



4th NFDI4Microbiota Annual Conference

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Poster Abstract Book

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#1 NFDI Basic Services as a Foundation for Cross-Disciplinary and FAIR Research Data Management

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The German National Research Data Infrastructure (NFDI) is developing basic services to make research data management (RDM) more effective, sustainable, and FAIR (Findable, Accessible, Interoperable, Reusable). These services cover federated identity management (IAM4NFDI), consultation on persistent identifiers (PID4NFDI), and RDM training programs (RDMT4NFDI). They address common challenges across scientific domains and are coordinated through the Base4NFDI initiative.

For researchers, these services open opportunities to work seamlessly across disciplinary borders within the national and international research landscape. Secure access to resources, reliable data citation, and interoperable solutions reduce technical and administrative hurdles, making it easier to share, reuse, and publish research data. By lowering barriers, the services foster collaboration between communities, strengthen reproducibility, and promote open science practices.

Development follows a requirement-driven approach. Research Software Engineers (RSEs) adapt and integrate technical solutions, while Service Stewards act as intermediaries between users and developers. This ensures that services remain practical, usable, and responsive to community needs, while also addressing legal and ethical requirements for responsible data handling.

At the poster, we invite discussion on how NFDI's basic services can support both researchers in their daily work and developers in creating new tools and services. If you are developing services or research software, you can also learn how to benefit from the shared infrastructure, connect with our initiatives, and align your solutions with the NFDI framework. By joining forces, researchers and service providers can build sustainable, interoperable tools that empower collaboration across disciplines and secure long-term value for the scientific community.

#2 VirJenDB: A Curated and Secure Virus Sequence Platform

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High-throughput sequencing has generated an unprecedented volume of data. However, researcher-submitted data in repositories requires extensive curation and quality control for reuse. These tasks are hindered by the multiplicity of repositories, the volume of the data, and the complexity of virus (meta)data curation. As part of the **NFDI4Microbiota** initiative, VirJenDB offers a user-friendly platform to facilitate versioned, community-driven curation and ontology development to address these challenges.

Virus metadata and sequences were ingested from 16 sources, including about 200 fields of metadata or standards, covering taxonomy, sample, and host information. Up to 85 metadata fields have undergone at least one round of curation and are linked to 15.4 million virus sequences, with 88% from those infecting eukaryotes and the remaining infecting prokaryotes. Subsets were created, including a novel collection of 0.91 million viral operational taxonomic unit (vOTU) sequences across all viruses, while keeping the original sequences from each vOTU to facilitate downstream analyses, e.g., sequence variation.

The VirJenDB web portal (<https://www.virjendb.org>) is built with FastAPI and Elasticsearch, and hosted on the de.NBI cloud service using Aruna object storage. It provides HTTPS and API access to the sequence datasets and metadata, offering search, filtering, download, visualization, and documentation. Data resilience is ensured by geolocation replication across de.NBI virtual machines and offline devices, as well as regular incremental backups of indices and curated metadata. All metadata changes are version-tracked to enable reproducibility and rollback, while the backend and frontend codebases are directly linked to a GitHub repository to ensure transparency and consistent versioning. VirJenDB aims to connect the phage and eukaryotic virus research communities by supporting webtool integration, meta-analyses, and metadata schema extensions, further solidifying its role as a key data infrastructure project within the NFDI.

#3 Reproducible workflows with Nextflow and nf-core (nf-workflows)

NFDI4Microbiota Flex Funds Project

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Reproducible, easily shareable data analysis workflows are essential for bioinformatics and microbiome research. Nextflow is an open-source workflow manager that makes bioinformatics workflows portable across different kinds of computing devices, from laptops to High Performance Computing (HPC) infrastructure. Nf-core (<https://nf-co.re/>) is a collection of community-developed and maintained Nextflow-based data analysis workflows, ready to be used by anyone. Microbiome-related workflows in nf-core include, for example, taxonomic profiling based on amplicons or shotgun metagenomics data, assembly of isolates or metagenomes, RNA-Seq analysis, and human decontamination of (microbial) sequencing data.

The project's primary objectives are to maintain and expand relevant nf-core workflows for the NFDI4Microbiota community. In addition, we offer specialized training sessions and ongoing support for Nextflow, particularly within the nf-core framework, to further empower the NFDI4Microbiota community.

A recent survey revealed interest in diverse analysis approaches within the NFDI4Microbiota community, and improvements to the corresponding analysis workflows have been or are in progress. Courses are offered via the NFDI4Microbiota events website. Real-time support on each nf-core workflow is available at <https://nf-co.re/join#slack>.

For additional details, please visit our poster. We provide comprehensive support for data analysis using Nextflow and nf-core workflows, along with expert guidance on building or modifying custom workflows. We also welcome feedback and suggestions for further improvements.

#4 metaTraits: a large-scale integration of microbial phenotypic trait information

NFDI4Microbiota Flex Funds Project

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Microbes differ greatly in their organismal structure, physiology, and environmental adaptation, yet information about these phenotypic traits is dispersed across multiple databases and is largely unavailable for taxa that remain uncultured. Here, we present metaTraits, a unified and accessible trait resource that integrates culture-derived trait information from BacDive, BV-BRC, JGI IMG, and GOLD with genome-based predictions for medium and high-quality isolate and metagenome-assembled genomes (MAGs) from proGenomes and SPIRE. metaTraits covers over 2.2 million genomes and more than 140 harmonized traits mapped to standardized ontologies, spanning cell morphology (e.g., shape, size, Gram staining), physiology (e.g., motility, sporulation), metabolic and enzymatic activities, environmental preferences (e.g., temperature, salinity, oxygen tolerance), and lifestyle categories. All records are linked to the original evidence, and species are cross-linked to NCBI and GTDB taxonomies. The interactive metaTraits website provides search and visualization tools, taxonomy-level summaries, and two workflows for annotating user-submitted genomes or community profiles. metaTraits substantially advances accessibility and interoperability of microbial trait data, enabling comprehensive trait-based analyses of microbiomes across diverse environments. metaTraits is accessible via <https://metatraits.embl.de>

#5 MetaProt-KG

NFDI4Microbiota Flex Funds Project

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Microbiomes play a pivotal role in environmental processes, human health, biotechnology, and agriculture. Their study spans multiple omics disciplines, including metagenomics, metaproteomics, and metabolomics. Integrating data across these domains is complex and time-intensive, necessitating systematic methods to improve interoperability, accessibility, and analysis efficiency.

Knowledge graphs (KGs) offer a powerful framework to represent, interconnect, and visualize the intricate relationships between genes, proteins, metabolites, and associated metadata. By providing a holistic and structured view, KGs enable the exploration of molecular mechanisms, regulatory networks, and metabolic pathways, as well as the identification of unknown proteins. When combined with computational approaches such as machine learning and network analysis, KGs facilitate pattern discovery, functional role prediction, and the identification of key biological nodes. Large language models (LLMs) can further enhance KGs by contextualizing queries, minimizing hallucinations, and extracting up-to-date insights from unstructured biomedical literature. Additionally, KGs support data sharing and collaborative research, ensuring reproducibility through standardized formats, data provenance tracking, and quality control.

The MetaProt-KG project focuses on developing a microbiome knowledge graph with an initial emphasis on metaproteomics, leveraging domain expertise to establish a scalable foundation for broader microbiome research. The project's primary objectives are: (a) compiling and curating an overview of all relevant metaproteomics databases, (b) constructing and deploying a metaproteomic knowledge graph as a web-based tool on the de.NBI cloud, and (c) implementing an accessible suite of queries and algorithms for user-friendly data analysis via KGs.

#6 EnterArchaeo

NFDI4Microbiota Flex Funds Project

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The NFDI4Microbiota 2025 FlexFund project ‘EnterArchaeo’ proposed to improve training, knowledge, and practical skills within the microbiome community through the creation and implementation of training in the development and usage of high-quality and reproducible Nextflow pipelines for metagenomics research, as well as introduce the community to ancient microbiome data.

This poster will show highlights of the three work packages so far: the fourth edition of practical bioinformatics summer school on ancient metagenomics, the project infrastructure built around the Genomic Standards Consortium MlxS metadata schema extension for ancient DNA (MInAS), and the latest updates to the nf-core Nextflow pipelines for modern and ancient metagenomics: nf-core/createtaxdb (taxonomic profiler database building), nf-core/taxprofiler (multi-tool taxonomic profiling), and nf-core/mag (metagenomic de novo assembly and binning).

#7 BEXIS2 – a user-friendly Platform for Metadata Management

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BEXIS2 is a free and open-source community-driven web-based research data management system [1]. It facilitates the rich metadata annotation, data provenance preservation, and semantic integration of different data types acquired in microbiological experiments or collected in the natural environment. The simultaneous support of multiple schemas and the data-agnostic architecture of the software alleviate the management of complex data, including those acquired by correlative methods, like correlative light-electron microscopy or spatial proteomics. The system modularity allows modification of its various components, from database schemes to the user interface. The OpenAPI-compliant APIs of the BEXIS2 simplify the development of new functions and integration with third-party software (e.g. OMERO).

In the Cluster of Excellence 'Balance of the Microverse', we are instantiating the BEXIS2 as a meta-data management platform that implements community-accepted minimal information standards (e.g. REMBI [2], MIAPE [3], MARGARITAS [4]) in conjunction with ontologies and controlled vocabularies. For that, we are developing a new interface module for external terminology services, such as TS4NFDI [5]. In this contribution, we will provide technical details on the BEXIS2 architecture, discuss recent advances, and demonstrate metadata management workflow for NMR and light microscopy data.

The work is supported by the grant provided by Deutsche Forschungsgemeinschaft (DFG, German Research Foundation), Projekt 'EXC 2051: Balance of the Microverse', No 390713860.

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#8 Integrative data management and analysis with the Microbial Signatures Database (MSD)

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A major challenge in microbiome research lies in integrating diverse experimental data and metadata to explore the community structure and functional potential of microbiotas across health and disease. However, tools for managing the extensive and diverse data, as for instance created by larger collaborations, such as the Collaborative Research Center 1371 for Microbiome Signatures, are scarce. We introduce the Microbial Signatures Database (MSD), a web application for data management and integration available at <https://www.misigdb.org/>. Beyond data management, MSD supports the analysis of 16S rRNA gene amplicon taxonomy profiling, metagenomics, and metabolomics. MSD offers users an interface for managing projects, organisms, and samples using standardized metadata. For 16S rRNA gene sequences, MSD integrates the UNOISE algorithm for sample-wise processing and the Taxonomy Informed Clustering (TIC) algorithm for taxonomy profiling of 16S rRNA gene amplicon data. Results can be integrated with metagenomics and metabolomics profiles. Additionally, users can export their analysis results and supporting data types to Namco (<https://exbio.wzw.tum.de/namco/>) for downstream analyses, such as functional profiling and multi-omics analysis. In the future, standardized processing methods for metagenomics and metabolomics will be integrated into MSD. This helps alleviate any variability introduced by different processing methods. Thus, results from these data types become more easily comparable. With a data management solution that consolidates various microbiome research perspectives into one place and offers integrated analysis solutions, researchers gain the advantage of enhanced integration and synergistic improvement in understanding gut microbial signatures.

Keywords: Microbiome, microbial signature, data integration, 16S rRNA, metagenomics, metabolomics

#9 Integrating Omics and Metadata to Identify Shared and Disease-Specific Microbiome Signatures

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In recent times, microbiome research has evolved thanks to advances on omics technologies along with methodologies for data analysis. Data analysis for microbiome data ranges from preprocessing sequences to produce the relevant feature tables to further statistical methods to e.g., increase the signal-to-noise-ratio and to machine learning (combined with additional data, e.g., disease associations) for the prediction of disease outcomes. These approaches are the means to expose the sample richness, the taxonomic compositions and after all the highly significant and most relevant taxa in the diseased versus the healthy groups. Here we present our project, which focuses on metadata-based analysis to identify shared and disease-specific microbial compositions, pathways across multiple gut and brain disorders. Pathways for standardizing the analysis have to take into consideration: the choice of reference databases, handling compositionality and normalization, diversity/differential abundance metrics, and reproducibility. To handle the complexity, metadata harmonization is key to establish and monitor the pipeline corresponding to a microbiome workflow. The existing efforts such as MIxS (Minimum Information about any (x) Sequence), STORMS (STandards for Reporting of Microbiome Studies), Workflow Provenance Standards, did not lead into solid adoption. We plan to build on top of such approaches targeting metadata harmonization aligned to other community-based efforts already accepted in the biomedical domain (e.g., Bioschemas) lowering the adoption barrier.

#10 Metadata matters: Structuring data in living materials research

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Living materials are modern composites in which biological and non-biological components are combined. The associated interdisciplinary approaches combine highly specialized processes and characterization to address complex living materials research questions. With the research advancements, the volume and complexity of data generated across various stages of research and development are increasing that requires strategies for knowledge exchange, data findability and reproducibility, and translate research description into machine readable formats. This involves not only storing and archiving data, but also extensive documentation, structuring and cross-linking of data for accessible and reproducible research. Despite the tremendous amount of information already available, the lack of standardized, interoperable metadata schema remains a significant bottleneck in the reuse, reproducibility and integration of living materials data. Moreover, advances in data-driven research increasingly demand high quality, annotated data for data science and machine learning approaches. This highlights the urgency for structured data in living materials research and therefore calls for a metadata schema that supports not only human readability but also machine-readable data, aligning with the FAIR data principles. To address this gap, we aim to establish metadata standards for living materials research that can be easily integrated into researchers' workflows. Therefore, to ensure usability and minimize additional efforts for researchers, we plan to develop a graphical user interface that enables efficient implementation of metadata schema and provides functionality to export into machine-readable formats.

#11 Life Cycle of Metagenomic Research Data Management

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Effective research data management (RDM) is essential for ensuring that data adheres to the FAIR principles of Findability, Accessibility, Interoperability, and reusability. In this session we will examine how these principles drive the life cycle of metagenomic data at the Uniklinikum University in Aachen, from data generation to long-term storage and reuse.

The process begins with the meeting of the researcher and data steward where the researcher shares the structure of the data. The data steward and researcher work together to create a metadata profile within AIMS. The profile FASTA and FASTQ are created using AIMS with the minimally NFDI4Microbiota recommended metadata standards. The profile is then made available for use in Coscine, the platform used at the UKA for storing and archiving research data along with its linked metadata profile.

In the next stage, the metagenomic data and its metadata are prepared for storage and reuse. This process is facilitated by the data steward using Python to automate the extraction of metadata from the CSV file. The extracted metadata is added to the metadata form and then both the file and metadata form are uploaded to specified resources in Coscine.

Each resource in Coscine has a unique persistent identifier, ensuring the findability of the data. Data stored in Coscine remains accessible for at least ten years following the conclusion of the research project, in accordance with good scientific practice. Researchers and collaborators can access the data using institutional credentials or ORCID. Project-based permissions enable secure sharing of data and collaboration.

By walking through each stage of this workflow—from data generation to archiving—this presentation demonstrates how the FAIR principles are applied in practice to support transparent, sustainable, and reusable metagenomic research at UKA.

#12 Unlocking the microbial potential of microalgae for sustainable biotechnological applications

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The Krohn Lab focuses on the molecular and functional exploration of microalgae and their associated microbiomes, with emphasis on microbial community interactions, novel bioactive compounds, and sustainable biotechnological applications. Supported by EU and BMBF funding, our work integrates molecular microbiology, bioinformatics, and biochemical analytics to address challenges at the inter-face of One Health, environmental sustainability, and microbial innovation. Our research portfolio includes:

1. **Natural bioactive compounds & molecular biotechnology** - Identification of antioxidant, and quorum-quenching enzymes, as well as novel biocatalysts for food preservation, sustainable bioenergy, and environmental applications.
2. **Applied microbial biotechnology** - Exploration of microalgae–bacteria consortia as alternative production platforms for functional proteins, pigments, and metabolites with potential in food, feed, and aquaculture.
3. **Citizen Science & biodiversity monitoring** - Projects on microbial diversity and ecological monitoring in aquatic and peatland ecosystems, linking microbiology, data analysis, and environmental sustainability.
4. **Data-driven biology & molecular analytics**: Applying metagenomics, transcriptomics, and functional screening to elucidate microbial community functions, strengthen analytical capacities, and refine 'omics-based research methods.

These efforts align with NFDI4Microbiota's mission to advance high-quality microbial data management and reuse. Specifically, we seek support to:

- Integrate diverse microbial datasets into standardized repositories
- Improve metadata annotation through advanced bioinformatics approaches
- Provide training in microbiome data analyses for interdisciplinary teams

The synergies between our lab's multidisciplinary expertise and NFDI4Microbiota's infrastructure vision will foster impactful collaborations, enabling enhanced data interoperability, reproducibility, and innovation in microbial ecology and biotechnology.

#13 Characterization of unexpected shared strains in metagenomic datasets with SameStr

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Strain-level microbiota analyses are becoming increasingly important in microbiome research, as they provide valuable insights into possible microbial transfer events in various biological contexts, such as fecal microbiota transplantation (FMT), pathogen transmission in hospital environments, or natural host-to-host exchange. Using the SameStr tool, we can detect shared strains between pairs of samples by comparing read alignments of MetaPhlAn 4 marker genes, allowing a high-resolution reconstruction of microbial strain sharing networks. However, recent analyses have frequently identified an unexpectedly high number of shared strains between biologically unrelated samples, which could suggest technical artefacts, limitations for this type of analysis or biological causes, such as cross-contamination during sample processing. To better understand the technical and biological factors influencing these findings, we aim to identify systematic patterns underlying the identification of these unrelated shared strains and to characterize diGerences in marker alignments that drive their detection. By investigating whether specific taxa, abundance patterns, or alignment characteristics are associated with false positive shared strain detections, we seek to establish criteria that allow us to distinguish biological and technical contributions to the shared strain detection with SameStr, thereby improving the accuracy and interpretability of strain-level analyses.

#14 golemMB: A Modular and Flexible Shiny Application for Analyzing and Visualizing 16S rRNA Gene Data

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A wide range of pipelines is available for the analysis of 16S rRNA gene amplicon sequencing data, each with different strengths. golemMB (<https://github.com/ktrrj/golemMB>) is a Shiny application that complements existing approaches with a particular focus on modularity, flexibility, and data visualization. It integrates common analysis steps from the count table onward into a user-friendly and reproducible framework.

The application supports seamless data import into phyloseq, filtering of OTU, ZOTU, or ASV tables, and normalization using several approaches such as CLR, log1p, and RLE. Core ecological analyses include alpha diversity estimation with six indices and beta diversity exploration with ordination and distance-based methods.

Differential abundance testing is performed in parallel across taxonomic levels via the microbiomeMarker package. Results are visualized through metacoder heattrees, which can represent both differential taxonomic profiles and functional pathway abundances derived from PICRUSt2. Functional profiling is complemented by BugBase for phenotype visualization, while correlation analyses link microbial taxa with numeric metadata.

All steps are reproducible through parameterized R Markdown scripts, with session bookmarking and automated PDF reporting to support transparency and collaboration. By combining robust methods with interactive visualization, golemMB provides an accessible platform for microbiologists to explore and interpret 16S rRNA gene sequencing data.

#15 Implementing a NextFlow workflow for on-the-fly analysis of 3C data using *Smoother* on CloWM

NFDI4Microbiota 2026 Flex Fund Proposal

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Nucleic acid interactome data, such as chromosome conformation capture and RNA-DNA interactome data are often analyzed via pipelines. This is disadvantageous: each change of parameters requires re-running the pipeline, making parameter tuning slow and tedious. Hence, analyses often use suboptimal default parameters, obscuring biologically relevant patterns or introducing artifacts, which may result in incorrect data interpretation. To address this, we have developed *Smoother*, an approach fast enough to process interactome data on-the-fly using a sparse prefix sum index. This on-the-fly processing allows users to adjust parameters interactively (i.e., while they are looking at their data), fostering more accurate and thorough data analysis.

Currently, the widespread adoption of *Smoother* is hampered by two limitations: 1) No easy-to-use workflow exists for building *Smoother*'s index, and 2) although the sparse matrix compression approach we currently use leads to small index sizes with many datasets, indices can become very large with some datasets. We aim to address these challenges by 1) implementing a NextFlow workflow for CloWM that allows building *Smoother* indices and launching its web interface. Additionally, the workflow will include common quality control metrics. 2) Further, to reduce index size, we propose replacing the sparse matrix compression with a kd-tree-based approach that we have designed and tested.

With these improvements, *Smoother* will be a convenient, scalable, and comprehensive tool to analyze chromosome conformation capture and RNA-DNA interactome data, particularly for the microbiota research community.

#16 Microbial xenobiotics biotransformation data – Perspective on FAIR data submission

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The microbiome significantly influences the global chemical landscape through interaction with xenobiotics, including pharmaceuticals, pesticides and environmental pollutants. These xenobiotics are chemically modified by the host microbiome, altering their bioavailability, activity, and toxicity. Comprehending the underlying molecular mechanisms of such biotransformations is important for advancing human health and improving environmental sustainability. However, there is a current lack of high-throughput, high-quality data on microbial biotransformations of xenobiotics. To address this research area and critical data scarcity, the Zimmermann group at EMBL Heidelberg, equipped with a high-throughput metabolomics platform to generate microbial biotransformation data, and has teamed up with the Chemical Biology Services team at EMBL-EBI, Hinxton – host of the database ChEMBL, to provide a standard data repository for submission of this data type. Together, we are aiming the following: (i) easier data submission to ChEMBL, (ii) data collection from literature and databases, and (iii) standardizing terminologies for microbial biotransformation data. To facilitate a FAIR-compliant, standardized data lifecycle, we're pursuing active involvement with NFDI4Microbiota. As a step towards collaboration, we have submitted a 2026 Flex Fund call project proposal, called BioXend, which is a computational framework focused specifically on improving the data submission stage of the microbial xenobiotics biotransformation data life-cycle. The submission and integration of this data type into ChEMBL, will enable increased findability and reusability, and facilitate both the formation of a collaborative community of microbial biotransformation researchers to further expand ChEMBL, and the research on chemical transformation pathways and their implications in biomedicine and environmental remediation. This effort proposes ChEMBL as the standard repository for microbial xenobiotic biotransformation data and NFDI4Microbiota as the consortium to provide the infrastructure and harmonize coordination across the research community.

#17 Workflow for profiling the tumor microbiome and its role in antitumor immunity: *Malassezia* and AHR in PDAC

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The role of intratumoral microbiota in tumor biology and anticancer immunity is increasingly recognized, with potential applications in diagnosis and therapeutic strategies such as immunotherapy. However, their effects are largely species-specific, and many of these relationships need to be elucidated. To address this, we are developing an automated pipeline to identify intratumoral microorganisms, characterize their composition, and discover potential immunoregulatory mechanisms. The workflow is implemented in Nextflow to ensure scalable and reproducible analyses. It processes reads not mapping to the host genome, that originate from metatranscriptomic or metagenomic sequencing. Taxonomic classification is performed with Centrifuge or Kraken2, followed by quality control of the alignment results using Recentrifuge. Contaminants in the test samples can be identified from negative control samples or based on DNA/RNA concentration using decontam by the inverse relationship between relative abundance and nucleic acid concentration. Downstream analyses include (1) calculation of alpha and beta diversity parameters, (2) identification of the core microbiome, (3) inference of microbial co-occurrence using SPIEC-EASI, and (4) metabolite prediction with HUMAnN3. From bulk RNA sequencing data various immune related parameters and immune cell fractions can be estimated and correlated with microbial abundance. Impact on survival can be assessed if clinical outcome data is available, and potential molecular mimicry can be assessed with known tumor-associated antigens.

We applied this pipeline to RNA sequencing data from pancreatic adenocarcinoma (PDAC) from The Cancer Genome Atlas. Analyses of microbial diversity revealed that bacterial species richness was greater than that of fungi and viruses. Among the identified fungal species were the well-known species *Malassezia restricta* and *Malassezia globosa*. We also inferred aryl hydrocarbon receptor (AHR) signature scores and found that, in combination with *Malassezia* abundance, this signature had a significant impact on patient survival.

In summary, this pipeline provides a framework for the characterization of the intratumoral microbiome and hypothesis generation regarding microbial-immune interactions, including the potential role of *Malassezia* and AHR in pancreatic cancer.

#18 Evaluation of DNA Extraction Methods for Detecting Antimicrobial Resistance Genes in Wastewater

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The global spread of antimicrobial resistance (AMR) demands precise, reproducible detection across environments. Wastewater is a key reservoir of AMR determinants, and effective monitoring of antibiotic resistance genes (ARGs) requires optimized DNA extraction yielding high-quality material for molecular analyses.

We evaluated the performance of five commercial DNA extraction kits: PowerFecal Pro (Qiagen), QIAamp DNA Microbiome (Qiagen), Wizard Enviro Total Nucleic Acid Kit (Promega), NucleoMag DNA Water Kit (Macherey-Nagel), and Quick-DNA/RNA Water Kit (Zymo Research) - using the supernatant and pellet fractions of six wastewater samples collected from distinct sites. The evaluation focused on total DNA yield and the sensitivity of digital polymerase chain reaction (dPCR) for quantifying selected ARGs. The impact of each DNA extraction method on microbial community composition was assessed by high-throughput full-length 16S rRNA gene amplicon sequencing.

Our results indicated that the Promega kit yielded significantly higher DNA concentrations from the supernatant and pellet compared to other kits. Using dPCR, the lowest concentrations of 16S rRNA and carbapenemase resistance gene *bla*VIM-1 were obtained with the Promega kit.

At the microbiome level, the choice of DNA extraction kit had a notable effect on the resulting microbial community profiles. For instance, extractions with Promega, and MN (supernatants) kits frequently resulted in complete depletion of detectable *Pseudomonas*. Additionally, sequencing depth showed a significant positive correlation with the detection of specific taxa, such as members of the order *Enterobacterales*.

This study demonstrates that the choice of DNA extraction method is a critical factor that can introduce systematic biases in the assessment of microbial community composition. These findings provide a robust framework for selecting appropriate DNA extraction protocols to improve the accuracy and comparability of AMR surveillance efforts in wastewater.

#19 Advancing Wastewater Surveillance of Carbapenem-Resistant *Enterobacteriaceae* through Media Optimization and High-Throughput Culturomics

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Carbapenem-resistant *Enterobacteriaceae* (CRE) pose a serious public health challenge due to their resistance to last-resort antibiotics and their ability to transfer carbapenemase-encoding genes to other bacterial species. Wastewater, as a convergence point for human, animal, and environmental microbiota, provides a valuable reservoir for monitoring CRE prevalence and the spread of antimicrobial resistance. Effective detection of CRE in wastewater is critical for early identification and for monitoring the spread of resistance in communities.

This project builds on prior evaluations of the performance of different selective agar media in isolating CRE from wastewater samples. Five commercially available chromogenic media; CHROMagar mSuperCARBA, BD CHROMagar CPE, CHROMID Carba, CHROMID Carba SMART CARB, and CHROMID Carba SMART OXA were systematically compared for their efficiency and selectivity. The analysis revealed 1,949 isolates across diverse genera showing substantial differences in recovery rates among the different test media. Notably, CHROMagar mSuperCARBA demonstrated superior performance by recovering a higher prevalence of public health-relevant genera such as *Escherichia*, *Klebsiella*, *Enterobacter* and *Citrobacter*, highlighting the critical role of media selection in shaping surveillance outcomes. Additionally, considerable variability in colony selection among laboratory analysts further highlighted the limitations of manual approaches and the urgent need for standardized, reproducible workflows.

Building on these findings, we aim to establish a standardized workflow that integrates optimized selective media with high-throughput culturomics for comprehensive CRE surveillance in wastewater. The workflow will incorporate an automated single-cell dispenser in combination with a semi-automated MALDI-TOF-based pipeline for bacterial identification. This approach is designed to minimize biases associated with manual colony selection, reduce labor-intensive processes, and improve the detection of low-abundance CRE populations. By coupling selective media optimization with automated culturomics, the workflow is expected to significantly enhance the efficiency, reproducibility, and resolution of wastewater-based antimicrobial resistance monitoring.

#20 L-arginine metabolism is crucial for the outcome of intestinal *Salmonella* Typhimurium infection

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Arginase 1 (Arg1) and inducible nitric oxide synthase (NOS2) compete for L-arginine as common substrate and exhibit various, sometimes opposing effects on immune responses, tissue regeneration and microbial survival¹. While the role of NOS2 in *Salmonella* Typhimurium (S. Tm) infection is well-characterized, the function of Arg1 is less understood.

Since S. Tm infection disrupts amino acid metabolism² and intestinal inflammation interferes with L-arginine metabolism³, we investigated the effects of Arg1 on colonization resistance in the well-established streptomycin S. Tm infection model. We assessed the composition of intestinal microbiota by 16S rRNA and shotgun sequencing, the metabolome by HPLC and the immune response by scRNAseq and flow cytometry.

Following oral S. Tm infection, predominantly myeloid cells expressed Arg1. Unexpectedly, Arg1 driven L-arginine depletion promoted intestinal S. Tm infection and colitis. A delayed recovery of intestinal microbiota, an altered intraluminal metabolome and an expansion of myeloid cells with enhanced inflammatory signatures accompanied this unexpected phenotype. Dietary L-arginine supplementation restored L-arginine levels, diversity and richness of intestinal microbiota, and thus, restrained S. Tm replication and colitis. Similarly, fecal microbiota transplants (FMTs) from donor mice with a deletion of Arg1 in myeloid cells into wild-type recipients restrained S. Tm infection, while FMTs from wild-type littermates into Arg1-deficient mice prevented an advanced recovery from colitis and infection.

In summary, the Arg1-mediated regulation of L-arginine availability and the subsequent consequences on microbiota composition determine the outcome of S. Tm infection and infection-driven colitis. The identification of specific, L-arginine dependent microbiota and/or metabolites opens new avenues for therapeutic intervention against infectious pathogens.

Literature:

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#21 Environmental controls on methylotrophic methanogenesis in sediments of the East China Sea

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Methylotrophic methanogenesis has recently been recognized as a key process driving cryptic methane cycling in sulfate-reducing sediments. We conducted biogeochemical analyses in two sediment cores to constrain the dynamics and controls of methylotrophic methanogenesis in coastal sediments. At both sites we detected micromolar concentrations of methane in the presence of sulfate, and much higher values (up to 4.2 mM) below the sulfate–methane transition zone (~150–170 cm). Methanogenic substrates, including H₂/CO₂, acetate, and methylated compounds, were also present. Radiotracer experiments confirmed methane production from multiple substrates, and the presence of sulfate did not inhibit methanogenesis at either site. At the coastal site, where marine organic matter predominated (TOC: 0.6%; C/N: ~6.4; δ₁₃C-TOC: –22‰), methane was mainly produced via hydrogenotrophic methanogenesis, consistent with the progressive ¹³C enrichment of dissolved inorganic carbon with depth below the transition zone. At the estuarine site (TOC: 0.5%; C/N: 7.4; δ₁₃C-TOC: –23‰), where a predominance of long-chain odd-carbon n-alkanes indicated elevated terrestrial organic matter input, methylotrophic methanogenesis from methanol and trimethylamine contributed up to 30.2% of methane production. Consistently with the rate measurements, 16S rRNA sequencing revealed that Methanofastidiosales and Methanomethyliales dominated throughout the sediment column at the estuarine site, reflecting higher diversity of methylotrophic methanogens under strong terrestrial input. Sulfate-reducing bacteria, mainly Desulfobacterota, occurred at both sites, whereas putative iron reducers (*Pseudomonas* and *Anaeromyxobacter*) were more abundant and diverse in estuarine sediments, likely due to greater iron oxide availability that may facilitate organic matter degradation and anaerobic methane oxidation. These results suggest that the source and composition of organic carbon, rather than sulfate, regulate methanogenic activity, providing evidence that terrestrial input can substantially enhance methylotrophic methanogenesis in coastal sediments.

#22 Material-Microbiome Interactions

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Estimations show that the anthropogenic mass, the technosphere, already exceeds the biomass on Earth. Understanding the technosphere-environment interactions and their impacts is key to safe and healthy ecosystems and human societies. At BAM, we aim at promoting awareness for microorganisms living and evolving in contact with human-made materials and technical systems. We are creating datasets of biofilms on plastic and other anthropogenic materials and in the context of important technical challenges (e.g. microbially influenced corrosion or biocide-induced antimicrobial resistance). This enables us to identify and examine key organisms in the investigated systems that potentially harbor new, also industrially relevant, species and traits. Further, we explore material effects on aquatic microbial communities, aiming at the development and establishment of innovative and environmentally relevant methodologies. By using bioinformatics and molecular tools and interdisciplinary research, we enhance the understanding of material-microbiome interactions for a safe and sustainable future.

#23 Impact of vendor-specific bacterial and fungal variations in the murine gastrointestinal tract on the colonization with *Candida albicans*

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Candida albicans establishes complex interactions with members of the gastrointestinal microbiota. A key aspect of these interactions is colonization resistance, where resident bacteria limit fungal overgrowth. Antibiotic-induced disruption of the microbiota is therefore a major risk factor for candidiasis. To explore bacterial taxa potentially contributing to colonization resistance against *C. albicans*, we examined natural microbial variation among laboratory mice from different breeding facilities. Fecal material from 20 C57BL/6 colonies was analyzed using 16S rRNA gene and ITS region sequencing. Colonies differing in microbial composition were selected, and five colonies were included in subsequent colonization experiments. Following antibiotic exposure and oral inoculation with *C. albicans*, fecal samples were longitudinally collected to track fungal burden and to assess variations in bacterial community structure.

Although the initial microbiota and mycobiota profiles varied considerably among colonies, colonization outcomes were remarkably consistent. Each mouse colony showed comparable *C. albicans* dynamics, with antibiotic treatment predictably enhancing fungal colonization. Surprisingly, sucrose supplementation alone was sufficient to promote persistent and elevated fungal levels, even in the absence of antibiotic treatment.

Our findings highlight that pronounced inter-colony variation in the microbial composition of laboratory mice does not necessarily translate into altered resistance against *C. albicans*. Furthermore, fecal microbiome analysis revealed a homogenization of bacterial composition during the acclimatization phase. As expected, even stronger shifts in bacterial composition emerged when antibiotic treatment was administered during the experimental period.

Together, these results provide important insight into the interplay between bacterial communities and *C. albicans* colonization. The dataset generated here will serve as a foundation for further analyses aimed at understanding colonization resistance and fungal dynamics in the gastrointestinal tract in greater detail.

#24 *Methanosphaerula subterranea* EG compromises cell defense systems in exchange for stable energy metabolism pathways under high CO₂ conditions

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Microbial adaptation to high CO₂, especially through evolution at the genomic level, can provide insights into the metabolism and survival strategies that may have been employed by the primitive life forms on early, CO₂-rich Earth. From the natural high CO₂ subsurface environment in Hartousov, Eger rift area, Czech Republic, we isolated an active methanogen strain, *Methanosphaerula subterranea* EG. Both *M. subterranea* EG and its sister species, *M. palustris* E1-9c, share a similar lacustrine origin but have since adapted to contrasting environments (ambient vs. high-CO₂) and therefore provide an ideal system for exploring genomic adaptation to high-CO₂ conditions. Pan-genomics analysis of *Methanosphaerula* and their closest relative, the uncultivated genus UBA288, reveals a significant genome reduction on the cell defense systems against viruses. Specifically, strain EG contains only one set of type IV restriction-modification system that relies on a standalone m5C specific restriction enzyme, a single set of type I-E CRISPR system, and limited toxin-antitoxin system, even though virus communities are as influential in high CO₂, as in low-CO₂ environments. By comparison, most of the reference genome and metagenome-assembled genomes (MAGs) possess more than one set of related systems. In contrast, energy metabolism pathways are much more conserved, as the pathway completeness involving energy harvesting and carbon fixation across the analyzed genomes and MAGs show far fewer differences. The persistence of the high CO₂-adapted strain EG in maintaining its methanogenesis and acetyl CoA pathway intact, while compromising its other functional systems, supports the hypothesis that these pathways represent the most ancient biological processes.

#25 The gut microbiota predicts and time-restricted feeding delays experimental colitis (paper title)

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The etiology of inflammatory bowel disease (IBD) remains unclear, treatment options unsatisfactory and disease development difficult to predict for individual patients. Dysbiosis of the gastrointestinal microbiota and disruption of the biological clock have been implicated and studied as diagnostic and therapeutic targets.

Here, we examine the relationship of IBD to biological clock and gut microbiota by using the IL-10 deficient (*IL-10*^{-/-}) mouse model for microbiota-dependent spontaneous colitis in combination with altered (4 h/4 h) light/dark cycles to disrupt and time restricted feeding (TRF) to restore circadian rhythmicity. Fecal samples were collected in 6 h intervals during the day, taxonomic microbiota compositions were determined by 16S rRNA gene amplicon sequencing and cosinor-based models were used for cosine wave correlations of the relative abundances of bacterial taxa.

We show that *IL-10*^{-/-} mice were characterized by altered microbiota composition and impaired microbiota rhythmicity irrespective of external clock disruption, which had no consistent colitis-promoting effect on *IL-10*^{-/-} mice. Notably, TRF delayed colitis onset and increased gut microbiota rhythmicity in *IL-10*^{-/-} mice. Compositional changes and reduced rhythmicity of the fecal microbiota preceded colitis and could predict colitis symptoms for individual *IL-10*^{-/-} mice across different experiments.

Our findings suggest clinical relevance of gut microbiota rhythmicity and composition as potential biomarkers with predictive potential for colitis development and of TRF as a promising dietary intervention to prevent colitis in high-risk individuals, which should be further studied.

#26 Contamination-controlled profiling reveals uGI microbiota types associated with opportunistic pathogens and TNF- α

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The upper gastrointestinal (uGI) microbiota has been implicated in infectious, metabolic, and immunological conditions, yet remains poorly characterized due to invasive sampling methods and low microbial biomass. We developed and validated a contamination-controlled 16S rRNA gene and transcript-based protocol to profile the murine and human uGI microbiota from low-biomass samples. We applied this protocol to murine esophageal, gastric, and duodenal tissues, and to human saliva, gastric, and duodenal aspirates from patients undergoing endoscopy for suspected food-related, mild GI symptoms. Our objective was to identify conserved compositional and structural uGI microbiota patterns and assess their clinical relevance in relation to pathogen burden and inflammation. In mice, we found evidence for transcriptionally inactive and active intestinal taxa along the uGI tract, supporting horizontal microbiota transfer. In humans, we identified two distinct, inversely correlated salivary microbiota types – one dominated by the *Prevotella* 7, the other by the *Neisseria* genus – which were conserved in the duodenum. The *Prevotella* 7-dominated uGI microbiota type was associated with lower relative abundances of gastrointestinal and extraintestinal opportunistic pathogens. These patterns were reproducible in an independent cohort and associated with lower systemic TNF- α levels. Our findings suggest that noninvasive salivary microbiota profiling can stratify individuals based on uGI microbiota composition and inflammation-associated risk traits, offering new opportunities for clinical applications and translational studies.

#27 Skin colonisation dynamics and immune interactions delineate commensal and pathogenic strains of *Staphylococcus epidermidis*

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Staphylococcus epidermidis is a beneficial coloniser of mammalian skin, though some pathogenic strains can cause sepsis, particularly in premature neonates. The widespread presence of *S. epidermidis* on the skin, combined with its increasing multidrug resistance, complicates treatment and calls for alternative strategies. The dynamics of skin *S. epidermidis* and its role in neonatal sepsis were analyzed in a longitudinal cohort of preterm infants. *Staphylococcus epidermidis* isolates from neonatal skin swabs were classified into 30 clonotypes based on genes related to multidrug resistance, biofilm formation, and quorum sensing. The most prevalent clonotype carried mobile elements for multidrug resistance and biofilm regulation. Blood culture-positive sepsis isolates matched this pathogenic clonotype, highlighting the carrier risk of pathogenic strains in the preterm skin microbiota. Human 3D epidermal and gnotobiotic mouse models were exposed to low biomass bacteria to reveal the distinct features of commensal and pathogenic strains in early-life colonization. Commensal strains rapidly expanded, colonized deeper skin layers, and triggered immune responses, while pathogenic strains failed to expand and formed silent biofilms on the epidermal surface. Long-term pathogen colonization triggers tissue inflammation, marked by cytokine secretion, neutrophil infiltration, and Langerhans cell migration to lymph nodes. During bacteremia, the pathogen spreads systemically and colonized the gut, as seen in infants with necrotizing enterocolitis. Over 80% of isolates from preterm skin and blood carried the multidrug resistance island. Therefore, bacteriophages were evaluated as targeted bioregulators. Our data highlight *S. epidermidis* as a significant threat on preterm infant skin and suggest further investigation of phages as a prophylactic strategy.

#28 Biodegradation of Polyurethane: A Co-culture synergy between *Bacillus subtilis* and Actinobacteria

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Polyurethane (PU) is a versatile polymer used in several technical applications as foams, coatings, binders, and elastomers. However, its persistence in the environment makes recycling challenging, particularly when combined with non-biodegradable fillers. This study explores biodegradation as a recycling strategy in which a PU film is degraded. A co-culture of *Bacillus subtilis* with three Actinobacteria, *Streptomyces violaceoruber*, *Rhodococcus ruber*, and *Micrococcus luteus*, respectively was used for PU biodegradation over 14 days in minimal medium. Biodegradation products were characterized by gas chromatography–mass spectrometry (GC–MS), while surface changes in PU films were analysed by colorimetry, contact angle analysis, and microscopy. After incubation, phthalates, aromatic intermediates, and aliphatic hydrocarbons were detected in the media, while visible cracks and surface damage were observed on the PU films. A discolouration of the PU films of up to ΔE 3.5 and ΔL -1.5 was observed. Surface energy increased by 48%, whereas wettability decreased by 43% in samples treated with *B. subtilis*+*S. violaceoruber* and *B. subtilis*+*R. ruber* co-cultures. Compared to employing single cultures such as *R. ruber* and *B. subtilis* which achieved lower wettability, the synergistic co-culture approach demonstrated enhanced polyurethane degradation within 14 days. Overall, this work demonstrates the feasibility of employing microbial consortia to accelerate PU degradation, offering a potential pathway for recycling PU-based components. Optimization of strain combinations and conditions could further enhance this microbial strategy for sustainable end-of-life material management.

#29 Legacy herbicides and coral reef health: atrazine as a case study

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Agrochemical pollution is increasingly impacting coastal marine ecosystems through continuous estuarine discharge and episodic surface runoff. Among these pollutants, legacy pesticides pose a substantial threat to marine communities due to their persistence in both sediments and seawater. Atrazine, a very persistent herbicide (DT50 > 365 days) that inhibits the photosystem II in phototrophs, remains one of the most commonly detected unapproved herbicides in marine environments. As such, atrazine also comprises the health and resilience of photosymbiotic metazoans by disrupting their symbiosis with phototrophs.

Despite these risks, the effects of atrazine on host-microbe interactions, including alterations in the gene expression of both partners, host development, and photosymbiotic function, remain poorly understood. To address this knowledge gap, we utilize the photosymbiotic anemone *Exaiptasia diaphana*, which lives in symbiosis with photosynthetic dinoflagellates (including Symbiodiniaceae), to investigate the ecotoxicological effects of atrazine under controlled laboratory conditions. *E. diaphana* was exposed for short (7 days) - and long-term (60 days) to various concentrations of atrazine, encompassing environmentally relevant, EU Environmental Quality Standards, and experimental concentrations (0.054 – 100 µg/L). We assess the effects of atrazine by analyzing the microbial community using 16S rRNA metabarcoding, host and symbiont responses through gene expression analyses, and monitoring the photophysiology and the development of *E. diaphana*.

Our study provides insight into the effects of various relevant concentrations of atrazine on host-symbiont interactions under short (7 days) - and long-term (60 days) exposure, potentially informing *ex-situ* management strategies and microbiome-informed conservation and restoration efforts for vulnerable coastal habitats.

#30 Uncoupling Microbial Composition and Function: Insights into Bile Acid Pathways During a Five-Day Fasting Protocol

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Bile acids are now recognized as key modulators of host–microbiome interactions, impacting diverse physiological systems such as glucose and lipid metabolism, immune function, and cardiovascular health. In addition to their well-known role in emulsifying dietary fats, bile acids serve as signaling molecules by activating receptors like FXR and TGR5, thereby linking microbial activity in the gut to systemic metabolic regulation. Although the transformation of primary to secondary bile acids—mainly via microbial enzymes such as bile salt hydrolase (BSH) and 7 α -dehydroxylase—is well characterized, the immediate effects of dietary interventions like fasting on this system remain insufficiently explored.

This study examined the effects of a five-day, low-calorie (250 kcal/day) vegetable juice fasting protocol on gut microbial composition and secondary bile acid metabolism in a cohort of 36 healthy adults. Fecal and plasma samples were collected before and after the fasting period. Using quantitative PCR (qPCR), we analyzed the abundance of four bacterial taxa relevant to bile acid metabolism and gut barrier function: *Akkermansia muciniphila*, *Bacteroides ovatus*, *Bacteroides fragilis*, and *Prevotella copri*. These species were chosen due to their established roles in mucin degradation, bile acid transformation, and metabolic health. The qPCR results showed no statistically significant alterations in the abundance of these microbes following the fasting intervention.

Despite the lack of compositional changes, previous research suggests that microbial functionality can shift independently of taxonomy. To investigate this possibility, shotgun metagenomic sequencing is being conducted to assess potential changes in genes involved in bile acid metabolism—specifically those associated with the bile acid-inducible (bai) operon and BSH activity. These initial results underscore the complexity of host–microbiome interactions, where functional adaptations may occur without significant taxonomic restructuring. Our findings highlight the value of integrating multi-omic approaches to gain deeper insights into microbiome responses to acute dietary changes. Notably, short-term fasting may influence bile acid signaling and metabolic flexibility through functional microbial shifts, even in the absence of notable changes in microbial composition—suggesting new directions for targeting bile acid pathways in metabolic and cardiovascular health interventions.

#31 Towards a circular economy through microbial stability and in situ cultivation in a photobioreactor - chicken coop system

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Finding sustainable systems that minimize waste and maximize resource efficiency especially in agriculture is a major challenge for circular economy. In this context, biological systems such as photobioreactors offer an attractive way to convert under-utilized nutrient streams such as CO₂ into useful biomass products, such as photosynthetic microorganisms (PMOs) like *Spirulina* (Cyanobacteriota).

In this context, we investigated microbial interactions and community stability in an integrated photobioreactor-chicken coop system, where waste and nutrients were retained, to enable closed loop operations.

For this, samples taken from the photobioreactor at different timepoints were analyzed by metagenomics to evaluate the taxonomic composition and metabolic potential. First analysis demonstrated the impact of chicken house exhaust on the cyanobacterial culture via CO₂ fixation and provided insights into NH₃ metabolism. Taxonomic classification of the microbial community suspended in the photobioreactor revealed a predominance of bacterial organisms before aeration, with a notable shift to a more diverse community, including eukaryotic species, after exposure to chicken coop exhaust.

In perspective we integrated a novel macroporous material into the system to allow direct monitoring of microbial dynamics. This will provide further valuable insights into microbial interactions in this particular context and allow us to directly assess community stability. Understanding community stability is essential to run integrated, closed-loop biological production systems. It illustrates how microbiome research can contribute to circular economy and serves as a precursor for possible integration into biological systems for resource efficient and sustainable solutions.

#32 Gut Microbiota as a Key Regulator of Systemic Immunity After Stroke

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Neuroinflammation plays a crucial role in determining outcomes after ischemic stroke. A growing body of evidence supports the concept of an excessive systemic immune response after stroke, which contributes to secondary brain degeneration. Emerging research highlights the gut microbiota (GM) as a key regulator of immune function, with significant implications for both basic biology and translational medicine. Acting as a bioreactor, the microbiome produces not only energy precursors but also immunomodulatory molecules. We have previously demonstrated that stroke leads to persistent dysbiosis of the GM, which exacerbates brain injury. Mechanistically, this dysbiotic state promotes long-term proinflammatory T-cell polarization in both the intestinal immune compartment and ischemic brain. In our latest work, we show that depletion of GM—either through broad-spectrum antibiotics or germ-free conditions—attenuates neutrophil activation after stroke. This disarming of neutrophil responses was associated with decreased expression of inflammatory genes in the brain, reduced vascular thrombosis, smaller infarct volumes, and improved behavioral outcomes. In summary, our findings reveal that GM plays a decisive role in post-stroke systemic immune activation and directly influences stroke severity. Targeting GM composition may offer a promising therapeutic option for modulating neuroinflammation and improving stroke outcomes.