected over 120 litres of water from a Swiss mountain stream which were filtered, concentrated, and screened against 40 bacterial species isolated from stream biofilms. This effort yielded 60 double-purified phage isolates infecting 15 bacterial species. Soft-agar assays and electron microscopy revealed diverse plaque morphologies and tailed virus structures, while DNA sequencing uncovered a wide range of genome sizes (~27 to 360 kbp). Genomic annotation also indicated the presence of putative temperate phages and numerous uncharacterized genes. We next challenged biofilm-forming bacteria with ALP phages and report that while static biofilms were largely unaffected, phages strongly inhibited biofilm growth under flow conditions in microfluidic devices, suggesting a context-dependent impact that alpine phages have on biofilm development. Collectively, the ALP collection highlights an untapped reservoir of phages in alpine streams with efforts ongoing to further expand the viral collection and to explore phage–biofilm interactions in lotic ecosystems.

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Aquatic viruses inhibit different salinity niches in the temperate mesotidal estuary

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Viruses are a vital part of the aquatic food web and hold a profound role in carbon availability at different trophic levels. Despite rising interest in aquatic viruses in recent years, very few studies were conducted in the estuaries, where freshwater and marine communities meet along the salinity gradient. Here, we present a paired analysis of metagenomics and metatranscriptomics data focused on the viral fraction derived from 2 years of seasonal sampling in one of Europe's largest estuaries, the temperate mesotidal Elbe below Hamburg. Our results reveal a sharp separation between the viral communities following salinity niches. Moreover, we showed that salinity can be well predicted based on our retrieved viral community data. We provide a detailed overview of the viral spatiotemporal distribution including taxonomy, hosts, and auxiliary genes. We identified salinity, total dissolved phosphate and temperature as

the main drivers of estuarine viral communities, thereby the salinity effect seems to be independent of the spatial component. This is the first detailed molecular study of viruses in the Elbe estuary shedding light on viral communities and infections dynamic on the dynamic interface between freshwater and marine environments.

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An ensemble of phylogenetically related giant viruses implicated in cryptophyte bloom collapse.

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Cryptophytes are dominant and ubiquitous in freshwaters, forming substantial periodic phytoplankton blooms that are in part collapsed by giant virus induced mortality. However, actual viral or host proteins that initiate strong contact resulting in a successful infection remain largely unknown. We isolated 12 giant viruses infecting Rhodomonas, from such a spring bloom of the cryptophyte. Their genomes ranged from 500-600kbp and phylogenomic analysis placed them within the order Imitervirales. All isolates were very similar to each other but still formed two distinct groups of six genomes each, Budvirus-like and Kafkavirus-like, and all are detectable during a Rhodomonas spring bloom. Collectively, this ensemble of viruses plays a major role in Rhodomonas bloom collapse. Using comparative genomics and metagenomic fragment recruitment analyses, we identified highly variable metagenomic islands in these viral genomes, likely encoding genes involved in host recognition (e.g. highly variable adhesins). If, as is the case with bacteria and phages, such regions of high variability are critical for host-virus interactions, then it is entirely plausible that genes in these islands are potential host-receptors. Furthermore, it may be expected that similar highly diverse regions in protist genomes would be prime candidates for encoding receptors for giant viruses.

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Temporal dynamics and strain-level host interactions of phages infecting Gammaproteobacteria Rheinheimera spp. in the Baltic Sea

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Phages (viruses that infect bacteria) are a non-negligible component in aquatic environments since they are the most abundant entities and drive nutrient turnover through infection, host re-programming, and lysis. However, there is limited knowledge on how seasonal dynamics and genetic variations among phages influence ecosystems. In this study, abundance data based on plaque formation by phages infecting multiple strains of Rheinheimera spp. were collected bi-weekly from the central Baltic Sea, starting in autumn 2022. More than 100 phage genomes, collected during 2020-2023, were sequenced and the candidate phages' host range was investigated. Furthermore, the dynamics of phage and host abundance were investigated by mapping reads of metagenomic data sampled weekly to monthly since 2012. The results showed that the abundance of Rheinheimera spp. and their phages had re-occurring peaks in July-August, while being below detection limit during spring. This coincides with the yearly occurring cyanobacterial blooms in the Baltic Sea. Viral genome identity formed discrete species clusters within the Barbavirus genus, as well as resolving 2 novel genera. Phages originally isolated from the same host tended to fall into the same cluster and have similar host range. This study highlights strain-level interactions among ecologically important bacterial-phage systems.

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Diversity and distribution of novel isolated phages of the genus Limnohabitans (Burkholderiales)

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Phages (viruses that infect bacteria) are the most prevalent entities in the biosphere, however those infecting major freshwater heterotrophic bacterial groups are still largely unknown. We report on isolation of 30 novel bacteriophages infecting different lineages of the genus Limnohabitans (Gammaproteobacteria, Burkholderiales), one of the major bacterial group widely prevalent in freshwater habitats. Using six different inocula of bacteria-free (97%) infecting strain 2KL-27 isolated from different depths and seasons of the year. Parallel genome sequencing of phage-infected and uninfected strains uncovered not one, but more phage genomes as-well-as latent phages hidden in genomes of strains Rim8, Rim28, and Hippo4. We also screened the relative

abundance of Limnohabitans phage sequences in metagenomes of diverse freshwater lakes and streams and revealed the environmental prevalence of their populations.

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Viral biodiversity in glacier-fed streams

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Viruses are well known to modulate the biomass, diversity and activity of microbial communities in many ecosystems, yet, little remains known about their role in stream biofilms. Here, we leverage metagenomic data collected by the Vanishing Glacier Project from 170 glacier-fed streams around world to unravel the diversity and biogeography of benthic dsDNA viral communities. Despite the extreme environmental conditions in glacier-fed streams, we uncover a large and previously unknown viral diversity. The biogeography of both host and phage communities is structured by spatial isolation, reflected by conspicuous regionality. The GFS microbiome is characterized by low cell abundances and few but well-adapted bacterial clades. Phages leverage high host specificity, elevated lysogeny and encode auxiliary metabolic genes to interact with their hosts, pointing to the prevalence of mutualistic interactions rather than mere lysis of host cells in GFS. Taken together, our work highlights the co-evolutionary history of phages and their hosts in a cryospheric ecosystem, now threatened by climate change.

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Isolation and characterization of oomycetes and their viruses using long-read sequencing

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Oomycetes are ecologically important aquatic eukaryotes that influence biodiversity, food webs, and organic matter cycling, yet they remain understudied in freshwater systems. Giant viruses, which regulate host populations and impact nutrient dynamics, have been detected as integrated elements in oomycete genomes. However, linking giant viruses to specific hosts via metagenomics remains difficult, making culture-based methods the only reliable approach. During the April 2025 spring bloom in the Římov freshwater reservoir (Czechia), we isolated oomycetes from epilimnetic biomass retained on 5.0 µm-pore filters and detrital leaves on PmTG agar at 15 °C. Twelve isolates were obtained—including Phytopythium litorale, Pythium coloratum, Pythium dissotocum, and Phytophthora gonapodyides—and identified via ITS1/ITS4 Sanger

sequencing. Cultures were exposed to viral fractions (0.2–0.8 μm) collected from the same location and monitored for pozitive infections. Long-read sequencing was used to generate high-quality host and viral genome assemblies, and assess population-level variation. Endogenous viral elements were identified via hallmark genes and GC-content signatures. This integrated cultivation-genomics approach will advance our understanding of virus–oomycete interactions, their roles in carbon and nutrient cycling, and establish model systems for aquatic virus ecology.

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The Viral Side of Summer: Rethinking Bathing Water Microbiology

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The microbial quality of public beaches is typically assessed by counting faecal indicator bacteria (FIB) such as Escherichia coli and intestinal enterococci. However, these indicators do not reliably reflect pathogenic viruses such as norovirus, which is environmentally more stable and a significant waterborne burden worldwide. In this study, we evaluate Pepper Mild Mottle Virus (PMMoV) as a surrogate marker for noroviruses and compare its performance with FIB. In addition, we explored the presence of other potential pathogenic viruses using viral enrichment panel (Twist) and compared it with 16S rDNA bacterial diversity. Sixty-nine water samples were collected from public beaches in Uppsala in 2024. According to traditional FIB, most samples met acceptable microbial quality standards. However, Norovirus was detected in 16 samples. The mean PMMoV concentration was significantly higher in norovirus-positive samples than in norovirus-negatives. PMMoV values also showed a weak-moderate correlation with FIB. Viral enrichment panel sequencing revealed further potential pathogens, while 16S rDNA sequencing showed poor predictive power for viral pathogens. This study demonstrates that recreational waters considered safe by FIB can still contain human pathogenic viruses and emphasizes the potential of PMMoV as a more effective indicator of norovirus contamination.

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From sediment to bloom: spatial patterns of algal resting stages and harmful algal bloom (HAB) risk in the Baltic Sea

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Besides Cyanobacteria, harmful algal blooms (HABs) of Eukaryotes are becoming increasingly prevalent in the Baltic Sea, altering phytoplankton diversity and negatively impacting higher trophic levels. Many HAB species form resting stages which may seed future blooms. To assess the bloom potential of HABs in the Baltic Sea and identify risk areas, we analysed spatial distribution patterns of resting stages in the sediment. Using a modified DNA extraction protocol, sediment samples from the German Bight and the Gulf of Finland enabled selective 18S rRNA gene metabarcoding of resting stages. These data highlighted strong spatial differences between western and eastern Baltic Sea sediments, but also regional differentiation potentially due to freshwater inflow. German sediments showed higher Shannon and Simpson diversities, with a presence of up to 40 % green algae sequences. Finnish sites were dominated by diatom and dinoflagellate resting stages, while the latter showed the highest relative presence offshore. HAB species were more frequently sequenced at German stations, indicating invasion of toxic algae into the Baltic Sea. To explore dispersal patterns of algal cells, we use numerical particle tracking simulations that will help identify risk areas of HAB development and promote a better understanding of HAB dynamics in the Baltic Sea.

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Size does matter: tiny microbes slipping out 0.2 µm pores may alter standard biodiversity assessment

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In the last decades, advances in sequencing technologies have enabled a better understanding of prokaryotic dynamics in the Ocean by providing unprecedented details about their biodiversity. In amplicon sequencing studies of planktonic prokaryotes, while we are still far from a global consensus protocol regarding target regions (e.g. choice of primers) and approach (long vs. short reads), biomass collection using 0.2 µm pore membranes for DNA extraction is an undisputed standard procedure. In this study, we challenged this dogma by analyzing the 0.2 µm filtrate of samples collected monthly over two years at a coastal station in the northern Adriatic Sea. Surprisingly, about 2.5% of the total free-living community escaped the standard 0.2 µm pores. The biodiversity of small microbes was limited, with the most abundant

eight Amplicon Sequence Variants accounting for 75% of the total, on average. These were made up of well-known small bacteria such as SAR11 clade I and II and C. Actinomarina as well as by members of the mostly uncultured NS5 group of the Flavobacteriaceae and Marine Group II archaea. Overall, our results suggest caution in the use of 0.2 μm pore membranes for the study of these taxa and for rare biosphere-oriented investigations.

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Comamonadaceae and Flavobacterium as bioindicators of River Run-Off in European Coastal Waters

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River run-off influences marine coastal ecosystems by discharging nutrients, sediments, contaminants, and microorganisms into the sea. Understanding the consequences of this advection is crucial for ecosystem monitoring and management. Environmental DNA (eDNA) can contribute to better describing microbial communities, and therefore it was used here to trace freshwater-origin bacteria diluted in marine waters, in order to define bioindicators of river run-offs. As part of the EU-OBAMA-NEXT project, this study combined data from the English Channel, Bay of Biscay, and Mediterranean Sea, encompassing river-influenced (mixed) to marine areas. Bacterial communities were analysed by sequencing V4-V5 16S rDNA amplicons. Indicator Species Analysis (IndVal), Analysis of Compositions of Microbiomes with Bias Correction (ANCOM-BC), and Threshold Indicator Taxa ANalysis (TITAN) enabled the identification of key taxa. Comamonadaceae and Flavobacterium showed a strong statistical correlation with freshwater influence at all sites, beyond local ecosystem variability and geographical boundaries. These findings highlight the tolerance of these bacterial groups in tolerating salinity gradients and demonstrate the effectiveness of eDNA for high-resolution marine microbial ecology studies. The results support the development of a standardized European framework for monitoring freshwater inputs and improving the management and protection of coastal ecosystems.

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Niche partitioning of aerobic anoxygenic phototrophs across free-living and particle-associated fractions in the Alboran Sea, SW Mediterranean

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Aerobic anoxygenic phototrophic (AAP) bacteria contribute to ocean biogeochemistry by degrading organic matter and capturing light energy via bacteriochlorophyll a. However, differences in their genetic and ecological traits according to lifestyle remain underexplored. We investigated free-living (FL) and particle-associated (PA) AAP communities and their response to environmental variability in the northern Alboran Sea. Surface seawater was collected during seven cruises conducted in fall, winter, and spring, each covering four coast-to-offshore transects. AAP abundance was quantified by infrared epifluorescence microscopy and diversity was assessed using pufM gene amplicon sequence variants (ASVs). Abundance was significantly higher in spring than in winter, though no consistent spatial pattern emerged. PA communities exhibited greater diversity, with ~65% of ASVs exclusive to this fraction. Distinct AAP phylogroups showed niche preferences: PA-associated groups were common in coastal waters while FL groups predominated offshore. Sample ordination showed grouping by season, while a spatial structuring by transect (west-to-east) was more clear for FL than for PA communities. Environmental variables driving each community differed, with FL communities correlating primarily with salinity and PA communities with chromophoric dissolved organic matter. Our findings demonstrate that lifestyle (FL vs. PA) shapes the diversity, spatiotemporal organization, and environmental responses of AAP bacteria.

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Unexpected presence of the fungus Gjaerumia minor in the bathypelagic ocean

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The basidiomycete Gjaerumia minor is a fungus known for causing white haze disease in apples. Surprisingly, it is present in the deep ocean. Analysis of 18S rDNA sequences from the Malaspina 2010 expedition reveals that G. minor's relative abundance increases with depth, with higher concentrations found in the Pacific

compared to the Atlantic. Using a newly designed TSA-FISH probe, we confirmed the presence of G. minor cells in bathypelagic samples, observing two distinct morphotypes: rounded yeast-like cells and elongated hyphae. G. minor biomass in the Pacific correlates inversely with Apparent Oxygen Utilization (a proxy for water age) and recalcitrant carbon, suggesting a potential role in refractory carbon degradation. These findings highlight the ecological significance of G. minor in the deep ocean carbon cycle and raise questions about its transport and adaptation to this extreme environment.

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Microbial communities in the South Orkney Islands, Antarctica

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Waters surrounding the South Orkney Islands are highly productive, contributing to Antarctic food webs and krill fisheries. However, despite their ecological significance, marine microbial communities in this region remain poorly understood. Here, we aimed to describe the marine microorganisms (eukaryotes and prokaryotes) in this area, their spatial patterns of variability, and how environment shapes those communities. For this, seawater was collected around the islands and at different depths (5 to 1,000 m), and community composition was determined by 16-18S rRNA gene sequencing. Microbial communities varied by station, depth, and size fraction (PERMANOVA test). Environmental variables that better explained variability in community composition were temperature, salinity and chlorophyll concentration. Microbial communities were dominated by Bacteria (90.35%), followed by Archaea (5.27%), and Eukarya (4.38%). Surface waters (5–30 m) were dominated by Bacteroidota, Alpha- and Gammaproteobacteria, while deep waters (< 100 m) were dominated by Planctomycetota, Crenarchaeota, and Deltaproteobacteria. While microbial abundances decreased with depth, microbial diversity and the complexity of co-ocurrence networks increased. The number of indicator species diversity was higher that in shallower waters. All together, our results indicate that, in this Antarctic area, microorganisms are strongly structured by depth which, in turn, is linked to water masses distribution.

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Pronounced fine-scale spatial structuring of coastal microbiomes at the land-sea interface

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Marine microbiomes are fundamental to coastal ecosystem functioning. Yet, the extent of fine-scale spatial structuring of microbiomes across the land-sea interface – if any – remains unexplored. We investigated bacterial community composition across 40 stations, each separated by ~500 m, in a grid from near-shore to offshore waters along a Baltic coastline in spring and summer. 16S rRNA gene amplicon sequencing (ASV analysis) showed that despite the close geographic proximity, microbial communities showed significant spatial structuring from near-shore to offshore waters. Moreover, community composition in two bays covered by the grid differed substantially (distance-decay analysis; $r=0.4-0.6$, $p<0.05$). Conversely, community composition in offshore waters was highly similar, although the area covered corresponded to the two bays. Dissolved organic carbon, temperature, and bacterial production strongly correlated with community structure during summer ($R^2=0.4-0.5$, $p<0.01$). Analysis of dominant ASVs revealed three distinct distribution patterns: i) bacteria abundant near-shore, decreasing offshore (e.g. filamentous cyanobacteria), ii) bacteria abundant offshore, decreasing towards the coast (e.g. Pseudohongiellaceae), and iii) populations evenly distributed across all samples (e.g. Burkholderiaceae). These pronounced short-distance shifts imply that ecosystem functions regulated by coastal transition zone microbiomes, e.g. carbon sequestration and nutrient catchment, are highly dependent on the differential distribution of individual bacterial populations.

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Microbial ecology of the seagrass seed

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Seagrass ecosystems provide important carbon stores, function as biodiversity hotspots and support 20% of the world's largest fisheries. In the UK, conservative estimates put the loss of seagrass extent at around 44% since the 1930s, with similar losses experienced worldwide. While seagrass restoration techniques are studied and improved, the molecular ecology of the seagrass seed, and how it could influence restoration and conservation efforts, remains underexplored. In terrestrial systems the seed microbiome can confer disease resistance and resilience against abiotic stressors – indispensable traits for a species in decline. To begin to unravel the ecology of the seagrass seed microbiome, seeds of Zostera marina, the main species targeted for restoration, were collected from two different locations in the UK. Seeds were first divided into viable or inviable, and then each had their tissues homogenized, with each

seed constituting one sample. Extracted DNA was amplified with primers targeting the ITS, 16S, and 18S regions, to allow assessment of fungal, bacterial, and microeukaryotic communities respectively. Results revealed significantly different microbial community compositions between seeds of different sites, highlighted potential indicator species for seed inviability and have raised many further lines of research and improvements to methods.

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Contrasting spatiotemporal patterns of bacterial and protist plankton diversity uncovered through DNA-based monitoring in the Baltic Sea area

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Planktonic microorganisms in coastal waters form the base of food webs and biogeochemical cycles. These environments are exposed to pronounced environmental gradients, including a strong salinity cline. Yet, commonplace ecological assessment overlooks most of their diversity. We analyzed protist and bacterial diversity from new and publicly available DNA metabarcoding data collected in the Baltic Sea and the Kattegat-Skagerrak. We show that salinity, unlike other environmental factors, had a stronger effect on bacterial than protist community composition. Meanwhile, bacterial alpha diversity primarily followed seasonal patterns that we linked to deep water taxa reaching the surface through convective vertical mixing. On the other hand, protists were more diverse in high salinities. Still, Bayesian modelling showed that bacterial lineages were less likely than protists to occur in both lower and higher brackish salinities. We propose that protists are ecologically less sensitive to salinity due to the disconnection of basic metabolic processes from the cell membrane through compartmentalization. Additionally, further and more frequent dispersal of bacteria might have impeded local adaptation following historic salinization events. Ultimately, incorporating DNA metabarcoding into an environmental monitoring program allowed us to connect ecological and biogeographic processes with understudied taxa and biodiversity dynamics.

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Phytoplankton biodiversity in the Skagerrak: patterns, trends and connectivity between fjords

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Understanding the diversity and community structure of phytoplankton is crucial for elucidating their ecological role in marine ecosystems and biogeochemical cycles. As part of a comprehensive investigation into pelagic microbial communities, we are conducting an extensive field sampling campaign along the Swedish West coast (Skagerrak) aboard R/V Svea in July 2025. A total of thirty stations, encompassing both fjord and offshore basins, will be sampled for a battery of biotic and abiotic parameters, including phytoplankton with the help of plankton nets and CTD rosette casts. Phytoplankton communities will subsequently be characterized using a combination of advanced imaging (PlanktoScope), traditional microscopy counts, pigments, and 18S rRNA gene amplicons and metagenomics. With our multi-method approach we aim at detailed assessments of spatial diversity patterns and variability across environmental gradients. Here, we will present the preliminary results on phytoplankton assemblages and connectivity features between fjords and offshore sites in the context of local environmental gradients and hydrography patterns. This study, thus, aims to contribute to a broader understanding of phytoplankton dynamics and ecological patterns in temperate coastal and open marine systems.

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Biological interactions are driving bacterial community assembly under stable temperature conditions

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Temperature strongly influences marine microbial community composition and gene expression, altering the abundance and activity of key bacterial taxa. However, the specific mechanisms by which temperature shapes bacterial community assembly and mediates metabolic exchanges among community members remain poorly understood. For this study, we implemented a microbial time-series using aquaria tanks as a model system. In collaboration with the Maritime Museum Aquarium in Sweden, we sampled the bacterial biodiversity of four aquaria weekly over a year to generate ecological understanding on mechanisms of bacterial community assembly and its persistence under stable temperature conditions. The collected amplicon dataset revealed pronounced bacterial community succession dynamics in all tanks, although with sporadic patterns. In addition, the assemblies within the temperate aquaria displayed a steady increase in community dissimilarity over time, while the tropical communities initially showed a diverging behaviour followed by a period of stabilization or re-convergence. These observations indicate that biological interactions are driving the

bacterial community assembly under stable temperature conditions. Our findings will help us to explain the long-term community dynamics that we observe in natural communities but struggle to understand due to the high complexity of natural environments.

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Ecological success in freshwater lakes: insights from novel cultivated lineages of the abundant Nanopelagicales order

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The Nanopelagicales order is the most abundant bacterioplankton lineage in freshwater lakes, yet strain-level diversity and ecological strategies remain poorly understood due to the lack of stable cultures and reliance on incomplete metagenome-assembled genomes. Here, we present 72 newly cultivated and sequenced Nanopelagicales isolates, including 24 novel Planktophila species and four from the previously uncultured genus Aquilimus. Complete genomes revealed high inter- and intra-species diversity, particularly within Planktophila, likely enabling the coexistence of closely related taxa. Functional analyses uncovered diverse transporters and carbohydrate-active enzymes, supporting flexible substrate use from plant and algal sources. Global metagenomic recruitment showed that few species dominate freshwater ecosystems, with seasonal peaks following algal blooms. A repeated loss of the oxidative part of the pentose phosphate pathway during Planktophila diversification suggests a shift toward energy-efficient metabolism, while nitrate respiration genes in P. vernalis indicate adaptation to anoxic conditions. This culture collection provides a foundation for understanding the ecological success of Nanopelagicales in freshwater environments.

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Decoding the Oysterome: Environmental drivers of microbial and resistance profiles in oyster farming

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Aquaculture is considered an impactful human activities on coastal marine ecosystems, particularly when practiced intensively, as it can cause significant disturbances to natural communities and ecosystem functioning. These impacts include nutrient imbalance and, even the release of pharmaceuticals into surrounding waters. Among the diverse forms of aquaculture, oyster farming is one of the greatest in terms of both tonnage and added economic value and is part of a rapidly growing market. However, it remains among the least studied in terms of its ecological impact. In this study, we analyzed the microbiome and antibiotic resistome of four oyster farms in the Atlantic and Mediterranean using shotgun metagenomics, amplicon sequencing, and genome enrichment techniques. We examined microbial communities in water, sediments, farmed and wild oysters, as well as in associated species: clams, mussels, gastropods, tunicates, crabs, seabirds, and fish. Our findings show that oyster farming has a relatively low ecological footprint. Compared to other filter feeders, oysters harbor fewer allochthonous and pathogenic bacteria and antibiotic resistance genes. However, the oyster microbiome (by us termed the "oysterome") is highly influenced by environmental quality and external anthropogenic and climatic factors, rather than by the farming process itself.

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Impact of environmental variability on microbial community dynamics in surface waters along the coast of France

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Mediterranean shallow coastal lagoons typically experience significant disturbances due to seasonally variable freshwater inflow and high summer evaporation rates, which can lead to large-scale salinity fluctuations. With the ongoing climate crisis, such short and long-term environmental disturbances are expected to become even more prominent, disrupting the structure and function of microbial communities. To better understand this effect, surface waters from the Mediterranean Sea and lagoons along the Gulf of Lyon were monitored during two consecutive years. We investigated prokaryotic community-based changes using 16S rRNA metabarcoding and metagenomic analyses with respect to environmental variability. Overall, community composition differed between the Mediterranean Sea and lagoon waters. Temperature and concentrations of labile organic matter (approximated by protein-like fluorescence) appeared to be the main drivers shaping the community. Interestingly, sites with lower environmental variability (Mediterranean Sea) had a similar species turnover over time as sites with larger environmental variability (lagoons). More detailed analyses of the community and its genetic repertoire will enable us to detect trends in the functional capabilities throughout the year in response to disturbances. With our study, we offer valuable insights into changing communities in coastal environments that are largely affected by seasonal and climate change-induced environmental disturbances.

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High-frequency time-series sampling details diel gene expression patterns of marine prokaryotes in the western North Pacific subtropical region

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Marine microbial communities are known to exhibit diel oscillations in metabolic activity, especially in surface waters. These patterns may reflect temporal partitioning of transcriptional activity, which allows species to coexist by reducing competition. However, the vertical extent and ecological significance of these rhythms remain unclear. To investigate depth-related metabolic variation, we conducted high-frequency sampling at three-hour intervals over a 72-hour period at the Kuroshio Extension Observatory site. Seawater samples were collected at 12 depths between 10 and 300 m to analyze 16S rRNA amplicon metabarcoding, metagenomes, and metatranscriptomes. Community structure and metagenomic analyses revealed depth-related changes, including increased α-diversity and a distinct assemblage at 200 m; however, no clear diel variation was observed across the four depths (10, 70, 130, and 200 m). Metatranscriptomic data at 10 m depth showed pronounced diel oscillations in the expression of 3,007 genes, including those involved in ATP synthesis and photosynthesis, consistent with previous studies. We are analyzing metatranscriptomic data from deeper layers to determine whether diel gene expression patterns extend below the surface. These analyses are expected to provide new insights into the temporal and vertical distribution of microbial metabolic activity throughout the water column.

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Seasonal dynamics and thermal vulnerability of marine microbial communities in Uruguayan coastal waters

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In an era of accelerating ocean warming, deciphering the drivers of microbial community composition becomes more urgent than ever. Long-term time-series studies are particularly powerful for this purpose. However, most previous works remain confined to the Northern Hemisphere and focus solely on taxonomy, leaving the Southern Hemisphere underrepresented and microorganisms' functional dimensions underexplored. Further, phenomena such as current-driven community shifts and microbial vulnerability to ocean warming are rarely understood. In this work, we sought to bridge these knowledge gaps by investigating microbial communities using metagenomic (shotgun) and metataxonomic (16S rRNA) data collected over two years at the Southern Atlantic Microbial Observatory, off the coast of Uruguay. By applying a suite of different ecological analyses, we revealed strong seasonal shifts driven by environmental variability and the influence of the Brazil–Malvinas ocean currents, resulting in two community states (summer–autumn vs winter–spring). The communities from these semesters exhibited contrasting composition, diversity, and assembly mechanisms. Notably, these results also showed that taxonomic and functional diversity trends diverge between these periods. Crucially, our work suggests that the predicted tropicalization of Uruguay's coast is likely to boost taxonomic richness but erode functional repertoires, while also increasing vulnerability to ocean warming, potentially undermining biogeochemical functioning.

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RosHAB: From monitoring cyanobacterial blooms to constructing genomes of bloom-forming species

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A great challenge in freshwater management when regarding the surveillance of cyanobacteria is the delays between water sampling and the confirmation of a potentially toxic bloom, which requires immediate decisions on water usage. Rapid on-site detection of Harmful Algal Blooms (RosHAB) represents the next generation of monitoring practices, incorporating an innovative, in-the-field applicable genomics workflow for real-time, reliable, accessible and cost-effective early detection of cyanobacteria harmful algal blooms (cHABs) outbreaks. In this context, we have developed a pipeline for the Rapid on-site detection of Harmful Algal Blooms (RosHAB)

using Oxford Nanopore Technologies via academic and governmental collaboration. We will present the latest advancements in the RosHAB pipeline. Currently, the advancements allow results to be obtained within three hours from sampling to sequencing with ONT. In parallel to the pipeline, to improve taxonomic assignments, we are also building the International Cyanobacterial toxin (ICYATOX) database, with whole-genome sequences of cyanobacterial strains worldwide. We will additionally show updated pangenomes of important bloom-forming species composing ICYATOX and their contribution in understanding cyanobacterial ecological and evolutionary success. Together, RosHAB and ICYATOX aim to improve the efficiency of bloom surveillance and investigate genomic evidence of the success of cyanobacterial proliferation.

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FUNACTION: Spatial and Structural Drivers of Fungal Diversity Across Europen Freshwaters

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Fungi comprise taxonomically and evolutionarily diverse taxa and are ubiquitous in various aquatic ecosystems worldwide, including many aquatic lineages. As saprotrophs, parasites, and symbionts they play essential roles in food web dynamics and cycling of organic matter and nutrients, ensuring ecosystem health and stability. However, their biodiversity and functional roles in the aquatic realm remain elusive, therefore, they are almost completely excluded from mainstream conservation and monitoring efforts. A multinational initiative, FUNACTION, aims to address these gaps by testing the efficacy of existing nature protection sites and generating the necessary knowledge, tools, and networks to monitor, and protect fungi in aquatic ecosystems. By utilizing Third-Generation sequencing approaches, rich metadata, and pan-European sampling with centralized analysis, FUNACTION relates fungal diversity to environmental and anthropogenic drivers in a systematic manner. Seasonal characterization of fungal diversity across watersheds from above the Arctic Circle to northern Portugal, as well as a multinational coordinated leaf litter decomposition study

have now been completed. A ‘hot off the press’ view on aquatic fungal diversity within Europe will be presented. By this collaborative effort, we are generating critical knowledge on pan-European fungal diversity in aquatic ecosystems by utilizing advanced molecular techniques and data integration. We are, ultimately aiming to include fungi in routine monitoring programs and develop effective conservation strategies for these vital, yet overlooked, components of aquatic ecosystems.

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Digital PCR as a tool for targeted phytoplankton monitoring

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Monitoring phytoplankton seasonal dynamics is critical for understanding the functioning of marine coastal environments. Diatoms are among the most successful taxa and their diversity, biology, and seasonality have been at the center of research, specifically for large-sized species. At the SOMLIT-Astan station (Roscoff, Western English Channel), previous studies have demonstrated the significance and seasonal variations of diatoms by combining ribosomal DNA metabarcoding and microscopy counts Yet, these methods are labour-intensive or can be biased by operator or PCR efficiency. As an alternative, here we investigated the potential of digital polymerase chain reaction (dPCR) for quantifying a complex of three bloom-forming diatom species Guinardia sp. We first designed primers and probes located in the V4-rRNA gene specific for G. delicatula, G. flaccida and G. striata, respectively, and then developed a multiplex dPCR to target these 3 species at once. We then quantified each of these 3 species in samples collected bimonthly over 2 years (2015-2016) and compared our results to metabarcoding data (OTU from 18S V4 rDNA-derived taxa) and microscopy counts.

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Spatio-temporal shifts in microbial communities across bloom and non-bloom water samples in the oder river system

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Harmful Algal Blooms (HABs) in freshwater systems represent a growing ecological and public health threat. Following the unprecedented toxic Prymnesium parvum bloom in the Oder River in 2022, microbial community dynamics within the river were

investigated by molecular tools. The study focused on bacteria, cyanobacteria, fungi, and other eukaryotes, comparing bloom and non-bloom water samples. Sampling was conducted across 16 spatially distributed sites, including tributaries and ecologically buffered floodplain sites (refugees), between June 2023 and June 2024. Using a combined Illumina and Nanopore sequencing approach, high-resolution taxonomic profiling was carried out. This dual-platform strategy captured both short-read accuracy and long-read continuity, which improved assembly quality and functional annotation, particularly for complex and low-abundance taxa. This effort also led to the development of a gene catalog to better characterize overall gene abundances. The comparisons reveal marked shifts in microbial diversity and community composition during bloom events, alongside distinct functional signatures within a hydrological context. This integrated metagenomic approach facilitated better identification of microbial taxa and functional genes associated with bloom events, offering potential indicators for early detection. Linking the microbial community shifts with functional responses across spatial gradients, results in deeper ecological understanding of bloom dynamics.

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Microbial Community Assembly Mechanisms in an Interconnected Karst System

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Karst landscapes develop through the dissolution of soluble bedrock and often comprise interconnected surface- and subsurface-flow paths. On the north shore of Lake Thun (Switzerland), a mixed limestone–sandstone catchment drains via both overland streams and a subsurface karst network into the lake, providing a natural framework for studying bacterial community assembly in a complex, connected environment. We profiled 16S-rRNA genes across soils, sediments, surface and subsurface streams and pools, along with different lake strata. Each environment type except the lake displayed high β-diversity. Null-model analysis indicated that homogeneous environmental selection dominated community assembly in all compartments, whereas communities in the lake hypolimnion were additionally shaped by high dispersal and low compositional turnover. Sandstone soil was the most isolated environment type, sharing taxa mainly with adjacent limestone soils and sediments, likely a consequence of both hydrological separation and strong variable selection. Limestone soils and sediments shared more taxa with both surface and subsurface waters. Subsurface waters, in turn, shared the greatest number of taxa with the lake. Together, our results show that environmental filtering is the primary driver of bacterial community structure in complex karst catchments, influenced locally by hydrological connectivity and dispersal.

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Microbial biogeography across domains of life and plankton size-fraction in the Pacific Ocean

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Symbioses play a key role in ocean ecosystem functioning, yet the large-scale ecological drivers of the interactions that structure planktonic communities remain poorly understood. The goal of this study was to identify possible associations between eukaryotes and prokaryotes across size fractions, and identify their structuring factors. We analysed 1,200 surface plankton communities, from four size classes (0.2-3 µm, 3-20 µm, 20-2000 µm, >300 µm), sampled across 32 islands and 249 oceanic locations during the Tara Pacific expedition (2016-2018). Using 16SrRNA (prokaryotes) and 18SrRNA (eukaryotes), we studied the factors explaining the biogeography of microbial communities in different size fractions, in particular the effect of islands and associated changes in environmental parameters. We found differences in community composition between different types of water (coastal, open ocean, island-influenced) that varied depending on the size fractions. More precisely, the variability in community composition increased with organism size, and differences in community composition between coastal and island-influenced environments were more pronounced for higher size fractions. These patterns appear to be largely driven by the bacterial component of the communities, which is the most abundant. By identifying large-scale patterns of planktonic interactions, we aim at proposing new hypotheses about the factor structuring plankton globally.

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From field to plates: Investigating the changes in bacterial community composition of brown algae from the environment to the laboratory

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Marine macroalgae are ecosystem engineers that provide habitats for diverse microbes. They secrete carbohydrates on their surfaces, creating an environment favorable for bacterial growth. The enigmatic phyla Planctomycetota and Verrucomicrobiota are persistent groups on the surfaces of brown macroalgae throughout the year. Nonetheless, the reasons behind the stable association between

these specialized bacteria with algae remain unexplored. Here, we investigate how the bacterial community composition changes in the brown algae (e.g., Ectocarpus sp, among others) we collected from the field and cultivated in the lab. For each, we sub-sampled biomass at four different time points to capture changes in the bacterial community throughout the transition into laboratory conditions. Using high-throughput full-length 16S rRNA sequencing (PacBio Kinnex) for all samples, we tracked the changes in the relative abundance of the bacterial phyla. After one month of cultivation, there was an increase in the relative abundance of the phyla Pseudomonadota, Bacteroidota, Planctomycetota, and Verrucomicrobiota. Our analyses can be further used to investigate whether the changes in community composition are specific to the different species of algae. Finally, our results will lead to a better understanding of the diversity of specialized bacteria involved in the degradation of complex glycans.

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New lineage of scuticociliates dominates the ciliate community and bacterivory in hypolimnetic waters of a freshwater reservoir

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Deep, cold and dark hypolimnia represent the largest volume of water in freshwater lakes with the limited phototrophs' occurrence. However, the presence of prokaryotes support populations of bacterivorous ciliates and heterotrophic nanoflagellates (HNF). Nevertheless, protistan bacterivory rates and the major hypolimnetic ciliate bacterivores are poorly documented. We conducted a high frequency sampling (three-times a week) in the oxic hypolimnion of a stratified mesoeutrophic reservoir during summer, characterized by stable physicochemical conditions and low water temperature. Using fluorescently labeled bacteria we estimated that ciliates and HNF contributed, on average, 30% and 70% to aggregated protistan bacterivory, respectively, and collectively removed about two thirds of daily hypolimnetic bacterial production. The ciliate community was analyzed by the quantitative protargol staining method. One scuticociliate morphotype dominated the hypolimnetic ciliate community, accounting for 82% of total ciliates and over 98% of total ciliate bacterivory, with average cell-specific uptake rate of 202 bacteria per hour. Moreover, long-amplicon sequencing revealed that the scuticociliate belongs to an unidentified clade closely related to the Ctedoctematidae and Eurystomatellidae families. The high-resolution sampling, microscopic and sequencing methods allowed uncovering indigenous microbial food webs in the hypolimnetic environment and revealed a functional simplification of ciliate communities, dominated by a new bacterivorous scuticociliate lineage.

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Long-term metabarcoding-based diversity patterns of diatom-infecting zoosporic parasites in a coastal marine ecosystem

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Marine diatoms are a key phytoplankton group, accounting for ~40% of ocean primary productivity, sustaining food webs and carbon export from the surface ocean to depth. Many marine diatoms are infected by zoosporic parasites which have the potential to impact host dynamics and ecosystem functioning. Currently, we have a limited understanding of zoosporic parasite diversity in marine ecosystems, especially from long-term time-series. Here, we used a 20-year 18S rRNA metabarcoding dataset from samples collected from the coastal monitoring site, Station L4, off Plymouth (UK). The dataset was screened for established zoosporic parasites using the recently developed ParAquaSeq database, a library of annotated rRNA sequences from zoosporic parasites infecting aquatic primary producers. Potential parasite metabarcodes from the dataset were calibrated against reference zoosporic parasite barcodes. A diversity of major zoosporic parasite groups were identified, including Labyrinthulomycota, Cryothecomonas, and Pirsonia, with links to known diatom hosts. For example, several Pirsonia species showed distinctive inter- and intra-annual distributions and co-occurrences with associated diatom hosts. Discrepancies in these patterns were observed at distinct thermal ranges, as shown in freshwater systems. This study highlights the value of long-term molecular time-series for determining zoosporic parasite-host dynamics and potential environmental drivers of infection in a coastal ecosystem.

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Mechanosensing and buoyancy regulation in diatoms

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Diatoms are a key phytoplankton group in the marine ecosystem, responsible for approximately 40% of total marine primary production. In the past decades, it has been shown that they are able to regulate their buoyancy in the water column in order to cope with scarce resources and a fast-fluctuating environment. While cells are frequently subject to hydromechanical stress, the effect of this major environmental cue on buoyancy regulation has never been explored experimentally. To fill this gap, we have built an experimental set up allowing to track several diatoms species in millifluidic channels and get detailed statistical analysis of single cell sinking speeds

under controlled shear rates. Measurements on the species Coscinodiscus granii show that hydromechanical stimulus induces an increase in cell density which translates in higher sinking speeds within really short timescales (few seconds). Moreover, the relaxation of this response is observed to depend on the intensity and timespan of the applied stress, suggesting that diatoms cells perform some signal integration over time. These findings shed light on a new regulation mechanism in diatoms species, paving the way for a better understanding of diatoms spatio-temporal distribution at the ecosystem level, which should improve biological carbon pump quantifications.

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Environmental drivers of protist trophic networks in Iberian alpine wetlands

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Mountain wetlands are formed by an intricate mosaic of microhabitats with different waterlogging conditions and contrasting hydrological regimes, these conditions sustain high levels of protist biodiversity. Understanding the ecological drivers of this biodiversity is crucial for predicting how these communities will respond to environmental changes. These changes may drive the community species turnover and this, in turn, may affect the trophic network structure. To investigate this, we sampled 53 wetlands across five mountain national parks in the Iberian Peninsula, each comprising 10–12 wetlands along their elevational gradients. Within each wetland, we surveyed 10 plots including: vegetation structure, microhabitats variables (water table depth, pH…), and collected topsoil samples. With the top soil samples, protist communities were characterized through 18S rRNA metabarcoding. We then used a previously trained boosted regression tree model to infer trophic interactions between protists based on their functional traits. For each plot, we calculated network metrics describing trophic network structure. Our results suggest that microhabitat variables, plant cover and water table depth, better explain variation in food web properties than macroclimatic factors such as mean temperature or precipitation. This suggests that fine-scale environmental filtering plays an important role in shaping protist trophic networks in alpine mountain wetlands.

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Host Interactions and Seasonal Dynamics of Legionella Pathogens in a Stratified Freshwater Lake

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Legionella are predominantly recognized as waterborne pathogens in man-made water systems. However, Legionella species inhabit diverse natural aquatic environments, including marine ecosystems, freshwater lakes and rivers, where they interact with various protozoan hosts, across different environmental conditions. Yet, Legionella ecology in natural environments remains poorly understood. To address this knowledge gap, we conducted molecular analyses utilizing both Legionella-specific qPCR and amplicon-based sequencing to study Legionella abundance, diversity, community composition, and co-occurrence with potential protozoan hosts in a stratified freshwater lake. Our study identified significant seasonal patterns in Legionella absolute abundance, with peaks occurring during and immediately after lake mixing events. Genus specific amplicon-based sequencing revealed shifts in Legionella community composition, associated with sampling month and key environmental factors, including temperature, dissolved oxygen, chlorophyll, and dinoflagellate biomass. Surprisingly, our work identified a number of Legionella genotypes present only under oxygen limiting conditions. Network analysis highlighted interactions between Legionella and potential microbial hosts, including ciliates and dinoflagellates. The current study is the first comprehensive analysis of Legionella ecology in a stratified freshwater lake. We show that changes in Legionella persistence distribution, and community composition are related to physicochemical factors, mixing events and associations with potential hosts.

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Late spring protist communities along a coastal transect in southern Norway: spatial and interannual variability using a metabarcoding approach

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Estuarine and coastal environments are known as areas of high biodiversity. Understanding protist community composition and its spatial distribution is essential for a better understanding of ecosystem dynamics, and for informed management of the region. This study focuses on spring protist communities in the coastal region of the Skagerrak-North Sea. Our main objectives were to determine spatial and inter-annual variability, as well as functional diversity, in particular trophic strategies. The18S rRNA

gene was used for metabarcoding of protists in water samples collected from 12 different stations of a transect along the coast of South Norway in April 2021 and 2022. We observed differences between inner and outer coastal sites, as well as between eastern and western sites located within two different fjords. Interannual variation was also evident, mainly driven by differences in water column stratification and bloom timing. Deep-water samples, on the other hand, had similar community composition across sites and years, indicating a protist community adapted to life below the euphotic zone. Parasitic and mixotrophic forms dominated along the transect, especially in the deep samples but also in the surface ones, highlighting the importance of these trophic strategies within the environment.

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Identifying diverse protist consumers of Limnohabitans planktonicus in a lake and its main inlet through quantitative stable-isotope probing

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Protists are crucial bacterioplankton grazers in freshwater ecosystems, influencing food web dynamics and biogeochemical cycles. While most studies have focused on bulk grazing rates, the diversity of actively feeding protists remains largely unexplored at fine taxonomic levels. Here, we aimed to identify protist grazers of the cosmopolitan freshwater bacterium Limnohabitans planktonicus in a Swedish lake and its main inlet. We conducted a 36-hour replicated bottle experiment, amending water with 13C, 15N - L. planktonicus cells, and employed quantitative stable-isotope probing coupled with 18S amplicon sequencing. Additionally, the composition of bacterial communities was determined with 16S amplicon sequencing and cell abundances were monitored by flow cytometry. Our results show that lake protists incorporated on average more isotopic material compared to the inlet, indicating higher grazing intensity. Active protist consumers comprised 123 and 186 OTUs in the inlet and lake respectively, with 45 OTUs shared across habitats. These grazers predominantly belonged to choanoflagellates, cercozoans, chrysophytes, dictyochophytes and ciliates, with fewer taxa from cryptophytes and dinoflagellates. Additionally, perkinsid parasitic protists were detected, likely interacting with L. planktonicus consumers. Our study directly links protist diversity to specific bacterial prey, highlighting distinct quantitative contributions of microbial grazers to the microbial loop in hydrologically connected habitats.

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Experimental assessment of interactions between marine bacteria and model protists: from predator-prey relationships to bacterial-mediated lysis

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Bacteria in aquatic environments are a principal food source for predatory protists. Whereas interactions between bacteria and protists are recognized to determine the pathogenesis and epidemiology of several human pathogens, few studies have systematically characterized the interactions between specific aquatic bacteria and protists beyond the prey-predator relation. We therefore surveyed individual co-cultures between 18 different genome-sequenced marine bacteria with known virulence gene repertoires and three model protist species widely used for assessing bacteria-protist interactions. Strikingly, 10, 5, and 3 bacterial isolates were capable of lysing the protists Acanthamoeba polyphaga, Tetrahymena pyriformis and Euglena gracilis, respectively. A majority of the bacteria were able to grow and/or maintain viable populations in presence of viable protists. Some bacteria survived longer with viable protists but not heat-killed protists, and were observed in protist vacuoles; 5 isolates depended on viable amoebae to grow. In this respect, marine bacteria are similar to several protist-dependent human pathogens. The remarkable capability of these marine bacteria to survive encounters with, and even actively kill, model predatory protists under laboratory conditions suggests that diverse bacterial defense strategies and virulence mechanisms to access nutrients may be important in shaping microbial interactions.

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How environment shapes sreshwater protists communities: a multi-year study from the mazurian lakeland

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Unicellular eukaryotes (protists) play a crucial role in freshwater ecosystems, contributing to nutrient cycling and food web dynamics. Despite their ecological importance, freshwater protists remain less studied than their marine counterparts. In particular, little is known about how environmental factors shape their diversity and trophic strategies across seasons. This study aims to investigate the influence of environmental factors on the taxonomic and functional composition of protistan communities in freshwater lakes. Over a three-year period, water samples were collected from 35 chemically diverse lakes in northern Poland. We obtained over 300 samples and using 18S rRNA sequencing, we assessed the taxonomic and trophic

composition of microeukaryotic communities. Temperature was a primary driver of community composition, with clear seasonal clustering, especially among photoautotrophs. Mixotrophs, as expected, were less seasonally and chemically influenced. Autumn samples showed the greatest divergence; trophic state of the lake explained 65% of community variation, while chloride ion concentration explained 10%. Across all seasons, chloride was the most significant chemical factor, while oxygen levels contributed marginally (up to 5% in summer). Protistan communities are shaped by seasonal and chemical factors. The strong influence of chloride - often linked to anthropogenic pressure - suggests high impact of human activity on freshwater ecosystems.

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Effect of microplastics on microbial communities from the coastal Baltic Sea

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Increasing microplastic (MP) pollution is greatly affecting the aquatic environment. Thus, it is vital to know its effects on the ecosystem. The current studies focus mostly on its toxicity in marine organisms, mostly animals such as fish, but little information is available regarding microbial communities. This study was designed to find out the effects of different concentrations of Polystyrene (PS), which is of smaller size and commonly found pollutant. The natural microbial community was collected from the coastal Baltic Sea and exposed to bacteria- sized PS and glass microspheres. In this experiment, we studied the effect of PS concentrations on the abundance and activity of heterotrophic nanoflagellates (HNF) and prokaryotes over five days. We observed a consistent negative effect of PS (that was more conspicuous at higher concentrations) on HNF abundance, while for bacteria the effect was negligible or, even positive. Moreover, the presence of glass and MPs particles lowered the respiration rate of the microbial community, indicating the importance of the physical effects of increased particle concentrations in the water. The changes in microbial abundance and activity were accompanied by shifts in bacterial and HNF community composition. These results indicate the complex nature the presence of MPs may have on microbial communities. The combined effect of toxicity and the physical presence of inedible particles may alter the primary microbial consumers and cause a disturbance in microbial aquatic food webs. Further experiments are planned to understand the observed patterns.

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Dominance of eutrophic prokaryotic community members after community coalescence events

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Microbial coalescence events, in which communities mix partially or fully, occur frequently in nature, yet how member life history traits influence interactions and community function remains poorly understood. We experimentally coalesced communities from two aquatic environments with contrasting temporal heterogeneity and trophic status (stable-oligotrophic and unstable-eutrophic) and incubated for 5 days. Additionally, unmixed communities were grown as control incubations. Eutrophic communities were characterized by low species diversity and by taxa with large genomes and high codon usage bias, which has been previously associated with generalists and opportunist organisms. Members of these eutrophic communities consistently dominated coalesced communities, suggesting that rather than species diversity, species life history influenced the community dynamics after coalescence. The dominance of eutrophic community members was reflected in community productivity with similar rates in the coalescence and eutrophic control incubations compared to lower productivity in the oligotrophic control incubations. In contrast we found that respiration rates in the coalescence communities exceeded that of all control incubations. This suggests that low-abundance members of the oligotrophic communities triggered a stress response in eutrophic community members, as indicated by elevated respiration rates, which on long term may enhance the influence of oligotrophic community members on community dynamics after coalescence.

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From depths to lab: isolation and cultivation of a novel Patescibacteria

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Patescibacteria, also known as the Candidate Phyla Radiation (CPR), comprise a diverse phylum of mostly uncultivated, ultrasmall bacteria that represent a substantial fraction of bacterial diversity. Cultivated members, primarily from the class Saccharimonadia, exhibit a unique lifestyle as obligate episymbionts or epiparasites on prokaryotic hosts. Due to the limited number of cultured representatives, much remains unknown about their host specificity, attachment mechanisms, and nutrient acquisition. Here, we report the isolation and stable cultivation of 'Candidatus Borromeicoccus

lacustris' from the hypolimnion of Lake Maggiore (Italy), the first cultured representative of class JAEDAM0, in co-culture with its betaproteobacterial host Undibacterium sp. The complete genome of 'Ca. B. lacustris' is 1.4 Mbp, encoding 1,432 genes and using an alternative genetic code where the TGA stop codon is reassigned to glycine. Although over 500 proteins lack annotations, more than 120 proteins are predicted to be secreted, potentially modulating host metabolism. Indeed, electron microscopy revealed that CPR attachment induces outer membrane vesicle formation in the host. Growth experiments showed that, despite a negative impact on the host, the co-culture remains stable in stationary phase for extended periods, especially at 8 °C, reflecting in situ conditions. This system offers new opportunities for detailed ecophysiological studies of CPRs.

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Genomic insights into the metabolic potential of marine particle-associated bacteria

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Prokaryotes are key contributors to the biological carbon pump, mediating the degradation of organic matter that sinks into the deep ocean. Particulate organic matter (POM) sediments in the form of marine particles creating enriched microenvironments that support specialized prokaryotic communities, often enriched in hydrolytic enzymes and well-equipped for the breakdown of POM. However, our understanding of the specific functional capabilities of these particle-attached microbes remains incomplete. We hypothesized that particle-attached bacteria (here taken as those in the fraction 53–200 µm) possess larger genomes, faster growth rates, and greater metabolic potential than their free-living counterparts. To test these hypotheses, we isolated nearly 500 bacteria from marine particles and performed whole-genome sequencing on 11 representative strains. These isolates, which belonged to several taxonomic orders (e.g., Pseudomonadales, Enterobacterales, Flavobacterales, and Sphingomonadales), were compared to known closely phylogenetically related free-living marine genomes to identify both shared and unique functional traits. While the isolates represented members of the rare biosphere at the genus level, in amplicon metabarcoding data, their broader taxonomic groups were commonly found in particle-associated communities. Our findings suggest that particle-associated bacteria possess distinctive genomic features that likely enhance their potential to degrade organic matter. Overall, the isolation and genomic characterization of these microbes not only uncovered novel taxa but also provided valuable insights into the ecological roles of the marine particle microbiome in carbon cycling and microbial diversity.

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Structured microbial colonization of chitin particles in the Ocean: changes across time and depth

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Marine particles represent organic-rich microbial niches, that play a relevant role in ocean organic matter cycling. Chitin, a globally abundant biopolymer, is a key substrate for microorganisms colonizing these particles. In this study, we tracked microbial succession on model chitin particles over 130 hours using seawater from three sites and two depths (the Deep Chlorophyll Maximum and 500 m). 16S rRNA metabarcoding revealed pronounced temporal shifts in particle community composition, dominated by three bacterial classes: Gammaproteobacteria, Alphaproteobacteria, and Bacteroidia. These groups exhibited ecological versatility across colonization stages, with distinct genera producing bloom-like dynamics. Early colonizers (0–48 h) included Pseudoalteromonas shioyasakiensis, Sphingobium yanoikuyae, and Sulfitobacter spp., each exceeding 10% relative abundance. In later stages (>48 h) unclassified Cryomorphaceae, unclassified Cellibrionaceae, Marinobacter spp., and Pseudophaeobacter spp. increased. Strikingly, Alteromonas macleodii emerged ubiquitously, dominating initial phases with up to 75% abundance before declining. These dynamics suggest that community assembly on these artificial particles is structured: Gammaproteobacteria drive early colonization, followed by specialized metabolite degradation by Bacteroidia and Alphaproteobacteria. Our findings reveal that temporal substrate specialization and metabolic flexibility, rather than spatial factors (i.e. depth or station), govern microbial life cycles and particle remineralization in the ocean.

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Leaf morphological traits and environmental gradients shape microbial diversity and community composition in the Zostera marina phyllosphere

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Seagrass meadows, including those formed by eelgrass (Zostera marina), are critical coastal ecosystems that support biodiversity and provide numerous ecosystem services. They harbor complex microbial communities in their phyllosphere, yet the drivers shaping this microbial diversity remain incompletely understood. This study integrates data from several seagrass meadow locations in the Baltic and North Seas

to investigate how morphological traits of Z. marina and environmental gradients influence microbial diversity and community composition using 16S and 18S rRNA gene amplicon sequencing. Morphological traits such as shoot length, leaf width, and number of leaves, as well as environmental variables including salinity, temperature, bathymetry, and wave exposure, were analyzed. Correlation analyses revealed a striking positive relationship between leaf length and bacterial and eukaryotic richness. Wave exposure (modelled orbital velocity) was also a key predictor of microbial diversity while salinity was strongly linked to community composition. Furthermore, bacterial communities demonstrate a more conserved core community across datasets than eukaryotic communities, highlighting differential stability of these microbial domains in the seagrass phyllosphere. The multivariate approach enhances our understanding of microbial ecology in Z. marina meadows and provides valuable insights for conservation and restoration strategies of seagrass ecosystems under changing environmental conditions.

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Exploring the abundance, diversity and occurrence of toxin-antitoxin systems in marine Alteromonas

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Dormancy is a bet-hedging strategy used by a wide variety of bacterial taxa to overcome unsuitable conditions for growth. It is defined as a reversible metabolic state where cells reduce their metabolism to the minimum and stop dividing. Toxin-antitoxin (TA) systems have been proposed as one of the mechanisms to enter and escape this state, but their functions are still not well understood. They consist of two genes, one that encodes for a toxin that affects an essential function of the cell, leading to growth arrest, and another gene that encodes for an antitoxin that inhibits the action of the toxin. In this work we looked for TA systems in different species of Alteromonas, a predominant marine copiotroph with a boom-and-bust lifestyle. We analysed the genomes of different strains of A. macleodii and A. mediterranea in search of these systems, classified them and compared their presence in the different strains. Our results show that TA systems are more abundant in deep sea ecotypes than in their surface counterparts and appear to be more conserved. This suggests that dormancy may be more prevalent in deep sea environments, likely due to the intermittent nature of organic carbon inputs through sinking particles.

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Metagenomic characterization of the microbial community of salt marshes sediments over the diel cycle

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The diel cycle - the 24-hour rhythm of alternating light and dark phases - drives daily fluctuations in solar radiation, temperature and nutrients availability, strongly influencing the biogeochemical processes of marine ecosystems. Microbial communities are particularly sensitive to such fluctuations, playing a key role in mediating nutrient cycling, organic matter degradation, and overall ecosystem stability. Understanding the role of microbial communities in daily environmental changes is crucial for gaining insight into the functioning and resilience of dynamic coastal ecosystems. In this study, we aimed to characterize metagenome-assembled genomes (MAGs) of the microbial community of natural and restored salt marshes during the diel cycle. A set of 194 non-redundant medium-quality MAGs was obtained (> 50% completeness, < 10% contamination), most of them belonging to Pseudomonadota (68), Bacteroidota (38) and Desulfobacterota (31) phyla. Genes for carbon assimilation, known to be influenced by the diel cycle, were found in most Gammaproteobacteria, Alphaproteobacteria and Cyanobacteria MAGs. Genes for bacteriorhodopsin-like proteins were identified in 19 MAGs from different phyla. The presence of kai genes, known to regulate the diel cycle in Cyanobacteria, was also studied. These results will contribute to a better understanding of the regulation of the diel cycle in sediment microbial communities.

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Engineering an open culture system to investigate the role of microbial communities from Lake Geneva in enteric virus inactivation

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Lakewater (LW) microbes challenge the environmental stability of enteric viruses in aquatic ecosystems. We have shown that individual bacteria and bacterial communities from Lake Geneva reduced the infectivity of enteric viruses. Moreover, we found that high bacterial species richness in lakewater is positively correlated to viral inactivation. Here, we used chemostat cultures to assess the impact of LW microbial diversity in the inactivation of enteric viruses. Chemostats provide a constant inflow of nutrients and maintain bacteria in exponential growth phase throughout our assays. To manipulate

microbial diversity inside the chemostats, we used three approaches: dilution-to-extinction (1, 1:10 and 1:100), flow dynamics (0.062 – 0.125 ml/min) and nutrient supplementation (phosphate and nitrogen). 16S rRNA sequencing was used to compare bacterial diversity. Dilution-to-extinction and flow dynamics yielded communities with similar richness but different composition. Nine microbial communities grown using dilution-to-extinction were spiked with Coxsackievirus B5 (CVB5) and Human Adenovirus 2 (HAdV2). These communities decreased the infectivity of both viruses > 2 log. On the other hand, nutrient supplementation decreased viral inactivation compared to non-supplemented LW. Ongoing work focuses on identifying key members associated with inactivation. All in all, we used multidisciplinary approaches to grow lakewater communities with inactivating capacity against enteric viruses.

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Sediment resuspension impacting seasonal protist recruitment in shallow coastal areas

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Resting stage formation represents an important trait of many microorganisms, as it ensures the organism's survival during unfavorable environmental conditions. Germination from resting stages is often inhibited by burial and anoxic conditions in the sediment. Resuspension of surface sediments by storms might, therefore, represents an important mechanism that facilitates germination. We investigated the impact of sediment resuspension on planktonic protist community composition, assessed through 18S rRNA gene metabarcoding, by incubating sediment cores from the Baltic Sea either undisturbed or with regular resuspension events for four months at rising temperatures. In this experiment, the planktonic eukaryotic community composition was mostly characterized by temporal species succession likely driven by the gradual increase in water temperature. Furthermore, NO3- concentrations and sediment resuspension were identified as important factors shaping the eukaryotic community composition. Nitrate concentrations were significantly higher in the control than in the mixed sediment cores, as resuspension likely caused a significant loss of nitrate to the sediment. Unexpectedly, eukaryotic alpha diversity was not significantly altered by mixing, but the community composition changed due to germination of previously buried species. These results highlight the importance of sediment resuspension for germination and nutrient concentrations in the water column impacting planktonic microbial community composition.

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Links between the morphological and rank abundance structures of bacterioplankton communities and the optical and molecular structure of dissolved organic matter across Canadian lakes.

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Bacterioplankton and dissolved organic matter (DOM) are fundamental components in aquatic ecosystems and unravelling how they relate along major environmental gradients is a central goal to our understanding of ecosystem function. Here we explore the potential links between two aspects of bacterioplankton structure, i.e. rank abundance structure, derived from 16SrRNA sequencing approach, and the morphological structure, derived from a cytometric approach, and the optical and molecular structure of DOM. These links were explored across 393 lakes in the context of the pan-Canadian LakePulse project, the first standardized national lake survey that cover wide environmental, climatic and human impact gradients. Lakes dominated by terrestrially derived DOM and associated optical and molecular components have bacterioplankton communities with flatter rank abundance curves (i.e. less marked dominance) and with simpler morphological structures (i.e. oblate shape with low-DNA structure and single cytometric population). The opposite pattern was found in lakes with a larger fraction of autochthonous derived DOM and its associated DOM components, where bacterioplankton communities have steeper rank abundances curves (i.e. more marked taxa dominance) with more complex morphological structures (i.e. more diffuse, prolate shapes with distinct cytometric populations). Overall, key aspects of bacterioplankton and DOM structure appear to covary along broad environmental gradients.

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Microplastics as drivers of community changes in the Baltic Sea

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Microplastics (MPs) play a crucial role in shaping microbial community dynamics, particularly in aquatic environments. Variation in concentrations and microplastic types can significantly influence microbial abundance, respiration, and community composition. Here, we employed a genome-centric metagenomic approach to identify the response of microbial communities to the presence of bacteria-sized plastic particles from polystyrene (PS) and polyethylene (PE) with glass spheres and no particles as a control. We observed that bacterial abundance increased in the presence

of PS compared to both controls. In contrast, the response in PE treatment and glass control was similar, indicating a negative effect of particle presence rather than a toxic PE effect. To understand how these responses were driven, we obtained ~800 metagenome assembled genomes (MAGs) that were medium to high quality (>70% completeness and <10% contamination). We found that bacterial community composition may shift in response to MP exposure duration, while other microbial groups remain unchanged. Moreover, differences in metabolic pathways suggest that bacterial taxa may exhibit distinct adaptations to environments enriched in polyethylene and polystyrene. These early findings highlight that MP pollution may have a higher potential for shifts in microbial communities, with possible implications for microbial food web dynamics.

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Fungi in the Fronds: Exploring the Ecology of the Marine Hyphomycete Fungus Paradendryphiella salina in Seaweed Ecosystems.

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The roles of fungi in marine ecosystems are still largely unknown, including the ecology of fungi associated with seaweed. Even though fungi are commonly found in association with seaweeds, we know very little about their interaction. We have established an experimental system to study fungi-seaweed interaction using the common seaweed-associated marine hyphomycete Paradendryphiella salina. The fungus was grown with different seaweed polysaccharides to assess how the type of seaweed might influence adaptability, and also on living and dead seaweed to understand the niche occupied (i.e. biotrophic vs. saprotrophic) and at a range of temperatures. The nutritional content of P. salina and seaweed was assessed, to understand impact on palatability and nutritional content. Grow occurred with a variety of seaweed polysaccharides, but was increased on ulvan and fucoidan compared to others. The fungus demonstrates saprotrophic rather than biotrophic tendencies, proliferating on dead seaweed, with increased growth in warmer temperatures possibly indicating higher growth in summer. Fungus increase seaweed protein content, improving value for both grazing marine organisms in natural ecosystems and potentially humans. This study highlights the response flexibility of marine hyphomycete growth on seaweeds and begins to uncover the role of seaweed-associated marine fungi.

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Anchialine cave microbiomes reveal hidden resistome diversity and mobile genetic elements

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Antibiotic resistance is a pressing global issue, threatening human health and clinical efficacy. Investigating antibiotic resistance genes (ARGs) is crucial not only in medical contexts but also across diverse ecosystems. Yet, ARGs in unique environments like anchialine caves— transitional habitats linking freshwater and seawater systems —are still poorly studied despite their unique ecological features. This study provides the first comprehensive assessment of ARGs and mobile genetic elements in an anchialine cave. Using metagenomic sequencing and complementary computational approaches, we identified a diverse set of ARGs, with bacitracin and multidrug resistance genes being the most prevalent. The microbial community and resistome composition were significantly influenced by the salinity gradient. Notably, we detected novel β-lactamase variants, highlighting the cave's role as a potential reservoir of previously undetected resistance genes. ARGs in the cave demonstrated horizontal transfer potential via plasmids, unveiling ecological implications. These findings underscore the importance of exploring the resistome in unique environments like anchialine caves. The interplay between ARGs and mobile genetic elements provides insights into potential reservoirs and resistance mechanisms in natural ecosystems. This study advances our understanding and emphasizes the need for integrated strategies to address antibiotic resistance across diverse habitats.

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Long-term analysis of antibiotic resistance genes in a coastal Mediterranean microbial community

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The widespread use of antibiotics in human health, agriculture, and aquaculture has, over the past decades, led to the release of resistant bacteria into marine environments, promoting the selection of antibiotic resistance genes (ARGs) in European marine microbial communities. Here, we investigate the temporal dynamics of ARGs in the Blanes Bay Microbial Observatory (BBMO), located in the northwestern Mediterranean Sea, over a 15-year period to identify patterns of variation and temporal trends. ARGs were identified by querying metagenomic sequences from the BBMO (2008–2022; monthly samples) against the Comprehensive Antibiotic Resistance Database (CARD). Furthermore, Mobile Genetic Elements (MGEs) were characterised using the mobileOG-db database. The results suggest a seasonal variation in the presence of ARGs between warm and cold months, based on atmospheric temperature, a pattern that appears to have been disrupted in the years 2020 and 2021, when more constant abundances were observed. Furthermore, the results may suggest a possible correlation between ARGs and mobile elements. This study contributes to a deeper understanding of the long-term temporal variation of ARGs in coastal communities and their relationship with anthropogenic impacts.

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Thermal discharge effects on microbial populations and copepod gut content in the Baltic Sea

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Climate change is expected to increase water temperatures, potentially reshaping microbial communities and their interactions. This study assessed the composition and abundances of microbial populations in two Baltic Sea bays (warm and control bays) from winter to late spring and investigated the gut microbiomes of copepods from the respective bays. The warm bay has experienced approximately 50 years of artificial warming due to thermal discharge from a nuclear power plant, serving as a natural laboratory for studying temperature effects on ecosystems. The bacterial and Eukaryotic community composition differed significantly between the bays. For the phytoplankton, the diatom bloom differed in timing, occurring earlier in the control bay

and later in the warm bay. Abundances of photosynthetic nanoplankton, photosynthetic picoeukaryotes, and phycocyanin-rich picocyanobacteria were generally higher in the control bay, while zooplankton abundances were higher in the warm bay from early March to early April. Compositional differences in the surrounding water influenced the enrichment of photoautotrophic prey in the copepod gut microbiome between the bays. On the other hand, differences in the prokaryotic gut microbiome of copepods between the bays suggest environmentally induced variation in host regulation, along with changes in the composition of rare rather than abundant ASVs present in ambient seawater. Our findings illustrate how warming reshapes coastal microbial communities and highlight the need to assess interactions to predict climate change impacts.

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Impact of intensive coastal aquaculture on the resident microbial communities, the spread of antimicrobial resistances and potential pathogenic bacteria

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Aquaculture can significantly influence aquatic microbial ecosystems, particularly through the massive release of nutrients and pharmaceuticals in coastal waters and sediments. This study examined the microbial communities and their associated antibiotic resistome in water and sediment of three sites in the northern Euboea Peninsula (Greece) characterized by active, fallowed, and absent intensive aquaculture activities. Shotgun and amplicon sequencing revealed that aquatic microbial communities were only slightly affected by aquaculture, with no significant differences in microbiomes or resistomes observed among the sites. In contrast, the sediment communities clearly exhibited the impact of aquaculture, displaying distinct microbial structures, resistomes, and pathobiomes. Resistance genes, along with opportunistic and potentially pathogenic bacteria, were particularly prominent in sediments from sites with active or historical aquaculture, indicating a marked disturbance and a diminished capacity for natural remediation. These results suggest that, while waterborne microbial assemblages recover rapidly or are less affected by intensive aquaculture, sediments act as long-term reservoirs of potential pathogenic bacteria and ARGs. The findings underscore the need to reassess fallowing strategies and highlight the importance of monitoring benthic microbial dynamics as part of sustainable aquaculture management.

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Microbial responses to contaminant exposure and acidification: insights from mesocosm experiments

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Contaminants of Emerging Concern (CECs) are a diverse group of chemicals recognized for their potential toxicity in aquatic environments. Despite their growing prevalence, the impacts of CECs on marine microbial communities remain poorly understood. Here, we present the outcomes of three mesocosm experiments aimed at evaluating the effects of CEC exposure on nearshore microbial communities under conditions of warming and acidification. To this end, a mixture of 12 representative CECs was selected, including plasticizers, industrial chemicals, pesticides, antifungal agents, ultraviolet light stabilizers, and pharmaceuticals such as antibiotics. The experiments include CEC addition, acidification and their combination, conducted sequentially at three different temperature regimes. Each trial involved monitoring of chemical parameters (pH, alkalinity, and chlorophyll a) and biological responses (microbial abundance, bacterial production, and extracellular enzyme activity) during 10 days. Our results indicate a marked metabolic stimulation of bacterial communities following the addition of contaminants, as evidenced by their production and extracellular enzyme activities. In contrast, the abundance of small picoplankton (Synechococcus) was consistently lower in these treatments. Yet, we did not observe a clear effect of acidification among treatments. This work sheds light on the interactive effects of multiple stressors on the abundance and composition of the ocean microbiome.

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Interactive effects of thermal and dietary-induced stress in the physiology of marine heterotrophic and mixotrophic protists

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Although temperature and inorganic nutrient availability are closely linked in marine ecosystems, their combined effects on key functional parameters of plankton have been poorly studied. We investigated the responses of three protists, Oxyrrhis marina (heterotrophic dinoflagellate), Karlodinium armiger (mixotrophic dinoflagellate), and Strombidium arenicola (heterotrophic ciliate), to chronic warming and acute heat stress (both +3°C), under nitrogen (N) or phosphorus (P) limitation. Protists consumed prey with either balanced (nutrient-replete) or imbalanced (nutrient-limited) cellular stoichiometry. Across all species, physiological rates increased under both warming treatments when prey were nutrient-balanced. Under N limitation, chronic warming increased ingestion and growth rates in heterotrophs; only K. armiger benefited from acute heat stress boosting its growth rate and doubling efficiencies. In contrast, P limitation caused lower growth rates in heterotrophs, with S. arenicola being particularly sensitive, contrary to K. armiger, that showed similar growth rates to nutrient-balanced conditions. Cell volume decreased under both warming treatments in nutrient-replete conditions; under nutrient limitation, some differences arose among protists. Under P limitation, the protists C:P and N:P ratios increased; however, the extent to which temperature and nutrient stress affected stoichiometric ratios overall differed among species. This study highlights the ecological advantage of mixotrophy over heterotrophy under combined thermal and nutrient stress (especially P limitation).

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Exploring the ARG-senal of select Pseudomonas strains isolated from freshwater beach sand

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The transfer and spread of antimicrobial resistance genes (ARGs) from the environment to clinical settings has been cause of increasing concern. Recent work has highlighted the role of mobile genetic elements (MGEs) in the spread of ARGs and

their ties to phylogenic and ecological distribution. In this study, we isolated 200 putative Pseudomonas strains from sand samples collected along the beaches of Lake Maggiore, identified through 16S sequencing and selected nine Pseudomonas spp strains for whole genomes sequencing. Several common ARGs were identified, including sul1, CRP, mexF, mexW, and oqxB and species-specific PAM-MBL variants, and several MGE markers like Type IV Secretion System genes (T4SS) and intI1 integron intregrases. By taking a deeper look into these isolates, and exploiting well-known microbial taxa, their phylogeny, genotypes, and antimicrobial arsenal, we could evaluate the presence of mobile genetic elements (MGEs) and ARGs and compare genomic findings with phenotypic resistance profiles that are often missed with a metagenomic focus.

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Exploring microbial adaptations to seasonal anoxia in the coastal Baltic Sea

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Many coastal ecosystems experience a higher frequency of seasonally occurring anoxia, which impacts marine nitrogen and carbon cycling. We investigated microbial adaptations to seasonal anoxia at the coastal Baltic Sea time-series station Boknis Eck. During seasonal anoxia in October and oxic conditions in December and January, we measured nitrate reduction rates, heterotrophic production, and dark carbon fixation rates at two contrasting depths under in situ O2 conditions and experimentally-induced anoxia. Nitrate reduction rates suggested a widespread potential for nitrate respiration under induced anoxia at both depths. The observed decrease in heterotrophic production at anoxic compared to oxic conditions was less pronounced at 25 m depth (85.11 %, seasonally anoxic) than at 15 m (171.19 %, year-round oxic) in January, indicating a potential microbial adaptation to seasonally fluctuating oxygen levels. Dark carbon fixation rates exceeded heterotrophic production at both depths, ranging from 7.94 to 101.69 nM d-1. During seasonal anoxia, nitrate-reducing, sulfate-reducing and sulfur-oxidizing bacteria of the families Vibrionaceae, Desulfatiglandaceae, and Thioglobaceae were primarily present at 25 m. Our results suggest that seasonal anoxia promotes the activities of anaerobic taxa in bottom waters, yet the potential for anaerobic metabolisms can be present in microbial communities throughout the water column.

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Antimicrobial Resistance by metaGenomics Overview in a Sewage system (ARGOS)

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Antimicrobial resistance (AMR) poses a significant threat to global public health, yet detailed knowledge of AMR dynamics within Spanish sewage networks remains limited. Here, we present the ARGOS (Antimicrobial Resistance by metaGenomics Overview in a Sewage system) project—the first large-scale metagenomic investigation of Madrid´s sewage network, managed by Canal de Isabel II. This study will apply high-throughput sequencing and advanced molecular approaches to characterize the resistome across multiple sampling stations of the sewage infrastructure. By quantifying the diversity and abundance of antimicrobial resistance genes (ARGs), identifying AMR hotspots, and assessing the effectiveness of wastewater treatment plants, we aim to build a comprehensive map of resistance dissemination across the network. We hypothesize that the resistome of most sampling stations will be similar, resembling profiles observed in other European and North American cities. Nonetheless, differences may emerge among stations due to factors including demographic parameters, intensive antibiotic use (e.g., hospitals, farms), or proximity to wastewater treatment plants. The insights generated by ARGOS will inform targeted AMR surveillance strategies and contribute essential baseline data for policy development aimed at mitigating the environmental spread of AMR. Ultimately, this work will position Madrid as a national and international reference site for resistome monitoring.

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Microbial diversity in Irish marine coastal waters and sediments with contrasting pollution

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The rise in contamination of marine environments due to anthropogenic activities presents an escalating problem affecting the biodiversity of the aquatic systems, including microbial communities. We analyzed microbial diversity along the Irish coast (surface seawater and sediments). The sites ranged from pristine to polluted stations (near estuaries and ports), where the concentration of certain Contaminants of

Emerging Concern (CECs) was higher. We analyzed variations in the composition of the microbial community through metabarcoding. Observed patterns were related to the concentrations of a suite of contaminants. Our results show a general decrease in diversity and a selection of particular taxa in contaminated sites. Specific taxa likely thrive in polluted systems by replacing more sensitive taxa. Our work highlights the ecological shifts driven by pollution and emphasizes the broader environmental consequences of human activity along the coastline. Understanding these microbial responses is essential for informing conservation strategies and managing the health of marine ecosystems.

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Land use impacts coastal porewater microbiota and submarine groundwater discharge effects in a coastal lagoon

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Groundwater from coastal aquifers flows into coastal waters as Submarine Groundwater Discharge (SGD), constituting a significant source of nutrients and solutes for marine environments. Changes in coastal groundwater systems caused by land use and anthropogenic activities (e.g., nutrient or metal contamination, microbial pollution) can alter the structure and function of groundwater microbial communities, thus shaping the chemical composition of SGD and its impact on coastal ecosystems. Yet, how human-derived activities impact groundwater communities and SGD effects on marine systems is still unknown. We studied the composition and activity of porewater prokaryotic communities at four beach sites around the Mar Menor coastal lagoon, each influenced by different land uses, and evaluated the response of lagoon microbial communities to SGD through in situ experimental incubations. Our findings revealed strong site-specificity of porewater communities across the four sites studied, shaped by different environmental conditions. Land use influenced porewater microbial composition to some extent, with metal-associated bacteria being more abundant at the mining-influenced site or wastewater-related bacteria at the urban-impacted site. We also found site-specific effects of SGD on lagoon communities likely caused by differences in local discharge rates and chemical composition of groundwater. Overall, our results point to localized responses of groundwater ecosystems to climatic and anthropogenic stressors.

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Building coastal ecological baselines: a time series approach to phytoplankton and environmental monitoring in the Farsund archipelago

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Coastal ecosystems are increasingly impacted by the combined pressures of anthropogenic activities and climatic change. In particular, the coastal regions of the Skagerrak and North Sea are influenced by densely populated catchments that contribute to eutrophication, brownification and pollution. In 2019, the University of Agder (UiA) established a long-term coastal monitoring station in the Farsund archipelago (South Norway) with the aim to improve our understanding of how coastal planktonic communities respond to both natural variability and anthropogenic pressures. Monthly sampling is conducted to measure water column profiles of temperature, salinity, algal fluorescence and oxygen, along with collection of water samples for nutrient concentrations, algal biomass, and the composition of phytoplankton and zooplankton communities. The key research questions include how nutrient inputs from land, particularly nitrogen and phosphorus, affect microbial eukaryotes composition and bloom development. The project also investigates the ecological consequences of coastal darkening, a growing phenomenon in Norwegian fjords linked to increased terrestrial runoff. The time series will be presented with the aim to provide a valuable framework for investigating long-term ecological trends related to climate change, planktonic community composition, coastal darkening and eutrophication in coastal ecosystems.

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Exploring the plastic degradation potential of open ocean fungi

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Plastic pollution in marine environments presents a persistent ecological threat, with synthetic polymers such as polystyrene and polyurethane contributing significantly to long-term contamination. Marine fungi are increasingly recognized for their ability to interact with synthetic pollutants, but little is known about their plastic-degrading potential, particularly concerning open ocean fungi. Therefore, the current study aims to identify marine fungal strains capable of utilizing polystyrene and polyurethane. A collection of 260 marine fungi was isolated from the open-ocean waters (Arctic,

Atlantic, and Antarctic Oceans). This collection was initially screened on artificial seawater agar media supplemented with polystyrene and polyurethane as sole carbon sources. Fungal isolates exhibiting enhanced growth relative to controls were selected as potential degraders. These candidates were subsequently subjected to a secondary screening on freshwater-based media with the same plastic substrates to evaluate their degradative activity under low salinity conditions. Isolates showing good growth on polystyrene and a clear halo on polyurethane media were selected. A total of 14 fungal strains, including both filamentous fungi and yeasts, demonstrated evidence of plastic utilization with salinity tolerance by pelagic open-ocean fungi. These findings expand our understanding of the ecological role of marine fungi in biotechnological applications for plastic waste mitigation.

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Microbial communities and traits in the bioplastisphere of European seas

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Conventional fossil fuel plastics take centuries to decompose in the ocean, and even bio-based plastics may do so very slowly. We studied the biodegradation of six bio-based plastics (CA, CAP, PBS, PHB, PA and PE) in four European seas (Baltic, Mediterranean, Irish and Norwegian) under natural and standardized conditions (multi-year in situ mesocosm and standardised in vitro incubations, respectively). Only CA, PBS and PHB degraded substantially, with variable rates between materials, sites and incubation conditions – up to 27.8% in situ weight attrition (CA, Baltic Sea) and 56.0% in vitro C loss (PBS, Baltic Sea). Metagenome and metatranscriptome analysis of Baltic Sea data revealed that, despite great differences in polymer properties, the three biodegradable bioplastics selected for similar microbial communities (the bioplastisphere), that differed from those in the water column and in the non-biodegradable materials. Population-level metabolic reconstruction revealed several traits that were more prevalent in the bioplastisphere, including an increased potential for denitrification. Our results show that not all bio-based plastics are biodegradable in the marine environment and some may degrade only in specific conditions. Ongoing analysis will disentangle the effects of environmental constraints on plastic biodegradation and characterise the taxonomic and functional diversity of the bioplastisphere.

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Uncovering the Microbiome of Salpa thompsoni in the Context of Global Change in the Southern Ocean

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In recent decades, Global Change has substantially altered Antarctic ecosystems, with one of the most notable effects being the massive proliferation of Salpa thompsoni. The rapid expansion of this gelatinous marine animal is leading to relevant alterations in trophic networks and marine biogeochemical cycles in the Southern Ocean. As microbes are key in marine systems, conducting a detailed analysis of the salp microbiome contributes to understand more about the role of salps in the Southern Ocean. In this study, we described the microbiome associated with S. thompsoni, using high-throughput sequencing of 16S and 18S rRNA gene amplicons, which allows us to describe the microbial diversity of both prokaryotes and eukaryotes. Individuals were collected from the Western Antarctic Peninsula, in Maxwell Bay and in South Bay. Our results revealed that the bacterial classes Gammaproteobacteria, Alphaproteobacteria, and Bacteroidia dominate the prokaryotic communities, while the class Gregarinomorphea predominates among the eukaryotic ones. Overall, the salp microbiome differed between locations and varied according to body size and specific body parts. This study provides for the first time a detailed analysis of the salp microbiome, contributing to unveil the role of microbes in the dynamic of the Antarctic marine ecosystem.

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The role of microbial genomes as centiles of water quality on a semi-enclosed bay in northwest Spain

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With the advancement of new sequencing technologies, researchers have gained new and valuable insights into the uncultured microbial majority present in aquatic

ecosystems. Moreover, the microbial genomes recovered directly from the environment can be used as centiles of water quality and human impact. With the goal of investigating understudied aquatic ecosystems and contribute to environmental monitoring, our work focuses on the microbial diversity of a semi-enclosed bay in the city of Vigo (northwest of Spain), a water body vulnerable to the anthropogenic impact of a large city. In total, eight water samples were collected at various depths across three different locations of the sea inlet. After shotgun sequencing, we reconstructed 199 metagenome-assembled genomes (MAGs) of medium-to-high quality, and were later grouped into 80 species-level clusters according to an average nucleotide identity (ANI) threshold >95%. After mapping the species-clusters representative genomes against the metagenomic reads, we found that they accrue approximately 35% of the mapped reads, and are mainly represented by the bacterial orders Flavobacteriales and Pseudomonadales, and the archaeal order Poseidoniales. Future work will include an assessment of antimicrobial resistance genes and an exploration of the microbial communities across depth and environmental profiles (including temperature, dissolved oxygen and salinity).

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Ecological Stability in Tropical Freshwater Microbial Communities Under Climate Change

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Climate change is altering freshwater ecosystems, but the stability of tropical microbial communities remains poorly understood. This study investigated these communities resistance, resilience, and vulnerability to climate variability, focusing on structural and functional responses. A 160-day mesocosm experiment was conducted with constant and variable warming treatments (+4°C), nutrient enrichment, and reduced detritus inputs. Microbial community structure (via 18S rRNA gene sequencing) and ecosystem function were assessed across different ecological levels. Phototrophic communities exhibited distinct responses to the treatments. Under ambient conditions, significant community shifts were observed early (days 7–80, $R^2 = 0.381$, $p < 0.001$). Constant warming delayed these shifts, appearing only later in the experiment (day 80 onwards). Variable warming, however, produced weaker phototrophic responses until a significant shift at day 160 ($R^2 = 0.228$, $p < 0.008$). Mixotrophic, heterotrophic, and parasitic groups showed delayed and more pronounced responses to variable warming (days 40–120, $p < 0.05$, R^2 ranging from 0.175 to 0.203), with inconsistent responses under constant warming. Ambient conditions led to less pronounced and intermittent shifts in these groups. These findings show that warming regimes distinctly shape microbial community structure and trophic dynamics in tropical freshwater ecosystems. Differential responses among functional groups highlight the need for multiscale ecological stability models to predict climate change impacts more accurately.

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High potential for methylphosphonate utilization associated with the Mediterranean seagrass Posidonia oceanica

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Large swaths of the world's coastlines are colonized by seagrasses, marine plants that constitute an important blue carbon ecosystem. Interestingly, seagrass meadows are often found in oligotrophic waters, raising the question of how seagrasses obtain nutrients like phosphorus (P) necessary for their growth. The phosphorus-depleted Mediterranean Sea hosts microbial communities capable of degrading organic P in the form of methylphosphonate (MPn). This process results in the release of methane, a potent greenhouse gas. In this study, we examined the potential of the Mediterranean seagrass Posidonia oceanica and its leaf-associated microbiome to use MPn as an alternative P source. Incubation experiments with 13C-labeled MPn revealed substantial methane production from seagrass leaves (average 41 nmol gDW^{-1} d^{-1}), indicating active MPn utilization. Methane production was highest in the fall, likely due to increased biofilm coverage, and significantly decreased with added inorganic phosphate, suggesting MPn use is phosphate-regulated. Molecular analysis showed that about 40% of the leaf biofilm community encoded the phnJ gene, essential for the C–P lyase pathway involved in MPn degradation. These findings suggest the P. oceanica microbiome plays a key role in organic phosphorus acquisition, potentially supporting seagrass persistence in nutrient-poor environments while contributing to marine methane emissions.

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Bioindicators and Ecological Indicators of Climate Change in Tropical Freshwater Systems Revealed by Microbial Co-occurrence Networks

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Climate change is a major global challenge, driving significant changes across multiple ecosystem levels. Microbial communities in tropical freshwater systems remain understudied, despite their key role in ecosystem functioning and sensitivity to environmental change. In this study, we evaluated the effects of warming on bacterial

communities from eight geothermal streams in Mato Grosso state, Brazil, using a space-for-time substitution (SFTS) approach. Water and sediment samples were analyzed through 16S rRNA gene amplicon sequencing to assess changes in diversity, taxonomic composition, and microbial co-occurrence networks. Alpha diversity (Hill numbers) decreased with increasing temperature, while beta diversity patterns indicated compositional homogenization under thermophilic conditions. LEfSe analysis identified thermotolerant Cyanobacteria, particularly Fischerella muscicola and Elainella saxicola, as potential bioindicators of warming. Co-occurrence networks showed temperature-driven structural changes: network density increased, and modularity decreased, suggesting more interconnected and less compartmentalized communities. Despite these structural shifts, network stability metrics (robustness and vulnerability) remained unaffected. Together, these findings highlight the value of microbial co-occurrence network topology as ecological indicators, alongside bioindicators, as sensitive tools for detecting early signs of climate change impacts in tropical freshwater ecosystems.

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Gelatinous zooplankton blooms affect the horizontal transmission of plasmid-borne antimicrobial resistance genes in marine environments

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Marine gelatinous zooplankton (GZ) often generate massive blooms, causing severe environmental impact. When these blooms collapse, their decaying biomass provides a substantial yet underrecognized source of labile organic matter (GZ-OM), offering nutrients and surfaces for microbial colonisation and interaction. Recent metagenomic analysis of GZ-OM degrading communities revealed high taxonomic diversity and up to fourfold enrichment of antimicrobial resistance genes (ARGs) and transferable plasmids compared to ambient seawater. This raises concerns about GZ-OM as a hotspot for ARG dissemination in marine environments. However, it remains unclear whether this ARG enrichment stems from selective colonisation by resistant microbes or increased plasmid transfer. To investigate if plasmid transfer indeed plays a role, we designed a plasmid transfer assay using marine bacteria. Vibrio and Pseudoalteromonas strains were isolated from Mnemiopsis leidyi OM, and the broad-host-range resistance plasmid pKJK5::gfp was introduced. Conjugation experiments in marine broth with and without GZ-OM assessed horizontal gene transfer potential. Most GZ-associated bacterial isolates were capable of acquiring plasmids carrying ARGs, with GZ-OM clearly affecting plasmid transfer rates between them. As GZ blooms become more frequent with climate change, their potential role in ARG transfer and plasmid dissemination in marine systems may have important implications for animal and human health.

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Biosurfactant production by deep-sea bacteria under lead and arsenic stress

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Extreme ecosystems are exposed to high environmental variability, making them key sites for exploring microbial adaptation to global change. This study investigated the ability of heavy metal-tolerant marine bacteria, namely Burkholderia and Bacillus species isolated from hydrothermal vents and Photobacterium species from the light organ of the deep-sea fish Hymenocephalus italicus, to produce biosurfactants, metal-chelating molecules with heavy metal bioremediation potential. Firstly, strains were cultured in deep-well plates in mineral medium supplemented with glycerol (1%, v/v) and salts of lead, cadmium, arsenic, and mercury (100 and 5000 ppm). Biosurfactant production was evaluated by standard screening tests, including emulsification index, surface tension measurement, methylene blue agar-CTAB, and hemolytic activity assays. Photobacterium isolates showed the best emulsifying activity at 100 ppm of lead and arsenic and were upscaled to 100 ml cultures to monitor biosurfactant production at 48-hour intervals. Emulsification activity peaked after the exponential growth phase, ranging from 20% to 33%. Crude extracts were preliminarily characterized by thin-layer chromatography. A final 500 ml culture was performed to improve the biosurfactant yield and for in-depth chemical characterization. Biosurfactant production by Photobacterium strains may represent an adaptive response to heavy metal accumulation in their deep-sea fish host, highlighting their ecological relevance and bioremediation potential.

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Bacterial communities under heatwave stress in Mediterranean freshwater and coastal systems

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Mediterranean freshwater and coastal ecosystems are highly vulnerable to increasingly frequent heatwaves, yet their impact on microbial communities remains poorly understood. In this study, we investigated bacterial community responses under natural and simulated thermal stress in two diverse aquatic ecosystems in Sardinia (Western Mediterranean): the artificial Bidighinzu Lake and the coastal Cabras Lagoon. Seasonal field samplings were conducted from January to December 2024, while controlled laboratory experiments were performed in July 2024 on both ecosystems to simulate a summer heatwave (+5 °C, 14 days). Field water samples collected at specific depths and stations were fractionated by sequential filtration (20 µm to 0.22 µm), while samples collected in the laboratory at different experimental times were 0.22 µm filtered. DNA was extracted from each planktonic fraction for 16S rRNA gene amplicon sequencing. Sequences were processed with DADA2 and taxonomic assignment performed using the SILVA database. Analyses are ongoing, but we expect that elevated temperatures will lead to a decrease in bacterial diversity and a shift toward potentially pathogenic and/or faecal bacteria. This integrated molecular and ecological approach, combining long-term monitoring with experimental simulation, will enhance our understanding of how climate change may reshape microbial assemblages and affect aquatic ecosystems and public health in Mediterranean waters.

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Antimicrobial resistance in the microbiome of marine animals of different trophic level.

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Antimicrobial resistance genes (AMRGs) pose a significant threat to public health. Recent findings indicate that wild aquatic organism, like oysters and fish, harbor high

levels of AMRGs in their gut microbiome. While the molecular mechanisms of resistance development are well documented, the role of marine food webs in AMRG dispersion, accumulation and diversification remains largely unexplored. Specifically, we study bioaccumulation through the food chain and the gut microbiome acting as vectors of AMRGs. This research employs several transects from polluted to less polluted marine environments, using gut samples from blue mussels, cod, and seals, incorporating metagenomics, and stable isotope analysis. Additionally, 20-year-long time series will provide insights into temporal changes in animal associated microbiomes and AMRGs. We anticipate discovering higher abundance and diversity of AMRGs near anthropogenic sources and notable shifts in their distribution over the two decades. We will also explore the diversity of the plasmidome, and its ecological role for the microbiome and antimicrobial resistance spread. Understanding AMRGs in marine food webs is vital for enhancing a "One Health" perspective, providing critical insights into antimicrobial resistance development.

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Allelic variations and Ggne cluster modularity act as nonlinear bottlenecks for cholera emergence

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The underlying factors that lead to specific strains within a species to emerge as human pathogens remain enigmatic. The diarrheal disease cholera is caused by strains from a phylogenetically confined group within the Vibrio cholerae species, the pandemic cholera group (PCG), making it an ideal model system to tackle this phenomenon. Comprehensive analyses of over 1,840 V. cholerae genomes, reveal that the species consists of eleven groups, with the PCG belonging to the largest and located within a lineage shared with environmental strains. This hierarchical classification provided us with a framework to unravel the ecoevolutionary dynamics of the genetic determinants associated with the emergence of toxigenic V. cholerae. Our analyses indicate that this phenomenon is largely dependent on the acquisition of unique modular gene clusters and allelic variations that confer a competitive advantage during intestinal colonization. We determined that certain PCG-associated alleles are essential for successful colonization whereas others provide a nonlinear competitive advantage, acting as a critical bottleneck that clarifies the isolated emergence of PCG. Our study uncovers the evolutionary roots of toxigenic V. cholerae, offering a tractable approach for investigating the emergence of pathogenic clones within an environmental population.

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Multi-Scale Patterns of Bacterial Diversity Across Compartments in Remote Arctic Lakes of East Greenland

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Lakes are complex ecosystems where aerosols, water, and sediments form interconnected compartments, each hosting microbial communities shaped by distinct environmental conditions. These habitats are often studied in isolation, limiting our understanding of microbial dynamics, transport, and diversity across spatial scales. This study explores bacterial diversity at multiple scales—within and between lake compartments (aerosols, ice cover, water, and sediments) and across a latitudinal gradient—using 12 remote glacial lakes in East Greenland. During a two-week campaign in August 2023, we conducted simultaneous sampling and environmental monitoring, followed by 16S rRNA gene amplicon sequencing. Bacterial abundances peaked in the ice cover (up to 10^6 genome copies/L), associated with elevated nitrate and ammonia concentrations, while lake water showed the highest taxonomic richness (Chao1 diversity). Beta diversity analyses revealed distinct bacterial communities in each compartment, though shared genera suggest possible microbial transport between environments. Latitudinal differences in community composition aligned with spatial heterogeneity in watershed geology and vegetation, indicating that catchment-scale features influence microbial patterns. These findings highlight the ecological role of ice cover, landscape heterogeneity, and cross-compartmental interactions, offering new insights into microbial biogeography in rapidly changing Arctic lake systems.

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At the beach with beta-lactamases: Chitinophagaceae as important carriers of antibiotic resistance genes in beach sand

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Freshwater environments serve as both reservoirs and conduits for antibiotic resistance genes. On one hand, these habitats naturally harbour a diverse genetic pool of resistance traits, which can evolve to counteract both current and future antibiotics. On the other hand, human activities introduce resistant bacteria into water systems, leading to genetic pollution from wastewater effluents. In fact, it is often unclear whether specific genes found in the environment are functional resistance genes or part of the core genome of bacteria. In this study we took a closer look at beta-lactamase genes (bla) from freshwater beach sand. We studied their distribution in the sand and identified the most common genes using metagenomics. We then assembled metagenome assembled genomes and found that the typical freshwater family

Chitinophagaceae was the main carrier of bla genes as well as arsenic resistance genes. Focusing on bla genes, in a further step we compared the sequences that we found in the sediments, to genes in published genomes and found that these genes were fairly common in this family. Using phylogenies, we then tried to reconstruct the evolutionary trajectories of various b, both in the environment and clinic.

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Large diversity in the O-chain biosynthetic cluster within populations of Pelagibacterales

Jose M. Haro-Moreno [1], Mario López-Pérez [1], Carmen Molina-Pardines [1], Francisco Rodriguez-Valera [1]

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Genomic diversity in prokaryotic species is due to the existence of extensive pangenomes, allowing different gene complements to be drawn depending on the strain. Here, we have studied the diversity of the O-chain polysaccharide biosynthesis cluster (OBC) in marine bacterial order Pelagibacterales as a proxy to measure such genetic diversity in a single population. The study of SAGs from the whole order found a pattern similar to that of other well-studied microbes, such as the Enterobacteriales or Alteromonas, where distinct OBCs represent strains containing different gene pools. We found that most OBC sharing happened among individuals of the same clonal frame (>99% average nucleotide identity). Moreover, given the parsimonious way this cluster changes, the diversity of the OBCs can be extrapolated to the size of the population’s pangenome. Through long-read metagenomics, we could detect 380 different OBCs at a single Mediterranean sampling site. Within a single population (single species and sample) of the endemic Ia.3/VII (gMED) genomospecies, we identified 158 OBCs, of which 130 were unique. These findings suggest that the gene pool within a single population might be substantial. While this figure is large, it aligns with the complexity of the dissolved organic matter that these organisms can potentially degrade.

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Comparative genomic insights into intra-clade diversity of SAR86 and SAR116

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The SAR86 and SAR116 clades are key lineages of marine bacterioplankton, known for their global distribution and ecological importance. Although their genomic features have been extensively studied through metagenomic and single-cell approaches, intra-clade diversity and subgroup-specific characteristics remain less explored. In this

study, we analyzed isolate libraries derived from East Sea and Garorim Bay seawater samples in Korea. Phylogenetic reconstruction using 16S rRNA gene sequences revealed multiple subclusters within each clade, suggesting genomic heterogeneity. To further investigate this, we selected 23 SAR86 and 16 SAR116 isolates representing distinct subgroups. Genomic DNA was obtained using multiple displacement amplification (MDA), enabling draft genome recovery for comparative analyses. Ongoing genome-level comparisons aim to reveal metabolic traits and adaptive strategies specific to each subgroup. This study provides new insights into the fine-scale diversity and ecological differentiation within SAR86 and SAR116 clades, based on MDA genomes directly derived from cultured environmental isolates.

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Extensive paralogism in the environmental pangenome: a key factor in the ecological success of natural SAR11 populations.

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SAR11 clade members dominate the oceanic microbiome. Despite their abundance, difficulties in recovering their full genetic diversity have hindered understanding of the eco-evolutionary mechanisms underlying intra-species variation. Here, we combined single-amplified genomes (SAGs) and long-read metagenomics to explore genomic diversity of natural populations within SAR11 genomospecies Ia.3/VII, the dominant group in the Mediterranean Sea. Reconstruction of the first complete genome of this genomospecies revealed that the core genome accounted for ~81%, while the flexible fraction was concentrated in small regions, typically single-gene islands at conserved positions which facilitates their exchange through homologous recombination. Using the flanking genes, we recovered the full diversity of these genomic islands by integrating SAGs and long-read data. Each variable region was associated with a specific set of genes that, although divergent, maintained equivalent biological functions. The environmental pangenome is large and enriched in genes involved in nutrient transport, as well as cell wall synthesis and modification, showing an extremely high degree of functional redundancy in the flexible genome (i.e. paralogisms). At the population level, this genomic architecture fosters polyclonality, preserving genetic variation within the population. This, in turn, mitigates intraspecific competition and enables the population to thrive under variable environmental conditions and selective pressures.

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Comparative genomics of coral photosymbionts and their free-living relatives

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Photosynthetic dinoflagellates are globally important primary producers that occupy a wide range of ecological niches. The dinoflagellate order Suessiales includes the family Symbiodiniaceae, commonly known as "zooxanthellae", which form intracellular associations as photosymbionts with corals and other marine invertebrates or protist hosts. These symbioses are vital for coral reef ecosystems and have been extensively studied. However, numerous Symbiodiniaceae genera also exhibit transient or exclusively free-living lifestyles, yet remain understudied, limiting our understanding of the group's ecological and evolutionary diversity. Despite the ubiquity of dinoflagellate symbioses, the evolutionary origins and molecular mechanisms underpinning these associations are still poorly understood. Genomic studies are key to tackle these questions, but they are often challenging to develop in dinoflagellates due to their large and complex genomes, resulting in limited genomic data for Suessiales. To address these knowledge gaps, we generated genomes and transcriptomes for both photosymbiotic dinoflagellates and their free-living relatives, allowing us to perform phylogenomic and comparative genomic analyses. This project aims to: (1) expand the limited genomic resources available for Suessiales; (2) reconstruct the evolutionary history of Suessiales, resolving the relationships between photosymbiotic and free-living taxa; and (3) elucidate the genomic signatures that enable zooxanthellae to establish diverse associations with marine hosts.

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Resolving the individuality of environmental bacterial cells: profiling freshwater SAR11 genomic hypervariable region through single-cell genomics

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The order Pelagibacterales (SAR11) consists of aquatic, free-living bacteria that are ubiquitous in freshwater and marine ecosystems. SAR11 genomes contain a hypervariable region (denoted as HVR2) with high levels of intraspecific diversity. While HVR2 may underpin SAR11's ubiquity, its pronounced sequence diversity hampers accurate reconstruction from metagenomic data. Additionally, freshwater SAR11 HVR2

diversity is less studied than marine ones. To elucidate this diversity and the mechanisms governing it, we applied single-cell genomics to SAR11 populations collected from different lakes (Biwa and Zurich) and years (2022 and 2024). In total, 196 complete HVR2s were recovered with an average length of 46 kb, consistent with the reported sizes of marine SAR11 HVR2. Singletons constituted at least 80% of the HVR2s at 96-100% average nucleotide identity (ANI) clustering thresholds, highlighting pervasive microdiversity. Yet, the remaining 20% of HVR2s were grouped into non-singleton clusters at 99% ANI with the largest cluster containing 7 members. Furthermore, we discovered nearly identical HVR2 pairs from Biwa in 2022 and 2024. More notably, a pair from Biwa and Zurich retained more than 90% amino acid identity and highly conserved synteny. Our results present the paradox that extremely diversified genomic regions can be highly conserved at the intercontinental scale.

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Taxonomic and Molecular Insights into Coccolithophore (Haptophyta) Community in the Coastal Ecosystem of the Northern Adriatic Sea

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Coccolithophores, a group of calcifying phytoplankton, play an important role in marine ecosystems and biogeochemical cycles. Yet, their diversity and community dynamics in coastal ecosystems are still insufficiently explored, especially through the integration of morphological and molecular approaches. This study investigates coccolithophore community in the semi-enclosed and ecologically sensitive Gulf of Trieste (northern Adriatic Sea). From October 2022 to September 2024, water samples were collected twice a month at two depths (surface and 15 m) at LTER site (approximately 1.2 Nautical Miles offshore, depth 22 m). Species were identified using light and scanning electron microscopy. Concurrently, molecular analyses targeting the 18S rRNA gene were conducted to reveal cryptic diversity and complement the morphological data. Preliminary results indicate a diverse community dominated by Gephyrocapsa huxley (formerly known as Emiliania huxleyi), with notable differences between the surface and deeper layers. The molecular findings revealed several previously unreported ASVs, highlighting considerable genetic diversity. These results emphasize the value of combining classical taxonomy with molecular tools for a more comprehensive understanding of phytoplankton communities. A deeper understanding of coccolithophore diversity in this region contributes to the comprehension of ecosystem function and the potential impact of climate-induced changes in the Mediterranean Sea.

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Microbiome reconstruction from Santa Pola crystallizer ponds introducing long read metagenomic sequencing

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The Santa Pola crystallizer solar ponds (Alicante, Spain) are hypersaline ecosystems hosting unique extremophile microbial communities, predominantly the archaeon Haloquadratum walsbyi. In this study, we applied third-generation metagenomics, combined with second-generation sequencing (hybrid approach), to analyze samples collected in 2023–2024 from ponds with 32% salinity. This strategy enabled improved genome continuity and resolution of complex genomic regions, essential for studying extremophiles. We reconstructed 120 high-quality metagenome-assembled genomes (MAGs) (>50% completeness, <5% contamination), 75% of which included at least one 16S rRNA gene, and six were recovered as complete, single-contig genomes. According to GTDB taxonomy, 78% of the MAGs correspond to putative novel species, several affiliated with the genus Nanosalinarum. The use of long-read data revealed intraspecies genomic diversity, structural variants, and allowed direct comparisons of metabolic potential across microorganisms with contrasting evolutionary strategies, such as H. walsbyi and Nanosalinarum-related genomes. These findings underscore the power of third-generation metagenomics to uncover the hidden taxonomic, evolutionary, and functional diversity of microbial life in extreme, hypersaline environments.

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Phytoplankton-associated bacteria mitigate sensitivity to diuron and copper in green flagellate and diatom isolated from contaminated natural costal waters

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In marine coastal ecosystems the role of bacteria associated with phytoplankton under toxic stress remains underexplored. Heterotrophic bacteria, with their ability to degrade, metabolize, and immobilize a variety of organic and inorganic compounds, may play a

crucial ecological role in protecting or facilitating phytoplankton communities, particularly in polluted environments. To assess whether bacteria mitigate toxics stress for the associated primary producers, controlled experiments were conducted using natural microbial communities sampled from Étang de l'Or, a heavily contaminated lagoonal ecosystem in France. A green-flagellate (Volvocales-like) and a diatom (Entomoneis sp.) were isolated from this field and axenised. The growth of xenic and axenic microalgal strains was measured after 72h of exposure to Diuron and Copper gradients. The toxics' concentration driving algal growth inhibition at 50% of controls (EC50) was assessed for xenic and axenic strains. The EC50 was 50% lower for the axenic green-flagellate exposed to Diuron and to Copper than for the xenic strain. Similarly, the EC50 for the axenic diatom to Copper was 50% lower than for xenic strain. These results, indicating a significantly greater toxics' sensitivity for phytoplanktonic axenized strains, support the hypothesis that algal-associated bacteria play a protective role on microalgal strain when submitted to toxic stress.

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PFAS exposure induces alterations in microbial communities associated with two key Arctic and sub-Arctic marine diatom species

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Perfluoroalkyl compounds (PFAS) are anthropogenic pollutants, also present in Arctic environments, where they cause toxic effects in resident biota, including marine diatoms, key components in global carbon cycling. We examined changes in diatom-associated microbial communities resulting from exposure to a mixture of six types of PFAS for 28 days at 900 pg/L. Metabarcoding was conducted on cultures of the benthic and pelagic diatoms Cylindrotheca closterium (CC) and Coscinodiscus granii (CG), respectively. Alpha diversity indices showed that PFAS exposure increased microbial richness and diversity in the benthic diatom (Chao1: 501.5, Shannon: 4.798) compared to controls (Chao1: 408.2, Shannon: 4.400). Also, the pelagic diatom showed increased richness (Chao1: 377.6 vs 335.5), but a slight decline in diversity (Shannon: 3.870 vs 3.397). The relative abundance of Alteromonas and Spingorhabdus was lower in both species, while Antarctobacter, Arenibacter, Ponticoccus, SM1A02, and Roseovarius increased in PFAS-treated benthic CC. Opportunistic genera like Muricauda and Arenibacter displayed adaptive behavior, remaining similar and abundant in pelagic CG, while SM1A02 and Oceanicaulis became enriched under PFAS stress in CG. SM1A02 was enriched by PFAS-treatment, indicating functional plasticity and a potential role in detoxification, nutrient remineralization, and carbon cycling. The genera Alteromonas, Sphingorhabdus, and Antarctobacter showed consistent reductions in both diatoms under PFAS exposure, indicating sensitivity. The microbial composition in the tested diatoms reflects a dynamic restructuring of the phycosphere microbiome under PFAS exposure.

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Marine microbial interactions are reflected in molecular composition of dissolved organic matter

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Heterotrophic bacteria contribute greatly to the turnover of marine organic matter via the microbial loop. Specifically in surface waters, a large quantity of labile dissolved organic matter is rapidly consumed by bacteria. Yet, it is still largely unknown how microbial interactions between heterotrophic bacteria impact the dissolved organic matter pool. To investigate this, we used a reductionist, full factory experiment with four isolates of the marine Roseobacter group using all possible co-culture combinations. Genomic markup of the bacteria affected substrate diversification and growth in monocultures. Co-cultures produced dissolved organic matter that were specific to the respective consortium and produced many novel molecular formulas. In contrast, consumed molecules were utilized by a majority of consortia and bacteria that were low abundant towards the end of the experiment contributed disproportionally to dissolved organic matter processing. Hence, in our setup, organic matter recalcitrance emerged as a property of community metabolism. Our findings highlight the importance of microbial interactions for the molecular diversity, composition and persistence of dissolved organic matter in the ocean.

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Insights on the different fieldworks from the SUMMIT project, a European Research Council Advanced Grant

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In 2019 the Simolab group, led by Dr. Rafel Simó at the Institute of Marine Sciences (ICM) in Barcelona, obtained a European Research Council (ERC) Advanced Grant to be executed during 5 years. The overall objective of the ERC, conducted during 2020-2025 and named SUMMIT, was to investigate the roles of DMSCs in shaping organism-organism interactions, fundamental for the functioning and evolution of marine ecosystems. Within this context, the SUMMIT-ERC project carried out several fieldworks: the BIOGAPS-Med cruise in 2019 and the SUMMIT-Med1 cruise in 2021, both in the Mediterranean Sea; and the SUMMIT-Moorea, a stay in the Richard Gump Research Station (Mo’orea, French Polynesia) to study tropical waters in 2024.

Moreover, within the advanced grant period, the Simolab group also participated, as co-leader, in the Antarctic cruise POLARCHANGE in 2023. With these fieldworks, together with laboratory experiments with cultured protists, the Simolab has given insights in microalgae and bacteria interactions, studying the roles of DMSCs in regulating N2 fixation by hetererophic diazotrophs, the DMSCs roles in prey selection by microzooplankton and the DMSP transporter and assimilation pathways in phytoplankton.

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Resilient hosts, dynamic waters: filter feeders' microbiomes and antimicrobial resistance dynamics in coastal ecosystems under freshwater runoff

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Antimicrobial resistance (AMR) is an escalating threat to human health. Despite advances under the One Health framework, major knowledge gaps persist due to the complex interactions among AMR drivers in natural ecosystems. The MARTRANSFER project investigates AMR dissemination risks in coastal environments, focusing on seawater and invertebrate filter feeders' microbiomes periodically exposed to freshwater inflow containing anthropogenic and agrochemical contaminants. Microbiomes in seawater and in two filter feeders formed three clearly separated clusters. Seawater microbiome responded strongly to freshwater inflow, while filter feeder microbiomes showed higher resilience to environmental changes. The genes' richness increased significantly in seawater following runoff, but no comparable changes were detected in the resistome or mobilome of filter feeders. However, both filter feeders' microbiomes harbored significantly higher abundances of mobile genetic elements than seawater, suggesting a key role in AMR persistence and potential spread. These results shed new light on the influence of episodic freshwater inputs on AMR dynamics in coastal seawater and underscore the role of invertebrates' microbiomes as potential AMR reservoirs. Within the One Health framework, these findings highlight the need for integrated seawater management strategies and comprehensive AMR risk assessments in invertebrates destined for human consumption.

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Light and inoculum source shape assembly of diatom phycosphere microbiomes in a co-culture experiment

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Diatoms account for about 40 % of the marine primary production. Their photosynthetic assimilates provide a carbon source for bacteria and promote a wide range of micro-scale interactions. Light can drive these interactions by regulating algal photosynthesis and therefore the release of organic carbon, potentially favouring taxa able to utilize these compounds. To study the assembly of phycosphere communities and their effect on algal growth, an axenic diatom (Conticribra weissflogii) was co-cultured with concentrated bacteria from two different natural seawater sources under different light intensities for 40 days. When inoculated with North Sea bacteria, algal cell growth was adversely affected in low light, whereas high light allowed for increased growth and more similar communities, based on 16S rRNA gene amplicon sequencing. Inoculation with Baltic Sea bacteria led to a more consistent algal growth response. Despite a large variability in taxa, the communities establishing from both water sources, mainly consisted of the core families Flavobacteriaceae, Rhodobacteraceae and Cyclobacteriaceae, previously described as associated with diatoms. The results highlight the importance of both mutualistic and antagonistic interactions between algae and bacteria, depending on growth conditions, and demonstrate the utility of co-cultivation experiments with complex bacterial inocula to study the assembly of phycosphere microbiomes.

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Microbiome dynamics in restored seagrass meadows: implications for ecosystem recovery

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Seagrass meadows are unique habitats, providing many ecosystem benefits. However, seagrass areas are declining worldwide and attempts to restore lost meadows achieved variable results. Seagrass-associated microorganisms can be beneficial by providing nutrients, plant-hormones, and pathogen-defense. Thus, root- and sediment microbiomes and their interactions may be critical for initial seagrass restoration success and the return of ecosystem services, yet their role is largely unresolved. We tracked shifts in Zostera marina meadow microbiomes, one, three, 12, and 24 months after transplanting shoots at newly restored- and unvegetated control sites in the German Baltic Sea, using 16S- and 18S rRNA gene amplicon sequencing. Seagrass establishment was successful with an increase in shoot densities of up to 25-fold. However, we found that prokaryotic communities in restored meadow sediments still resembled those of the unvegetated sediments rather than those of natural meadows, with higher abundances of clades comprising obligate anaerobe, potentially fermentative saccharolytic heterotrophs, as well as putative methanogens in natural meadow sediments. This suggests that plant influence on below ground microbial communities is mediated less by the formation of the root system, but indirectly by long term organic matter accumulation. Thus, prokaryotes could serve as proxies for defining natural meadow sediment state after restoration.

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Generalist Phyllosphere Taxa Dominate Microbial Communities on Macrophytes Across a Natural Salinity Gradient in the Baltic Sea

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Aquatic macrophytes serve as ecosystem engineers in shallow coastal waters, offering among others, surfaces for microbial colonization. This study investigated how salinity and host species influence the phyllosphere microbiome of macrophytes in the brackish Baltic Sea. Samples from Zostera, Myriophyllum, Chara, and Stuckenia were collected across a salinity gradient (6–15) in summer 2022 and analyzed using 16S and 18S rRNA gene sequencing. Results showed that phyllosphere microbiomes were distinct from surrounding seawater, with characteristic bacterial taxa such as Loktanella, Pseudorhodobacter, Methylotenera, and unclassified Synechococcales and Rhodobacteriaceae. Protists like Picochlorum were found across all hosts, while others like Cocconeis and Cyclotella were absent from Chara. Salinity and host species influenced microbial composition, but only 4–11% of taxa were specific to a particular salinity or host. These findings suggest that most phyllosphere taxa are generalists, adapted to the macrophyte surface environment rather than to species-specific conditions. This stability implies that the phyllosphere microbiome is resilient to moderate salinity changes, which maybe valuable for managing seagrass meadows in dynamic coastal ecosystems.

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Investigating the Marine Protist Interactome Using Single-Cell Genomics

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Marine microbial interactions are central to ocean ecosystem function, yet remain largely uncharacterized. We employed Single-Cell Genomics (SCG) to infer potential physical associations among uncultured marine protists and between protists and prokaryotes. We analyzed 3,081 Single Amplified Genomes (SAGs) from three marine regions: the Blanes Bay Microbial Observatory (BBMO), the Gulf of Maine (GoM), and the Tara Oceans expedition. Using both low-coverage and deeply sequenced SAGs, we identified 698 strong potential eukaryote–prokaryote interactions and 484 strong eukaryote–eukaryote interactions. Key protist taxa involved included MAST lineages, Chrysophytes, Micromonas, and Picozoa, while the most frequently associated prokaryotes belonged to the Flavobacteriales, Pelagibacterales, Planctomycetota, and Verrucomicrobiota. Notably, BBMO SAGs revealed more potential interactions in winter, suggesting seasonal interaction variation. Several potential interactions previously inferred from amplicon-based association networks were corroborated, while additional novel associations were uncovered, including putative parasitic and symbiotic relationships. Our findings support SCG as a powerful method for uncovering microbial associations and highlight the Mediterranean Sea as a potential hotspot of microbial interactions. This approach complements traditional marker-gene surveys and reveals associations involving poorly characterized taxa. The dataset and analytical framework presented here provide a valuable foundation for future investigations into the structure and dynamics of the marine microbial interactome, particularly in the context of global change.

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Model diatoms for interactions studies in the Western English Channel

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In the Western English Channel and more generally in permanently mixed coastal pelagic habitats, diatoms dominate phytoplankton communities in both diversity and abundance. Recent metabarcoding data have revealed that two nanodiatoms, Mediolabrus comicus and Minidiscus variabilis are present year-round, with M. comicus dominating in winter and M. variabilis exhibiting multiple peaks of abundance throughout the year. To better understand the biotic interactions driving these seasonal dynamics, an isolation strategy was implemented. Samples were collected biweekly over the course of a year at SOMLIT-Astan station in order to establish cultures of reference strains of M. comicus and M. variabilis along with their associated parasites. This intensive fieldwork led to the creation of a unique collection of over 100 new nanodiatom cultures, including 46 Mediolabrus and Minidiscus; one strain of each species was also axenized using antibiotics treatment. Additionally, 82 associated parasites were isolated, revealing for the first time the key role of biotic interactions in regulating nanodiatom populations. Among them, viruses were identified as persistent mortality agents, present year-round. Four viruses were further characterized revealing a remarkable genetic and functional diversity among novel RNA diatom viruses.

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Phycosphere microbiota composition in a community infected with zoosporic fungal parasites

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Among aquatic fungi in marine and freshwater environments, zoosporic parasite species belonging to Chytridiomycetes often target living phytoplankton. Host populations showed variable susceptibility in natural ecosystems as well as in ex situ cultures, pointing towards the potential function of host microbiome to provide resistance. In this study, we tested whether the infection of phytoplankton with zoosporic parasites influenced changes in the phycosphere microbiota. We sampled surface water at Port Ginesta (Barcelona, NW Mediterranean) and artificially infected three replicates of its phytoplankton community with active zoospores of the cultured chytrid Dinomyces arenysensis. We incubated infected samples along with three

replicates of untreated controls, and size-fractioned samples through 3μm and 0.2μm filters at three timepoints: days 3, 6 and 9 after infection. We used 16S rRNA metabarcoding to assess the bacterial diversity associated to the phycosphere or free-living, and performed microscopy observations to evaluate host abundance and infection prevalence. Preliminary observations highlighted that phytoplankton was infected by zoosporic parasites six days after the introduction of chytrids to the community, and we observed a complete absence of dinoflagellates nine days after infection. Data from the microbiota molecular diversity will potentially highlight changes in the phycosphere epibiotic community of infected and not infected phytoplankton.

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Microbial plankton networks complexity increases under river run off impact

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In estuarine ecosystems, river run-off greatly influences community composition and species interactions. Despite microorganisms playing major roles in the functioning of these ecosystems, microbial plankton community structure and interaction patterns due to river influence remain poorly understood and their spatial and temporal variabilities are often overlooked . As microbial interactions are not easily observable, co-occurrence networks inferred from eDNA data are classically used to infer putative interactions. To better understand spatiotemporal variability of microbial associations (bacteria and protists) across estuarine gradient influenced by run-off, impacted and non-impacted waters were sampled fortnightly for 3 years (2020-2023) in four French ecosystems. Sample-specific networks were constructed and their topological properties were summarized using eight graph metrics. Networks differed among ecosystems and between impacted and non-impacted waters at each site. Despite strong spatial and seasonal variabilities, a common pattern was identified, notably showing that microbial networks decreased in complexity along the land-sea gradient. This higher network complexity in freshwater-influenced seawater suggests a higher potential for cooperation and/or competition for resources among microorganisms in freshwater-influenced ecosystems. Specific microbial taxa were found to be associated either to impacted or non-impacted networks, suggesting their key role in ecological interactions in estuaries.

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Ammonium stimulates growth and morphological changes in a nitroplast-lacking strain of the microalgal species *Braarudosphaera bigelowii*

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The marine microalgal species *Braarudosphaera bigelowii* is the first nitrogen-fixing eukaryote described to date. The ability of *B. bigelowii* to fix nitrogen relies on the acquisition of nitrogen-fixing organelles (nitroplasts) that evolved from the endosymbiotic cyanobacterium UCYN-A, which acts as its main nitrogen provider in the natural environment. Intriguingly, one viable cultured strain of *B. bigelowii* (HR19-19) has lost the nitroplast under uncertain laboratory conditions, challenging the assumption of obligate nitroplast dependency in B. bigelowii. In order to better delineate the degree of dependency of *B. bigelowii* on nitroplast-derived nitrogen (or other biomolecules), we have analyzed the growth response of B. bigelowii HR19-19 after supplementing the culture media with cell extracts coming from different haptophyte and cyanobacterial taxa. Our flow cytometry counts show that the growth of the nitroplast-lacking B. bigelowii HR19-19 can be stimulated with ammonium-enriched additions coming from sources other than the nitroplast. Furthermore, epifluorescence and electron microscopy analyses revealed morphological changes triggered by cell extract additions that might be related with mechanisms involved in nutrient acquisition. These findings challenge our understanding of nitroplast metabolic integration in *B. bigelowii*, revealing unexpected plasticity in nutrient acquisition and providing novel insights into the early evolutionary trajectory of organellogenesis.

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The social life of marine cyanobacteria

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Marine ecosystems is driven by a multitude of biological interactions that regulate nutrient fluxes. Other marine bacteria have evolved various mechanisms of interaction with the ocean and neighboring cells, for example extracellular vesicles (1). Here, we show a new mechanism of direct interaction between marine cyanobacteria, the intercellular membrane nanotubes. Nanotubes are membrane-coated tubular structures connecting two cells that allow the transport of cytoplasmic components between them. A combination of transmission electron microscopy, scanning electron microscopy, fluorescence microscopy, and imaging flow cytometry (using the ImageStream MKII system) was used to observe and characterize nanotubes in several xenic and axenic cultures of Prochlorococcus and Synechococcus. We present evidence of inter-and intra-genus exchange of cytoplasmic material between neighboring and distant cells of cyanobacteria mediated by nanotubes. We show that nanotubes are produced between living cells, suggesting that this is a relevant system of exchange material in vivo (2). Our group is also interested in studying how extracellular vesicles and nanotubes can modulate the nutrient fluxes in the ocean. We are investigating how microvesicles might affect the growth of cyanobacteria when added to cultures under nutrient-limiting conditions; and whether nanotubes allow the transport of metabolites by using nanoscale secondary ion mass spectrometry (NanoSIMS). 1. Biller, S.J. et al., (2014) Science, 343. 2. Angulo-Cánovas, E et al., (2024) Sci. Adv. 10 (21). Funding TED2021-129142B-100 Ecological Transition and Digital Transition Projects (Agencia Estatal de Investigación, Spain), FEDER-UCO Project 1380795-F (Junta de Andalucía, Spain, cofunded by the FEDER programme of the European Union), P.P.2019 Submod 3.1 and BIO123 (Universidad de Córdoba, Programa Propio de Investigación) and PID2022-141370NA-100 (funded by MCIN).

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Prophage induction and superinfection exclusion in lysogenic cyanobacteria

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Prophage Induction and Superinfection Exclusion in Lysogenic Cyanobacteria Yara Nwatha, Esther Cattan-Tsaushu, Sarit Avrani Department of Evolutionary and Environmental Biology, and the Institute of Evolution, University of Haifa Abstract: cyanophages are viruses that infect cyanobacteria, typically via the lytic cycle; some can also enter the lysogenic cycle by integrating into the host genome. While integrated, the prophage remains inactive until induced by environmental signals. Prophage integration confers resistance to related phages through superinfection exclusion, which involves blocking infection at various stages. We investigated environmental triggers of prophage induction and superinfection exclusion in two cyanobacterial genera, each hosting a prophage from the same phage family but differing in integration sites, suggesting distinct integration mechanisms. Among the tested factors, nitrogen starvation most strongly induced prophage activation in both systems. Adsorption assays revealed that both phages could bind to their lysogen hosts, indicating that superinfection is blocked at an internal step. Interestingly, one lysogenic strain lost resistance to the wild-type phage. Genome sequencing revealed two unique mutations in the wild-type phage that were absent from the prophage genome, potentially explaining this escape from exclusion. Together, our findings shed light on the environmental regulation of prophage induction and the mechanisms of superinfection exclusion in cyanobacteria–phage systems, with implications for phage–host dynamics under changing nutrient conditions.

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Identifying antarctic krill-associated microbial communities in the Southern Ocean

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The Antarctic krill (*Euphausia superba*) is a key species in the Southern Ocean, but its populations may be declining due to global change, potentially disrupting food webs and carbon cycling. Microbes are essential for host health and adaptation, and this study aimed to characterize the krill microbiome. We analyzed 102 individuals collected from different latitudes, including South Georgia Islands (north) and Bransfield Strait (south). Microbial communities (prokaryotes and eukaryotes) were assessed in different body parts (stomach, hepatopancreas, exoskeleton) and expelled materials (molts, fecal pellets) using high-throughput sequencing. Results showed that major geographic areas had limited effect on microbial diversity, while differences between individuals were more relevant, revealing that life history of individuals are key in shaping krill microbiome. Additionally, different body parts hosted different communities, with some microbes showing high specificity to certain parts, while others were broadly distributed. The microbiome was dominated by prokaryotes (*Enterobacterales, Flavobacteriales*) and eukaryotes (*Eugregarinorida, Suctoria*). Indicator taxa included pathogens (*Clostridiales, Apostomatia*) and symbionts (*Enterobacterales, Suctoria*), with diet-related microbes like diatoms (*Bacillariophyta*) found in the digestive system and feces. Notably, krill from South Georgia Island hosted fewer diatoms and more pathogens, suggesting a link between environmental stress (higher temperatures and fishing pressure) and microbiome shifts.

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Zooplankton-associated fungal assemblages are diverse, host group-specific, and temporally dynamic

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The associations between microbes and planktonic invertebrates constitute key ecological interactions. While insights of the interplay of zooplankton and colonizing bacteria have been revealed in previous studies, nothing is known about the association of zooplankton and fungi. We analysed the association of fungi with >300 healthy individuals of three zooplankton groups (Cladocera, Copepoda, Rotifera) in a temperate hypertrophic shallow lake using ITS1-amplicons and a range of environmental factors. Our analysis included two to four species of each zooplankton group, individuals dissected into carapax and gut (*Daphnia spp.)* as well as adult and juveniles (copepods). Similarly to zooplankton-associated bacteria, we found that fungal communities were highly variable and had higher richness compared to the water column. While the high variability made it challenging to unravel patterns, we were able to identify characteristic fungal genera for each zooplankton group and found that host identity, rainfall and fungal spore sizes were the strongest drivers for fungal community composition. The relatively large number of soil-, litter-, wood-saprotrophic and plant-associated fungi further suggests a strong connectivity with the watershed. Taken together, our findings contribute to the understanding of limnetic trophic-web dynamics by addressing an important knowledge gap and identifying potential trophic interactions for future study.

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Decoding the genomes of industrial microalgae with long reads

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Microalgae are at the forefront of biotechnological research due to their potential in sustainable biomass production, biofuel generation, and synthesis of valuable bioproducts. The optimization of microalgae cultivation at an industrial scale requires a comprehensive understanding of the genomic underpinnings that control cell growth, metabolic pathways, and stress responses. Recent advancements in DNA sequencing technologies, particularly PacBio long-read sequencing, have improved our ability to reconstruct microalgal genomes. This advance in genomic resolution offers new opportunities to explore and harness the genetic diversity of microalgae for enhanced cultivation and productivity. Here, we have sequenced with PacBio Revio 11 microalgal genomes (nine eukaryotic and two prokaryotic) that are normally used for large-scale production. Preliminary results indicate that the assembly of two *Spirulina* cultures shielded single contigs of 6.4-6.7Mb, which are close to the already known genome size of this cyanobacterial species (6.7Mb). Seven of the eukaryotic microalgal assemblies had sizes between ~40 and ~110Mb, some of them featuring <20 contigs, pointing to low fragmentation. In most eukaryotic assemblies, we found genomic signal associated with bacteria that might interact with the microalgae. We analyzed the metabolic potential of these genomes, focusing on their biosynthetic pathways and adaptations to tolerate pathogens. The results contribute to a better understanding of the genomes of microalgae used in large-scale production systems and to optimizations in the production of microalgal biomass in large-scale outdoor systems.

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