Molecular Approaches in Microbial Ecology: Processes, Challenges, and Insights into Fish Microbiomes:

Molecular tools have revolutionized microbial ecology by enabling detailed investigation of fish-associated microbiomes.

This topic explores key microbial processes, methodological challenges, and current limitations in studying fish microbiome structure, function, and host–microbe interactions.

BASILI MARCO

CNR IRBIM ANCONA

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IRBIM
ISTITUTO PER LE
RISORSE BIOLOGICHE
E LE BIOTECNOLOGIE
MARINE

FishMed-PhD

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

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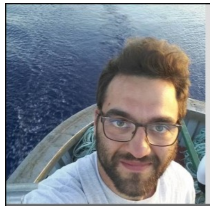
FiSh MICRO



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E LE BIOTECNOLOGIE
MARINE

Marco Basili
Microbial ecology Lab.
marco.basili@irbim.cnr.it

CNR – IRBIM
LAB. MICROBIAL ECOLOGY

Circles

ARENA

Aquatic
Pollutants

NATIONAL
BIODIVERSITY
FUTURE CENTER

Microbiomes, Symbiosis & Host–Microbe Interactions

- Molecular tools
(multiomics, sequencing)
- Host-associated microbiome theory
- Cross-host comparisons

Marine Organisms, Physiology & Adaptation

- Microbiomes influence on the host
(HOLOBIONT)
- Molecular tools overlap (omics, e-DNA)

FishMed-PhD

Ecosystems, Biodiversity & Environmental Change

- Microbiomes drive ecosystem processes
(Nutrient cycling, Food webs, Habitat
health,
- Environmental microbiology methods
- Indicators of ecosystem change

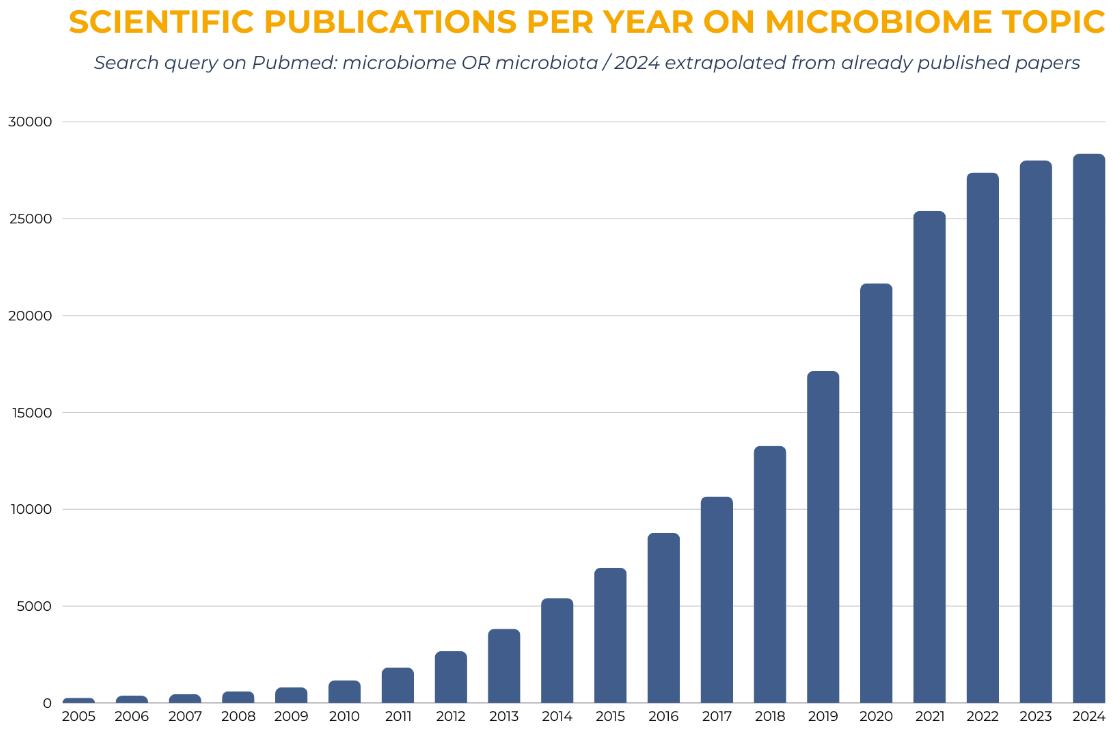
Fisheries & Aquaculture Applications

Fish microbiomes affect:
Growth rates, Feed efficiency,
Disease outbreaks, Stock health,
→ Aquaculture sustainability
→ Fishery management

Molecular ANALYSIS

Molecular approaches in ecology refer to the **use of DNA, RNA, proteins, and metabolites** to study organisms, communities, and ecosystems at the genetic and functional level.

These methods allow researchers to detect species, understand interactions, and uncover processes that are often invisible to traditional observation-based ecology.



SCIENTIFIC PUBLICATIONS PER YEAR ON MICROBIOME TOPIC

Search query on Pubmed: microbiome OR microbiota / 2024 extrapolated from already published papers

Molecular ANALYSIS

Molecular tools are applicable across systems

- High-throughput sequencing, metabarcoding, and meta-omics can be applied from host-associated microbiomes to entire ecosystems.

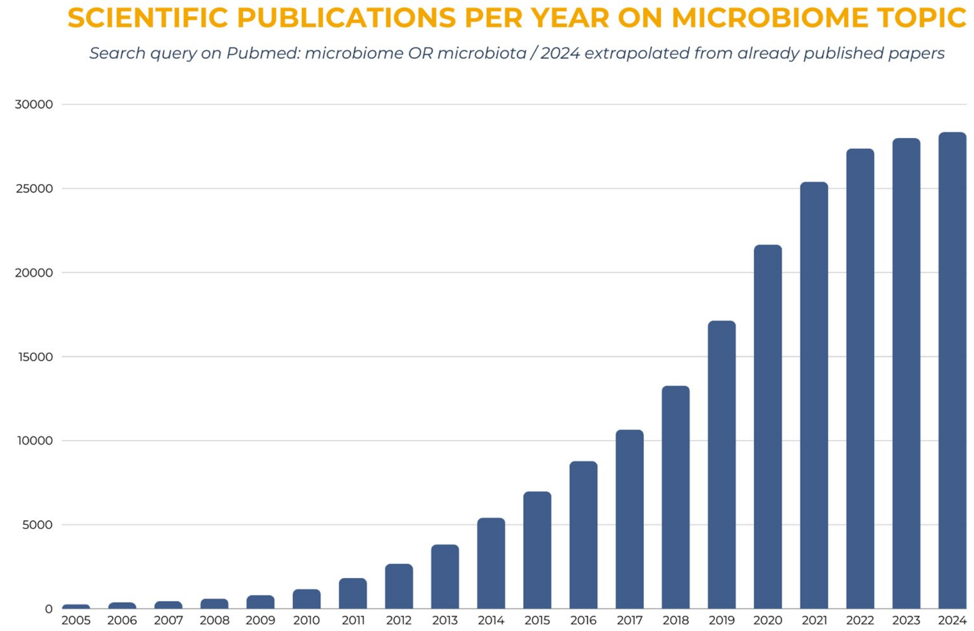
- These approaches reveal patterns invisible to traditional ecological methods.

Microbes as hidden drivers of ecology

- Understanding microbial processes helps explain ecosystem responses to environmental change.

Bridge between organismal biology and ecosystem science

- Molecular ecology links:
  - Host health & environmental quality
  - Population dynamics & microbial processes
  - Aquaculture practices & ecosystem impacts
- Supports a holistic “**holobiont**” perspective in ecological research.


SCIENTIFIC PUBLICATIONS PER YEAR ON MICROBIOME TOPIC

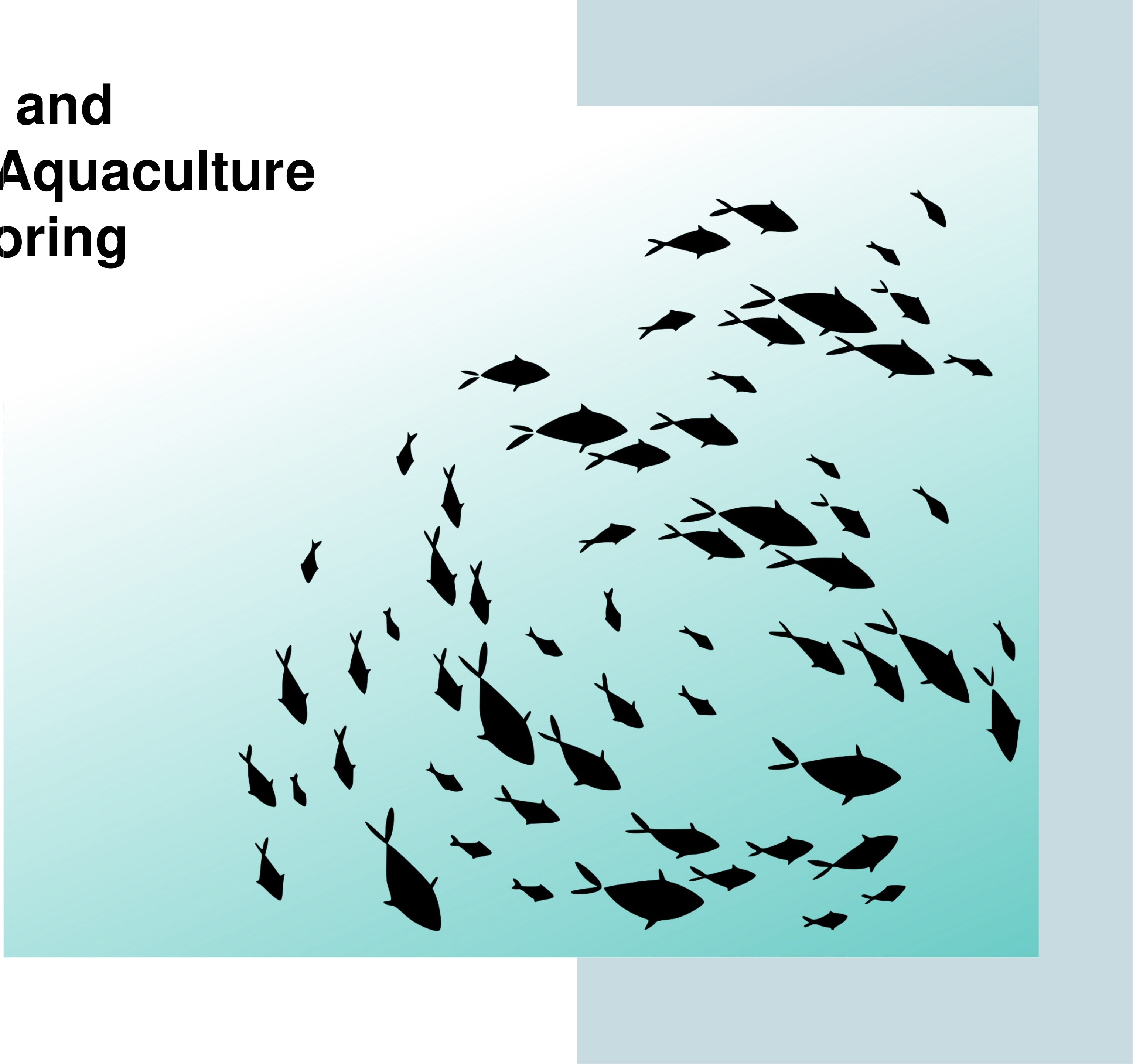
Search query on Pubmed: microbiome OR microbiota / 2024 extrapolated from already published papers

Applications for conservation and manageme

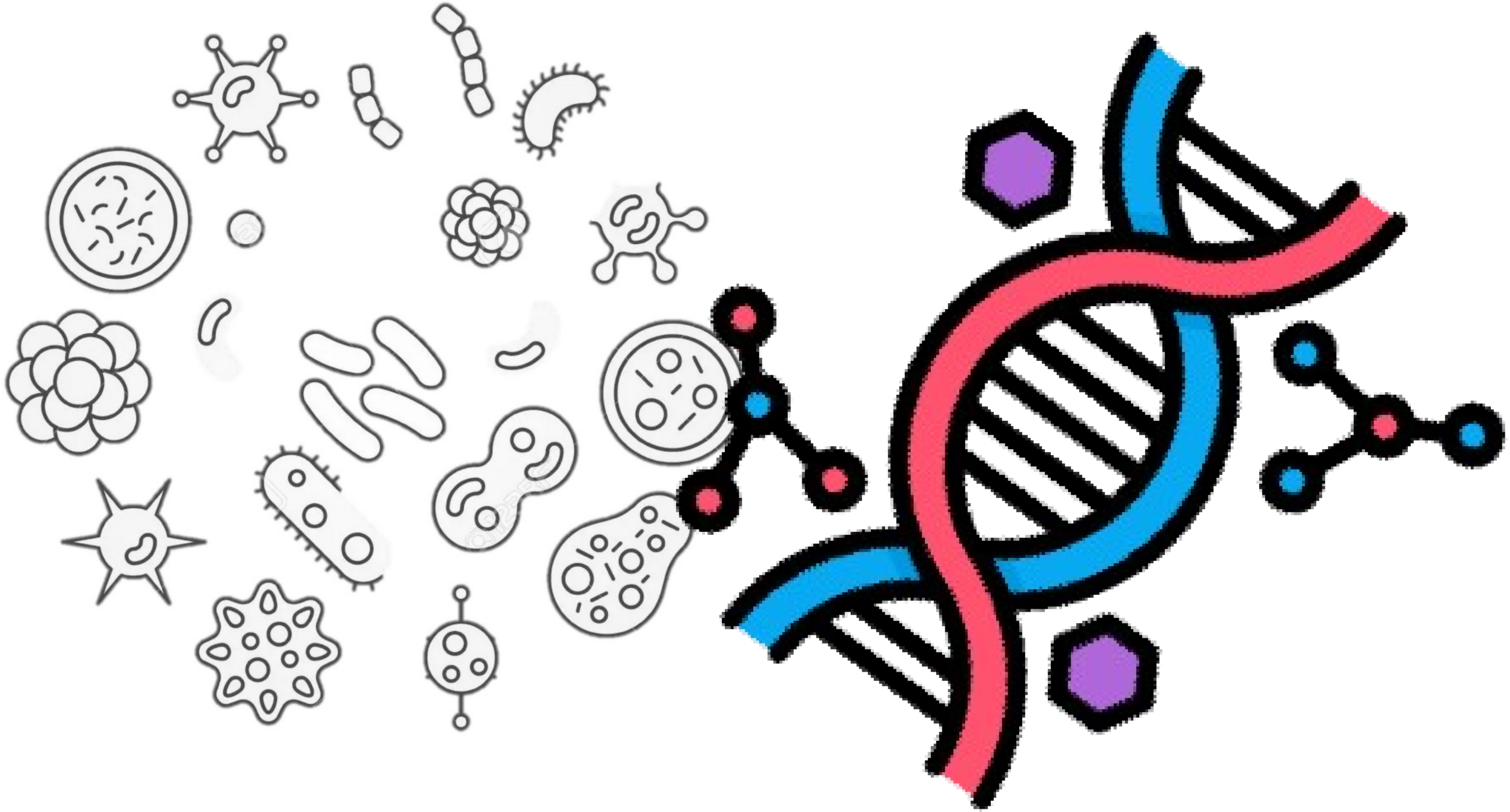
- Microbiome indicators for monitoring ecosystem health and pollution.

Microbiome, Biodiversity, and Application Strategies in Aquaculture and Environmental Monitoring

1- Introduction on Microbiome
2- Fish Microbiome
3- Application in Ecology
4- Bioinformatic tools
5- Conclusions



What is the MICROBIOTA–MICROBIOME?

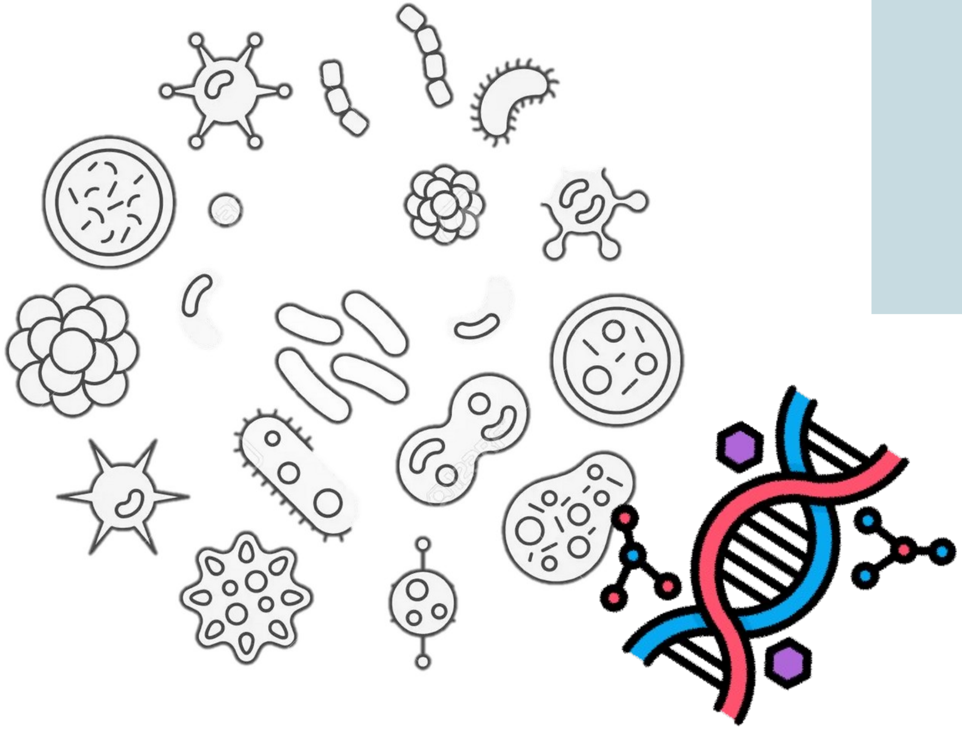


What is the
MICROBIOTA–MICROBIOME?

The microbiota is the assemblage of microorganisms inhabiting a defined environment or host.

- **It includes bacteria, archaea, fungi, protists, and viruses.**
- **The microbiome refers to the collective genetic material and functional potential of these microorganisms.**
- **Microbial communities drive biogeochemical processes and influence host physiology and ecosystem functioning.**

→ Understanding microbiota structure is essential for sustainable management of natural and aquatic resources.



What we can study with MICROBIOME?

Each microbiome contains diverse taxa with uneven abundance distributions

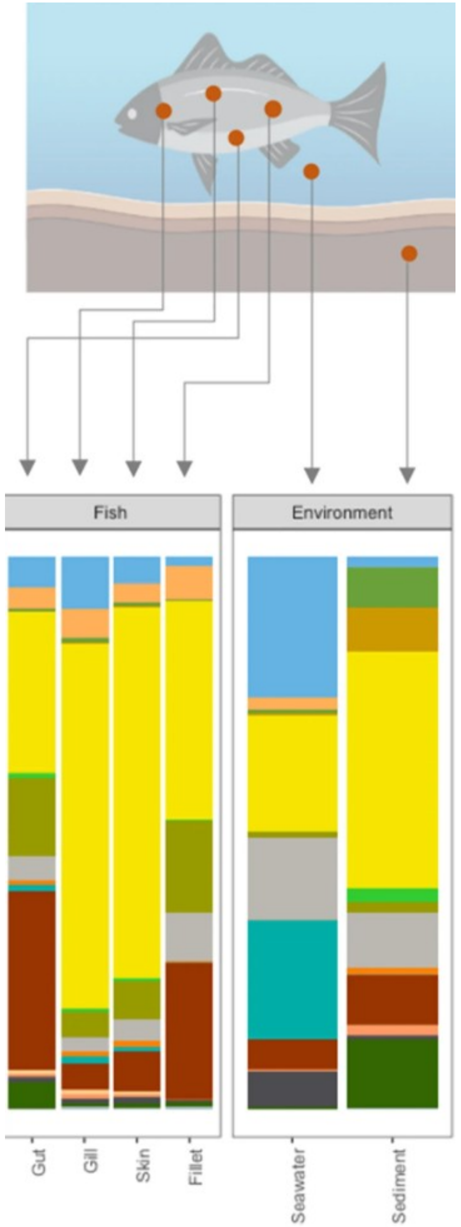
→ **Alpha Diversity**

Microbial communities form complex ecological networks shaped by cooperation, competition, and host interactions.

Community structure is influenced by environmental conditions, host factors, and stochastic processes.

→ **Beta diversity**

Composition can shift rapidly in response to environmental stressors and anthropogenic pressures.



What we can study with MICROBIOME?

Functional Redundancy in Microbial Ecosystems

Functional redundancy refers to the ability of multiple microbial taxa to perform similar **ecological functions.**

Ecosystem processes (e.g., nutrient cycling, decomposition, nitrification) can remain stable even when community composition changes.

This explains why ecosystems may appear **resilient** despite biodiversity loss or environmental disturbance.

Why It Matters?

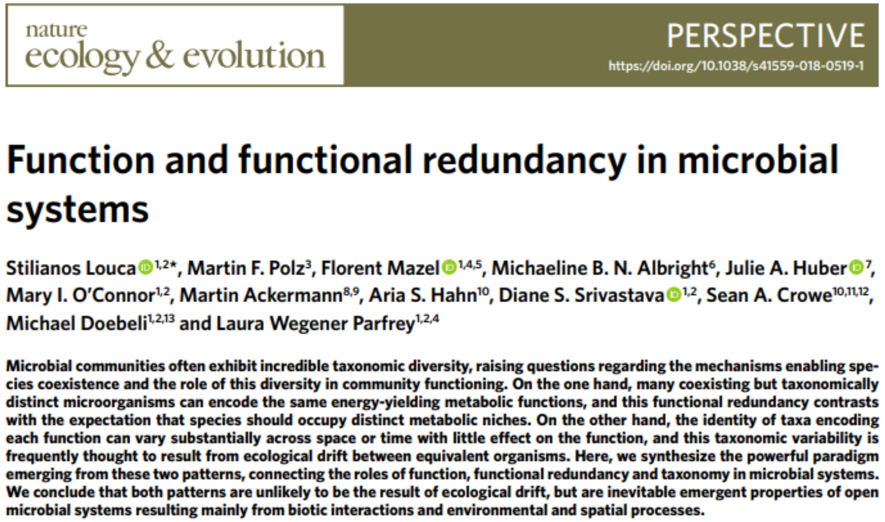
Provides ecosystem stability and resilience under stress (pollution, warming, salinity shifts).

Critical in aquatic systems where microbial turnover is rapid.

Marine & Aquaculture

Different bacterial taxa can perform ammonia oxidation in biofilters of recirculating aquaculture systems (RAS), maintaining water quality even if species composition shifts.

Functional Redundancy in Microbial Ecosystems



nature ecology & evolution

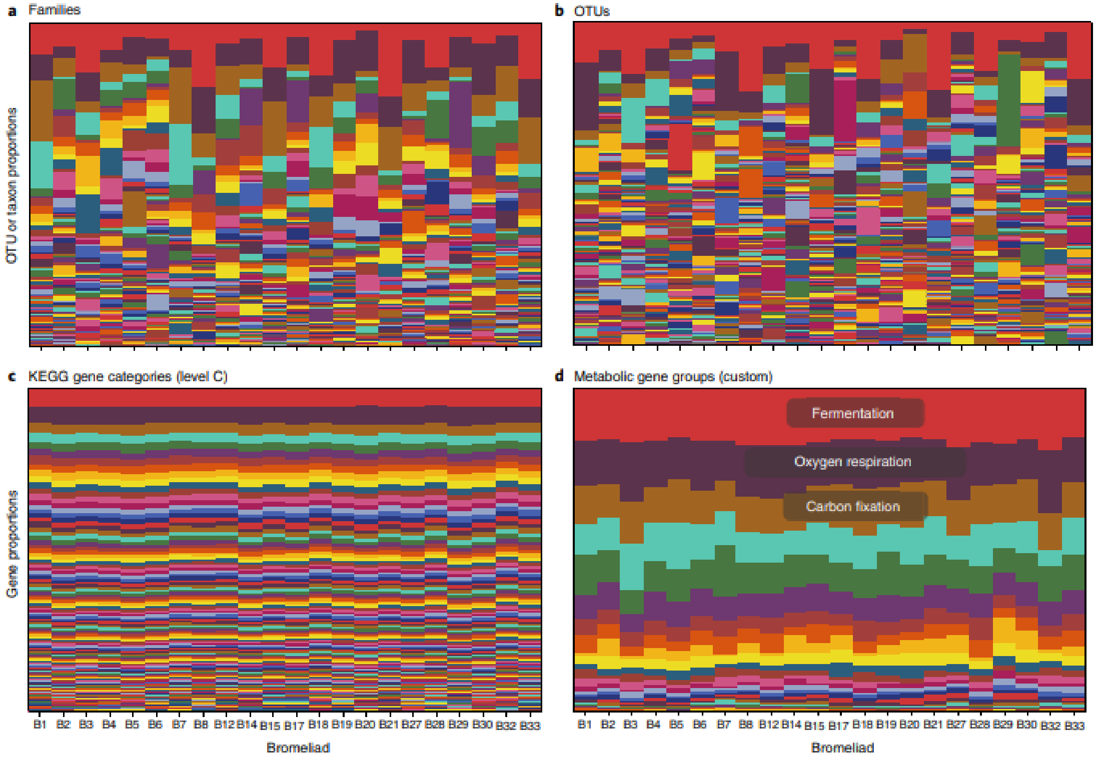
PERSPECTIVE

https://doi.org/10.1038/s41559-018-0519-1

Function and functional redundancy in microbial systems

Stilianos Louca[1,2]*, Martin F. Polz[3], Florent Mazel[1,4,5], Michaeline B. N. Albright[6], Julie A. Huber[7], Mary I. O'Connor[1,2], Martin Ackermann[8,9], Aria S. Hahn[10], Diane S. Srivastava[1,2], Sean A. Crowe[10,11,12], Michael Doebeli[1,2,13] and Laura Wegener Parfrey[1,2,4]

Microbial communities often exhibit incredible taxonomic diversity, raising questions regarding the mechanisms enabling species coexistence and the role of this diversity in community functioning. On the one hand, many coexisting but taxonomically distinct microorganisms can encode the same energy-yielding metabolic functions, and this functional redundancy contrasts with the expectation that species should occupy distinct metabolic niches. On the other hand, the identity of taxa encoding each function can vary substantially across space or time with little effect on the function, and this taxonomic variability is frequently thought to result from ecological drift between equivalent organisms. Here, we synthesize the powerful paradigm emerging from these two patterns, connecting the roles of function, functional redundancy and taxonomy in microbial systems. We conclude that both patterns are unlikely to be the result of ecological drift, but are inevitable emergent properties of open microbial systems resulting mainly from biotic interactions and environmental and spatial processes.



Keystone Taxa: Disproportionate Ecological Impact

Keystone taxa are microbial species that exert a disproportionately large influence on community structure and ecosystem function relative to their abundance.

Removal or decline of these taxa can trigger cascading ecological effects.

- Central nodes in microbial interaction networks

- Control of key processes (e.g., nitrogen cycling, pathogen suppression)

- Influence on host health in microbiomes

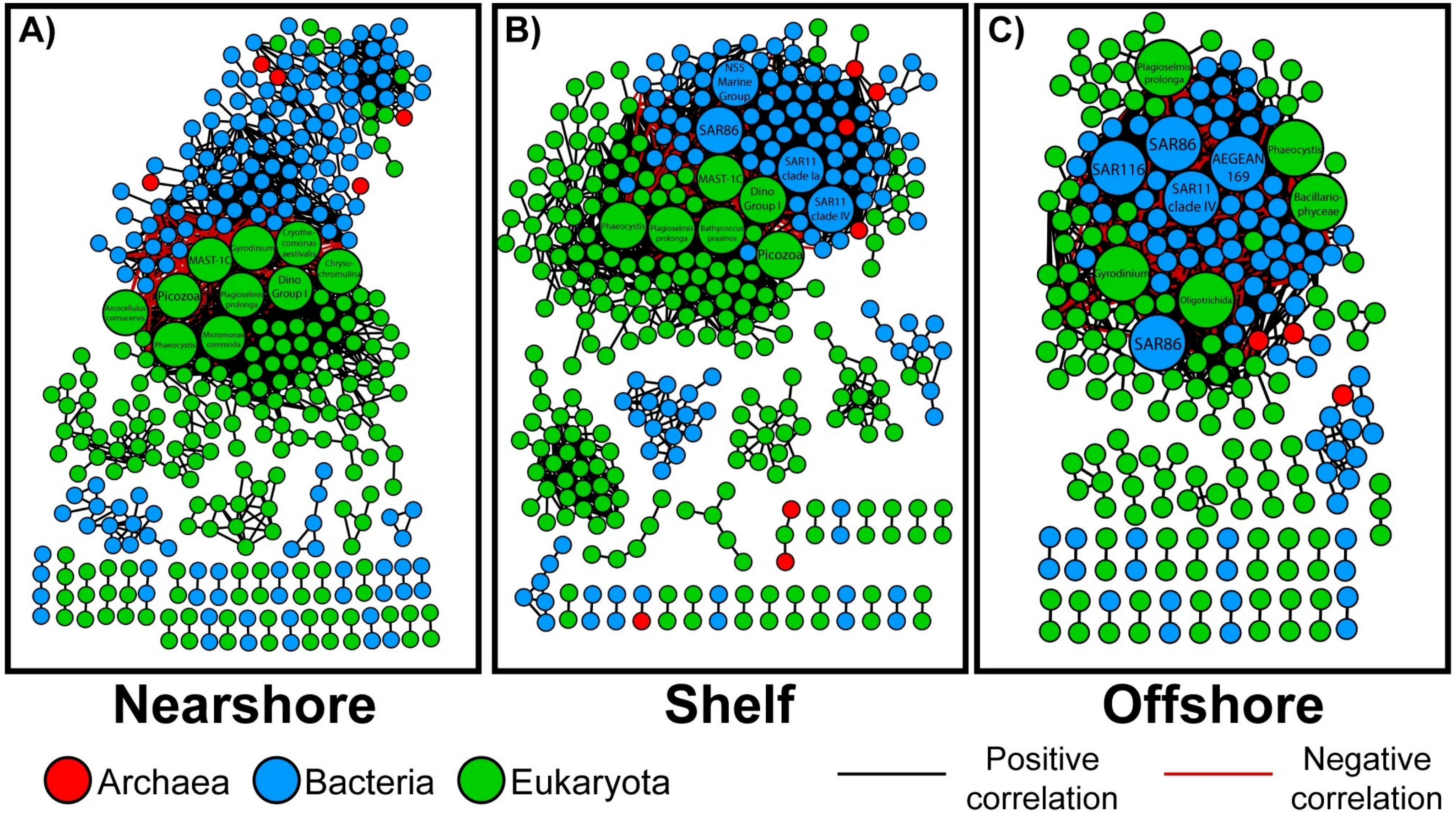
Fish Microbiome: Certain gut bacteria regulate immune responses and pathogen resistance in fish; loss may increase disease susceptibility.

Marine Ecosystem: Specific nitrifying or sulfur-cycling microbes maintain sediment biogeochemistry.

Network analyses increasingly identify keystone microbes using co-occurrence and interaction models.

Systems may be resilient due to redundancy but still vulnerable if keystone taxa are lost.

Keystone Taxa



Understanding redundancy and keystone roles transforms microbial ecology from descriptive to predictive science — essential for managing future environmental change.

Main Ecological Functions of Microorganisms

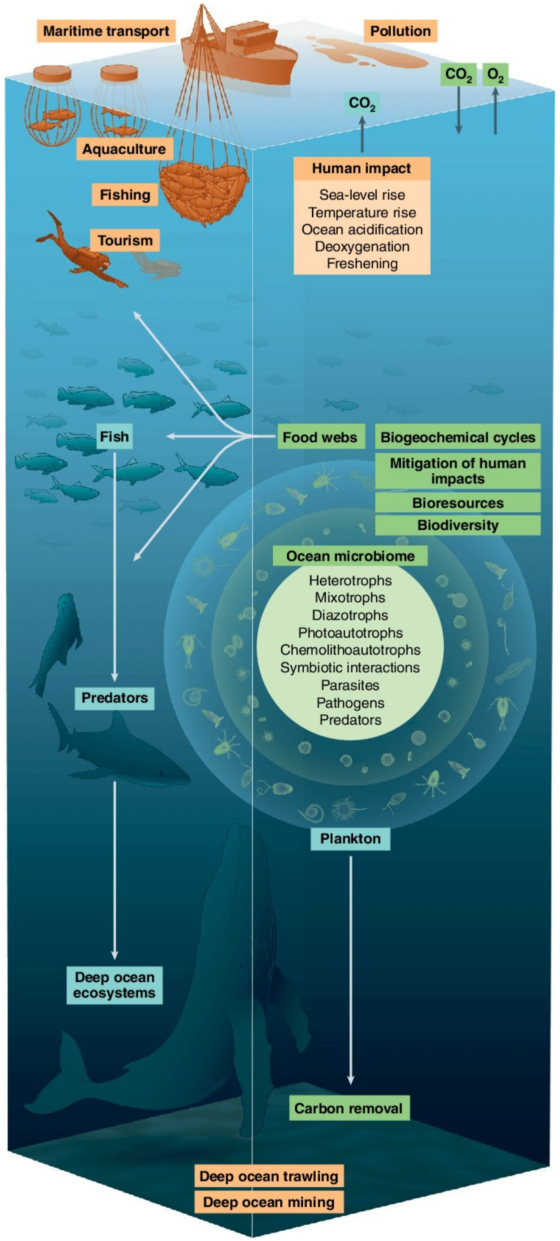
- Microorganisms drive the carbon, nitrogen, phosphorus, and sulfur cycles.

- They decompose organic matter and regulate nutrient availability across trophic levels.

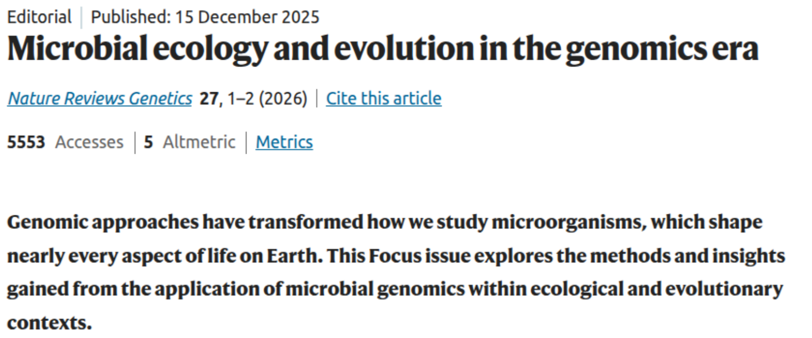
- Microbial metabolism influences primary productivity and food-web dynamics.

→ determine the productivity of aquatic systems that sustain fisheries.”

- Their activity is fundamental for ecosystem stability, resilience, and recovery after disturbance.



“Why microbial ecology will shape the next decade of marine research?”



Editorial | Published: 15 December 2025

Microbial ecology and evolution in the genomics era

Nature Reviews Genetics **27**, 1–2 (2026) | Cite this article

5553 Accesses | **5** Altmetric | Metrics

Genomic approaches have transformed how we study microorganisms, which shape nearly every aspect of life on Earth. This Focus issue explores the methods and insights gained from the application of microbial genomics within ecological and evolutionary contexts.

Comment | Published: 16 October 2025

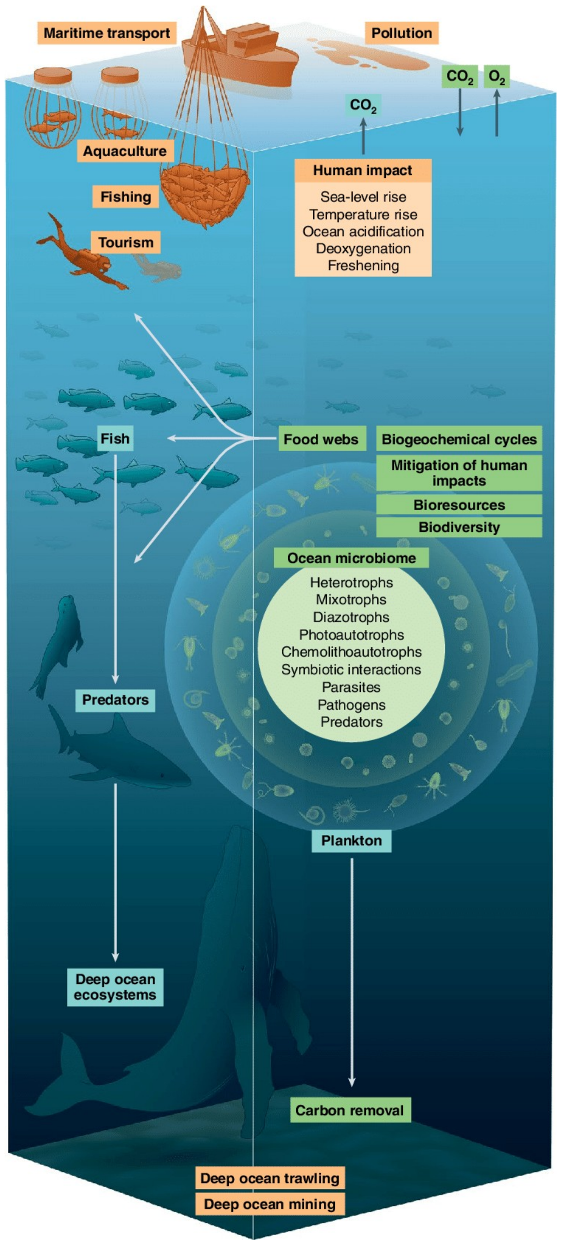
Microorganisms as architects of a sustainable future

Nicole S. Webster

Nature Reviews Genetics **27**, 5–6 (2026) | Cite this article

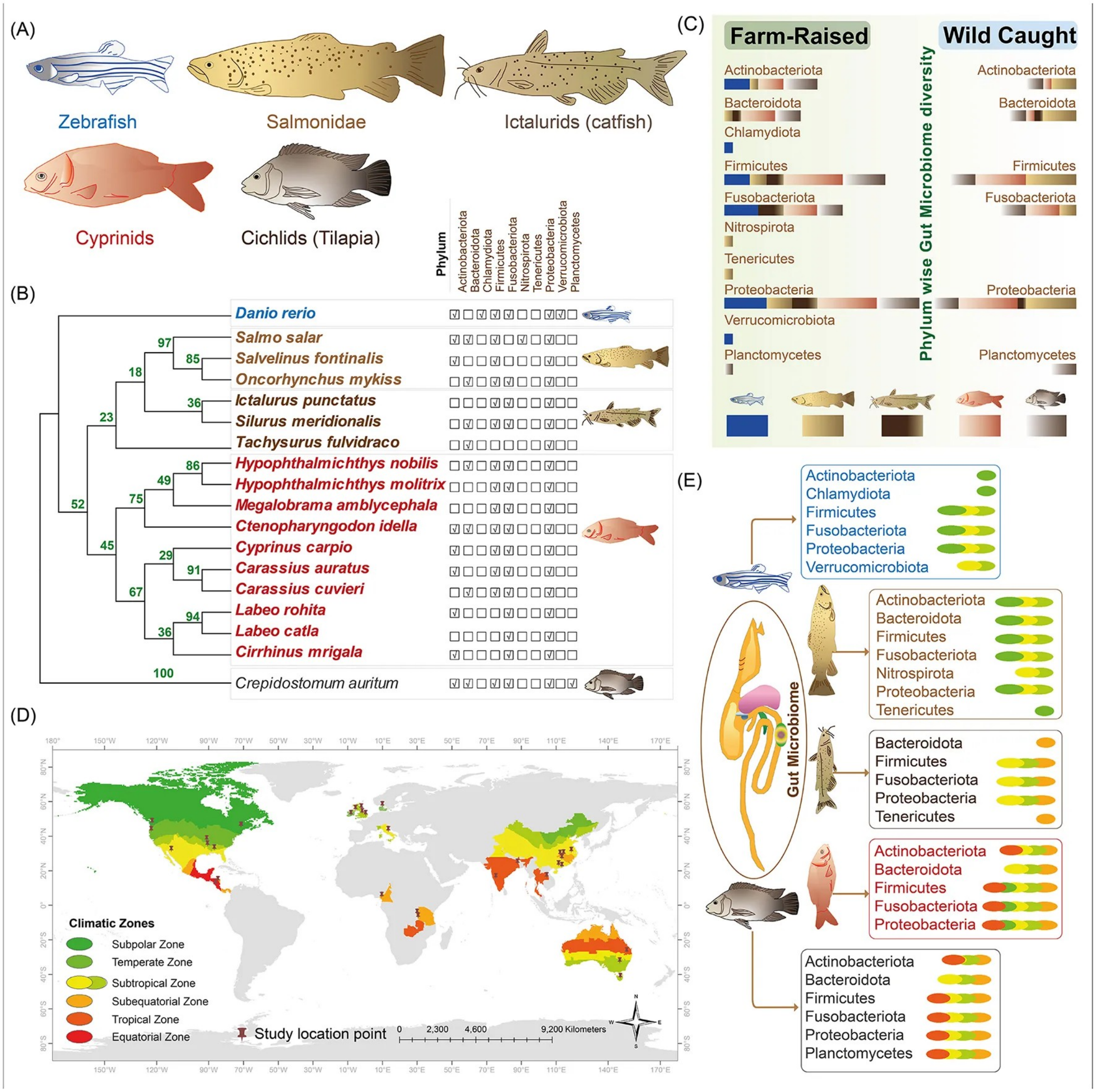
1130 Accesses | **1** Citations | **17** Altmetric | Metrics

Microorganisms are central to climate stability, food security and biodiversity, yet they remain absent from global sustainability frameworks. Recognizing and mobilizing their power is essential if we are to meet the challenges of the coming decades.



“Why microbial ecology will shape the next decade of marine research?”

in FISH host .....



MINI REVIEW article

Front. Microbiol., 07 January 2025

Sec. Microbial Symbioses

Volume 15 - 2024 | https://doi.org/10.3389/fmicb.2024.1521048

Fish gut microbiome and its application in aquaculture and biological conservation

Host Health and Disease

- Microbial communities are fundamental to immune function, nutrition, and disease resistance in animals, including fish.

- **Dysbiosis** (microbial imbalance) is linked to infections, reduced growth, and stress sensitivity.

- In aquaculture species (e.g., sea bass, sea bream), gut microbiome composition correlates with **growth performance** and pathogen resistance.

- Microbiome **manipulation** (probiotics, diet, environmental management) is emerging as a disease-prevention strategy.

Probiotic supplementation (e.g., Bacillus, lactic acid bacteria) improves survival and immune markers in farmed fish.

REVIEW | Full Access

Fish disease and intestinal microbiota: A close and indivisible relationship

Diana Medina-Félix, Estefanía Garibay-Valdez, Francisco Vargas-Albores, Marcel Martínez-Porchas

First published: 08 November 2022 | https://doi.org/10.1111/raq.12762 |

Environmental Monitoring

- Microbes **respond rapidly** to pollution, eutrophication, and habitat degradation.
- Microbial indicators can **detect ecosystem stress** earlier than macro-organism changes.
- Environmental microbiome monitoring supports water quality assessment and **fisheries management.**

Shifts toward pathogenic or opportunistic bacteria signal declining water quality in coastal systems.

Applications

- Early warning systems for harmful algal blooms
- Detection of contamination and anthropogenic impacts

MICROBIAL BIOTECHNOLOGY

Open Access

Special Issue Article | Open Access |

Monitoring and managing microbes in aquaculture – Towards a sustainable industry

Mikkel Bentzon-Tilia, Eva C. Sonnenschein, Lone Gram

First published: 24 July 2016 | https://doi.org/10.1111/1751-7915.12392

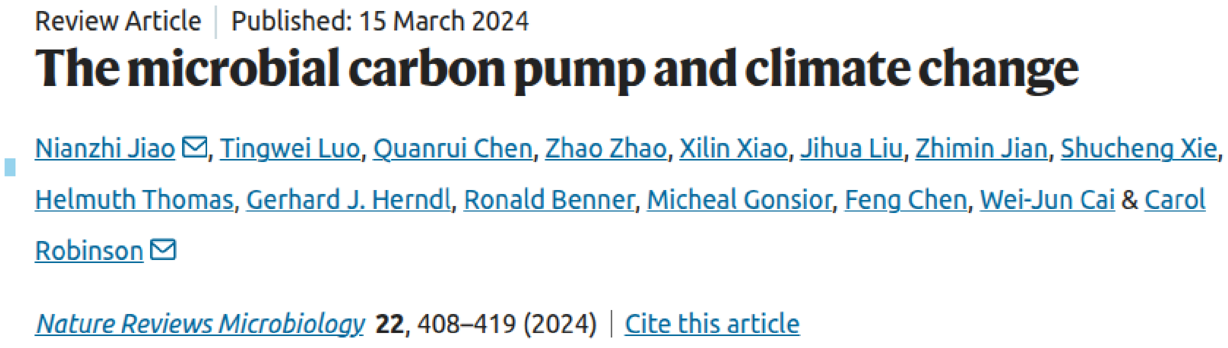
Climate Change Responses

- Temperature, acidification, and oxygen changes alter microbial processes and host–microbe interactions.
- Microbes mediate carbon cycling, nitrogen transformations, and greenhouse gas fluxes.
- Fish microbiomes shift under warming scenarios, influencing health and distribution.

Ocean warming increases Vibrio abundance, raising disease risks in marine organisms.

Implications

→ Microbial ecology is essential to predict ecosystem resilience and tipping points.



Review Article | Published: 15 March 2024

The microbial carbon pump and climate change

Nianzhi Jiao, Tingwei Luo, Quanrui Chen, Zhao Zhao, Xilin Xiao, Jihua Liu, Zhimin Jian, Shucheng Xie, Helmuth Thomas, Gerhard J. Herndl, Ronald Benner, Micheal Gonsior, Feng Chen, Wei-Jun Cai & Carol Robinson

Nature Reviews Microbiology **22**, 408–419 (2024) | Cite this article

Aquaculture Sustainability

- Microbiome management reduces antibiotic use and improves production efficiency.
- Water, sediment, and gut microbiomes influence disease outbreaks and growth rates.
- Sustainable aquaculture integrates microbial monitoring, probiotics, and biofilter management.

Recirculating aquaculture systems (RAS) rely on microbial biofilters for nitrogen removal.

Benefits

- Lower environmental impact
- Improved fish welfare
- Safer food production

EDITORIAL article

Front. Mar. Sci., 07 June 2023

Sec. Marine Fisheries, Aquaculture and Living Resources

Volume 10 - 2023 | https://doi.org/10.3389/fmars.2023.1227795

Editorial: Microbial diversity as a prerequisite for resilience and resistance in sustainable aquaculture

Andrea M. Tarnecki [1] Lior Guttman [2,3*]

Biodiversity Conservation

- Microbial diversity underpins ecosystem **stability and resilience**.
- Conservation strategies increasingly consider **host-associated microbiomes.**
- Microbiome-informed management can improve species recovery and **habitat restoration.**

Monitoring microbiomes of endangered species to assess health and adaptation capacity.

Applications

- Marine protected areas assessment
- Restoration of degraded habitats
- Tracking ecosystem recovery

Correspondence | Published: 12 September 2025

Launching the IUCN Microbial Conservation Specialist Group as a global safeguard for microbial biodiversity

Jack A. Gilbert, Raquel S. Peixoto, Amber Hartman Scholz, Maria Gloria Dominguez Bello, Lise Korsten, Gabriele Berg, Brajesh Singh, Antje Boetius, Fengping Wang, Chris Greening, Kelly Wrighton, Seth Bordenstein, Janet K. Jansson, Jay T. Lennon, Valeria Souza, Torsten Thomas, Don Cowan, Thomas W. Crowther, Nguyen Nguyen, Lucy Harper, Louis-Patrick Haraoui, Suzanne L. Ishaq & Kent Redford

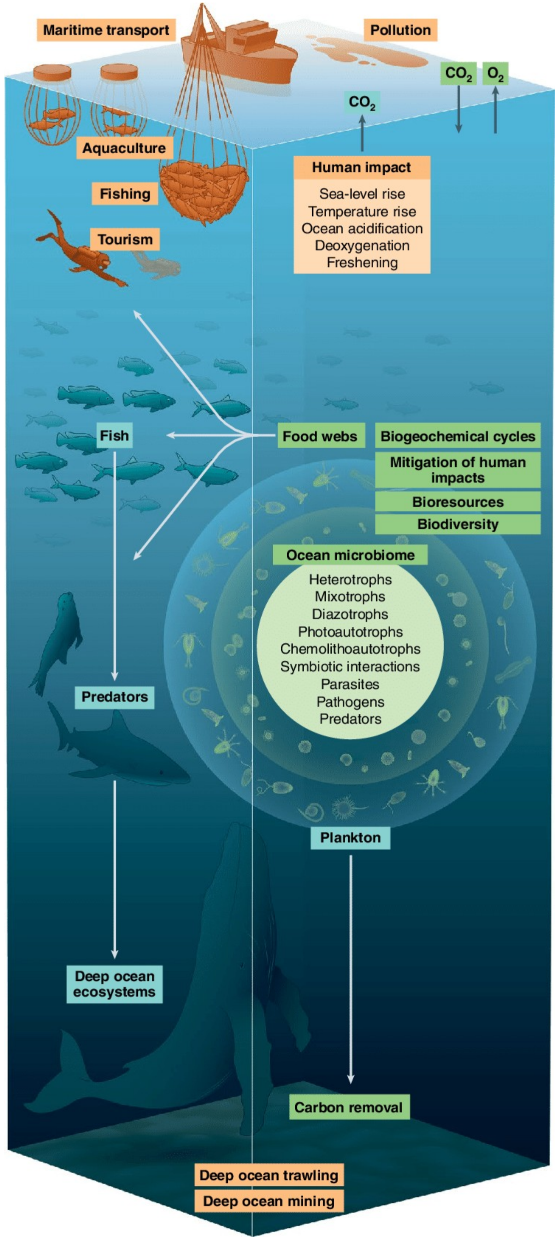
Nature Microbiology **10**, 2359–2360 (2025) | Cite this article

Environmental Responses of Microbial Communities

- Microbial communities respond dynamically to **temperature, pH, salinity, and nutrient availability.**

- Environmental changes can **favor some species** over others, potentially shifting ecosystem functions.

- Monitoring microbiomes provides **early indicators** of ecosystem health and stress.



Abreau et al., 2022

Environmental Responses of Microbial Communities

Microbial composition often changes before observable effects on higher organisms appear.

→ In marine systems, a rise of opportunistic bacteria often precedes **fish mortality** events or algal blooms.

→ Microbiome-based monitoring is increasingly used in aquaculture to **predict disease outbreaks** before fish show symptoms.

Microbes can act as **bioindicators of anthropogenic impact**, such as pollution or eutrophication.

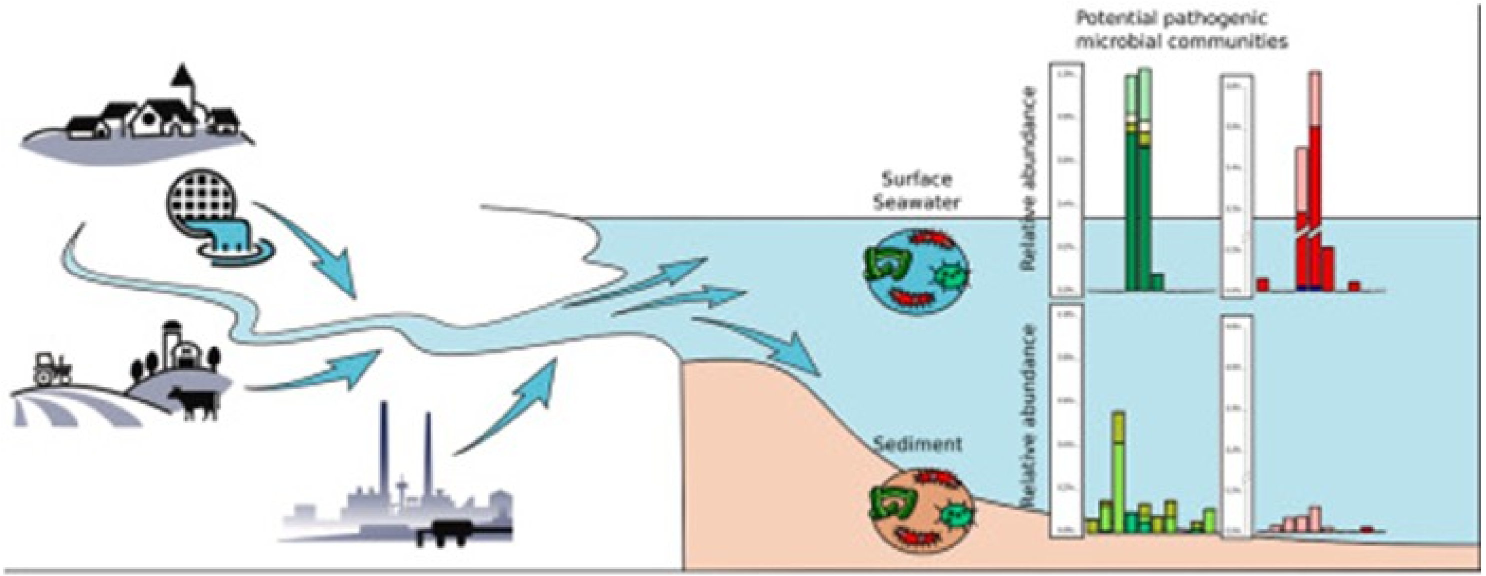
ELSEVIER

Environmental Pollution

Volume 285, 15 September 2021, 117672

Occurrence and distribution of microbial pollutants in coastal areas of the Adriatic Sea influenced by river discharge ☆

Marco Basili [a], Alessandra Campanelli [a], Emanuela Frapiccini [a], Gian Marco Luna [a], Grazia Marina Quero [a]

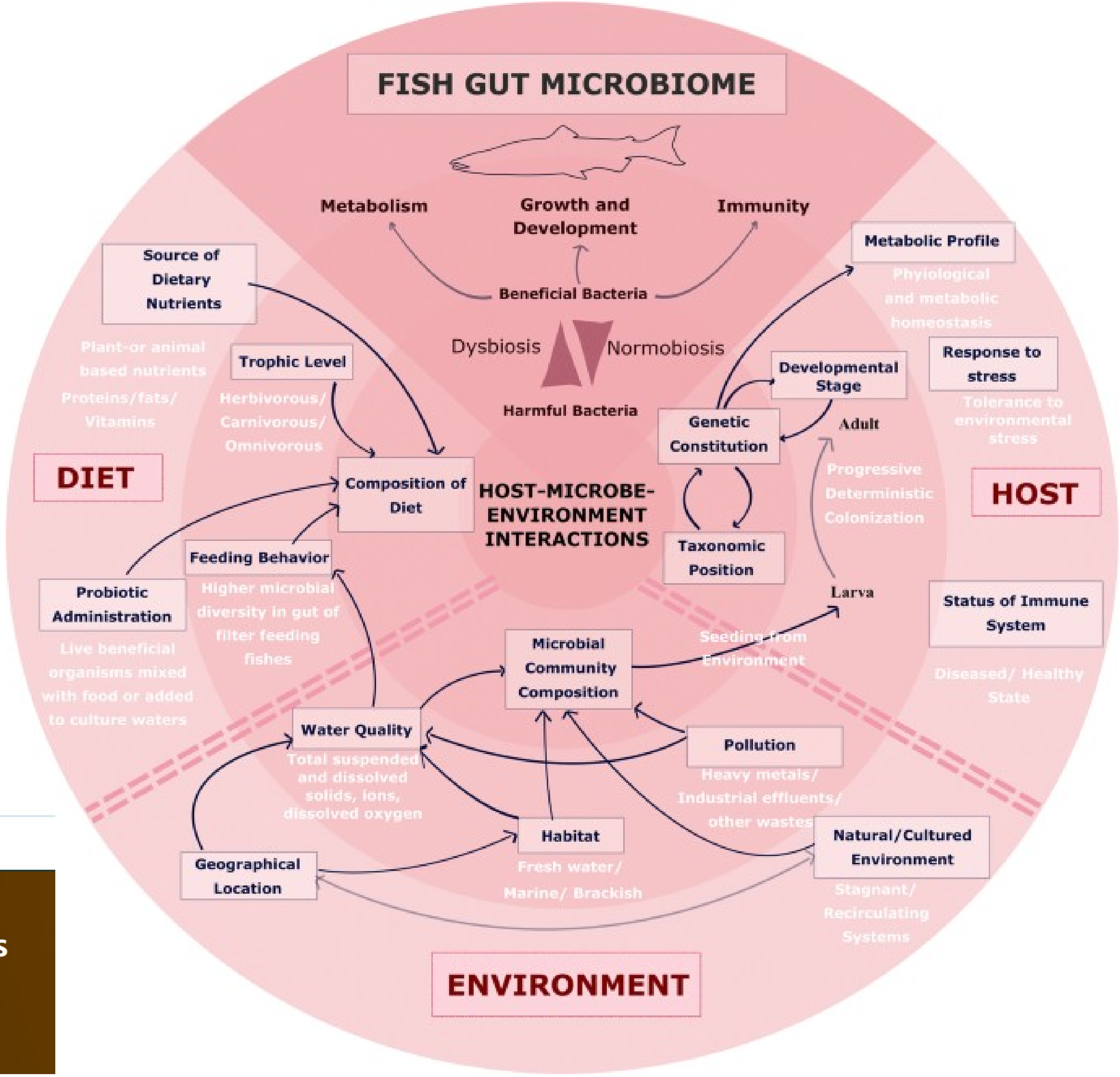


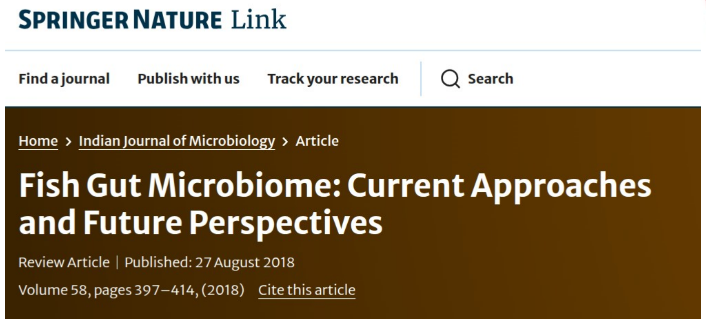
MICROBIOME and Marine hosts

Fish microbiomes are often more environmentally driven than mammalian ones because fish are constantly exposed to surrounding water microbes.

Fish are meta-organisms:

→ their health and adaptation rely on constant dialogue with their microbes.
HOLOBIOME





Home › Indian Journal of Microbiology › Article

Fish Gut Microbiome: Current Approaches and Future Perspectives

Review Article | Published: 27 August 2018

Volume 58, pages 397–414, (2018) Cite this article

Microbial Interactions with Fish Hosts

Fish microbiomes are tightly linked to host development, immunity, and metabolism.

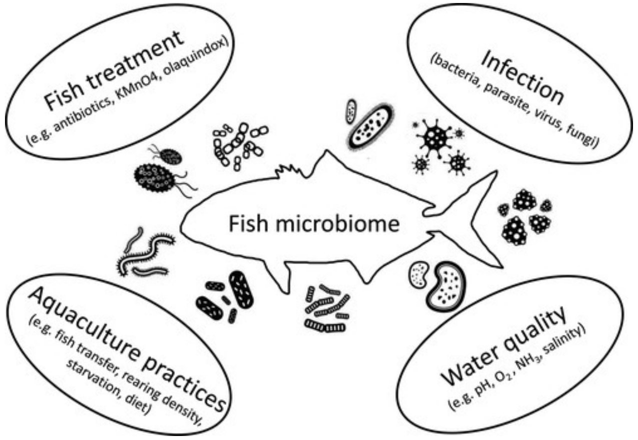
Microbes colonize gut, skin, gills, and mucus layers, forming specialized niches.

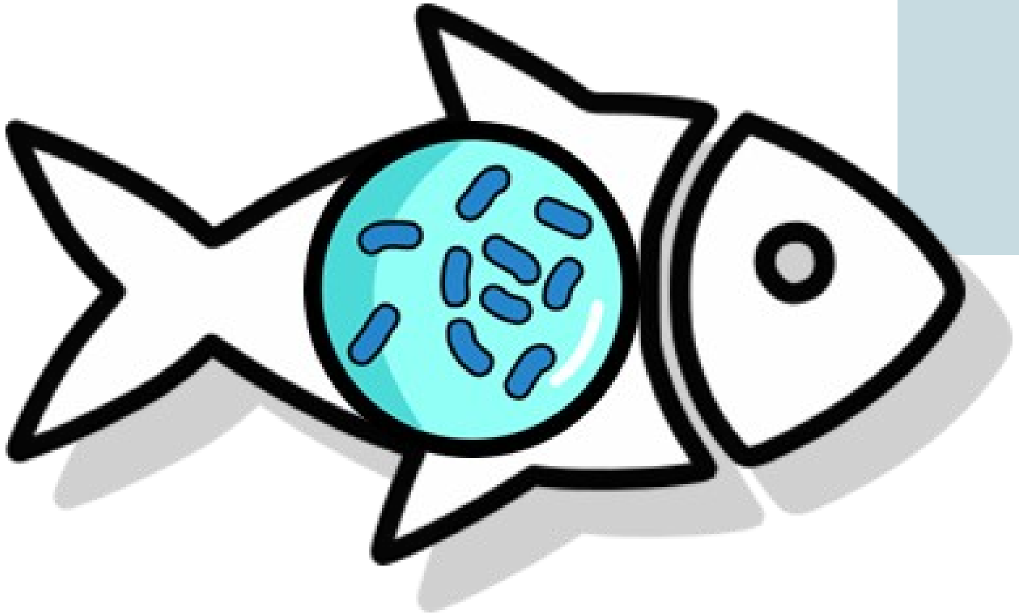
→ Gut: digestion, vitamin synthesis

→ Skin/gills: pathogen defense, environmental sensing

Host factors (genetics, age, diet) shape microbial composition, while microbes influence host physiology.

These interactions are bidirectional, creating a dynamic host–microbiome ecosystem.





Key Ecological and Physiological Functions

Microbes regulate nutrient absorption, metabolism, and detoxification.

They contribute to immune system priming and resistance to pathogens.

→ Some gut bacteria produce short-chain fatty acids that modulate fish immune responses.

Microbial communities help fish adapt to environmental changes, such as salinity fluctuations or temperature shifts.

Functional diversity of microbiomes supports ecosystem resilience.

→ **Functions matter more than species identity.**

Applications in Aquaculture and Conservation

- Microbiome monitoring is always a bioindicator for ecosystem health in farm and wild populations.

Tracking microbiome composition provides early warning of disease in fish farm or environmental stress.

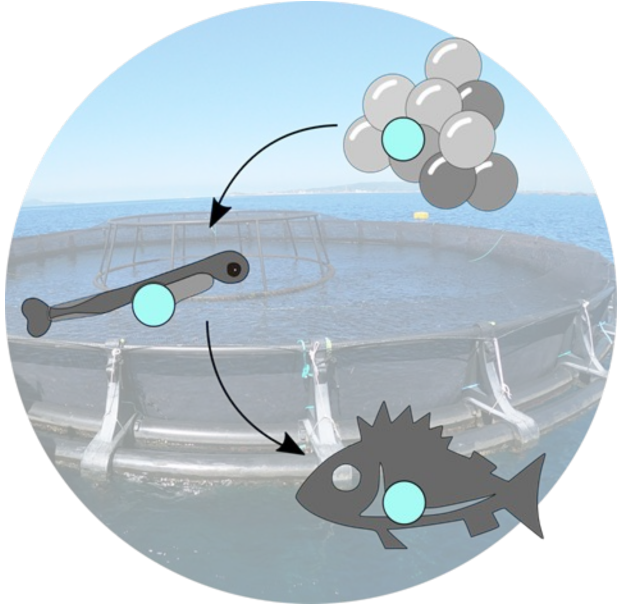
Microbial change and functional profiles can help in **feed formulations, probiotics, and management practices.**

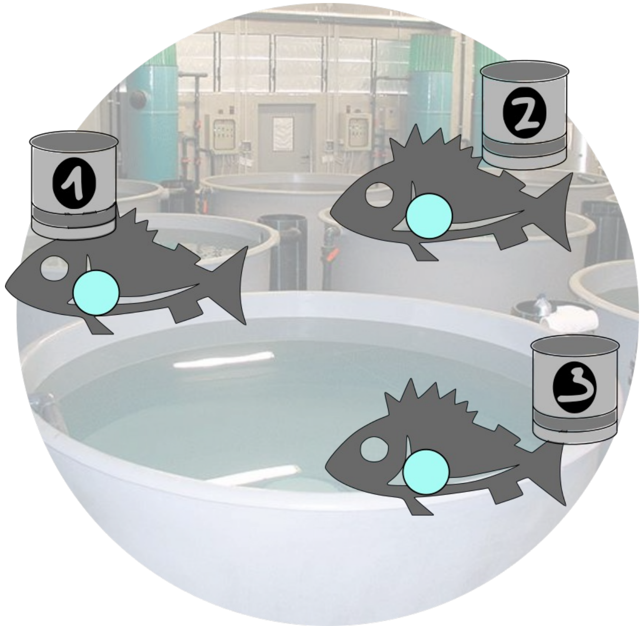
→ **Optimizing feed for growth and immune support**

- Mitigate organic carbon sedimentation beneath the open sea cages

Integrating **wild microbiome** data improves stock resilience and supports sustainable aquaculture.

→ **Microbial shifts indicate stress, pollution, eutrophication, or habitat change.**

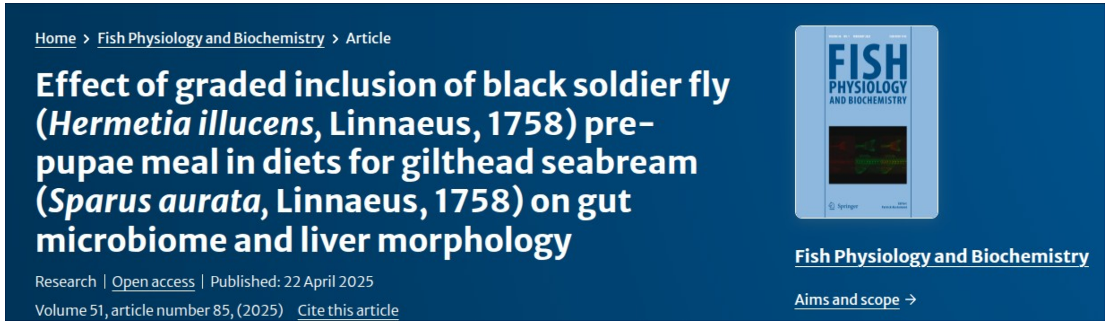




Applications in Aquaculture and Conservation

Limitations

- Diet–microbiome variability across species
- Short experimental durations
- Environmental confounding factors
- Taxonomic vs functional uncertainty
- Inter-individual variability
- Transient vs stable microbiome changes
- Feed-associated microbes
- Health and performance trade-offs
- Lack of standardized protocols



Home > Fish Physiology and Biochemistry > Article

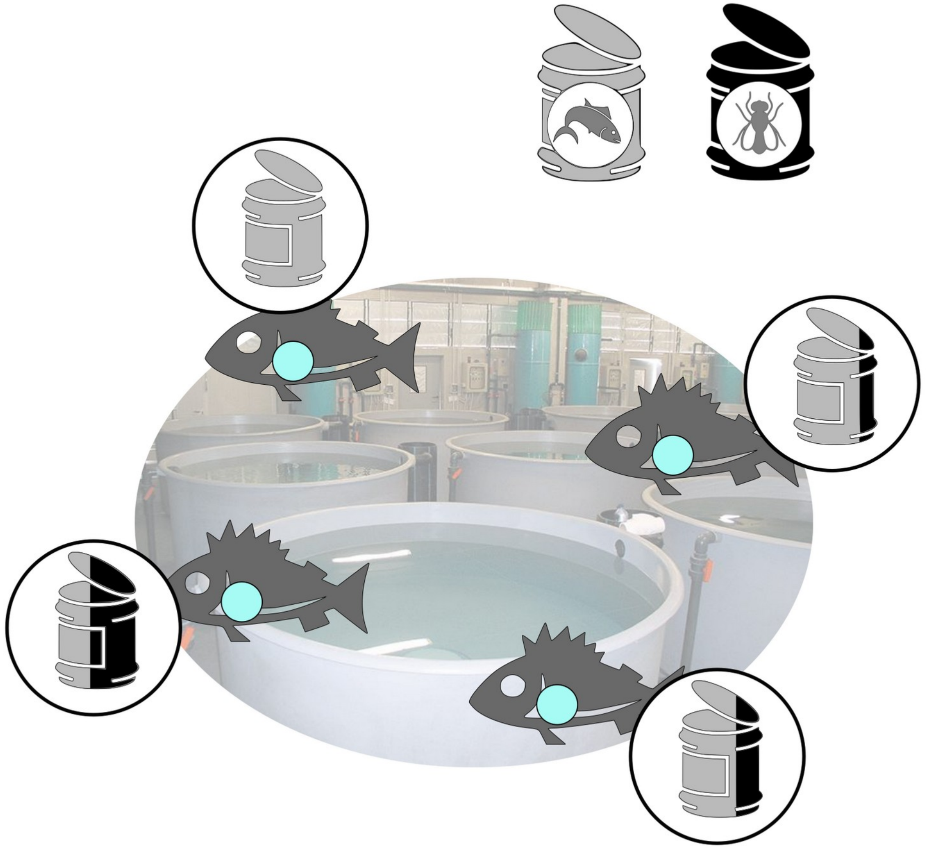
Effect of graded inclusion of black soldier fly (*Hermetia illucens*, Linnaeus, 1758) pre-pupae meal in diets for gilthead seabream (*Sparus aurata*, Linnaeus, 1758) on gut microbiome and liver morphology

Research | Open access | Published: 22 April 2025

Volume 51, article number 85, (2025) Cite this article

Fish Physiology and Biochemistry

Aims and scope →



Microbiome Composition and Water Health

- **Aquatic microbial communities** regulate nutrient cycling, organic matter decomposition, and water purification processes.
- A balanced microbiome acts as a natural biological filter, limiting the proliferation of pathogens and harmful algal blooms.
- Disruptions (pollution, eutrophication, temperature shifts) can favor opportunistic or **pathogenic taxa**, increasing disease risk for wild fish and other organisms.

Monitoring microbial composition provides an **early indicator of water quality** decline before visible ecological damage occurs.

Applications

- Detection of **bioindicator** (Fecal, sewage ...)
- Microbiome-based indicators support **management of coastal waters, lagoons, and aquaculture facilities.**

ELSEVIER

Science of The Total Environment

Volume 818, 20 April 2022, 151755

Partitioning and sources of microbial pollution in the Venice Lagoon

Marco Basili [a], Stephen M. Techtmann [b], Luca Zaggia [c], Gian Marco Luna [a], Grazia Marina Quero [a]

Microbiome Composition
and Water Health

- Analyzing microbial shifts provides critical information for assessing human impacts and planning corrective interventions.

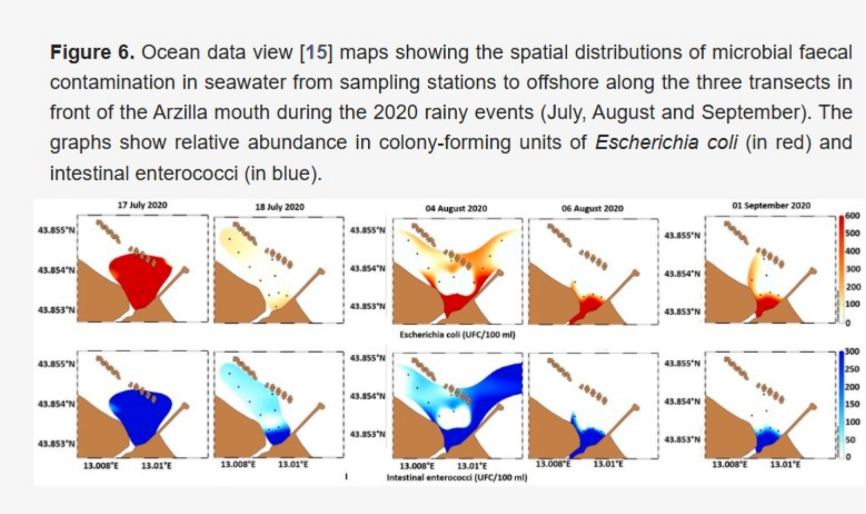
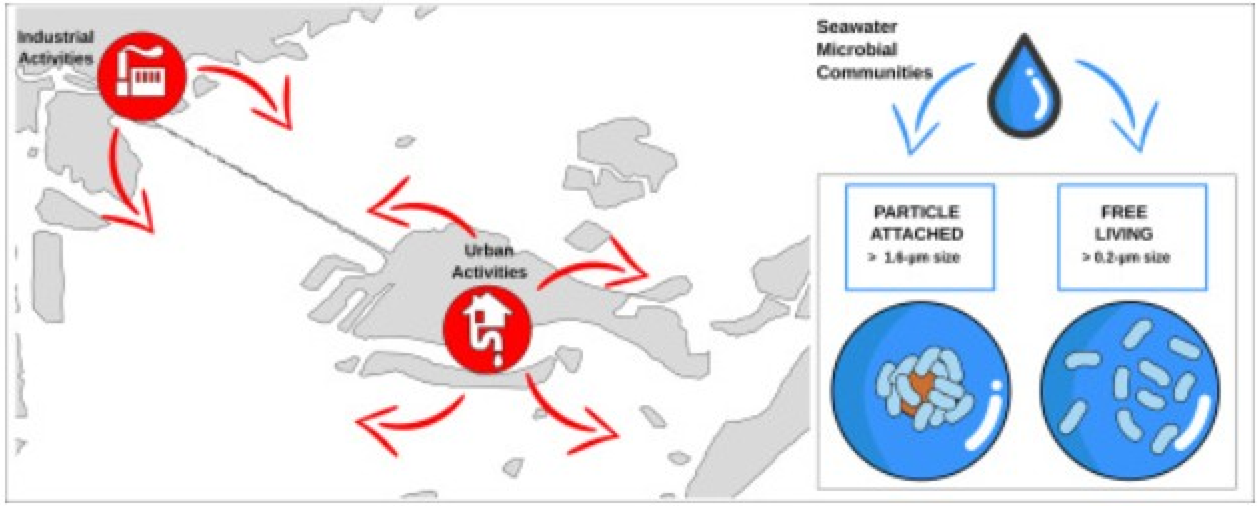
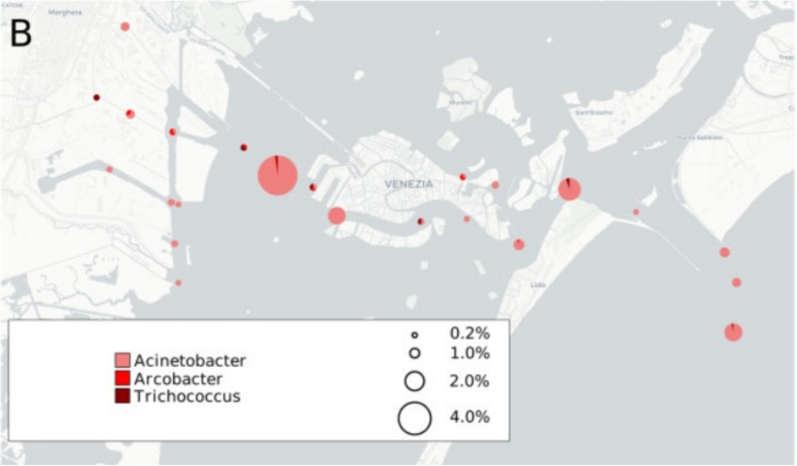


Figure 6. Ocean data view [15] maps showing the spatial distributions of microbial faecal contamination in seawater from sampling stations to offshore along the three transects in front of the Arzilla mouth during the 2020 rainy events (July, August and September). The graphs show relative abundance in colony-forming units of *Escherichia coli* (in red) and intestinal enterococci (in blue).





Microbial Communities in Sediments

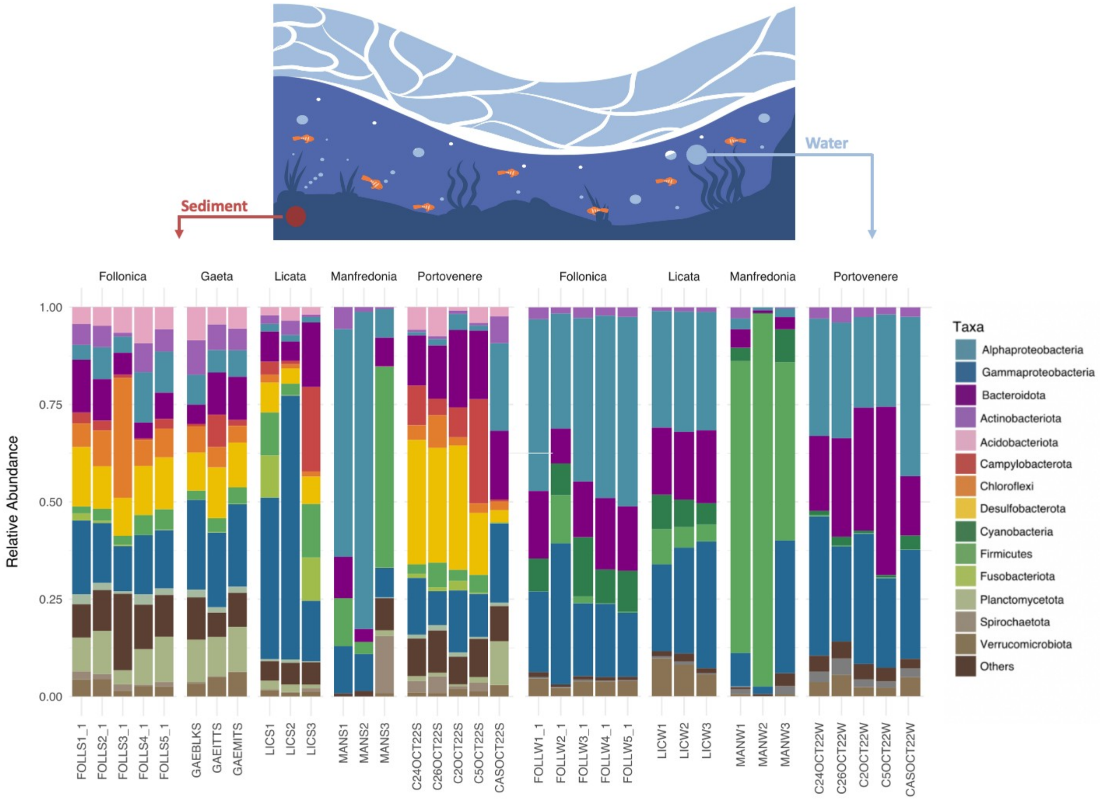
- Sediments host microbial communities distinct from pelagic waters.

- Sediment microbiomes can reveal historical ecosystem changes, acting as microbial “archives.”

These microbes regulate nutrient cycles (C, N, P) and decomposition processes.

→ essential for nutrient regeneration, especially in coastal and lagoon systems.

They strongly influence the health of benthic habitats and overall ecosystem productivity.



Microbial diversity as a Key for Ecosystem Stability

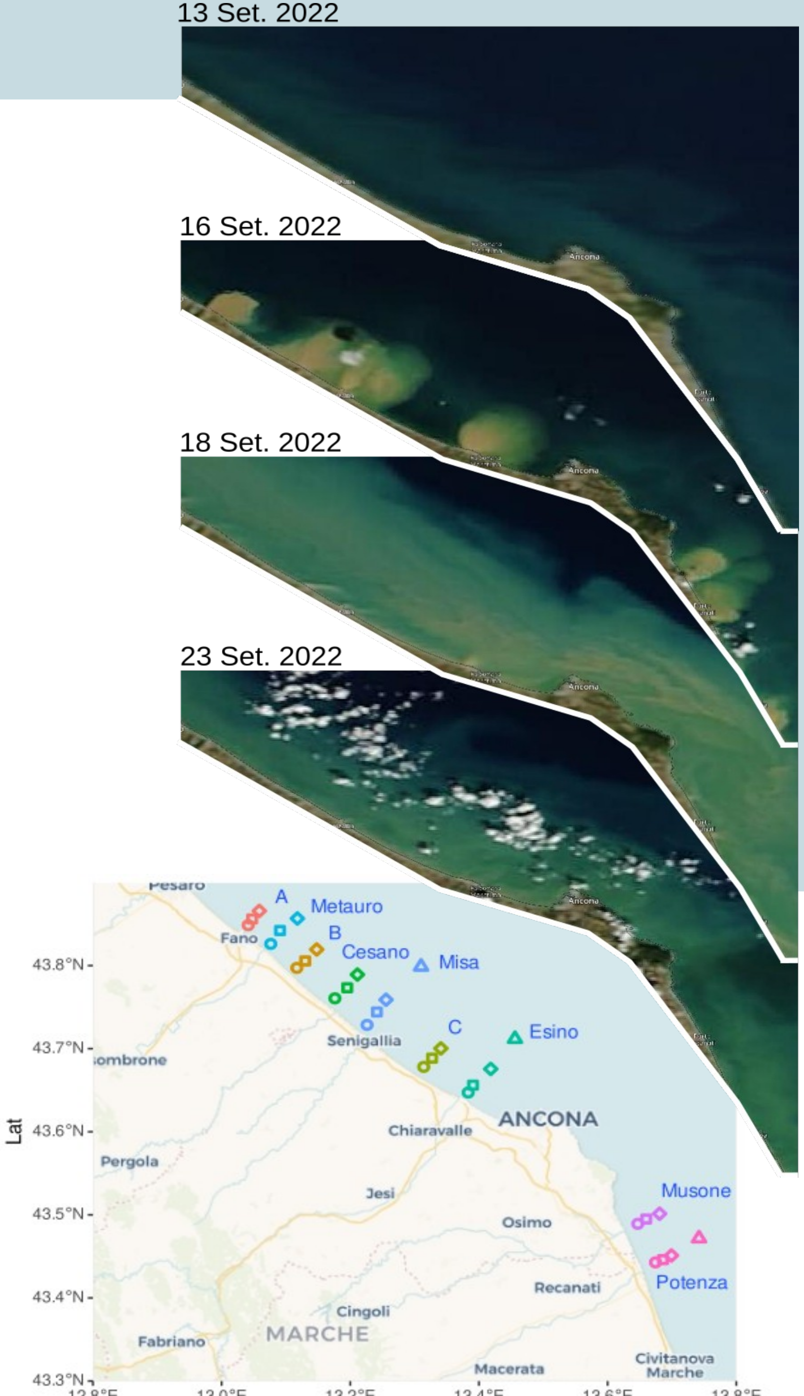
Diverse microbial communities enhance the ecosystem’s ability to respond to environmental stressors such as temperature shifts, salinity changes, or contaminants.

→ **ecosystems with higher microbial diversity recover faster from stress events.**

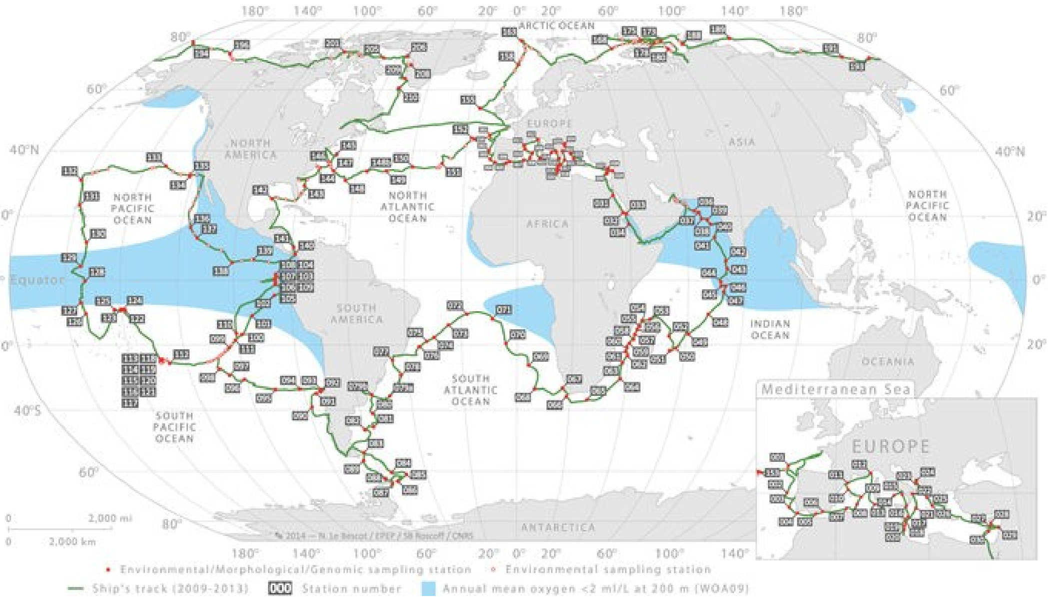
Higher diversity maintains critical ecological functions, even under disturbance.

Monitoring microbial diversity is therefore essential to understanding ecosystem health and stability.

→ **Environmental DNA (eDNA) and metabarcoding are powerful tools for rapid, non-invasive microbial monitoring.**



TARA OCEANS



Mission Ocean Microbiomes (Tara Microbiomes)

A major 5-year initiative building on the legacy of the Tara Oceans expeditions.

Focused on marine microbiomes, their functions, and their links to climate change and pollution.

Samples collected across Atlantic, Pacific, and polar regions help researchers understand how microbial communities vary globally with environmental gradients.

Earth Microbiome Project (EMP)

A global collaborative effort to map microbial diversity across earth’s environments (oceans, soils, freshwater, built environments).

Involves thousands of samples from very different biomes to build a standardized reference map of global microbial communities and their genes.

TREC — Traversing European Coastlines (Tara)

A recent Tara expedition sampling microbial communities across 22 European coastal countries.

Combines metagenomics, environmental data, and biogeochemical observations to study microbes at the land-sea interface.

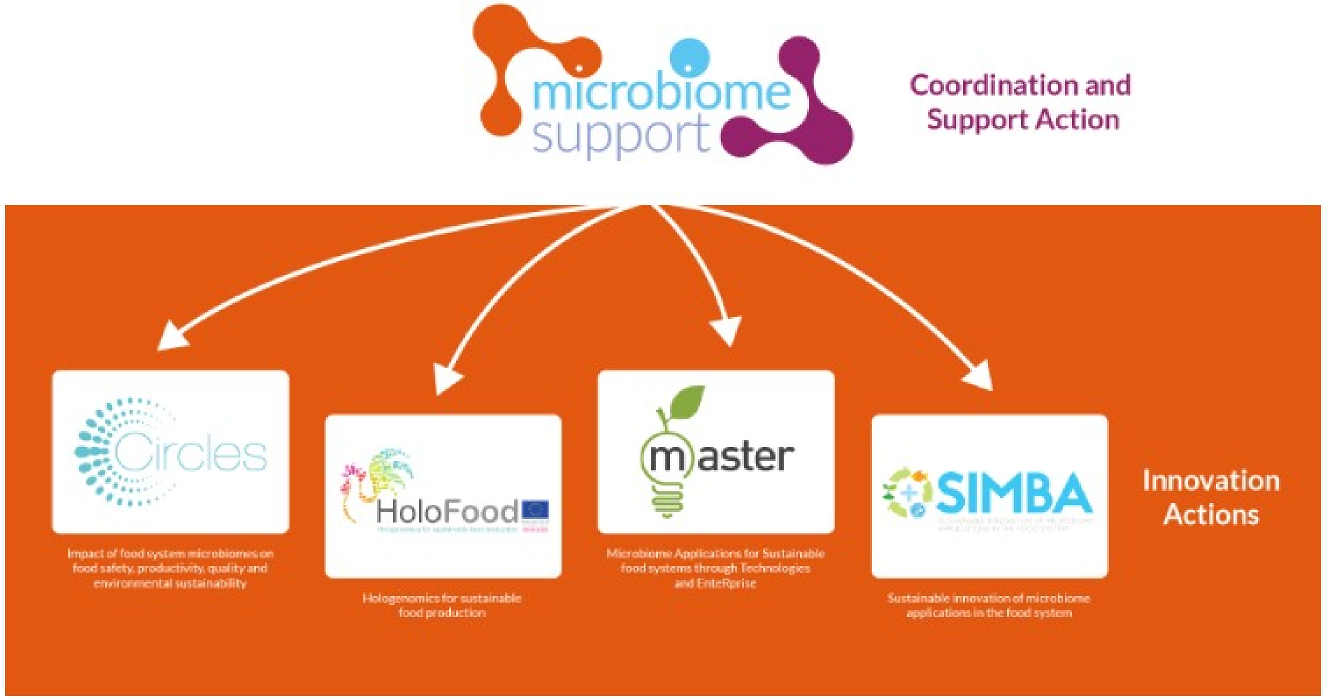
EU Horizon Projects: **Microbiomes for Sustainable Aquaculture & Ecosystems**

European research programmes such as Horizon 2020 and Horizon Europe have supported major research and innovation projects promoting microbiome-based approaches for sustainable aquaculture, blue bioeconomy, and environmental health.

→ EU explicitly recognizes microbiomes as key levers for achieving sustainability goals across food systems, aquaculture, and environmental monitoring.

→ Practical microbiome solutions across both terrestrial and aquatic food systems, including aquaculture sustainability.

→ Microbiome-based products/processes that protect aquatic ecosystems.



Coordination and Support Action

Impact of food system microbiomes on food safety, productivity, quality and environmental sustainability

Hologenomics for sustainable food production

Microbiome Applications for Sustainable food systems through Technologies and EnteRprise

Sustainable innovation of microbiome applications in the food system

Innovation Actions

FOOD SYSTEM MICROBIOMES

Microbiome research is embedded in EU sustainability agendas.

Standardization matters:

Projects like MicrobiomeSupport aim to align microbiome research methodologies and data infrastructures to make results actionable across sectors.

From data to practice:

Horizon Europe’s marine microbiome topics encourage development of microbiome analysis tools that support environmental services and ecosystem health assessment.

Cross-system integration:

MICROBIOMES4SOY links plant, animal, human, and aquaculture microbiomes, demonstrating the One Health nature of microbiome innovation.

Key Research highly competitive for EU calls:

- Role of microbes in nutrient cycling, carbon sequestration, pollutant transformation

- **Climate mitigation via microbial processes**

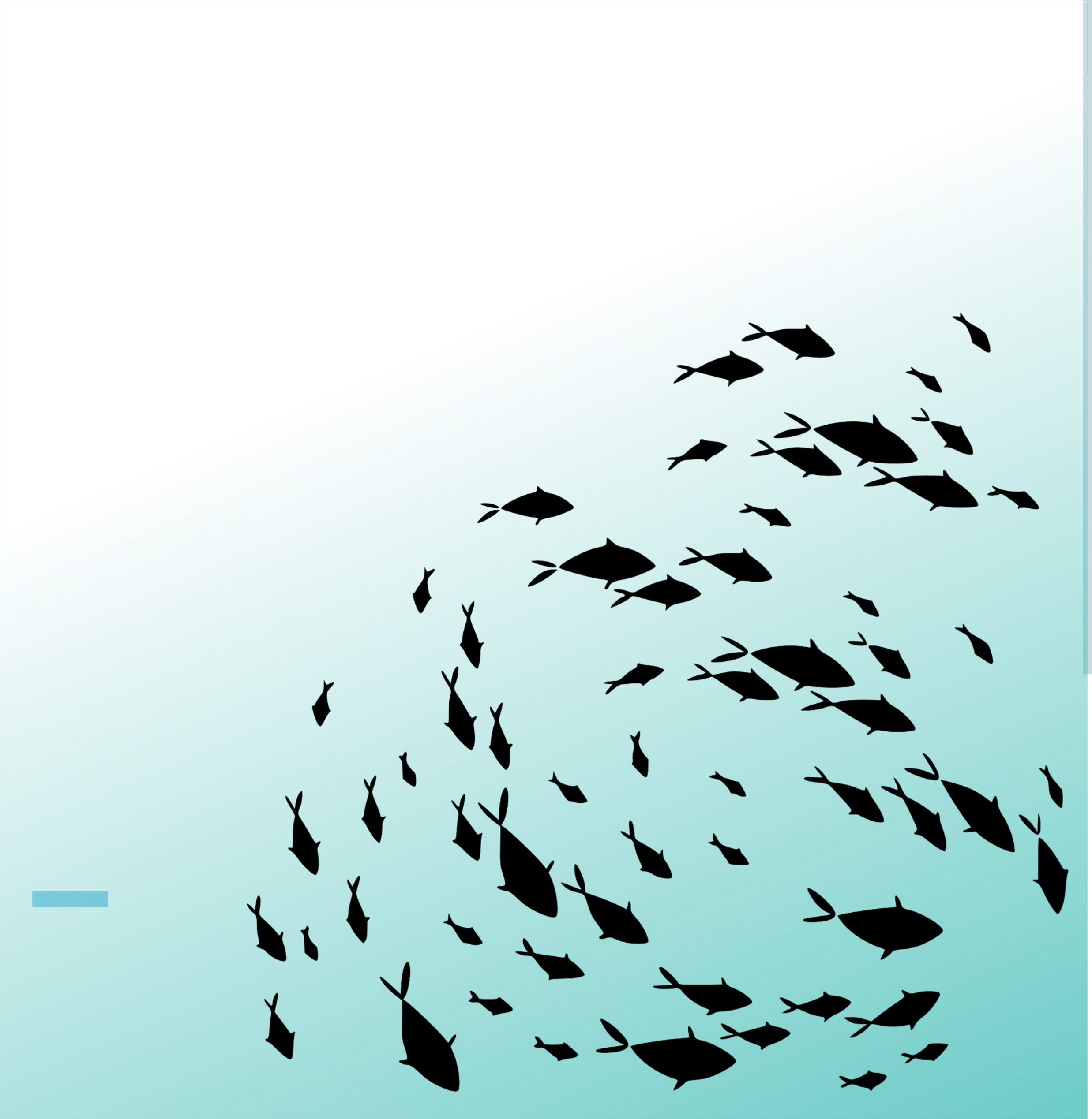
- **Host–microbe–environment dynamics**

- Microbial mediation of species resilience to stress

- Microbiome responses to warming, acidification, salinity change (early detection of environmental stress)

- **Development of microbial biomarkers for water quality, habitat health, biodiversity**

- Application of microbial consortia for contaminated site remediation, carbon sink enhancement



Methodological Strengths That Funders Like

European calls increasingly reward innovative molecular ecology approaches such as:

- **Integrative multi-omics (metagenomics + metatranscriptomics + metabolomics)**
- High-resolution temporal monitoring
- **Spatially explicit microbiome data (eDNA)**
- Machine learning for microbial indicators (multi-omics + AI)
- Microbiome ecological modelling and ecosystem forecasting
- Cross-domain data integration **(microbes + physics + biogeochemistry)**

- **FAIR data + open science commitment**
- **Societal benefits + policy relevance**
- **Feasible, well-structured work plan**
- **Strong, complementary multidisciplinary partners**

Take a Break

HOST (Fish -) Microbiome

Aquatic environments host enormous microbial diversity that constantly interacts with fish, and host habitat and habits are the major determinant of the gut microbiome of fish.

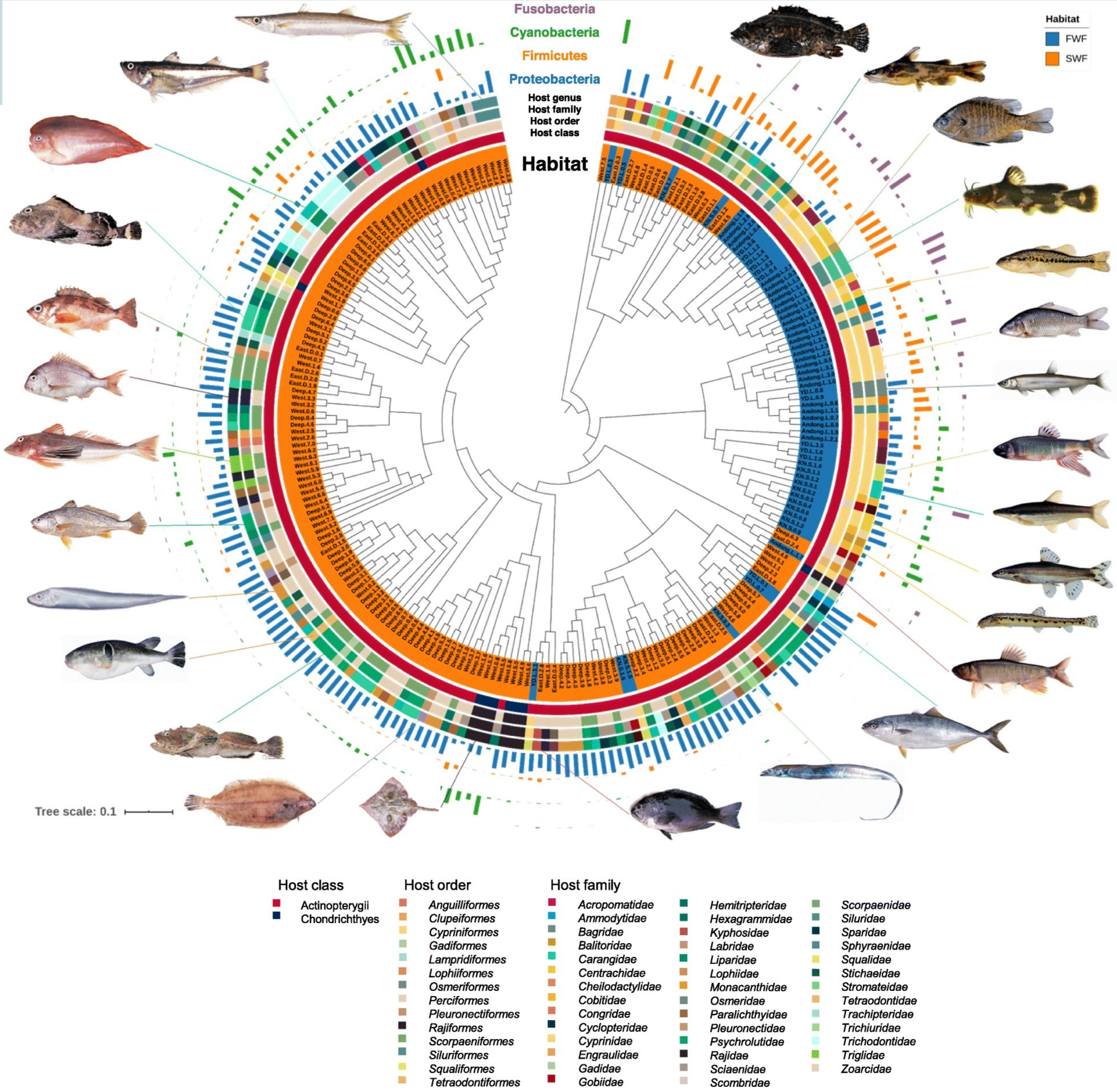
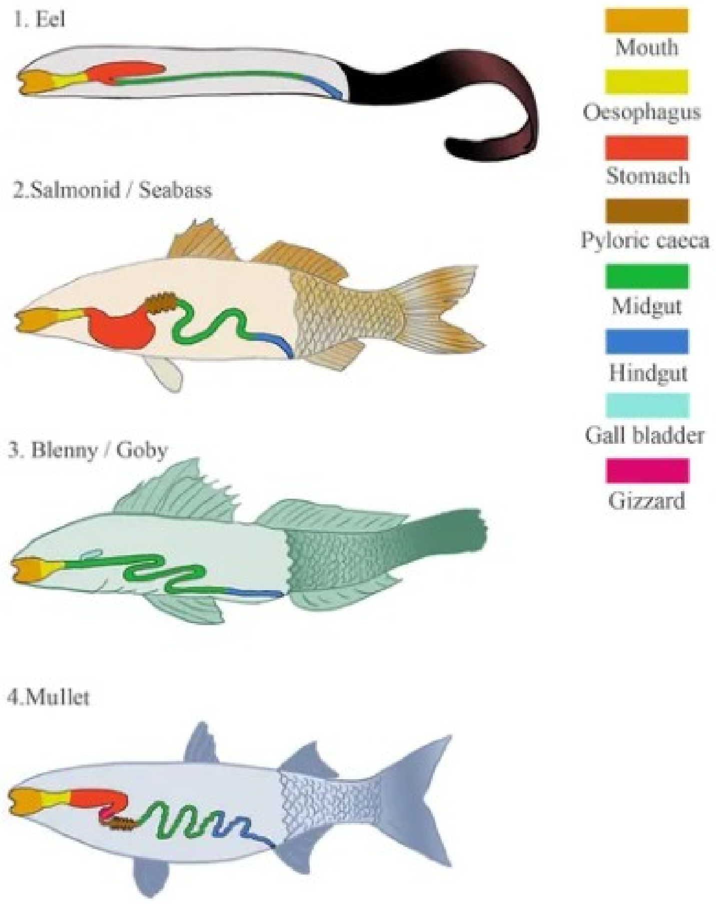


Figure 1



Introduction to Fish Microbiome

Dicriminate between **Transient vs stable** microbiome changes

Fish microbiomes include **internal (gut)** and **external (skin, gills, mucus) communities.**

Microbiomes influence host nutrition, immunity, metabolism, and environmental adaptation.

→ microbes are not passive passengers but active ecosystem components.

Egerton et al., 2018

Internal vs External Microbiomes

External microbiome: skin, gills, mucus — exposed to surrounding water and rapidly responsive to environmental change.

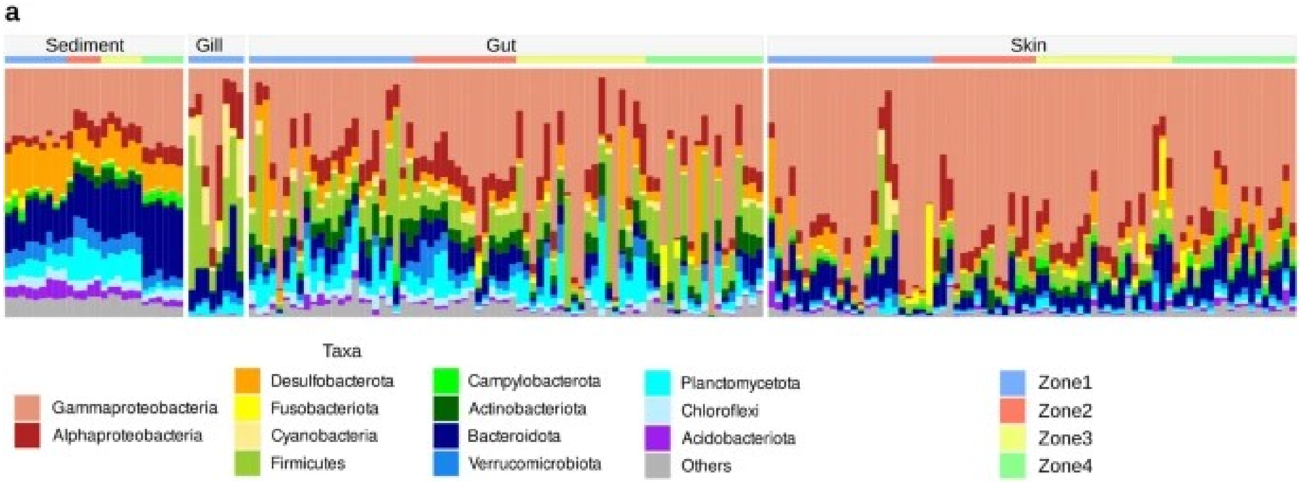
→ Fish mucosal surfaces (skin, gills) house distinct microbial assemblages that act as a **first immune barrier against pathogens.**

→ Skin and gill surfaces have **distinct microbial functional roles**

External communities differ significantly from gut communities.

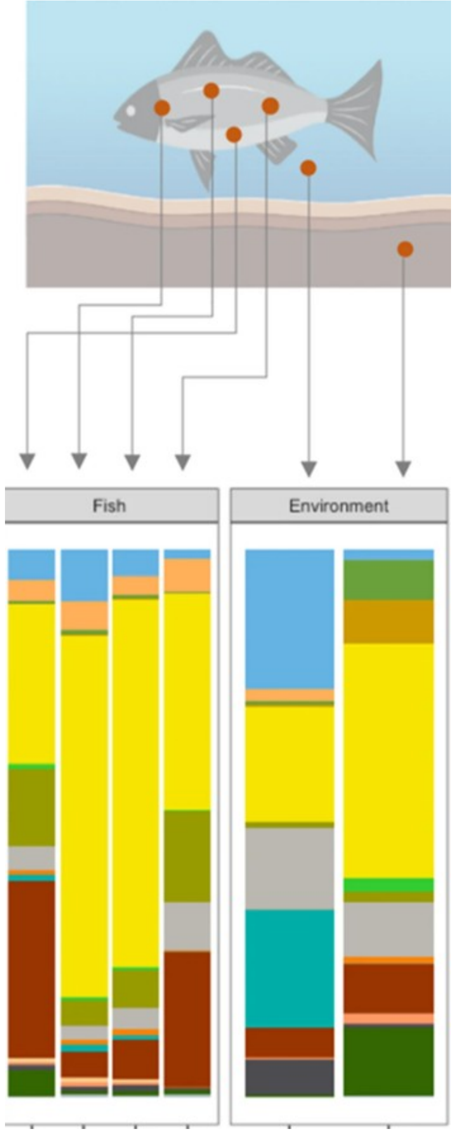
Internal microbiome: the gut shaped by diet, host physiology, and environment.

→ **gut communities are more host-selective**



Core Gut Microbiome Phyla

Fish gut microbiomes typically comprise a few dominant bacterial phyla:



Proteobacteria : often dominant and highly variable

→ Includes both commensals and potential pathogens (e.g., Vibrio).

Firmicutes : linked to carbohydrate metabolism

Bacteroidetes : polysaccharide degraders

→ The Firmicutes/Bacteroidetes ratio is a common ecological indicator of diet shifts.

Fusobacteria : common in many fish species

→ Cetobacterium somerae, a Fusobacteria, commonly dominates freshwater fish guts and produces vitamin B12, enhancing host nutrition.

Actinobacteria : potential antimicrobial producers

Tenericutes : variable presence across species

These phyla collectively often form ~90% of the gut microbiome.

Functional Roles of Fish Microbiomes

Fish microbiomes contribute to:

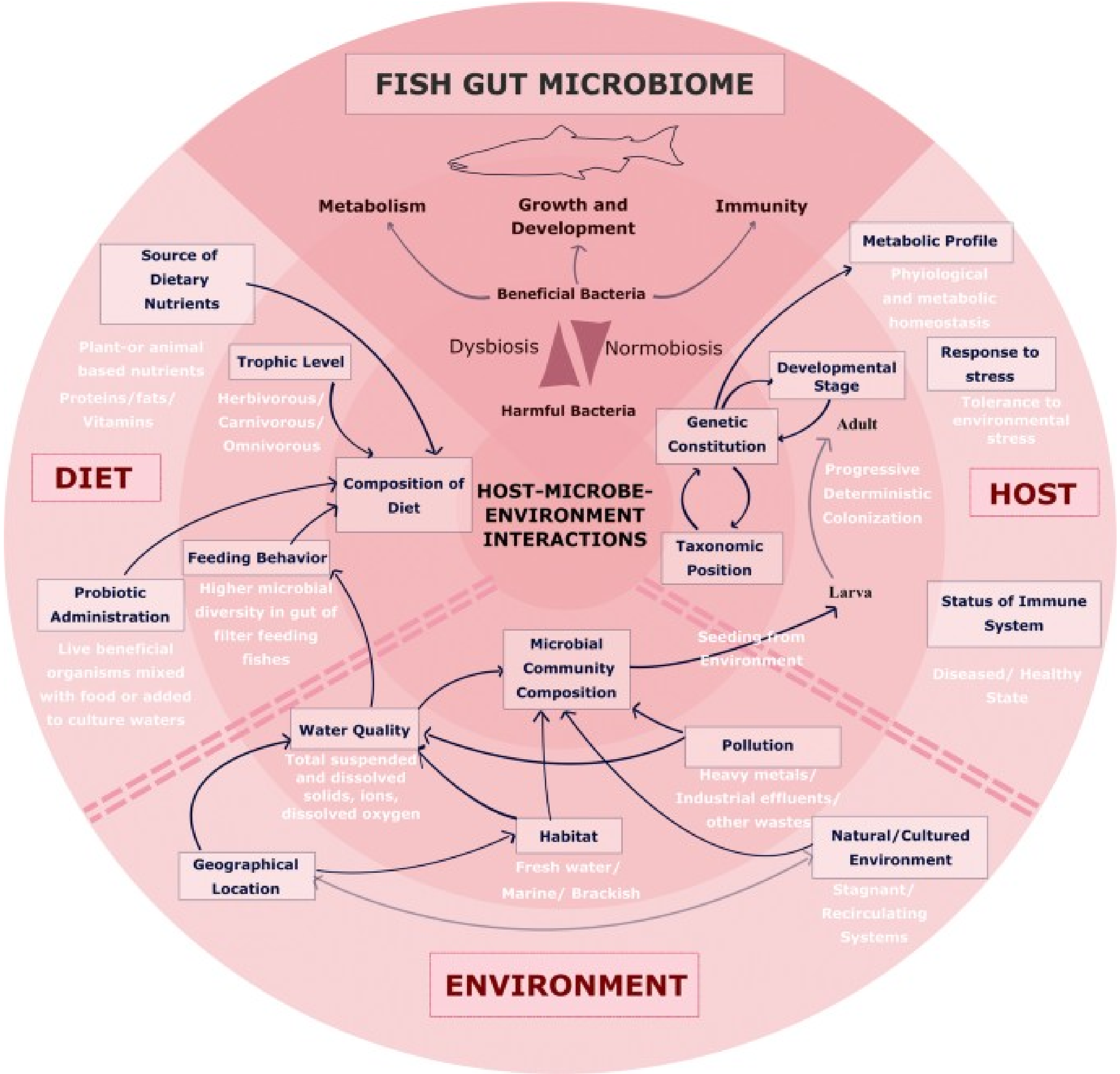
- Digestion and nutrient assimilation
- Vitamin synthesis (e.g., B12)
- Immune modulation and pathogen resistance
- Metabolic regulation and growth performance
- Host adaptation to diet and environment

REVIEWS IN Aquaculture

Review | Full Access

A microbial sea of possibilities: current knowledge and prospects for an improved understanding of the fish microbiome

Thibault P.R.A. Legrand, James W. Wynne, Laura S. Weyrich, Andrew P.A. Oxley



Practical Applications in Aquaculture

Bioindicators and Monitoring Potential

- **Microbiome profiles** can serve as **bioindicators** of health and environmental status.

- **Composition changes may signal stress, pollution, or disease before macro-indicators.**

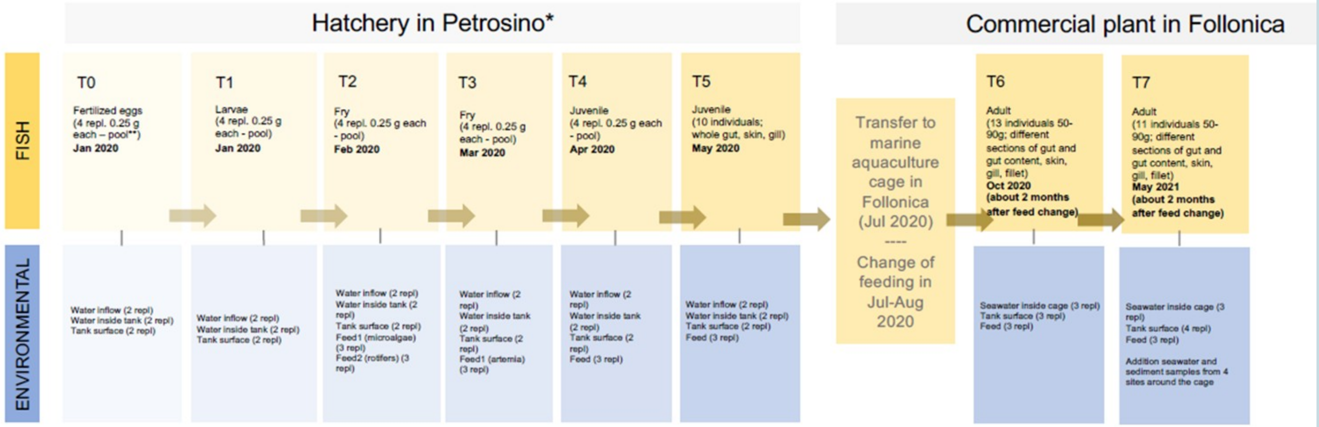
- Integrating environmental and gut microbial data provides **a holistic ecosystem view.**

Probiotics, prebiotics enhance fish health, feed conversion, and disease resistance.

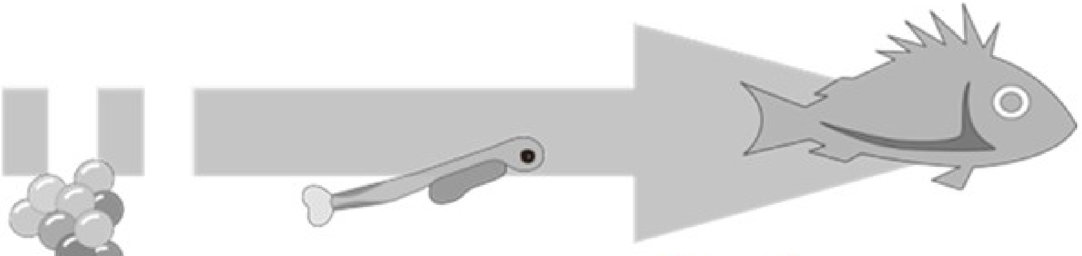
→ Microbiome management offers **alternatives to antibiotics.**

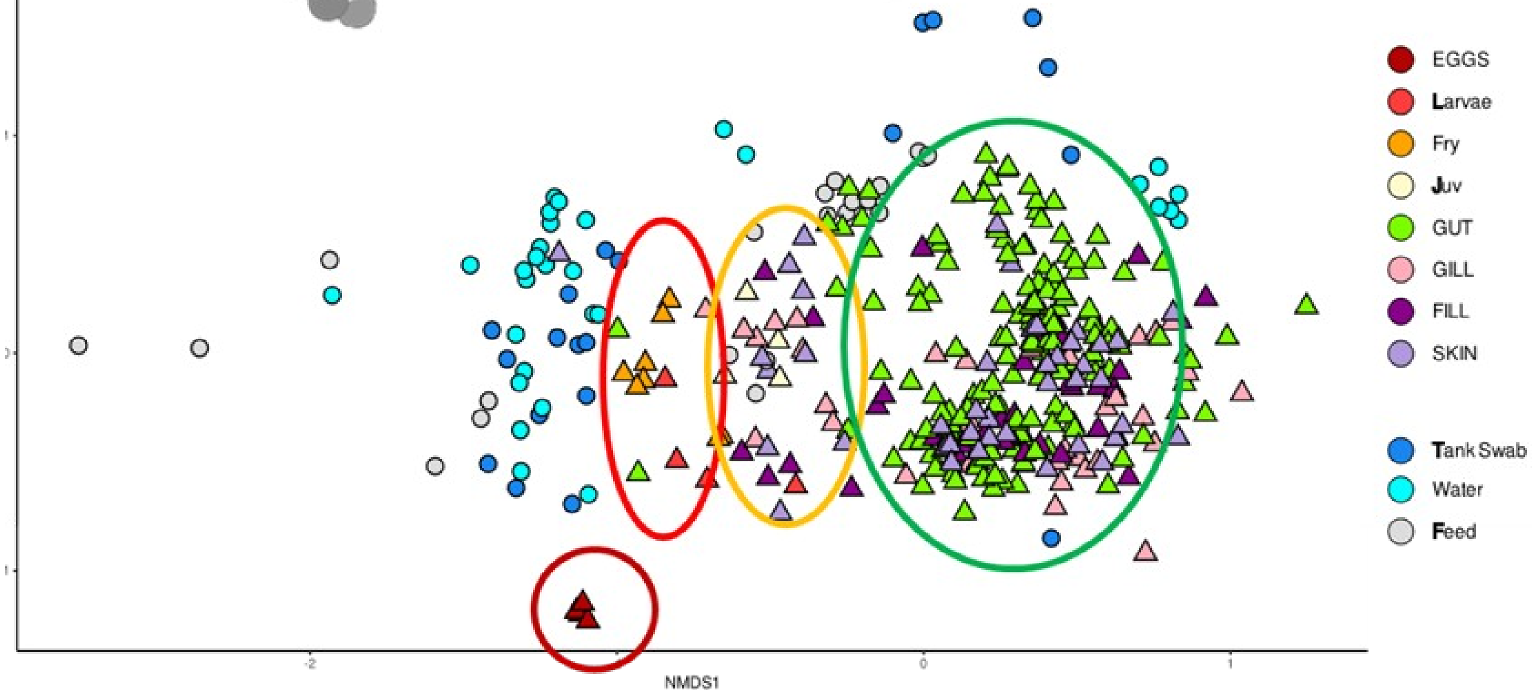
→ **Diet formulation** impacts gut microbiome composition and **host welfare and growth.**

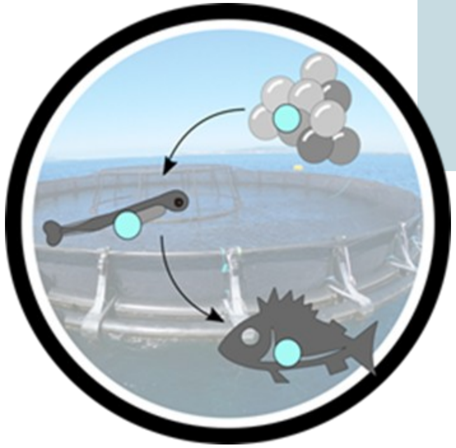
Practical Applications in Aquaculture

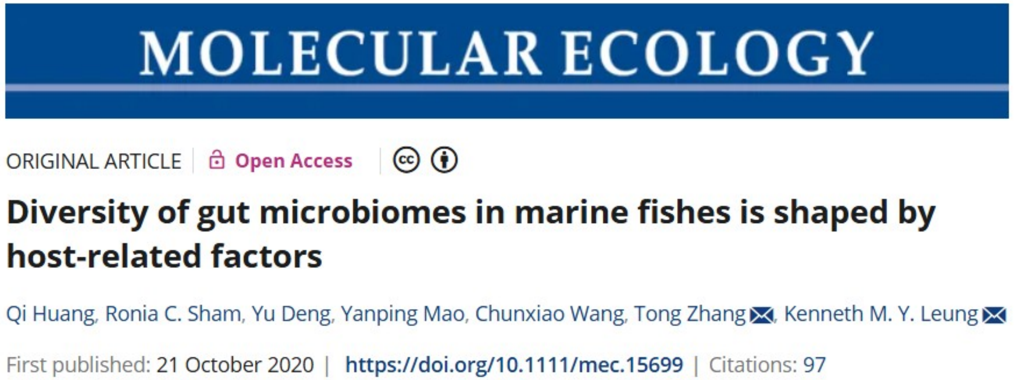


	Hatchery in Petrosino*						Commercial plant in Followica		
	T0	T1	T2	T3	T4	T5		T6	T7
FISH	Fertilized eggs (4 repl. 0.25 g each – pool**) **Jan 2020**	Larvae (4 repl. 0.25 g each - pool) **Jan 2020**	Fry (4 repl. 0.25 g each - pool) **Feb 2020**	Fry (4 repl. 0.25 g each - pool) **Mar 2020**	Juvenile (4 repl. 0.25 g each - pool) **Apr 2020**	Juvenile (10 individuals; whole gut, skin, gill) **May 2020**	Transfer to marine aquaculture cage in Follonica (Jul 2020) ---- Change of feeding in Jul-Aug 2020	Adult (13 individuals 50-90g; different sections of gut and gut content, skin, gill, fillet) **Oct 2020 (about 2 months after feed change)**	Adult (11 individuals 50-90g; different sections of gut and gut content, skin, gill, fillet) **May 2021 (about 2 months after feed change)**
ENVIRONMENTAL	Water inflow (2 repl) Water inside tank (2 repl) Tank surface (2 repl)	Water inflow (2 repl) Water inside tank (2 repl) Tank surface (2 repl)	Water inflow (2 repl) Water inside tank (2 repl) Tank surface (2 repl) Feed1 (microalgae) (3 repl) Feed2 (rotifers) (3 repl)	Water inflow (2 repl) Water inside tank (2 repl) Tank surface (2 repl) Feed1 (artemia) (3 repl)	Water inflow (2 repl) Water inside tank (2 repl) Tank surface (2 repl) Feed (3 repl)	Water inflow (2 repl) Water inside tank (2 repl) Tank surface (2 repl) Feed (3 repl)		Seawater inside cage (3 repl) Tank surface (3 repl) Feed (3 repl)	Seawater inside cage (3 repl) Tank surface (4 repl) Feed (3 repl) Addition seawater and sediment samples from 4 sites around the cage









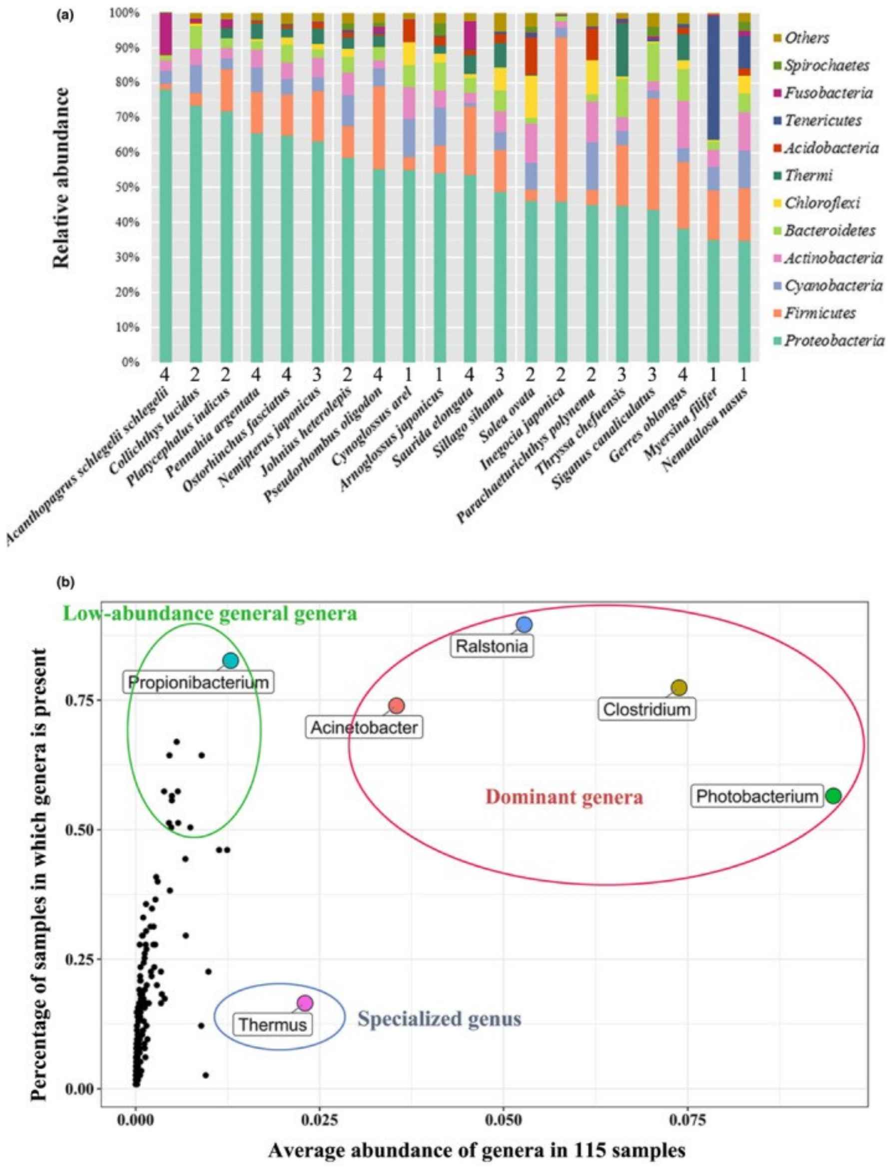
MOLECULAR ECOLOGY

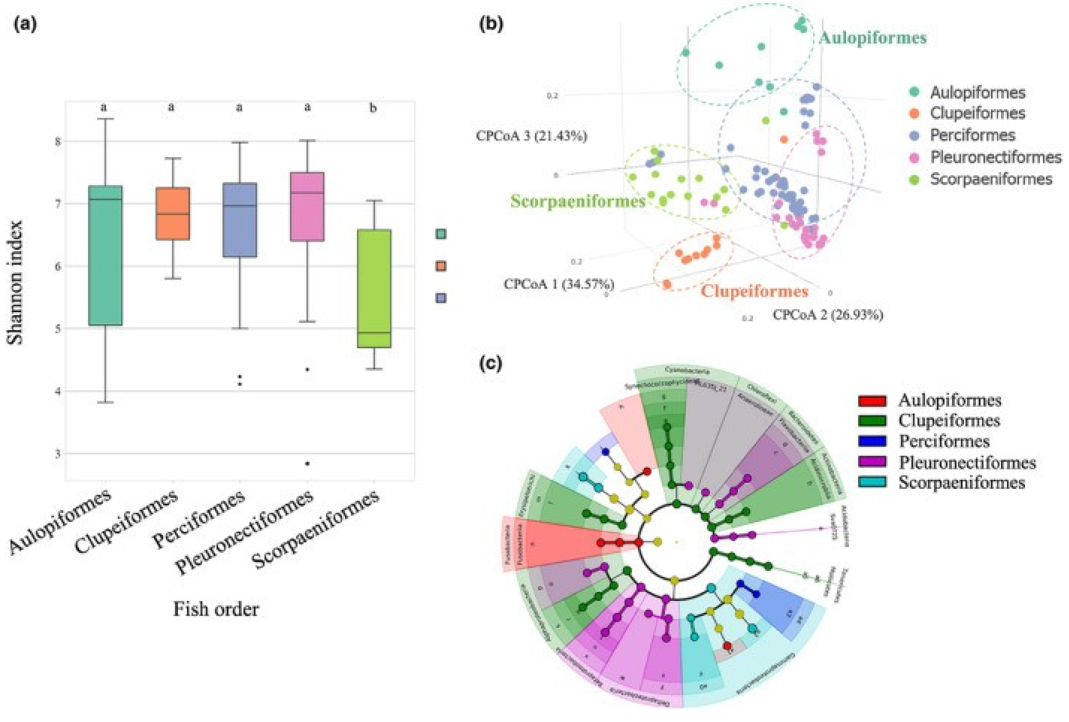
ORIGINAL ARTICLE | Open Access

Diversity of gut microbiomes in marine fishes is shaped by host-related factors

Qi Huang, Ronia C. Sham, Yu Deng, Yanping Mao, Chunxiao Wang, Tong Zhang, Kenneth M. Y. Leung

First published: 21 October 2020 | https://doi.org/10.1111/mec.15699 | Citations: 97



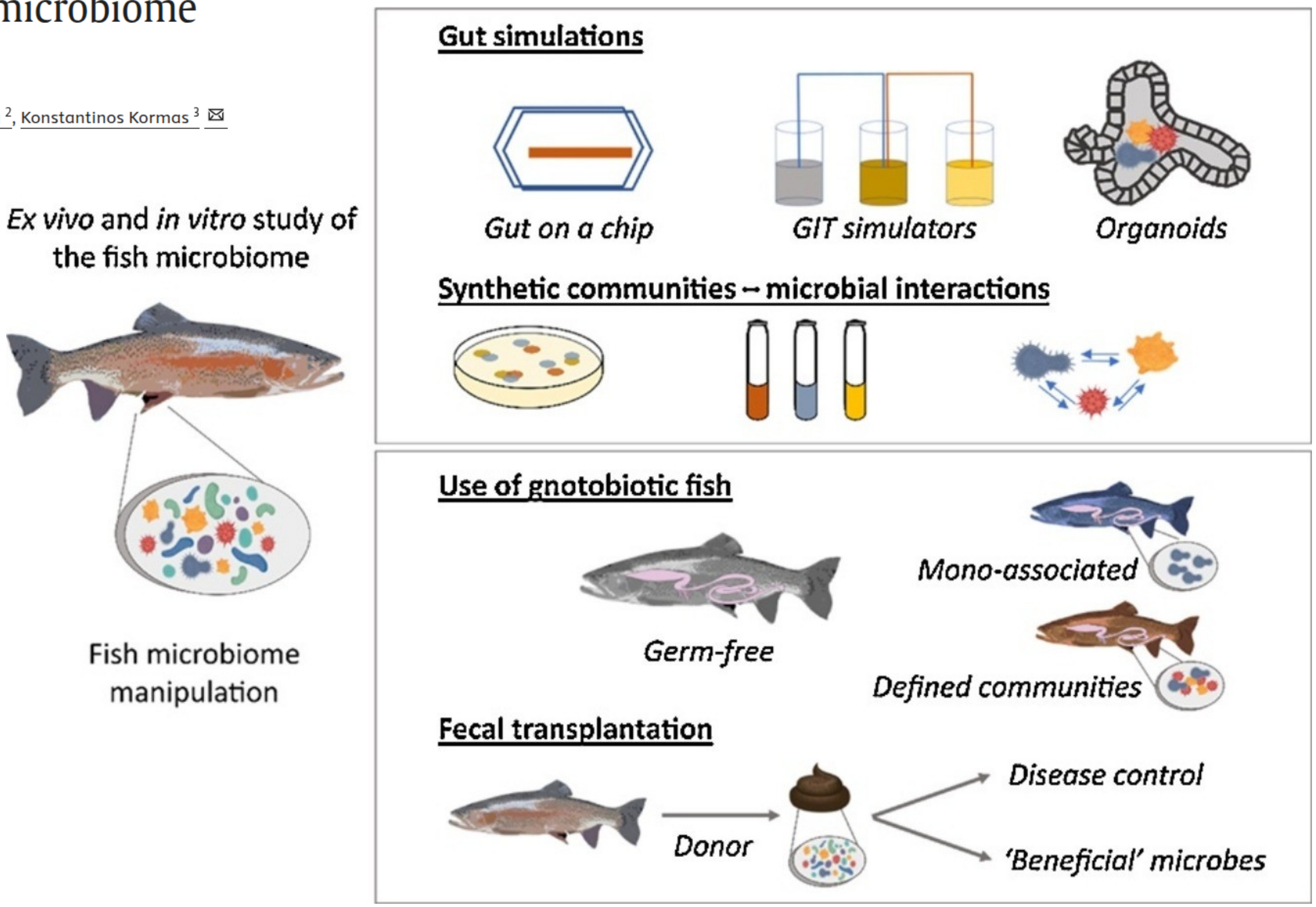


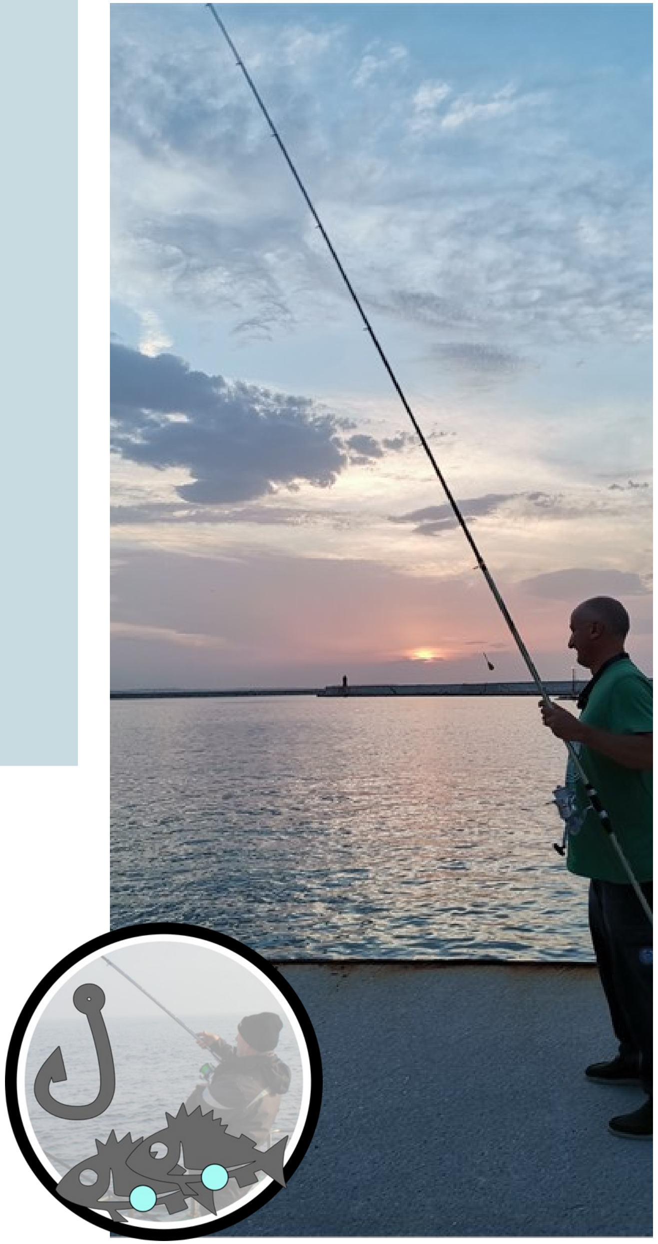
Time to integrate biotechnological approaches into fish gut microbiome research

Gian Marco Luna [1], Grazia Marina Quero [1], Fotini Kokou [2], Konstantinos Kormas [3] ✉

Practical Applications in Aquaculture

- Synthetic gut
- Gnotobiotic fish
- Fecal transplantation





Microbial Communities:

- Natural vs Farm systems

Natural marine environments host highly diverse microbiomes.

Intensive aquaculture systems often show reduced microbial diversity.

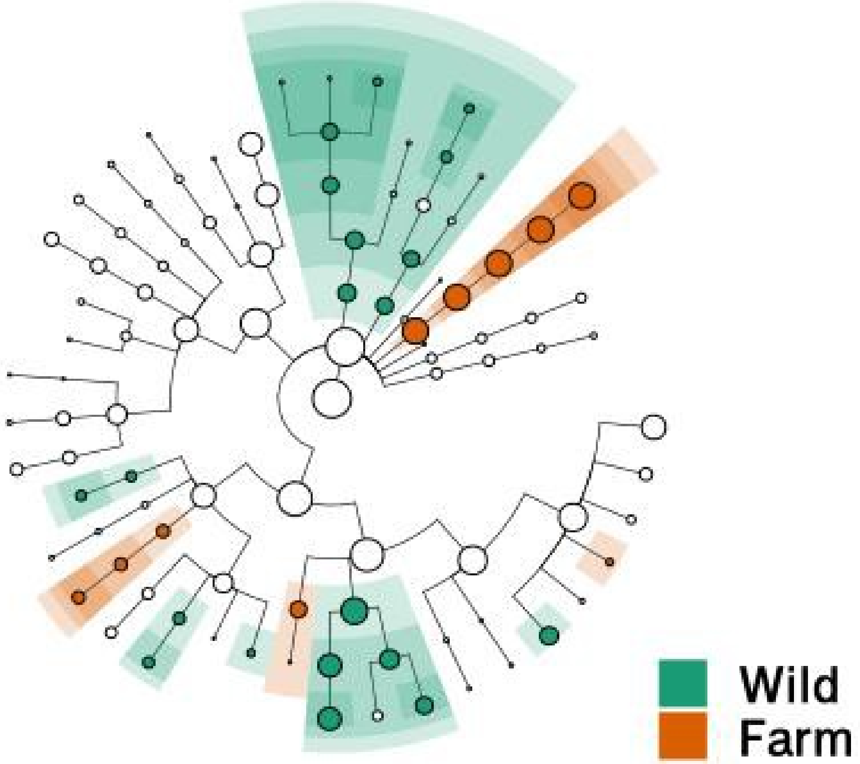
Tank management and biofilters strongly shape microbial composition.

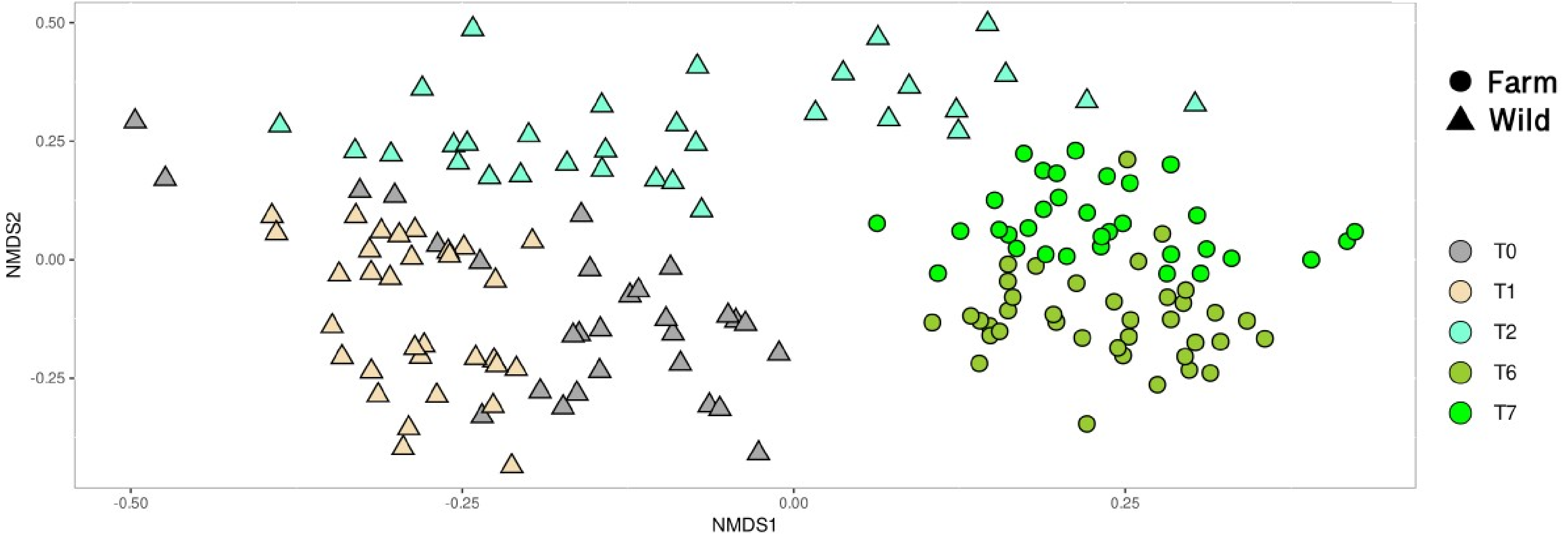
→ loss of ecological buffering capacity

Reduced diversity can increase instability and disease susceptibility.

→ higher risk of dysbiosis and pathogen outbreaks.

Microbial Communities:
Natural (WILD) vs Intensive (FARM) Systems





Microbial Engineering in Aquaculture Systems

Biofilters host nitrifying and denitrifying microbes essential for water quality.
→ Ammonia → Nitrite → Nitrate → Denitrification

Key taxa:

- **Nitrosomonas, Nitrobacter (bacteria)**
- **Thaumarchaeota (ammonia-oxidizing archaea)**
- **Heterotrophic bacteria**

Protists controlling biomass

Community composition determines system stability and fish health.

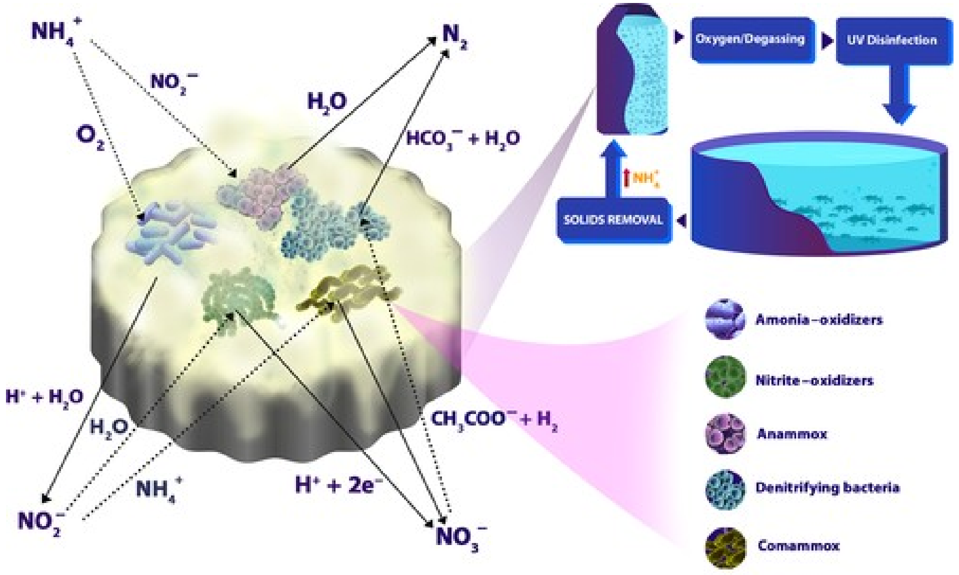
- **Biofilm structure integrates bacteria, archaea, and microbial eukaryotes.**
- **Biofilter efficiency in recirculating aquaculture systems depends on stable nitrifying bacteria.**

REVIEWS IN Aquaculture

Review 🔓 Full Access

Overview and future perspectives of nitrifying bacteria on biofilters for recirculating aquaculture systems

Pamela Ruiz, José Miguel Vidal, Daniela Sepúlveda, Claudia Torres, Gabriela Villouta, Carlos Carrasco, Felipe Aguilera, Nathaly Ruiz-Tagle, Homero Urrutia ✉



Microbiome as environmental monitoring Tool for living fish stocks

Aquatic microbiome analysis enables **real-time ecosystem assessment** for living fish populations in aquaculture and fisheries.

Microbial change often precede **fish mortality events** (e.g., oxygen depletion, pathogen blooms)

Early detection of:

- Pollution (chemical and organic)
- Eutrophication and harmful algal blooms
- Hypoxia and other environmental stressors

Functions as a biological **early-warning** system beyond traditional physicochemical monitoring

ELSEVIER

Environmental Pollution

Volume 314, 1 December 2022, 120222

Legacy metal contamination is reflected in the fish gut microbiome in an urbanised estuary ☆

Alessandra L. Suzzi [a], Michael Stat [a], Geoff R. MacFarlane [a], Justin R. Seymour [b], Nathan LR. Williams [b], Troy F. Gaston [a], Md Rushna Alam [a c], Megan J. Huggett [a d]

Integrated Approach

Comprehensive monitoring combines multiple microbiomes:

→ **Water microbiome** : immediate environmental conditions

→ **Sediment microbiome** : long-term ecosystem processes

→ **Host microbiome (fish skin, gill, gut)** : animal health status

Environmental DNA (eDNA)

- Captures microbes and eukaryotes from water samples
- Detects pathogens, invasive species, and biodiversity
- Enables non-invasive monitoring of living fish stocks

Gut content

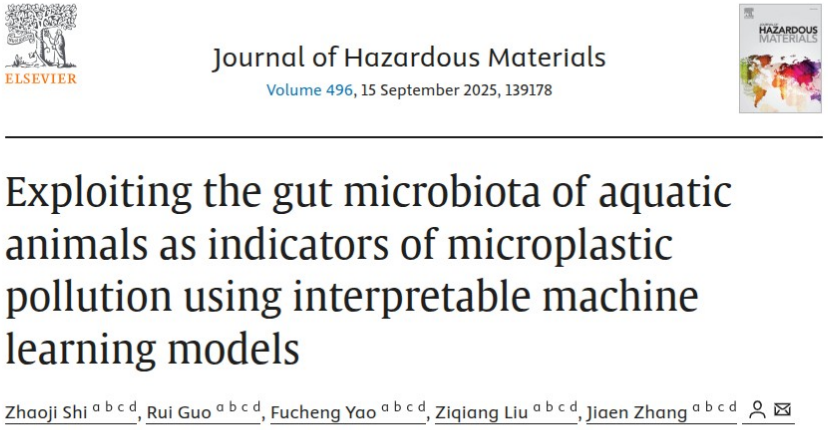
- Microbiome composition reflects recent feeding and dietary preferences.
- **Integration with metabarcoding of COI/18S allows identification of prey and eukaryotic food sources.**

Machine Learning & Predictive Ecology

Machine learning applied to microbial data predicts site ecological status.
→ Sampling → Sequencing → Feature extraction → Predictive models → Application

Microbial signatures act as ecological fingerprints.
→ Identify habitat degradation

Supports fisheries management decisions.

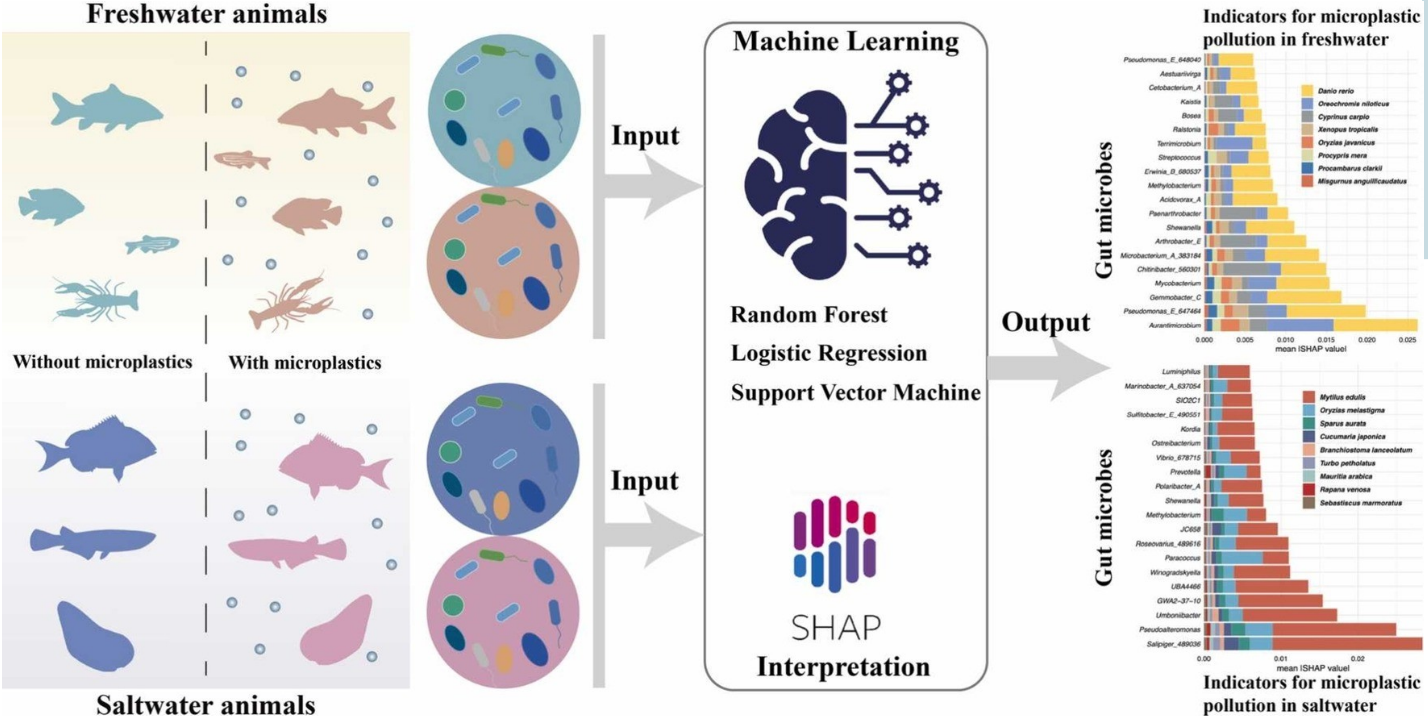


ELSEVIER

Journal of Hazardous Materials
Volume 496, 15 September 2025, 139178

Exploiting the gut microbiota of aquatic animals as indicators of microplastic pollution using interpretable machine learning models

Zhaoji Shi [a b c d], Rui Guo [a b c d], Fucheng Yao [a b c d], Ziqiang Liu [a b c d], Jiaen Zhang [a b c d]



Diet, Nutrients, and Microbiome
Nutrition as a Driver of Microbial Communities

Diet shapes both gut and environmental microbiomes.

- Plant-based feeds alter microbiomes compared to fishmeal diets.
- Microbiome optimization improves feed conversion ratio.

Balanced feeds promote beneficial taxa.
Include eukaryotic components:

- Microalgae in feeds
- Fungal fermentation products

Feed composition influences nutrient cycling in water

- Microbial Interventions in Aquaculture
- Probiotics enhance immunity and digestion
- Prebiotics stimulate beneficial microbes
- Synbiotics combine both approaches

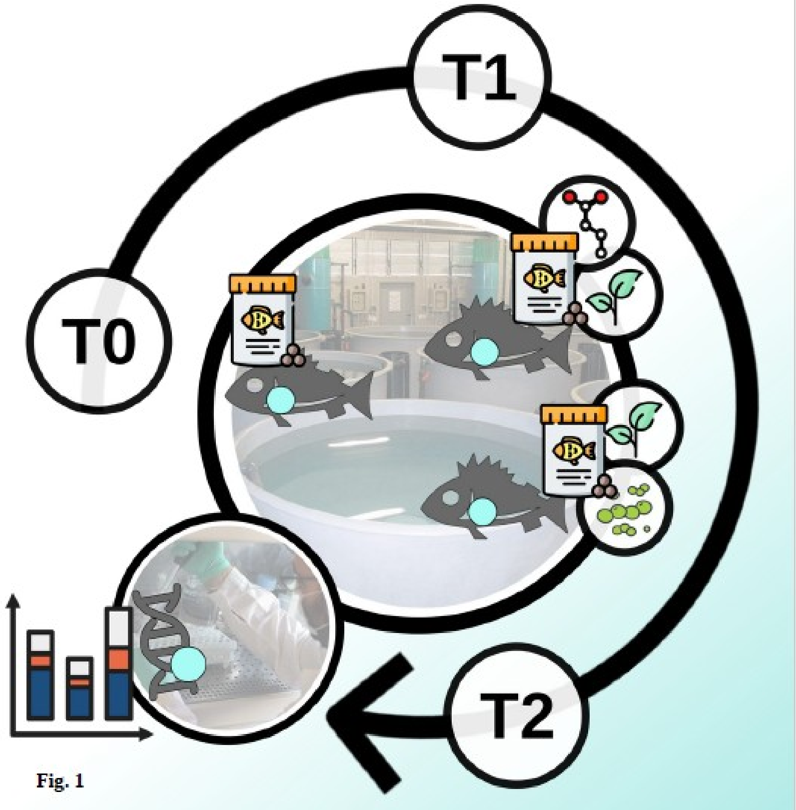


Fig. 1

Circular and Sustainable Aquaculture
Microbiomes in Circular Production Systems

Microbiome-informed strategies support circular aquaculture: Multi-trophic aquaculture (MTA)

Nutrient recycling
Waste reduction
Low-trophic species promotion
Sustainable feed innovation

REVIEWS IN Aquaculture

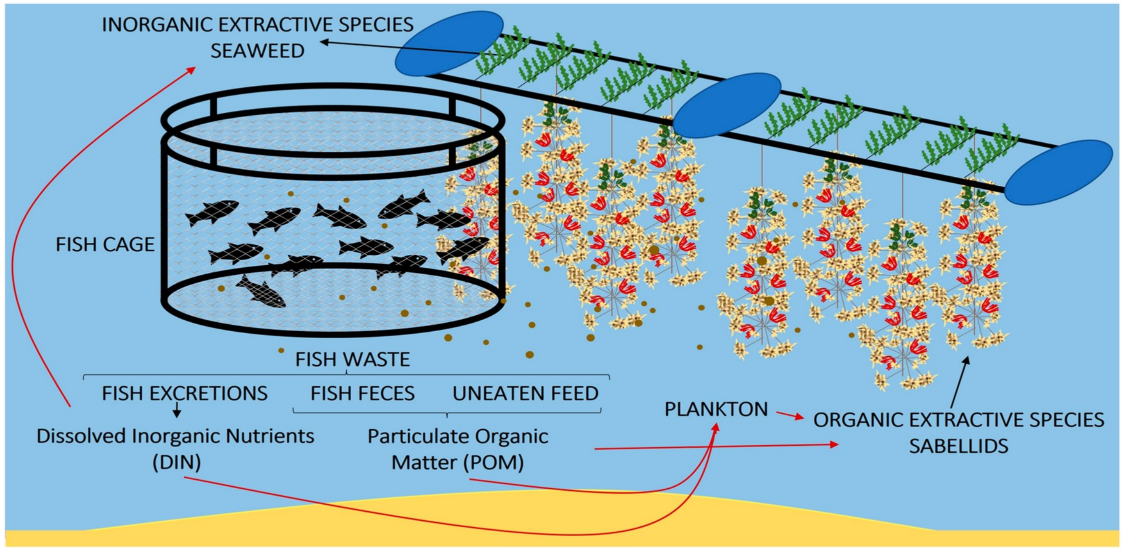
REVIEW | Open Access

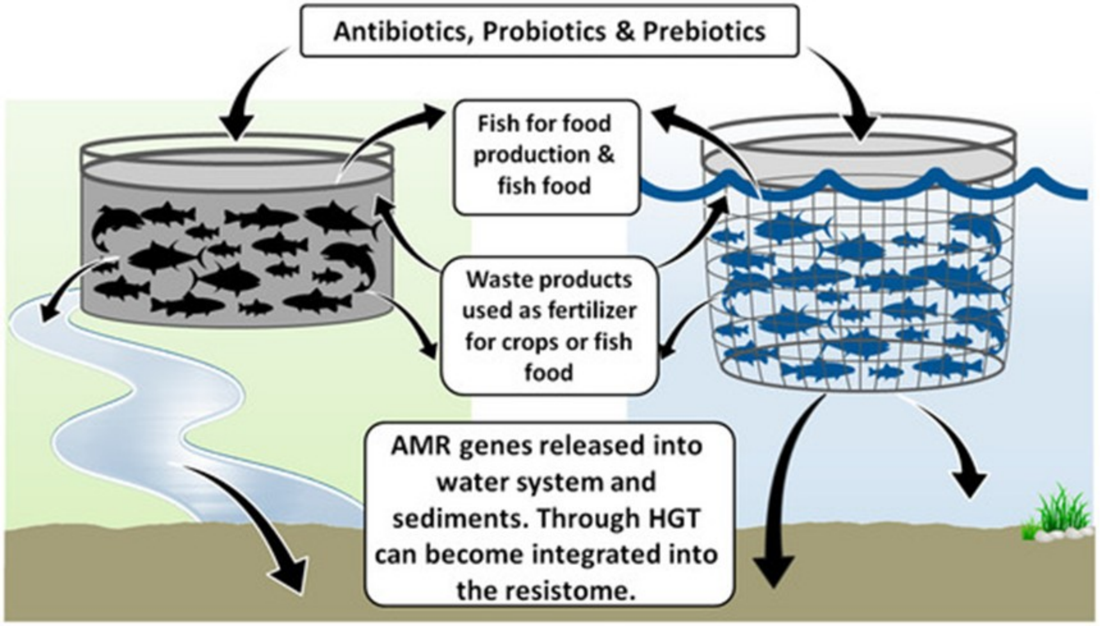
Transforming sustainable aquaculture by applying circularity principles

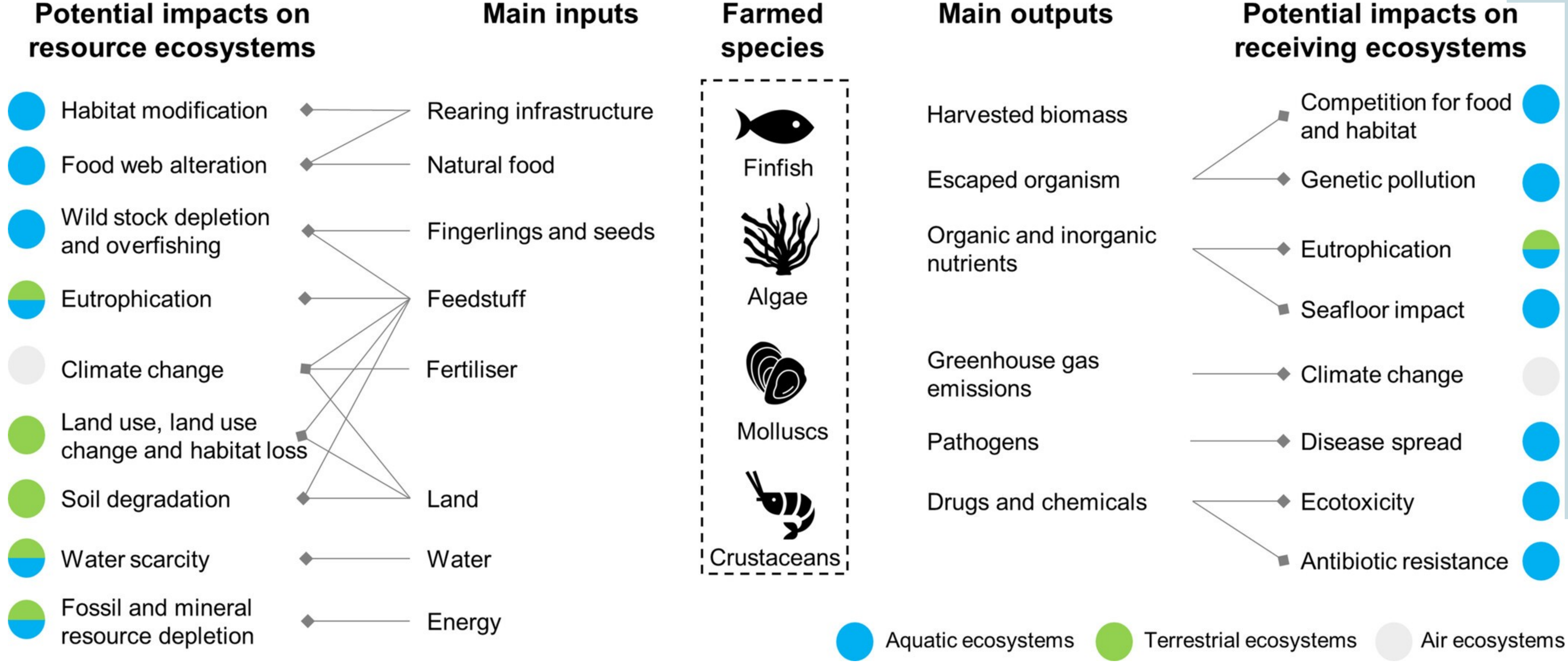
Killian Chary, Anne-Jo van Riel, Abigail Muscat, Aurélie Wilfart, Souhil Harchaoui, Marc Verdegem, Ramón Filgueira, Max Troell, Patrik J. G. Henriksson, Imke J. M. de Boer, Geert F. Wiegertjes

First published: 14 September 2023 | https://doi.org/10.1111/raq.12860 | Citations: 12

Imke J.M. de Boer and Geert F. Wiegertjes share last authorship.







Potential impacts on resource ecosystems

- Habitat modification
- Food web alteration
- Wild stock depletion and overfishing
- Eutrophication
- Climate change
- Land use, land use change and habitat loss
- Soil degradation
- Water scarcity
- Fossil and mineral resource depletion

Main inputs

- Rearing infrastructure
- Natural food
- Fingerlings and seeds
- Feedstuff
- Fertiliser
- Land
- Water
- Energy

Farmed species

- Finfish
- Algae
- Molluscs
- Crustaceans

Main outputs

- Harvested biomass
- Escaped organism
- Organic and inorganic nutrients
- Greenhouse gas emissions
- Pathogens
- Drugs and chemicals

Potential impacts on receiving ecosystems

- Competition for food and habitat
- Genetic pollution
- Eutrophication
- Seafloor impact
- Climate change
- Disease spread
- Ecotoxicity
- Antibiotic resistance

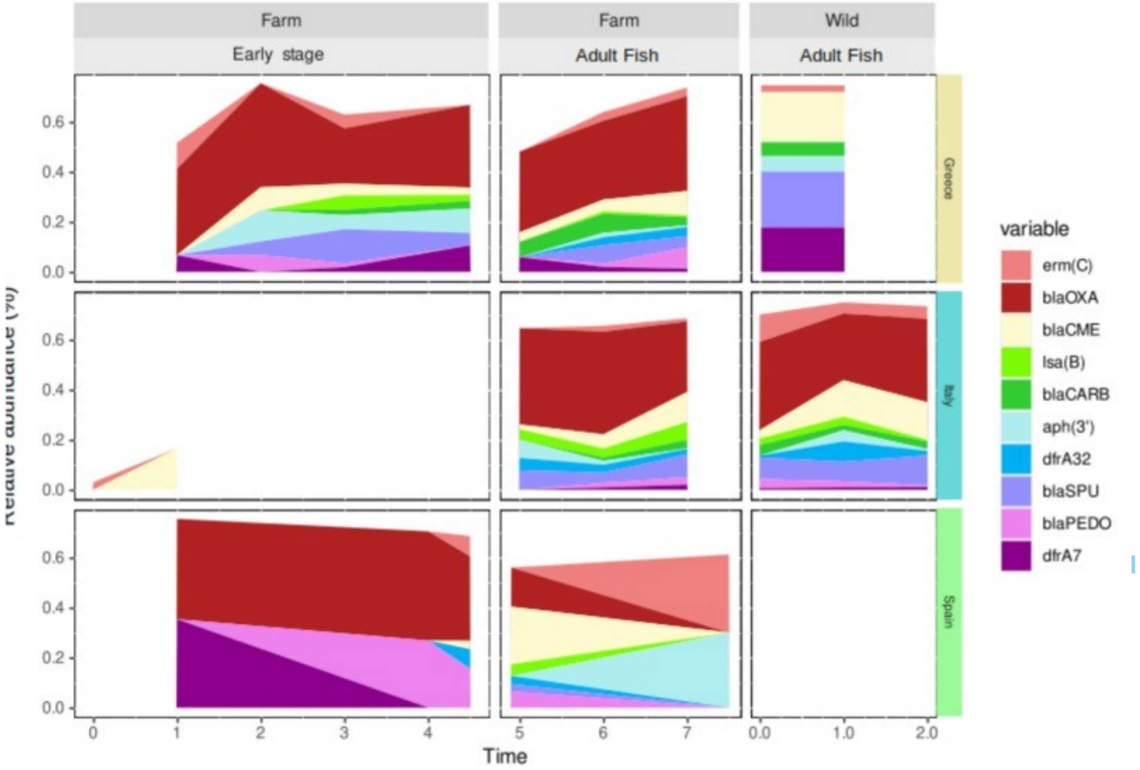
● Aquatic ecosystems ● Terrestrial ecosystems ○ Air ecosystems

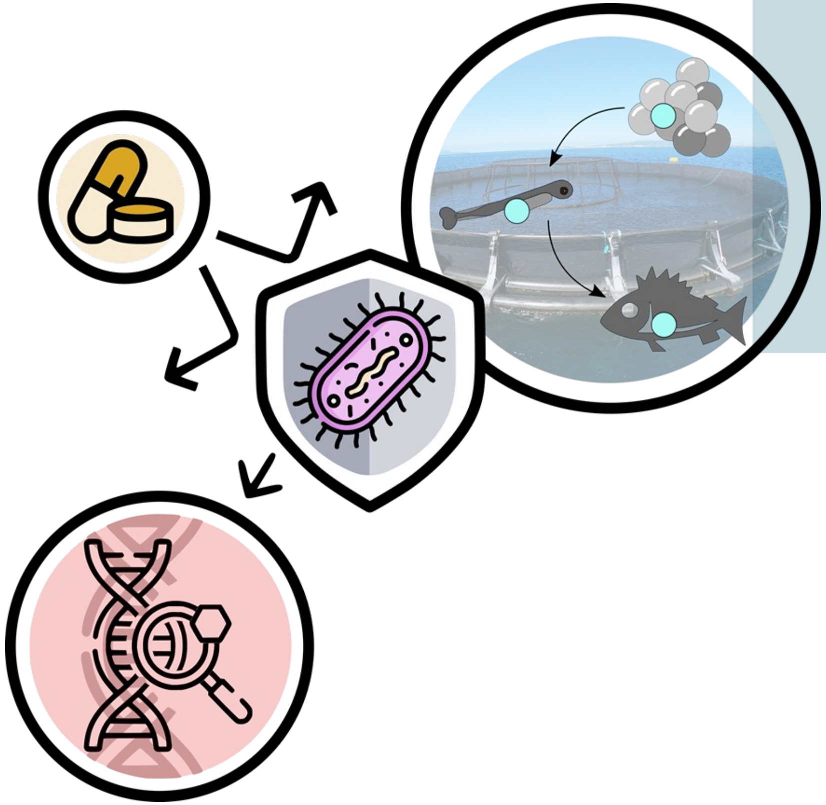
Antimicrobial resistance as a major global concern.

Microbiome manipulation is considered a key tool to **reduce antimicrobial resistance (AMR)** by limiting pathogen colonization and decreasing the need for antibiotic use.

By promoting beneficial microbial communities that outcompete resistant bacteria and disrupt resistance gene exchange

Microbiome-based approaches can curb the emergence, persistence, and transmission of AMR across hosts and environments.



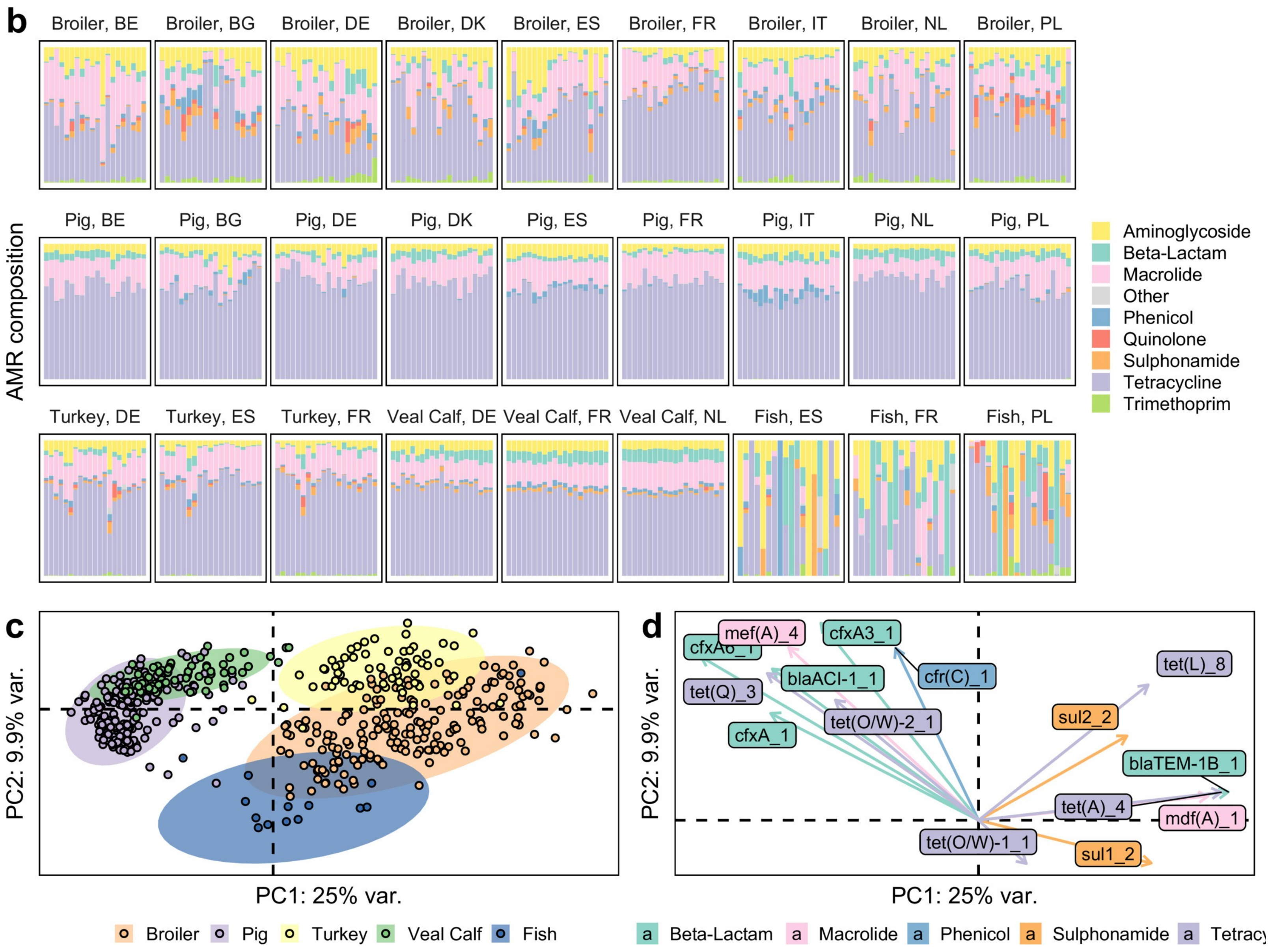


AMR

in aquaculture

Munk et al., 2024

Limited by the surrounding environment

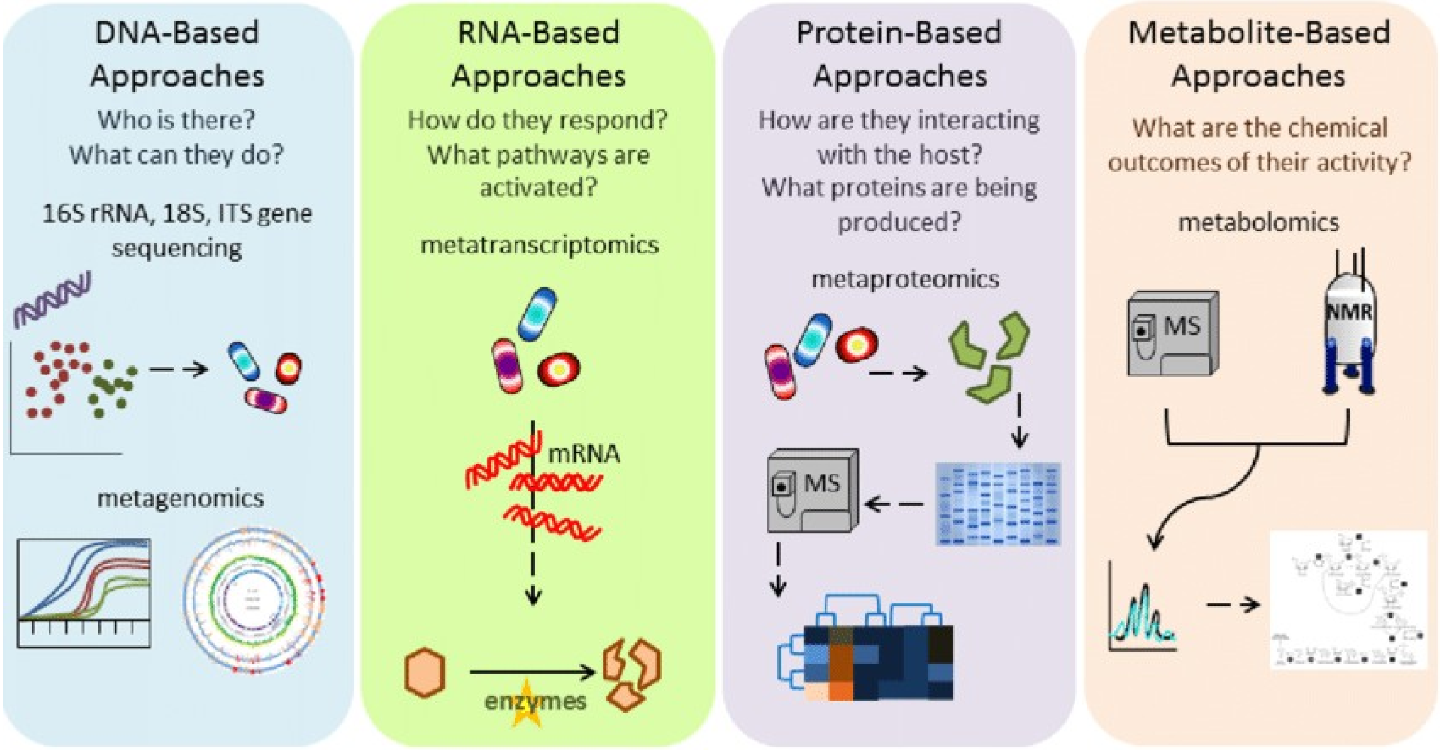


Take a Break

Microbiome Tools:
Molecular Approaches for Microbiome Analysis

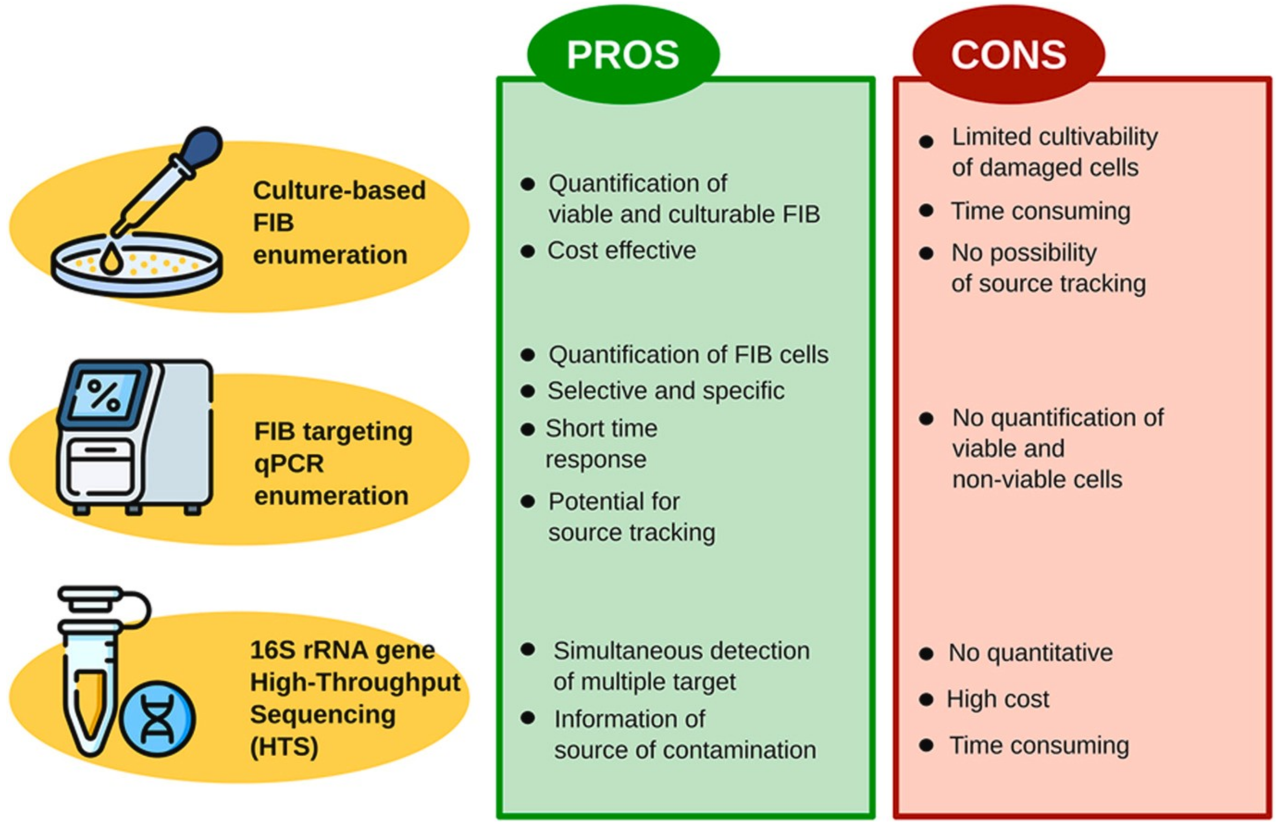
Molecular Approaches in Ecological Research

- **DNA-based tools** (e.g., metabarcoding, eDNA, population genetics) → identify species, biodiversity, and community structure.
- **Meta-omics approaches** (metagenomics, metatranscriptomics, metaproteomics, metabolomics) → reveal functional potential and activity of organisms and microbiomes.
- **Phylogenetic and genomic analyses** → reconstruct evolutionary relationships and adaptation mechanisms.



Integrating culture-based and molecular methods provides an improved assessment of microbial quality in a coastal lagoon ☆

Marco Basili [a], Laura Perini [b], Luca Zaggia [c], Gian Marco Luna [a], Grazia Marina Quero [a]



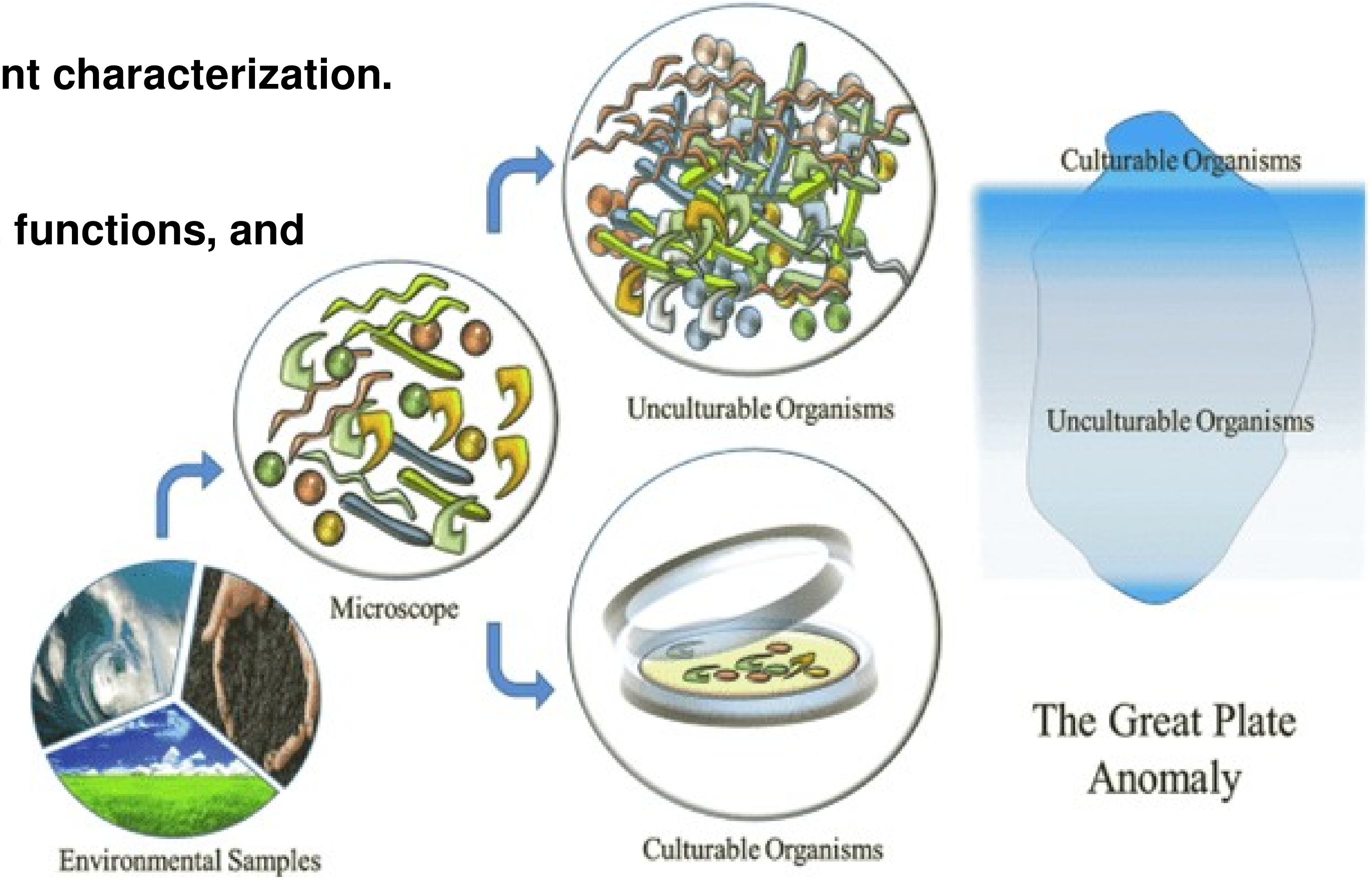
	PROS	**CONS**
Culture-based FIB enumeration	Quantification of viable and culturable FIB Cost effective	Limited cultivability of damaged cells Time consuming No possibility of source tracking
FIB targeting qPCR enumeration	Quantification of FIB cells Selective and specific Short time response Potential for source tracking	No quantification of viable and non-viable cells
16S rRNA gene High-Throughput Sequencing (HTS)	Simultaneous detection of multiple target Information of source of contamination	No quantitative High cost Time consuming

Why Molecular Approaches?

Most microbes are unculturable using traditional methods.
→ 90% of environmental microbes cannot be cultured.

Molecular tools allow culture-independent characterization.

Enable analysis of community structure, functions, and dynamics.



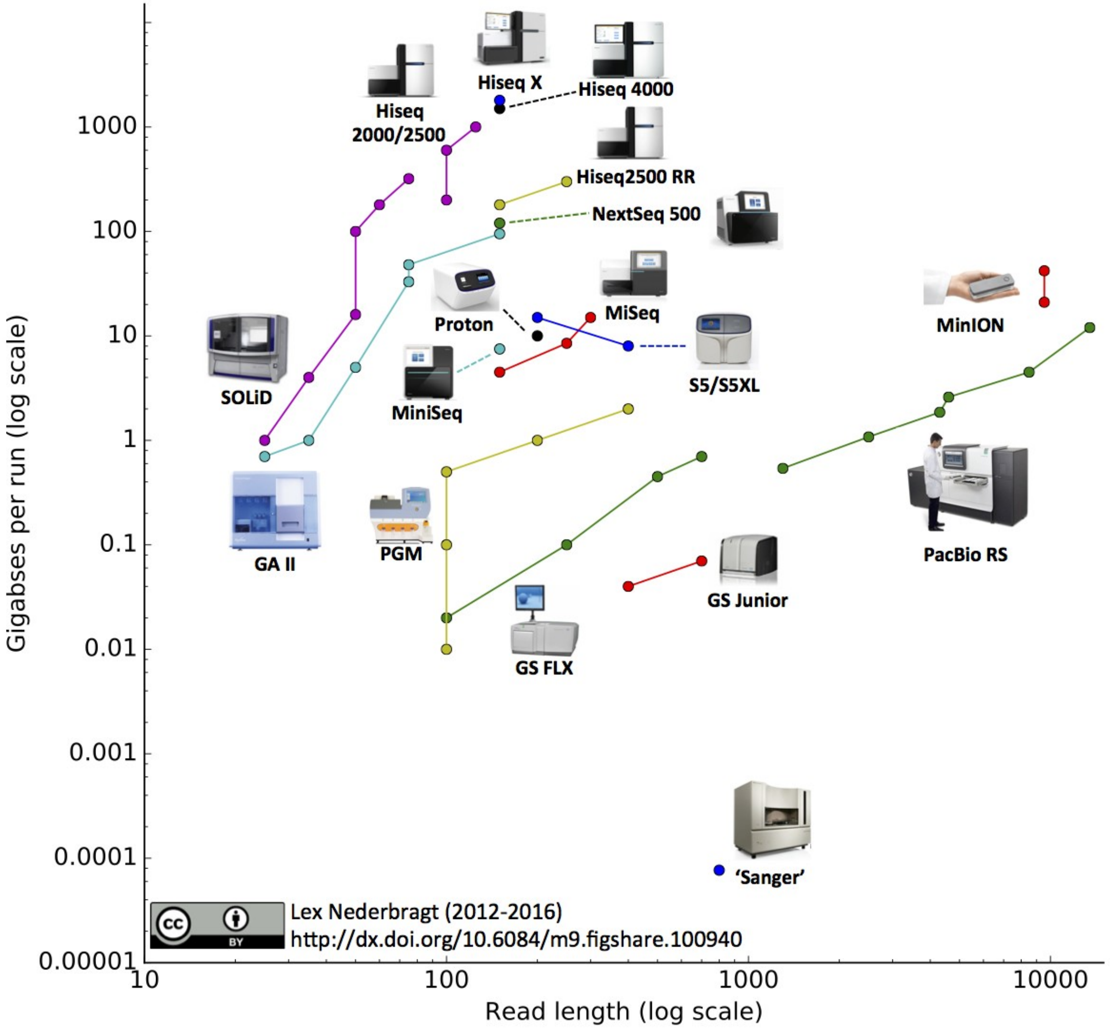
From Classical Microbiology to Multi-Omics (Next Generation Sequencing)

Evolution of approaches:

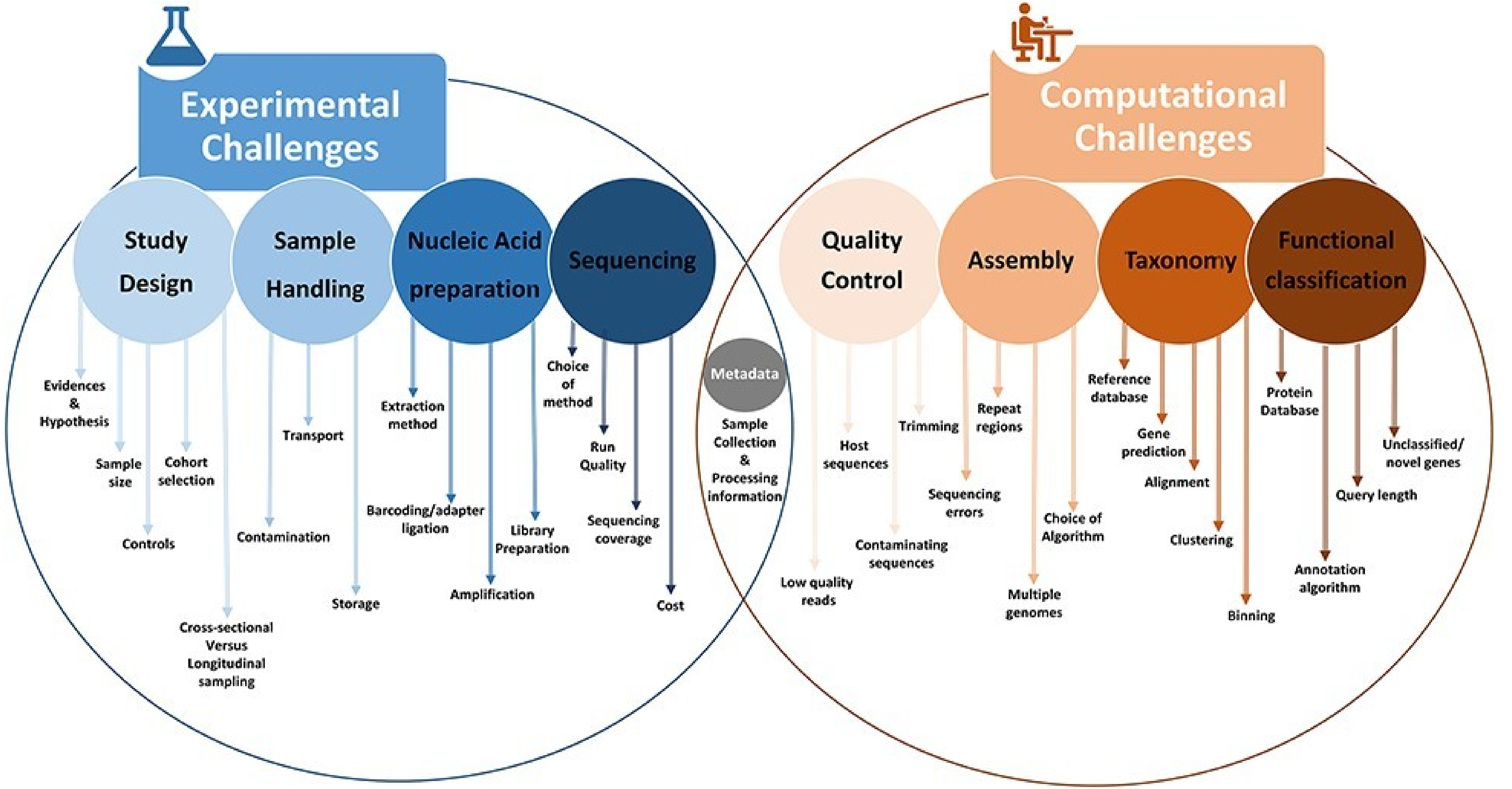
- Culturing
- PCR-based methods
- **Amplicon sequencing (Metabarcoding)**
- **Shotgun metagenomics**
- Integrated multi-omics

Molecular tools allow investigation of:

- Taxonomic composition
- Functional potential
- Active metabolic pathways
- Host–microbe interactions



Lex Nederbragt (2012-2016)
http://dx.doi.org/10.6084/m9.figshare.100940



Experimental Challenges

Study Design
- Evidences & Hypothesis
- Sample size
- Cohort selection
- Controls
- Cross-sectional Versus Longitudinal sampling

Sample Handling
- Transport
- Contamination
- Storage

Nucleic Acid preparation
- Extraction method
- Barcoding/adapter ligation
- Amplification
- Library Preparation

Sequencing
- Choice of method
- Run Quality
- Sequencing coverage
- Cost

Metadata
- Sample Collection & Processing information

Computational Challenges

Quality Control
- Host sequences
- Trimming
- Contaminating sequences
- Low quality reads

Assembly
- Repeat regions
- Sequencing errors
- Choice of Algorithm
- Multiple genomes

Taxonomy
- Reference database
- Gene prediction
- Alignment
- Clustering
- Binning

Functional classification
- Protein Database
- Unclassified/ novel genes
- Query length
- Annotation algorithm

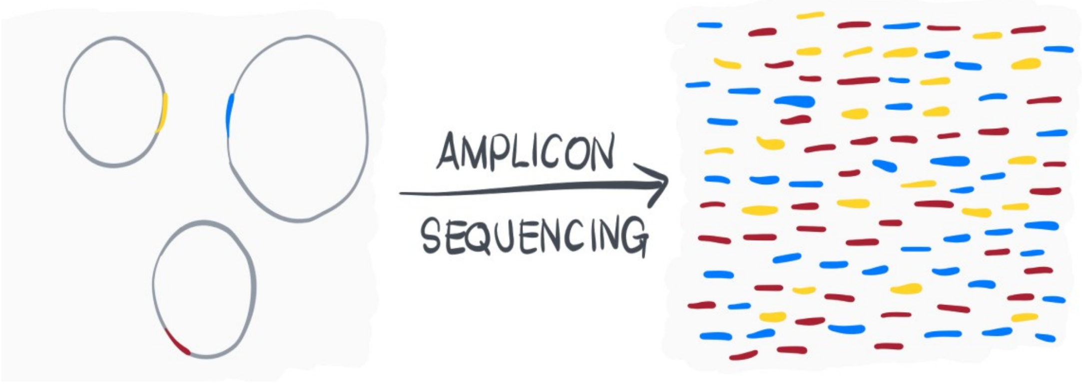
Metabarcoding and Marker Choice in Microbiome Studies

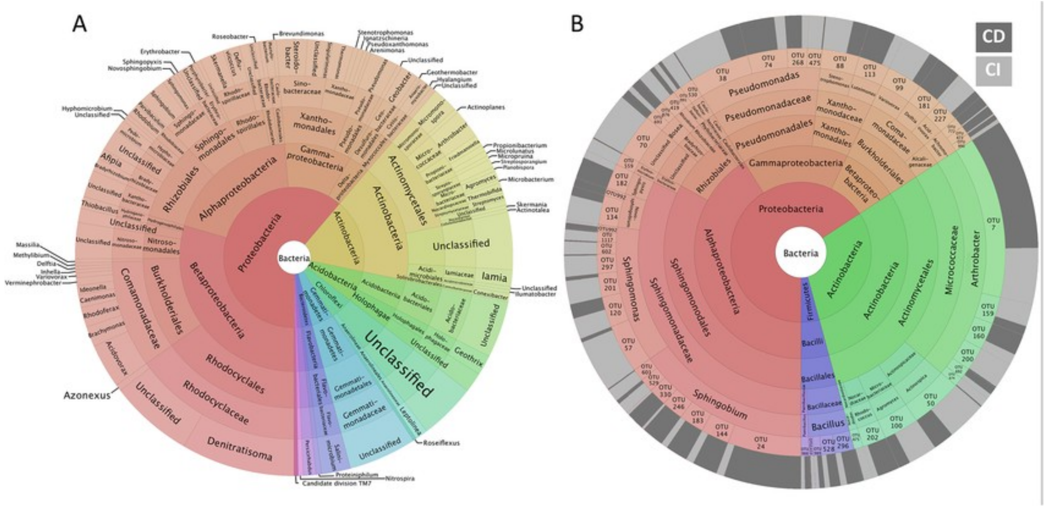
Metabarcoding identifies microbial communities using marker gene sequences.

Common markers:
- 16S rRNA (bacteria/archaea)
- ITS (fungi)
- COI (metazoans)

Enables high-throughput assessment of biodiversity and ecological change.

Marker choice determines taxonomic resolution and accuracy.





Public Databases for Microbiome Research

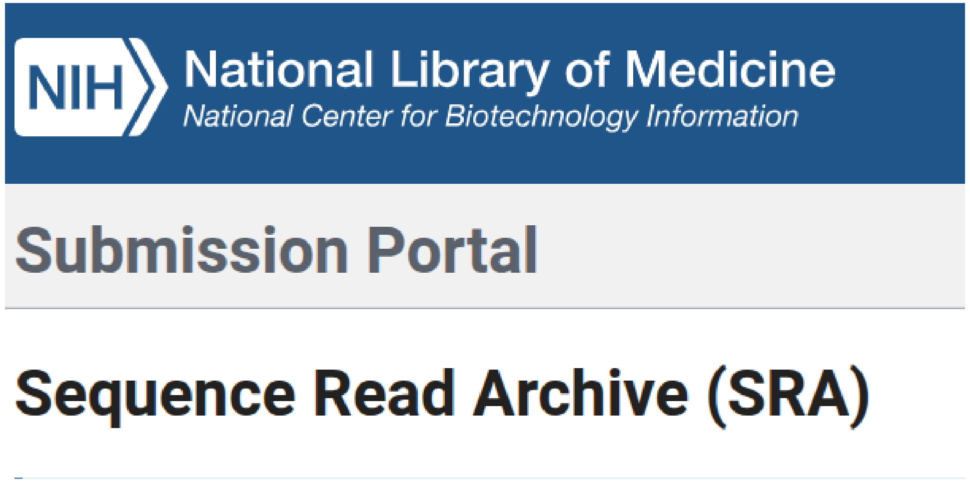
Open Repositories and Data Reuse in Microbial Ecology

Modern microbiome research increasingly relies on public international databases.

Major repositories:
NCBI, European Nucleotide Archive, DNA Data Bank of Japan, part of the International Nucleotide Sequence Database Collaboration (INSDC).

These platforms store raw sequencing data (FASTQ) and environmental metadata **(!!!!).**

Enable
- **reproducibility**
- **large-scale comparisons**
- **meta-analyses across ecosystems.**


NIH
National Library of Medicine
National Center for Biotechnology Information

Submission Portal

Sequence Read Archive (SRA)

- **Many high-impact microbiome papers now combine new data + public datasets.**
- **Learning to mine public databases is a key skill for future researchers.**

16S rRNA Gene: The Workhorse

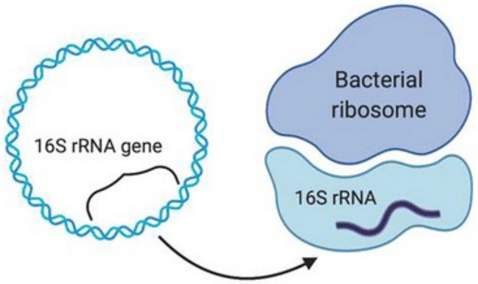
Marker gene for bacteria and archaea

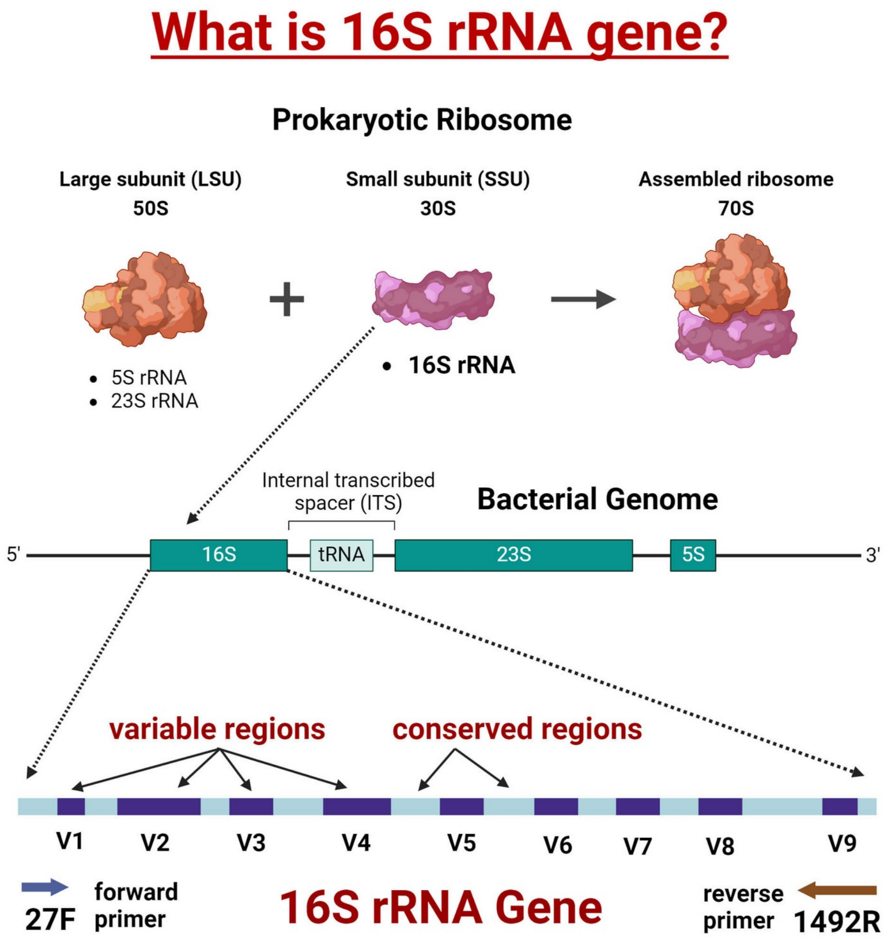
Conserved + variable regions
- Universal presence
- Evolutionary conservation
- Hypervariable regions (V1–V9)

Enables taxonomic profiling

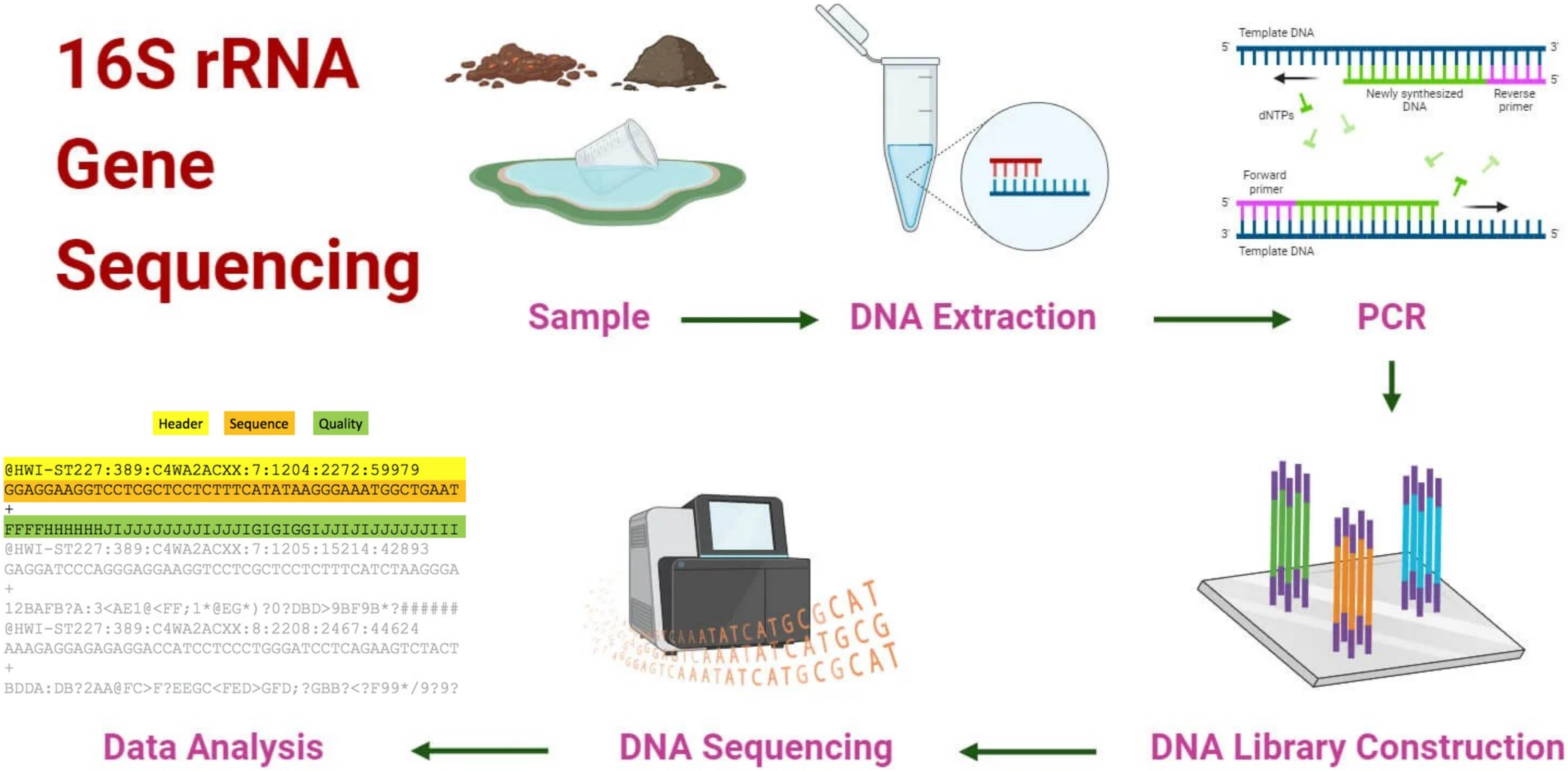
Sampling →
DNA extraction →
PCR →
Sequencing →
Bioinformatics

read → nucleotidic sequence
fasta/fastq → reads file with quality
OTUs (97% similarity clustering)
ASVs (100% exact sequence variants)





16S rRNA Gene Sequencing



Sample → DNA Extraction → PCR → DNA Library Construction → DNA Sequencing → Data Analysis

Header Sequence Quality

```
@HWI-ST227:389:C4WA2ACXX:7:1204:2272:59979
GGAGGAAGGTCCTCGCTCCTCTTTCATATAAGGGAAATGGCTGAAT
+
FFFFHHHHHHJIJJJJJJJJIJJJIGIGIGGIJJIJIJJJJJJIII
@HWI-ST227:389:C4WA2ACXX:7:1205:15214:42893
GAGGATCCCAGGGAGGAAGGTCCTCGCTCCTCTTTCATCTAAGGGA
+
12BAFB?A:3<AE1@<FF;1*@EG*)?0?DBD>9BF9B*?######
@HWI-ST227:389:C4WA2ACXX:8:2208:2467:44624
AAAGAGGAGAGAGGACCATCCTCCCTGGGATCCTCAGAAGTCTACT
+
BDDA:DB?2AA@FC>F?EEGC<FED>GFD;?GBB?<?F99*/9?9?
```

16S Limitations

Limited taxonomic resolution
- Due to short lenght of the fragment

PCR bias

Cannot directly infer function

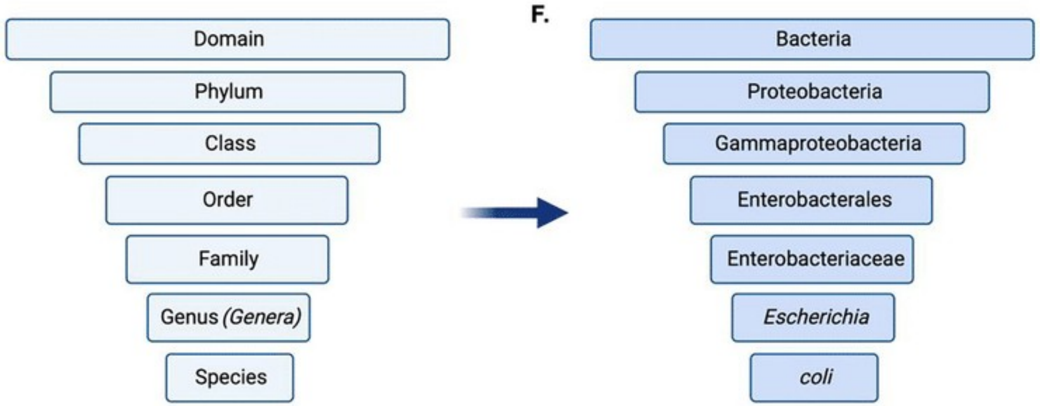
Misses viruses and most eukaryotes
- Not univeral

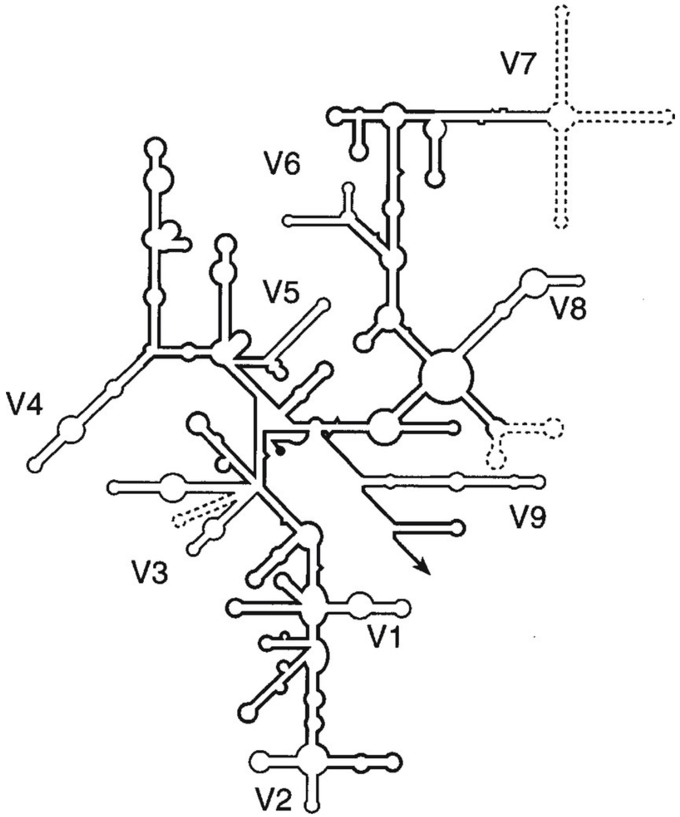
Not a single copy genes (SCGs)
- form 1 to 15 copies

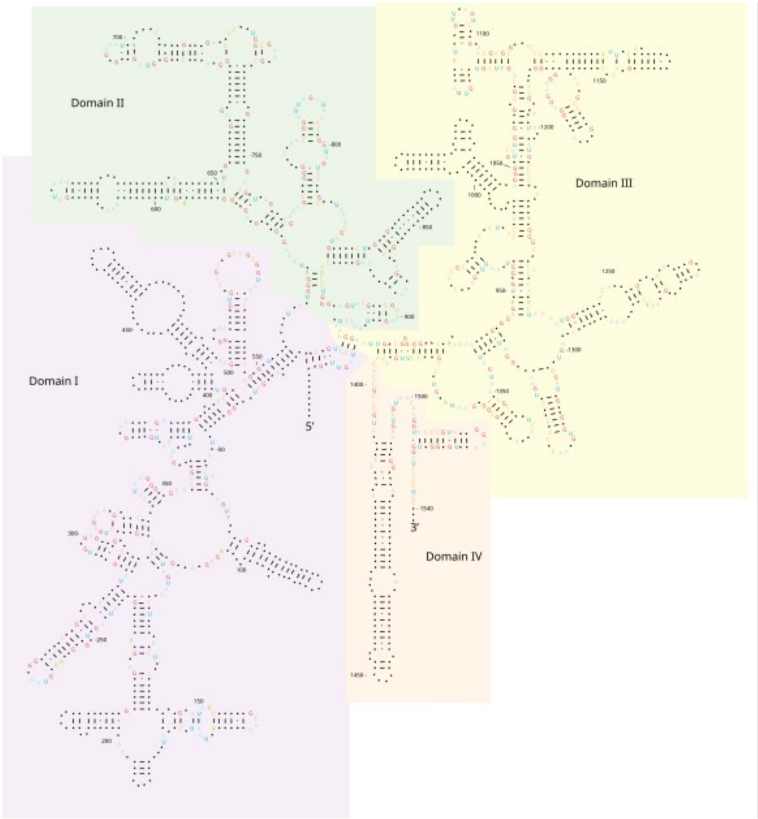
Not standard analysis
- but reproducible

National Library of Medicine
National Center for Biotechnology Information

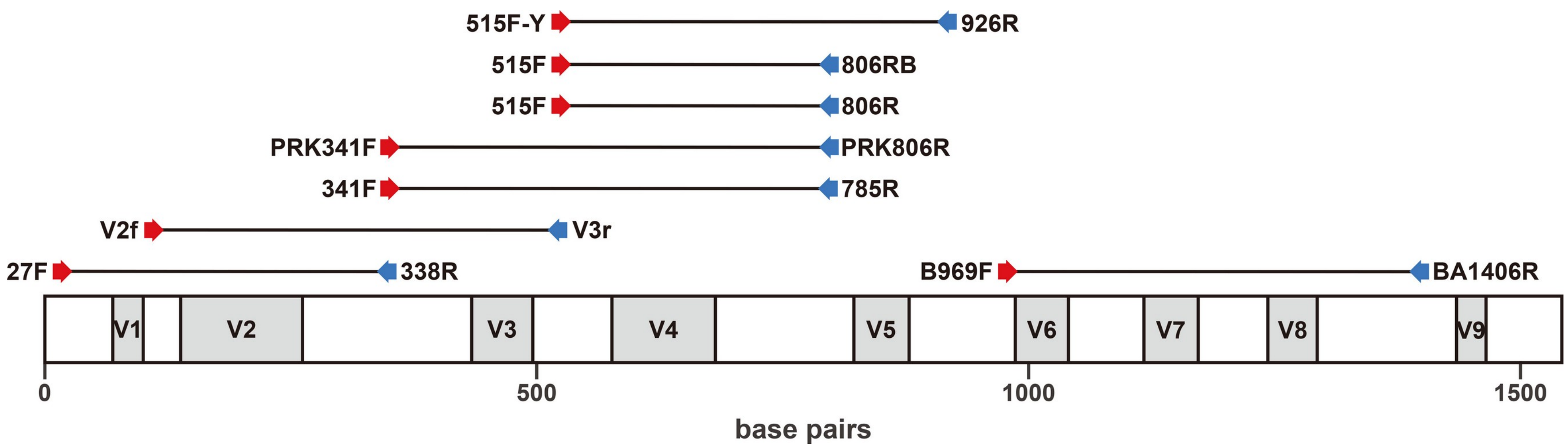
ENA
European Nucleotide Archive

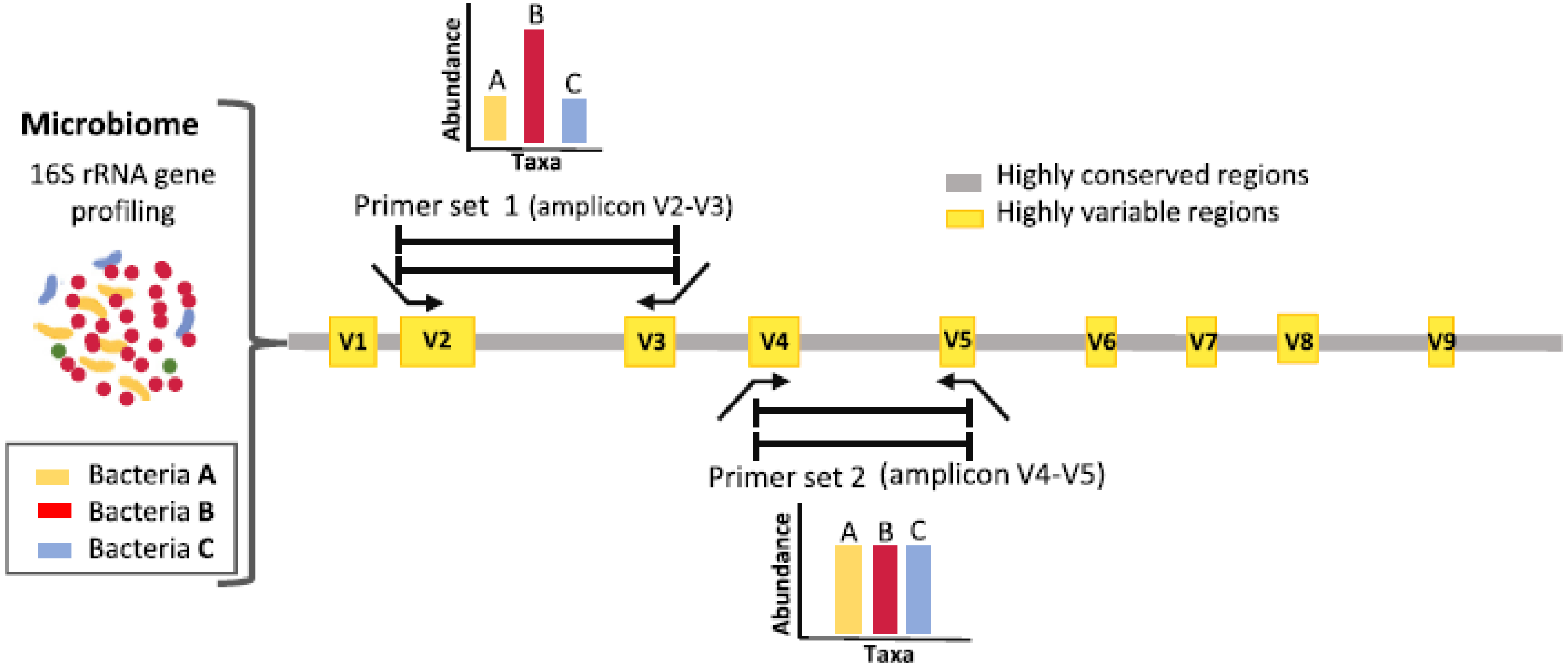




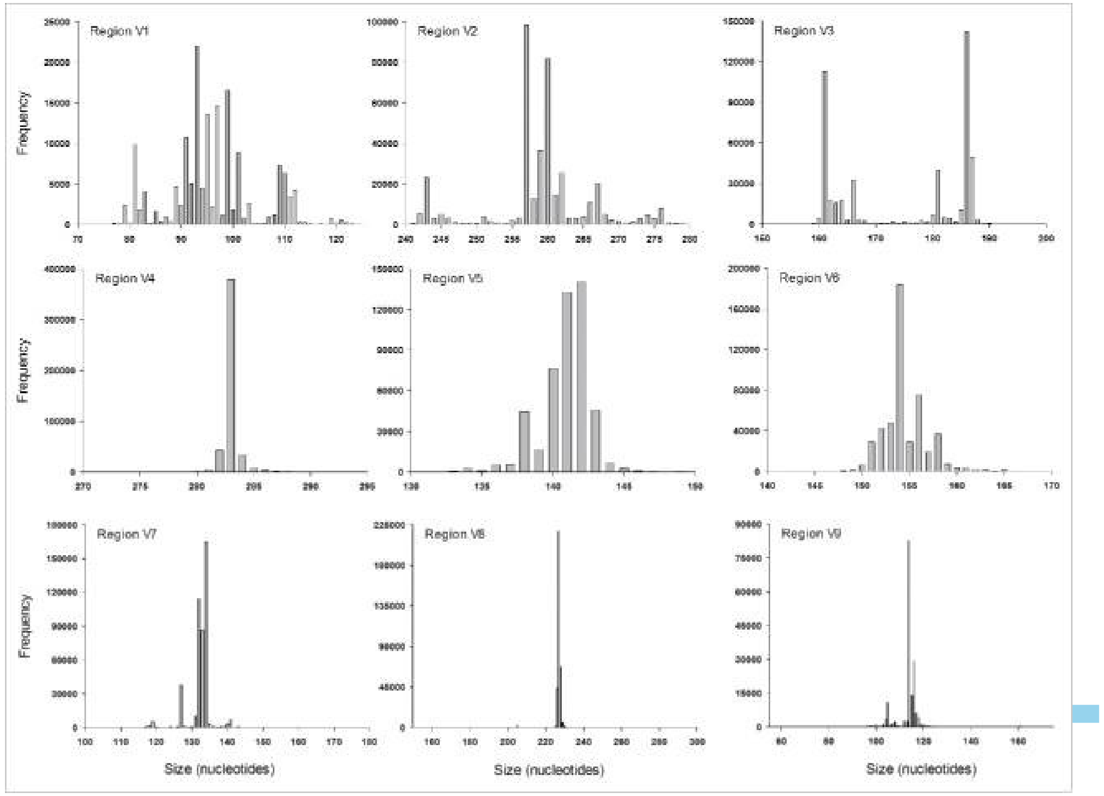


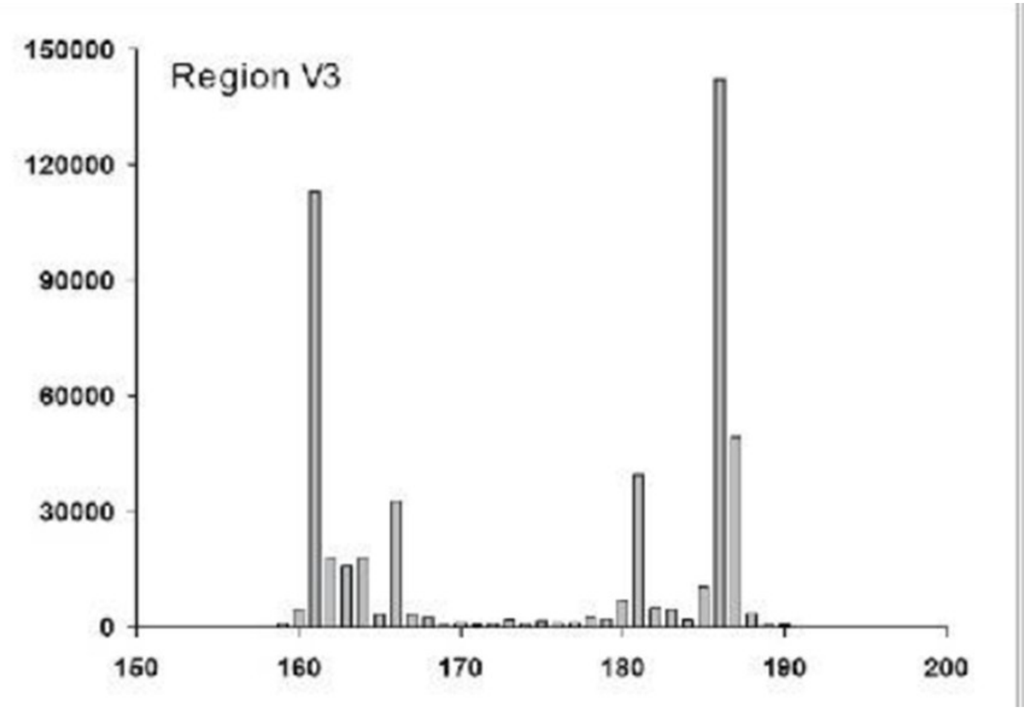
16S Primer selection

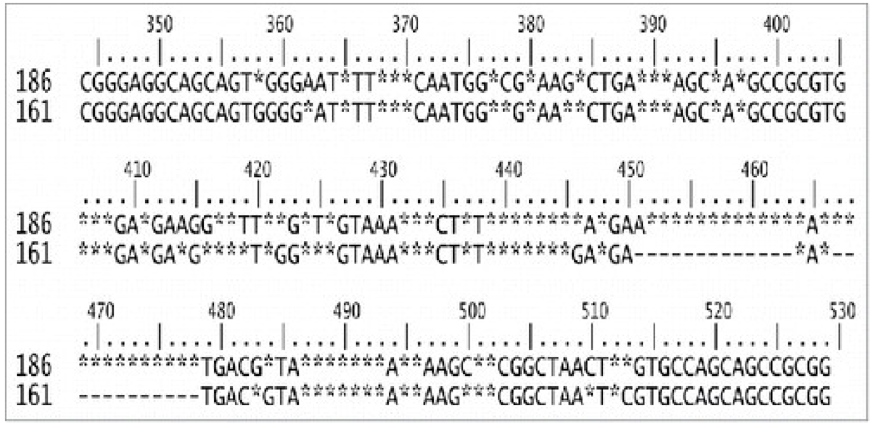




16S Primer selection







```
          350       360       370       380       390       400
      |....|....|....|....|....|....|....|....|....|....|....|....|
186  CGGGAGGCAGCAGT*GGGAAT*TT***CAATGG*CG*AAG*CTGA***AGC*A*GCCGCGTG
161  CGGGAGGCAGCAGTGGGG*AT*TT***CAATGG**G*AA**CTGA***AGC*A*GCCGCGTG

        410       420       430       440       450       460
     ....|....|....|....|....|....|....|....|....|....|....|....|...
186  ***GA*GAAGG**TT**G*T*GTAAA***CT*T********A*GAA*************A***
161  ***GA*GA*G****T*GG***GTAAA***CT*T*******GA*GA-------------*A*--

     470       480       490       500       510       520       530
     .|....|....|....|....|....|....|....|....|....|....|....|....|
186  **********TGACG*TA*******A**AAGC**CGGCTAACT**GTGCCAGCAGCCGCGG
161  ----------TGAC*GTA*******A**AAG***CGGCTAA*T*CGTGCCAGCAGCCGCGG
```

DNA Extraction for Microbiome Analysis (16S rRNA)

Importance of extraction quality

DNA yield, integrity, and purity directly determine the reliability of downstream analyses. Co-extracted inhibitors (e.g., humic acids, polysaccharides) may impair PCR amplification and sequencing.

Choice of extraction method

Chemical protocols (e.g., phenol–chloroform) and commercial kits must ensure high recovery and reproducibility.
Standardized kits improve cross-study comparability.

Cell lysis bias

Gram-positive, spore-forming, or otherwise resilient bacteria require stronger mechanical or enzymatic lysis (e.g., bead-beating, lysozyme).

Controls and protocol comparison

Method comparisons and extraction blanks are essential to detect contamination and quantify technical bias.

Best practices

Use protocols validated for the specific sample matrix

Include extraction blanks and replicates

Minimize DNA degradation during handling

Maintain protocol consistency across samples

Advantages

More representative recovery of microbial diversity

Improved sensitivity for rare taxa detection

Limitations

Differential lysis introduces taxonomic bias

Possible co-extraction of non-target DNA or inhibitors

Best practice for wildlife gut microbiome research: A comprehensive review of methodology for 16S rRNA gene investigations

Leigh Combrink[1,2,3]*, Ian R. Humphreys[1], Quinn Washburn[1], Holly K. Arnold[1,2], Keaton Stagaman[1], Kristin D. Kasschau[1], Anna E. Jolles[2,4], Brianna R. Beechler[2] and Thomas J. Sharpton[1,5]

PCR Amplification and Sequencing of the 16S rRNA Gene (Illumina)

Marker gene amplification

PCR targets variable regions of the 16S rRNA gene to enable high-throughput sequencing of bacterial communities.

Primer-induced bias

Primer sets may preferentially amplify certain taxa, altering the apparent microbiome structure.
Testing alternative primer pairs can mitigate this effect.

Quality controls

Negative controls detect contamination; mock communities assess accuracy, amplification bias, and pipeline performance.

Best practices

Limit PCR cycle number to reduce artifacts
Include technical replicates
Apply stringent sequence quality filtering

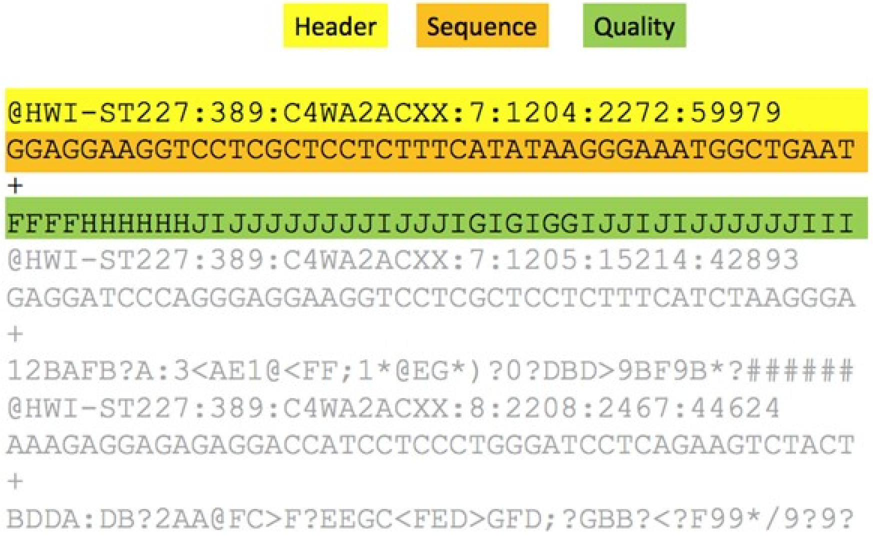
Advantages

Standard method for bacterial community profiling
High throughput and cost-effective
Compatible with large comparative studies

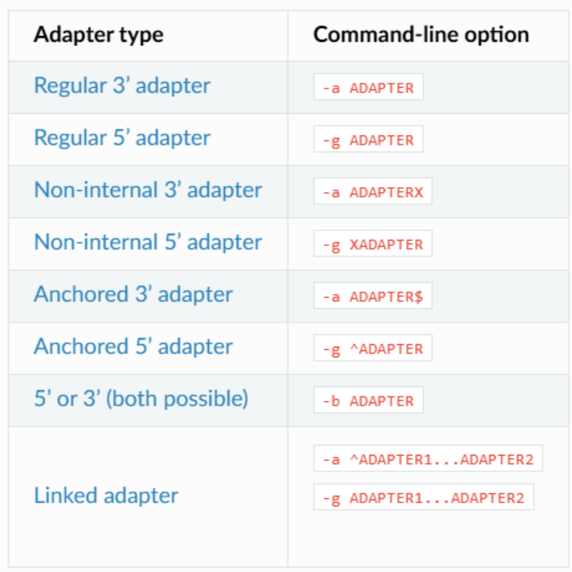
After receiving the sequencing files:

How should FASTQ data be handled?

Primer, adapter removal
CUTADAPT
source.https://github.com/marcelm/cutadapt/



```
@HWI-ST227:389:C4WA2ACXX:7:1204:2272:59979
GGAGGAAGGTCCTCGCTCCTCTTTCATATAAGGGAAATGGCTGAAT
+
FFFFHHHHHJIJJJJJJJIJJJIGIGIGGIJJIJIJJJJJJIII
@HWI-ST227:389:C4WA2ACXX:7:1205:15214:42893
GAGGATCCCAGGGAGGAAGGTCCTCGCTCCTCTTTCATCTAAGGGA
+
12BAFB?A:3<AE1@<FF;1*@EG*)?0?DBD>9BF9B*?######
@HWI-ST227:389:C4WA2ACXX:8:2208:2467:44624
AAAGAGGAGAGAGGACCATCCTCCCTGGGATCCTCAGAAGTCTACT
+
BDDA:DB?2AA@FC>F?EEGC<FED>GFD;?GBB?<?F99*/9?9?
```



Adapter type	Command-line option
Regular 3' adapter	`-a ADAPTER`
Regular 5' adapter	`-g ADAPTER`
Non-internal 3' adapter	`-a ADAPTERX`
Non-internal 5' adapter	`-g XADAPTER`
Anchored 3' adapter	`-a ADAPTER$`
Anchored 5' adapter	`-g ^ADAPTER`
5' or 3' (both possible)	`-b ADAPTER`
Linked adapter	`-a ^ADAPTER1...ADAPTER2` `-g ADAPTER1...ADAPTER2`

Sequence processing
DADA2

https://benjjneb.github.io/dada2/tutorial.html

- **Quality check**
- **Filt and trim**

→ could introduce Bias

- **Sequence inference – Denoising**

→ identify ASVs

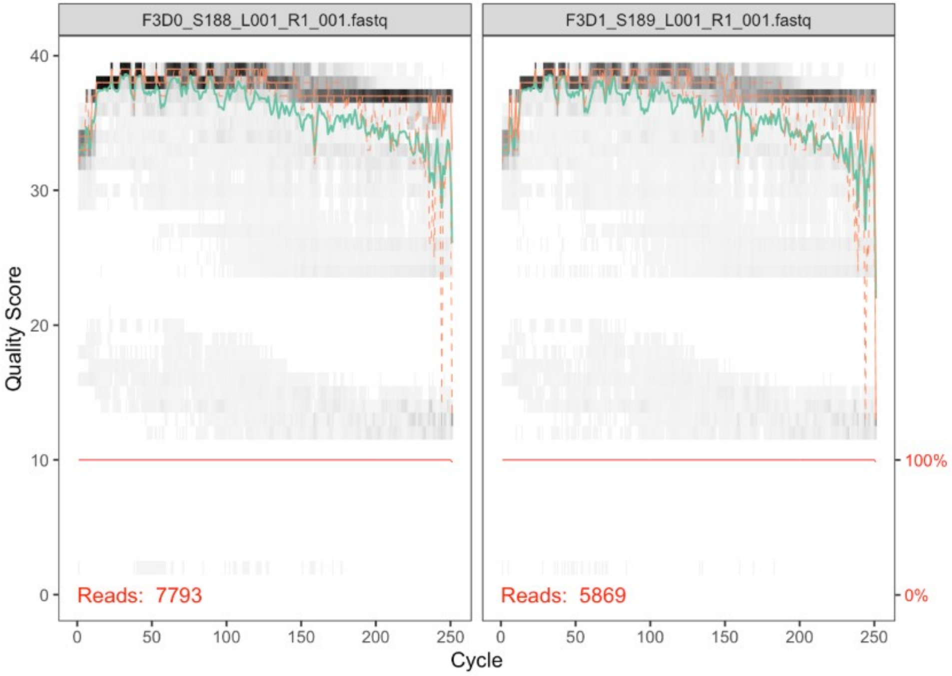
- **Merge paired end F and R**

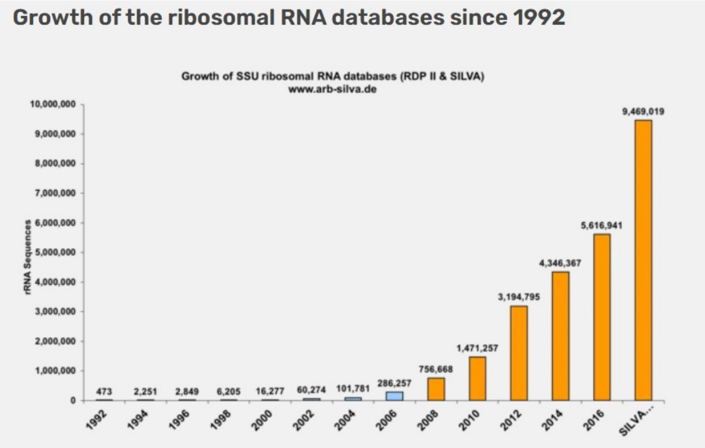
→ removal of Chimeric pair

- **Produce Sequence abundance table**
- **Taxonomical identification**

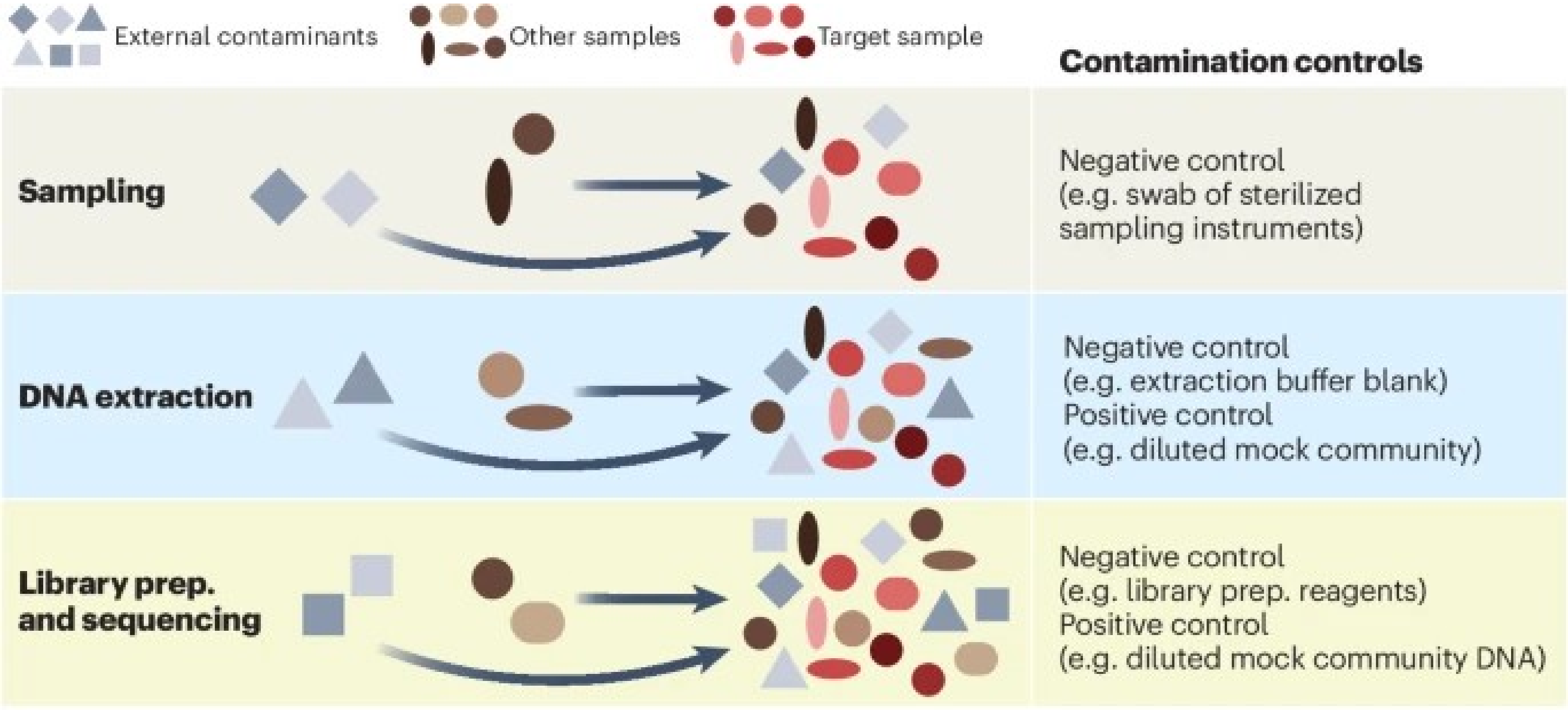
→ limitation of database and ASV length

Amplicon Sequencing. **Exactly.**




Growth of the ribosomal RNA databases since 1992

Evaluate Accuracy introducing negative control



Eukaryotic Markers

18S rRNA Gene

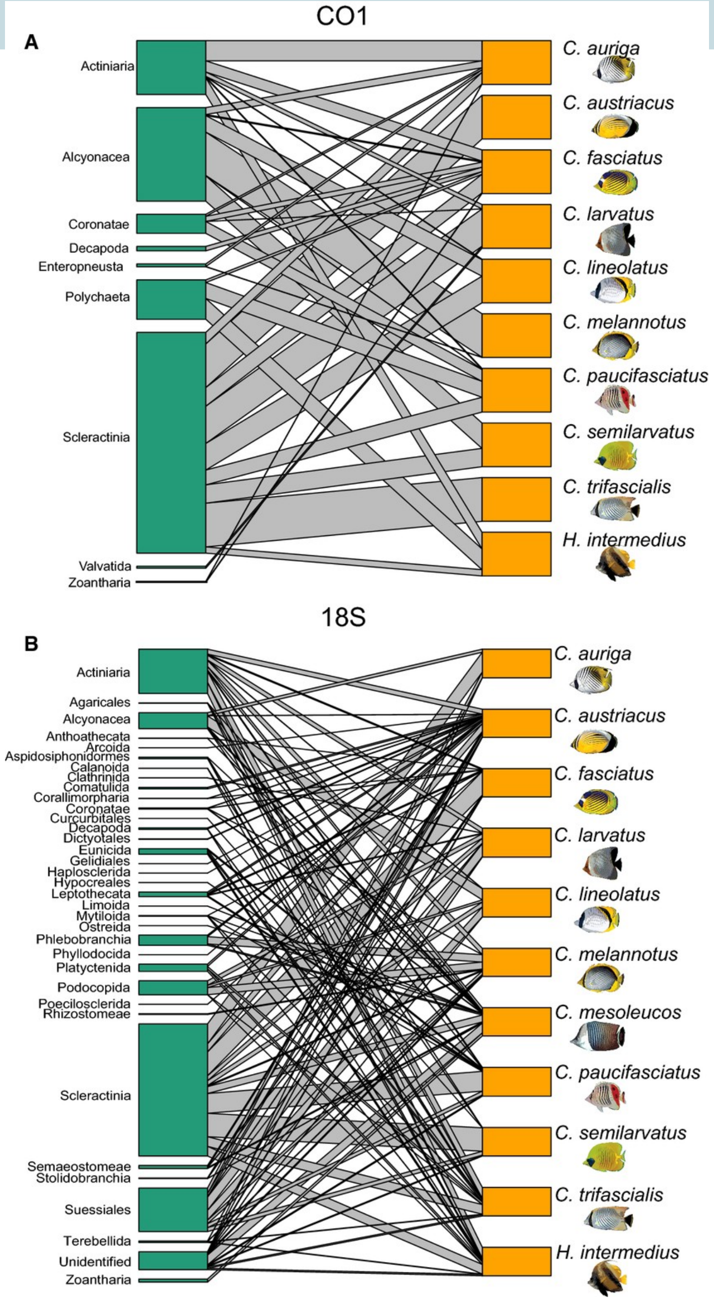
- Differences in gene structure, variability, amplicon length, and reference databases.
- Marker for eukaryotic : Protists, microalgae, parasites
- Eukaryotic rRNA gene copy number variation complicates abundance estimates.
- co-amplify host DNA (e.g., fish)

COI Marker : Cytochrome oxidase I gene

- DNA barcoding for animals
- Analysis requires careful filtering of pseudogenes (NUMTs), sequencing errors, and dependence on comprehensive reference databases (e.g., BOLD) for accurate taxonomy.

Multi-Kingdom Profiling

Combining: 16S + 18S + ITS + COI to reconstruct whole ecosystems.



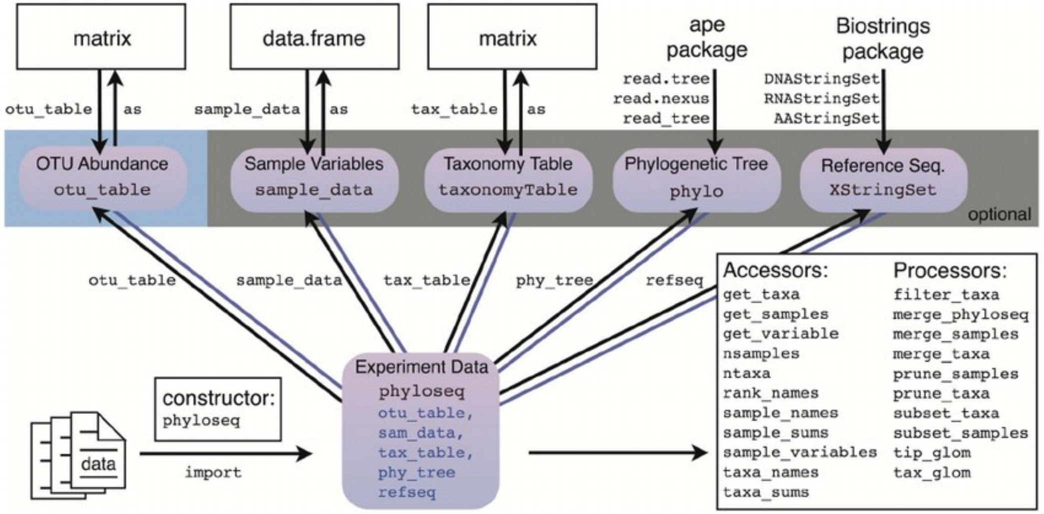
Diversity Analysis: Overview

Diversity analysis describes structure and complexity of microbial communities.

Two main components**: alpha diversity (within-sample) and beta diversity (between-sample).**

Essential to compare microbiomes across environments, treatments, or host conditions.

Importance of Metadata



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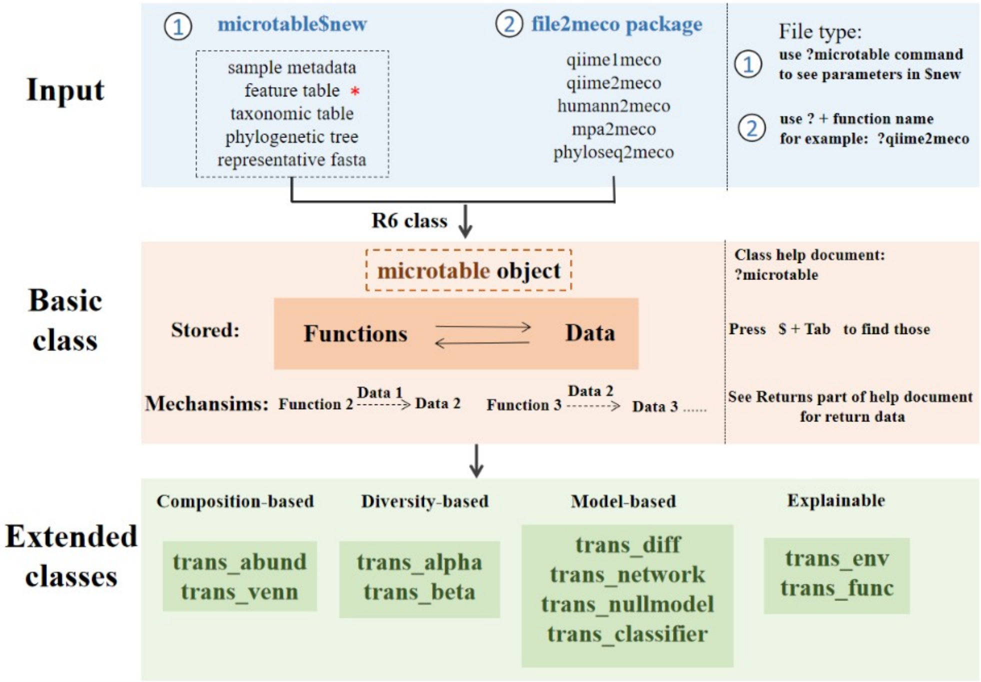


Alpha Diversity (Within-Sample)

Article | Open access | Published: 03 January 2025

Key features and guidelines for the application of microbial alpha diversity metrics

Ignacio Cassol, Mauro Ibañez & Juan Pablo Bustamante

mia

microbiome.github.io

Measures richness and evenness of taxa in a single sample.

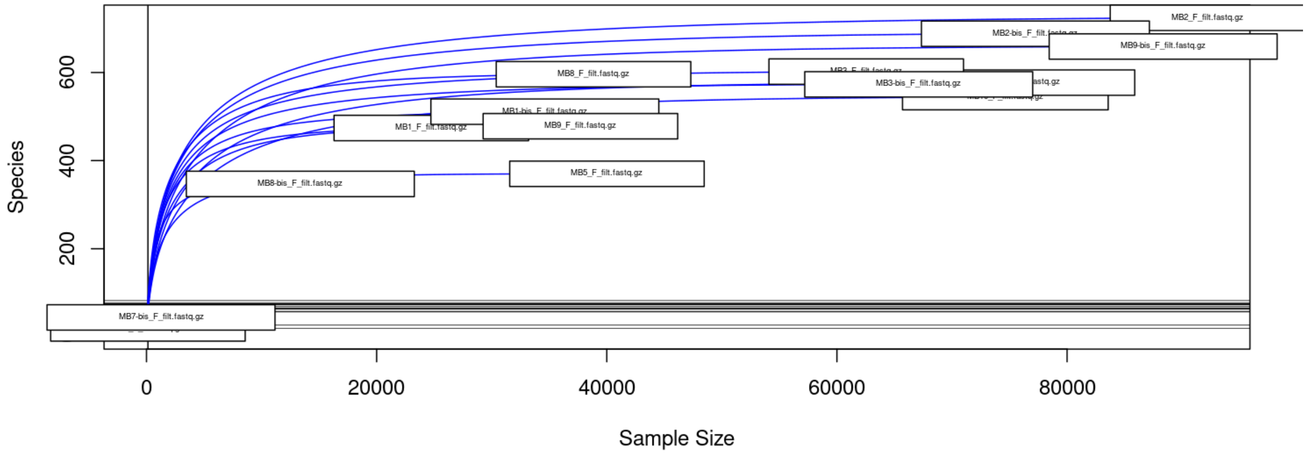
Common indices:

Observed ASVs/OTUs — raw richness

Shannon index — richness + evenness

Simpson index — dominance structure

Sensitive to sequencing depth
→ normalization required
(with correct method
es. relative abundance,
rarefaction,
log transformation...)



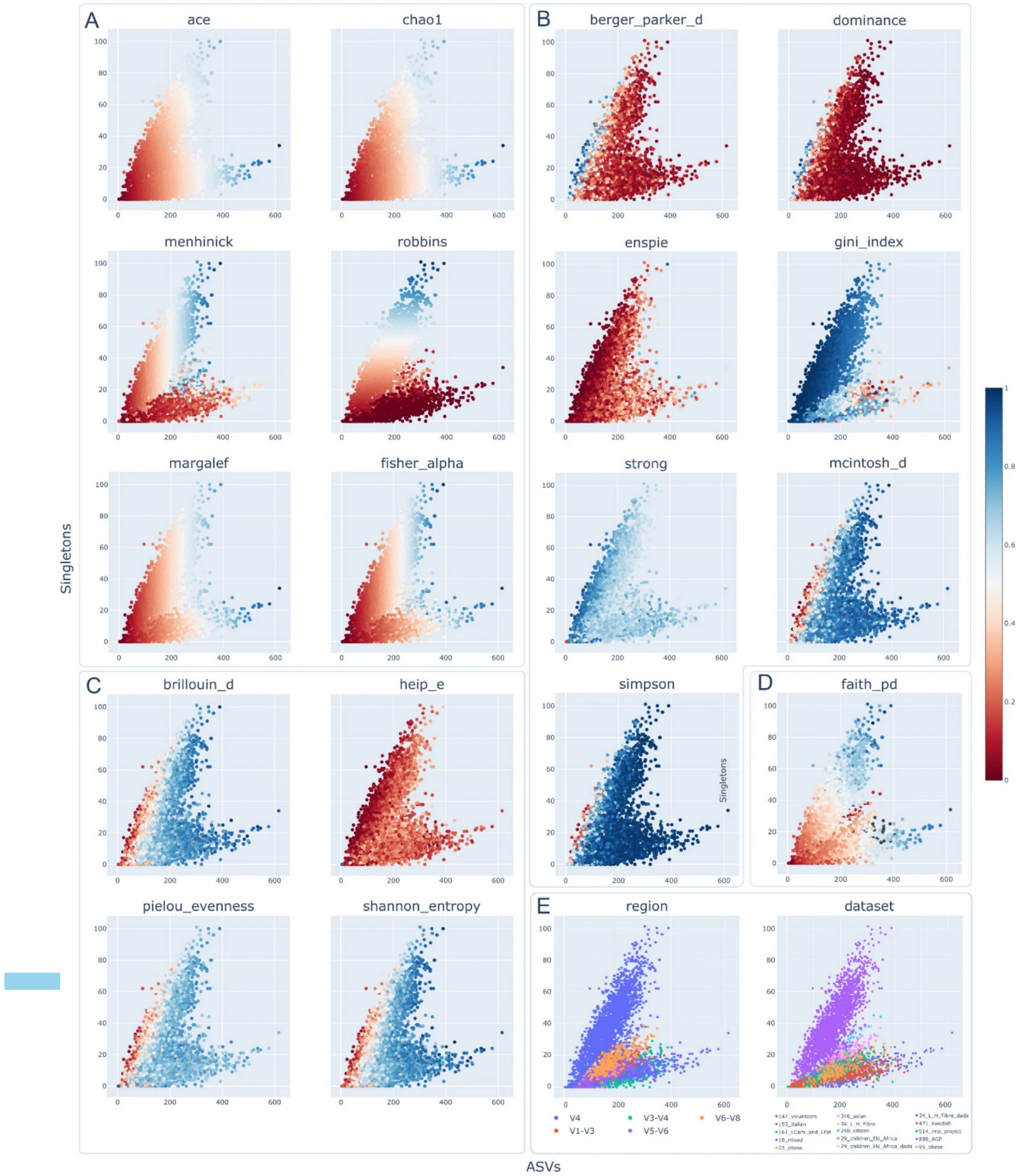
Alpha Diversity (Within-Sample)

Richness:
Chao1, ACE, Fisher, Margalef, Menhinick, Observed, and Robbins.

Dominance (also known as evenness):
Berger-Parker, Dominance, Simpson, ENSPIE, Gini, McIntosh, and Strong.

Phylogenetics:
Faith.

Information:
Shannon, Brillouin, Heip and Pielou..



Beta Diversity (Between-Sample)

Compares community composition among samples.

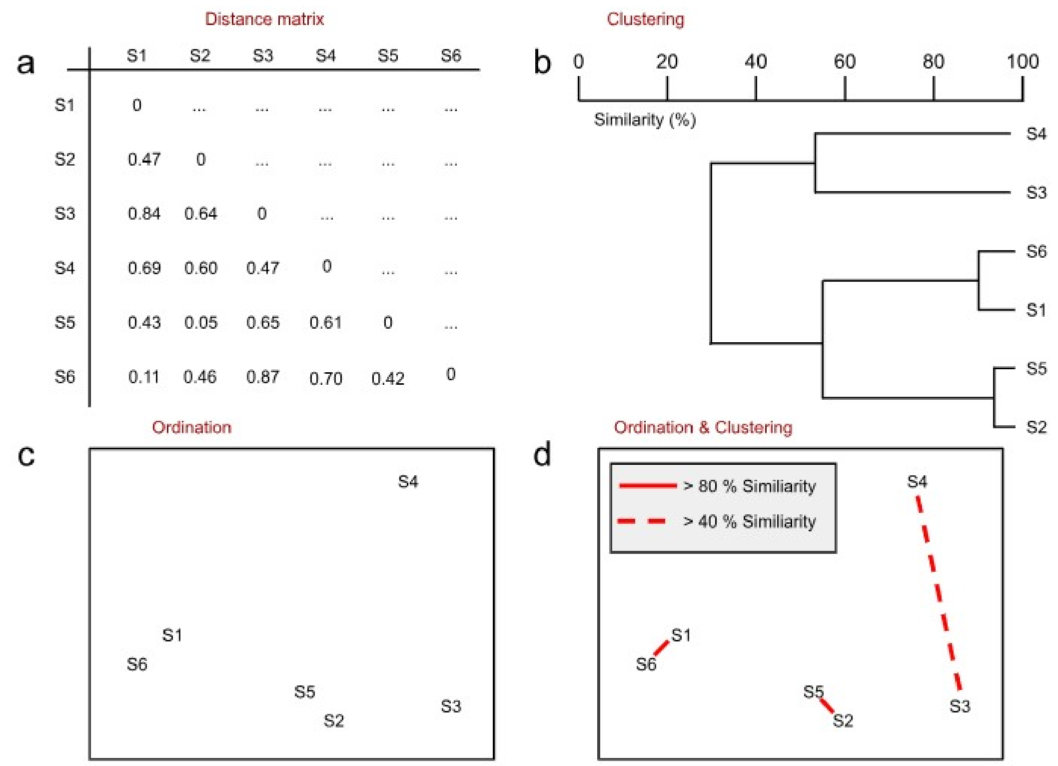
Distance metrics:

Bray–Curtis — abundance-based

Jaccard — presence/absence

UniFrac — phylogeny-based (weighted/unweighted)

Reveals ecological gradients and treatment effects.



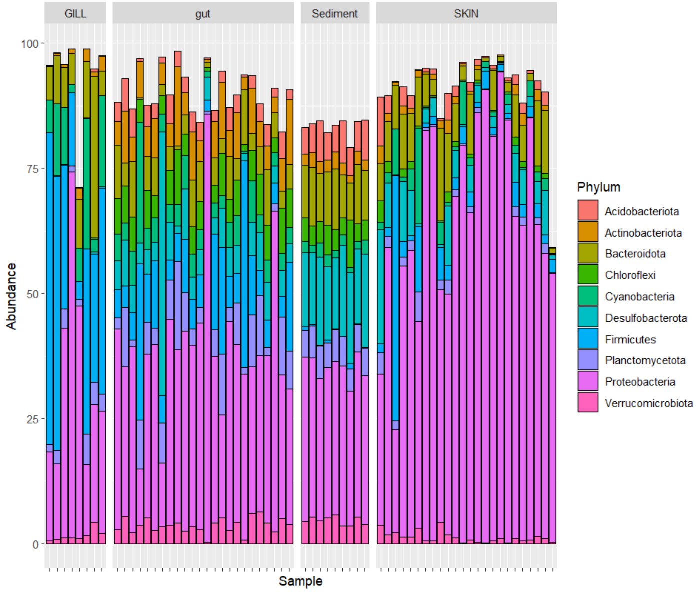
	S1	S2	S3	S4	S5	S6
S1	0	...	...	...	...	...
S2	0.47	0	...	...	...	...
S3	0.84	0.64	0	...	...	...
S4	0.69	0.60	0.47	0	...	...
S5	0.43	0.05	0.65	0.61	0	...
S6	0.11	0.46	0.87	0.70	0.42	0

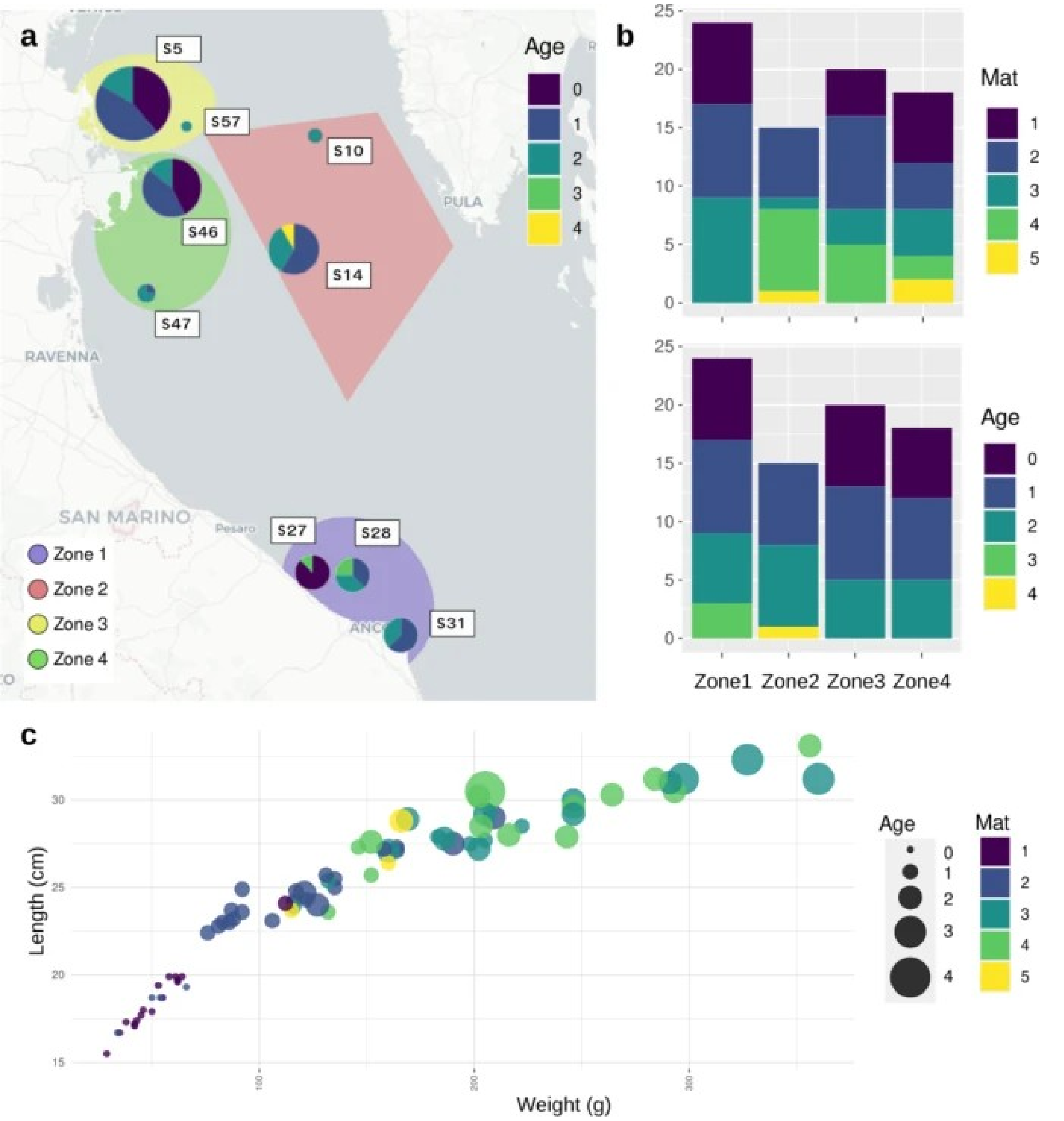
Taxonomic Visualization

Visual summaries of taxonomic composition.

Common formats:

- **Stacked bar plots (relative abundance)**
- **Pie charts (overview)**
- **Heatmaps (detailed patterns)**
- **Useful for identifying dominant taxa and dysbiosis signals.**





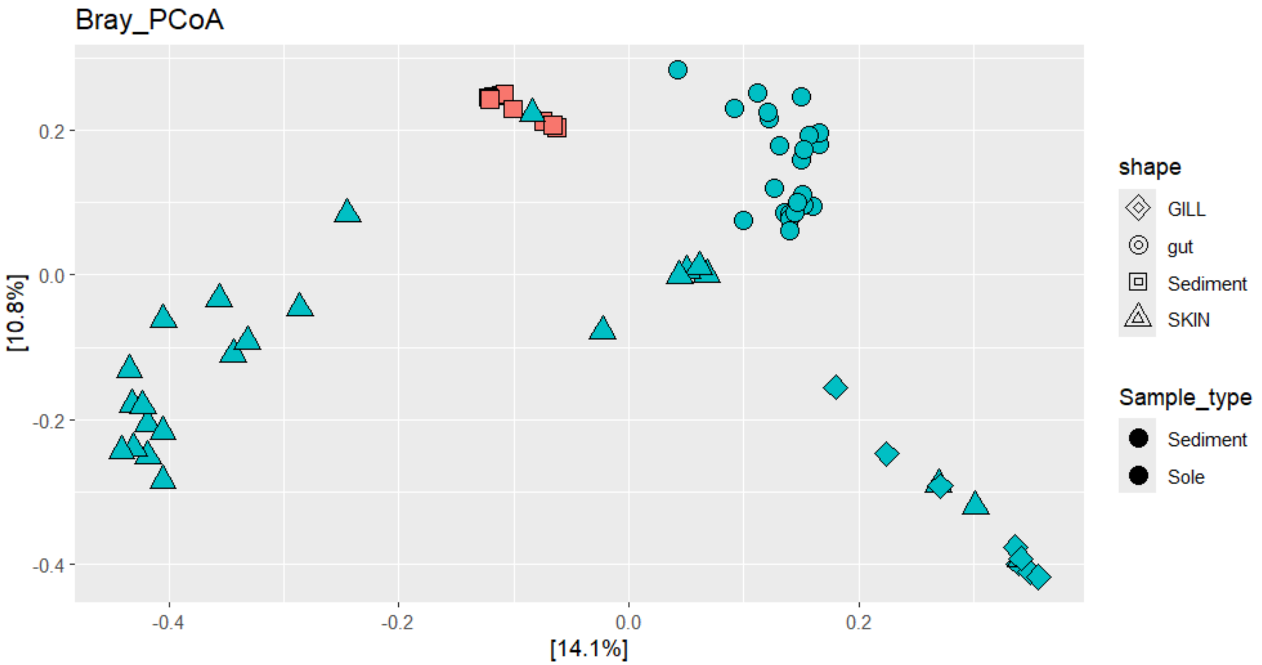
PCA (Principal Component Analysis)

- Linear ordination method reducing multidimensional data.

- Highlights main gradients explaining variance.

- Useful for detecting clustering by environment, host species, or treatment.

- Assumes linear relationships.



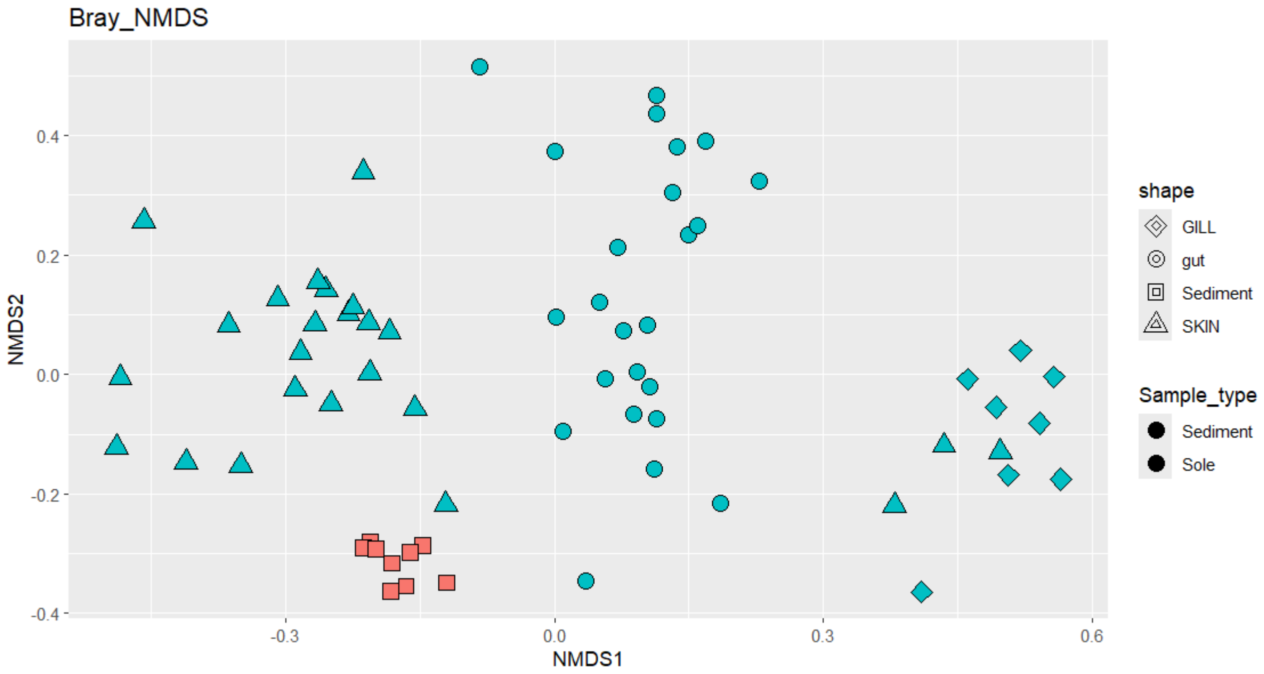
NMDS (Non-metric Multidimensional Scaling)

Non-parametric ordination based on rank distances.

Well suited for ecological data with non-linear relationships.

Stress value indicates goodness of representation.

Widely used for microbiome beta diversity visualization.



Microbiome–Environment Correlations

Links microbial patterns to environmental parameters.

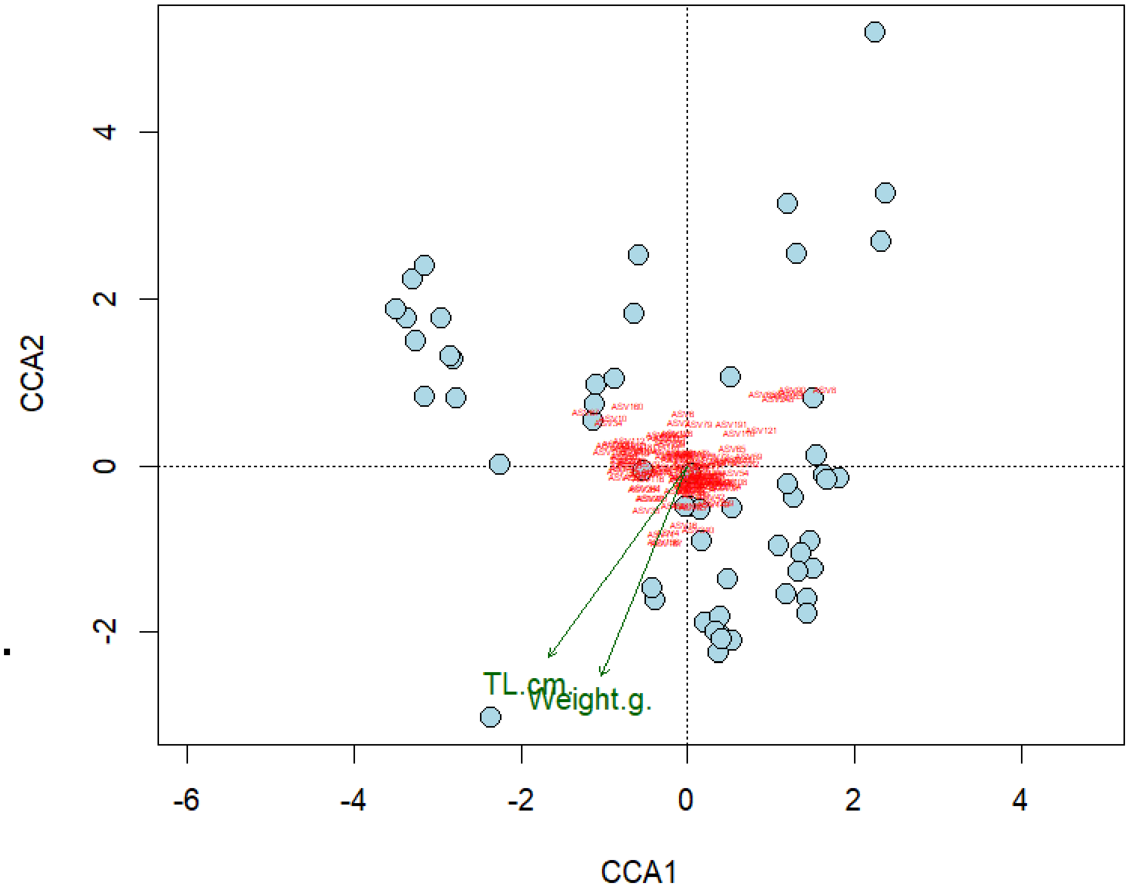
Methods:

Mantel tests

Redundancy Analysis (RDA)

Canonical Correspondence Analysis (CCA)

Identifies drivers such as fish size, age, maturity..



Differential Abundance Analysis

Detects taxa significantly associated with conditions.

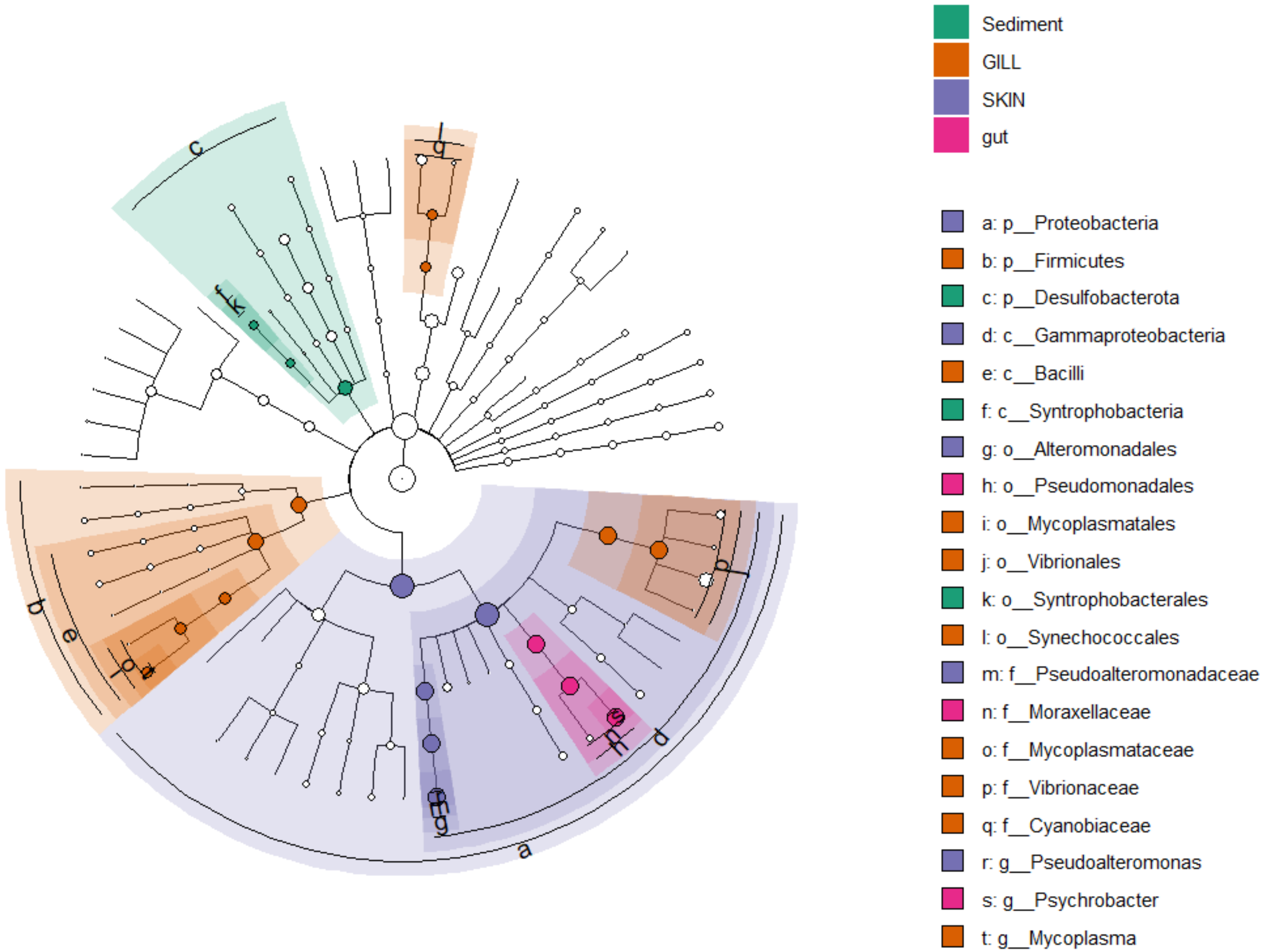
Tools:

DESeq2

ANCOM

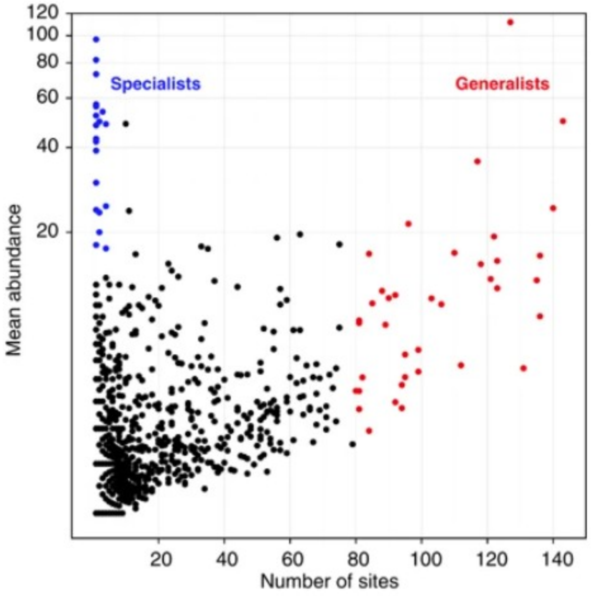
LEfSe

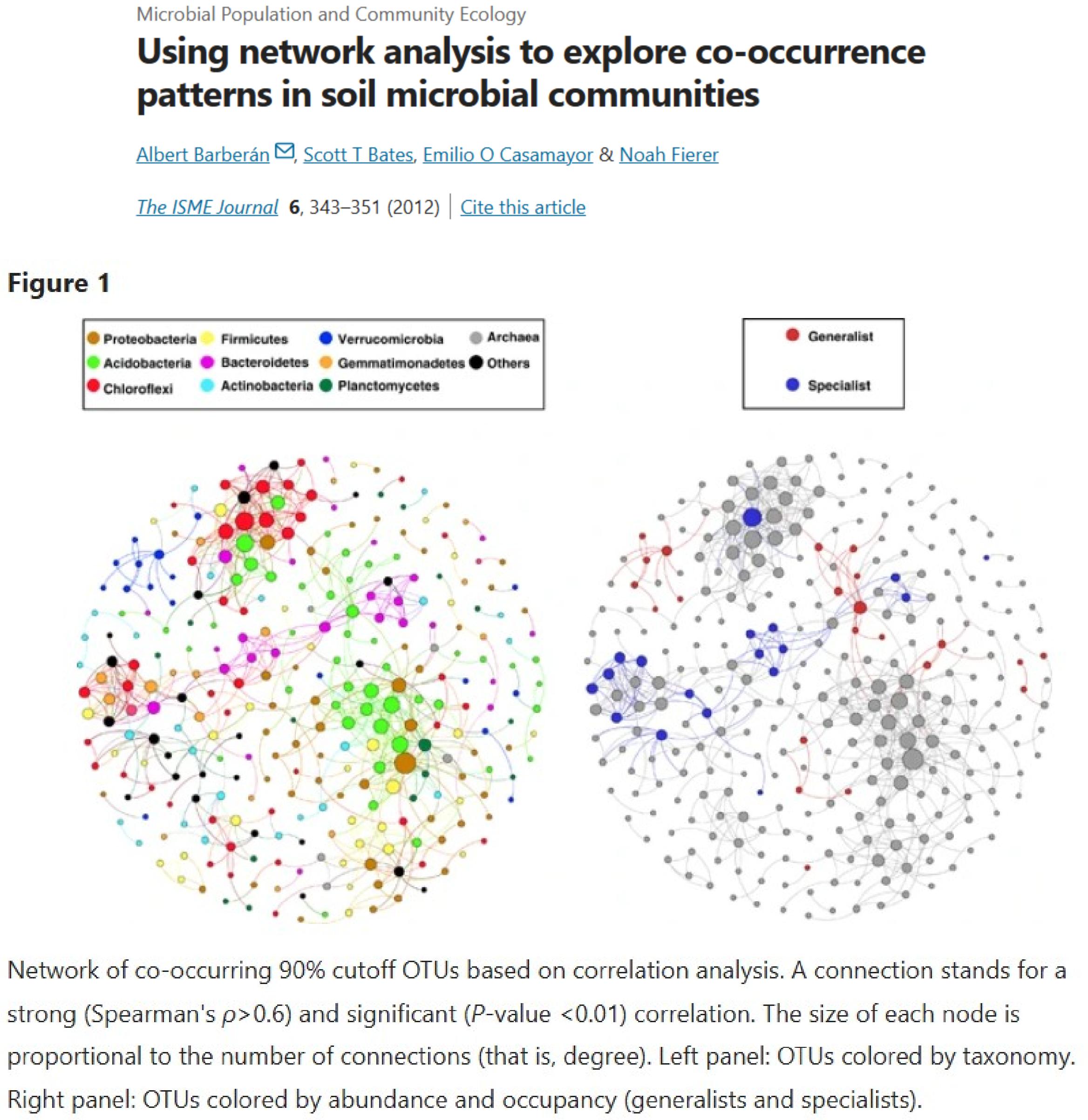
Supports biomarker discovery and ecologica interpretation.



Network and Co-occurrence Analysis

- Infers interactions among taxa (cooperation, competition).
- Based on correlation or probabilistic models.
- Identifies keystone species and community stability patterns.
- Important for ecosystem function understanding.





Microbial Population and Community Ecology

Using network analysis to explore co-occurrence patterns in soil microbial communities

Albert Barberán, Scott T Bates, Emilio O Casamayor & Noah Fierer

The ISME Journal **6**, 343–351 (2012) | Cite this article

Figure 1

Network of co-occurring 90% cutoff OTUs based on correlation analysis. A connection stands for a strong (Spearman's $\rho>0.6$) and significant (P-value <0.01) correlation. The size of each node is proportional to the number of connections (that is, degree). Left panel: OTUs colored by taxonomy. Right panel: OTUs colored by abundance and occupancy (generalists and specialists).

Integration and Ecological Interpretation

- **Combining diversity, ordination, correlations, and networks provides a holistic view.**

- Enables linking microbiome structure to function and ecosystem health.

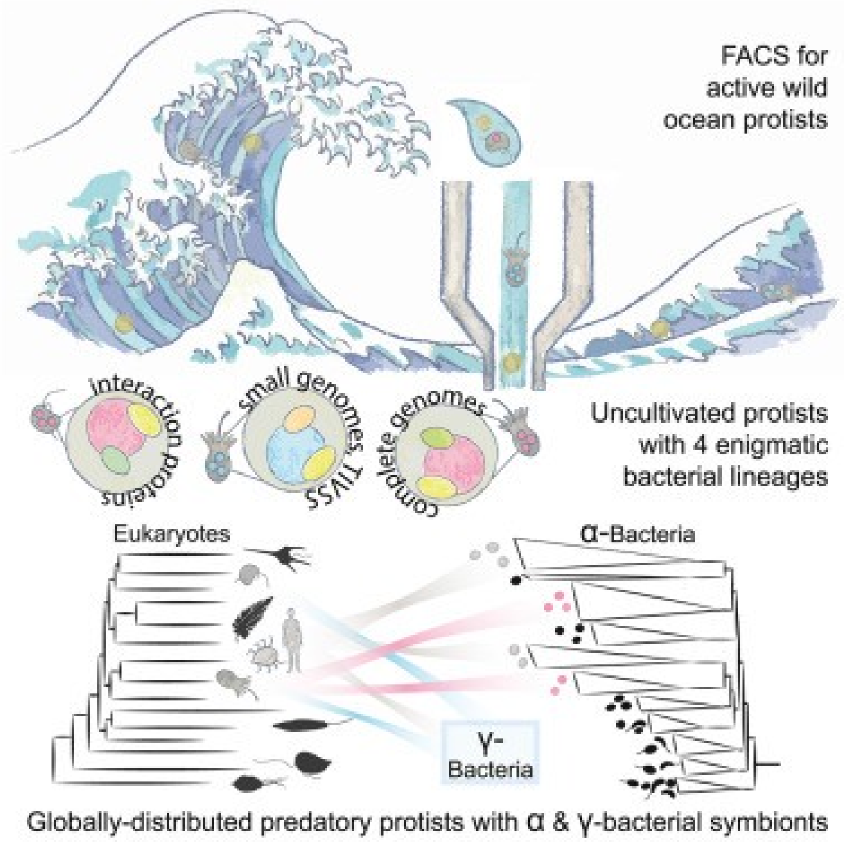
- **Supports applications in aquaculture management, conservation environmental monitoring.**

- Use of Bioindicator

Limits

- **Complex relation between taxa**
- **correlation not mean casuality**
- **functional redundancy**
- **Study comparison limited by methods**

GitHub



Wittmers e al., 2025

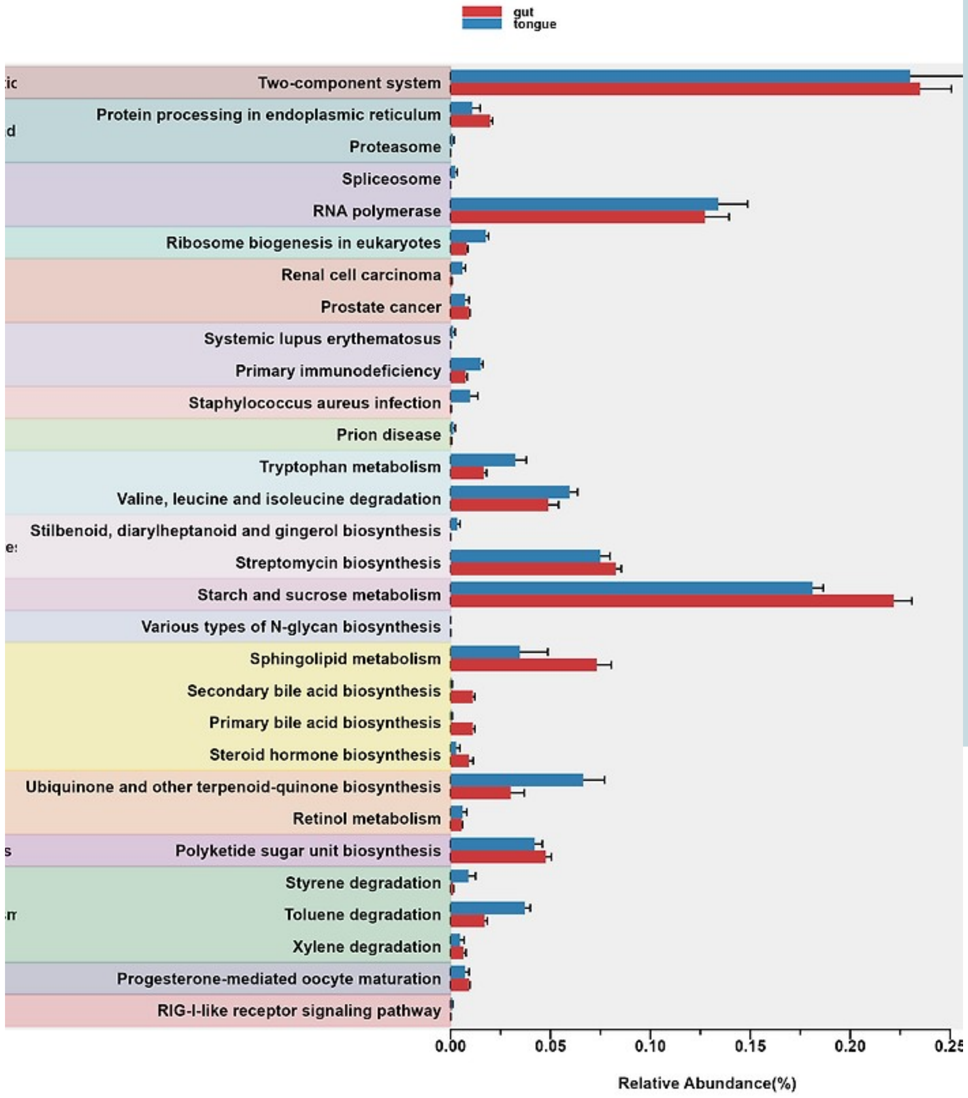
Functional Limits of 16S Sequencing

16S rRNA sequencing describes taxonomic composition, not gene content or metabolic functions.

Functional insights require:
Shotgun metagenomics (direct gene sequencing)

Predictive tools based on reference genomes (e.g., PICRUSt)
- 16S-based predictions rely on available reference genomes → incomplete coverage of microbial diversity.

These approaches integrate taxonomic profiles with genomic databases to infer potential pathways.
→ Be interpreted cautiously and validated using:
- Shotgun metagenomics
- Metatranscriptomics (gene expression)
- Metaproteomics (protein activity)

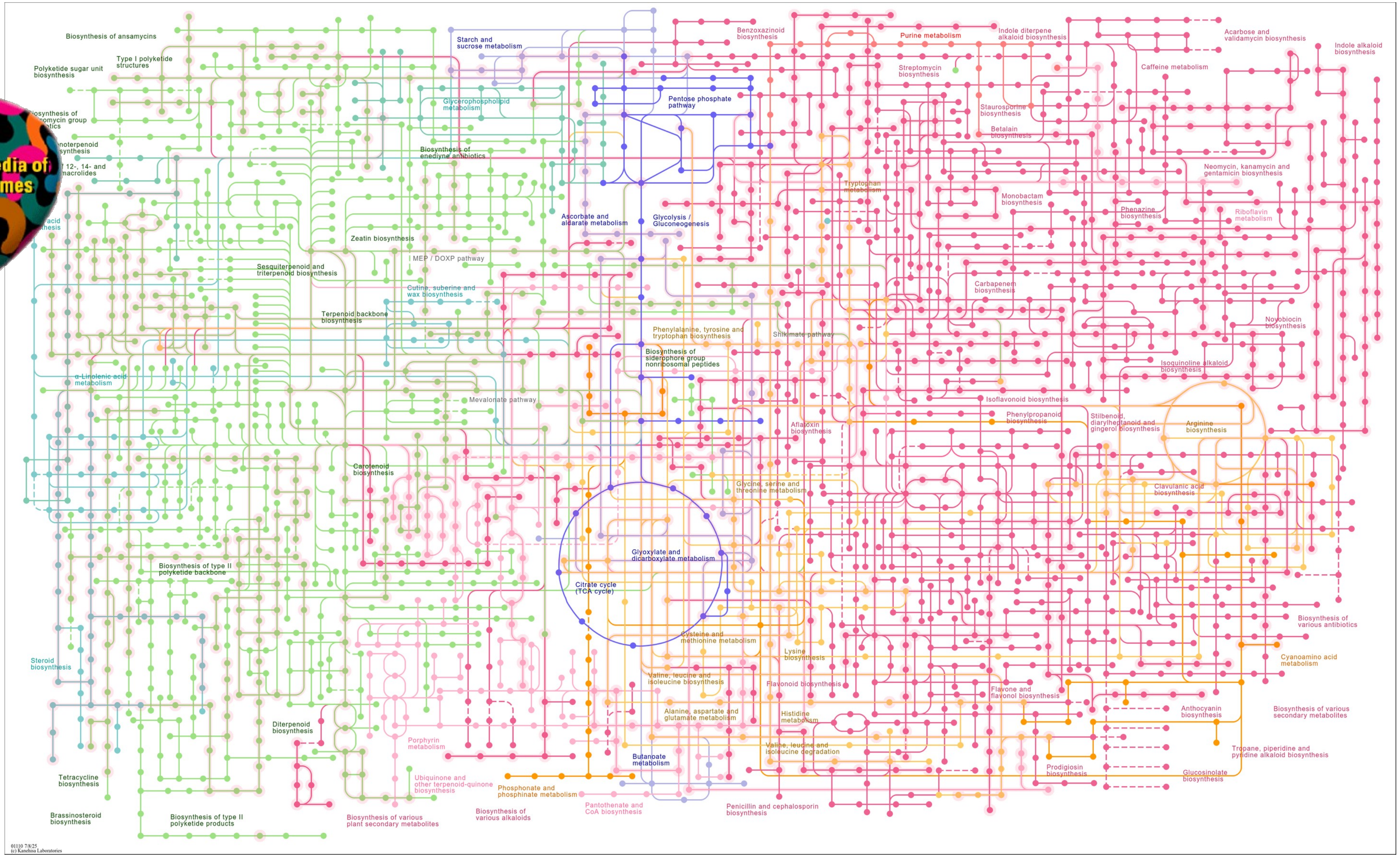


Output provides a preliminary functional landscape useful for hypothesis generation and experimental design.

Take a Break

Metagenomics sequencing

KEGG Kyoto Encyclopedia of Genes and Genomes



Shotgun Metagenomics?

Sequencing all DNA in a sample

No PCR targeting

Captures bacteria, archaea, viruses, eukaryotes

Structure vs Function

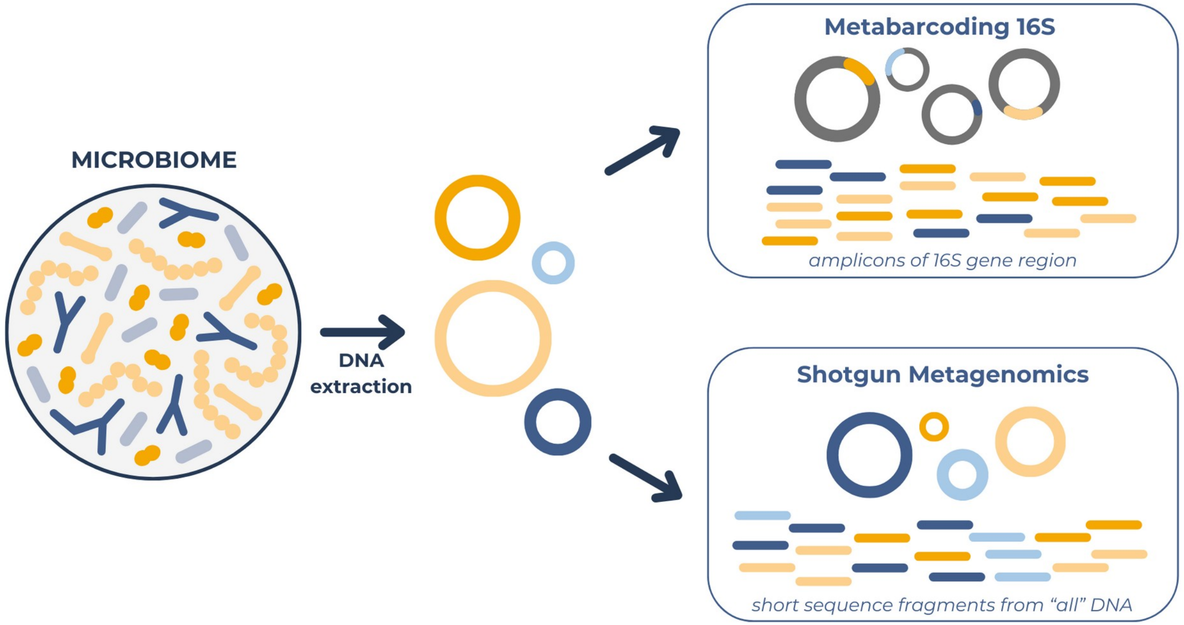
Metagenomics provides:

Taxonomy

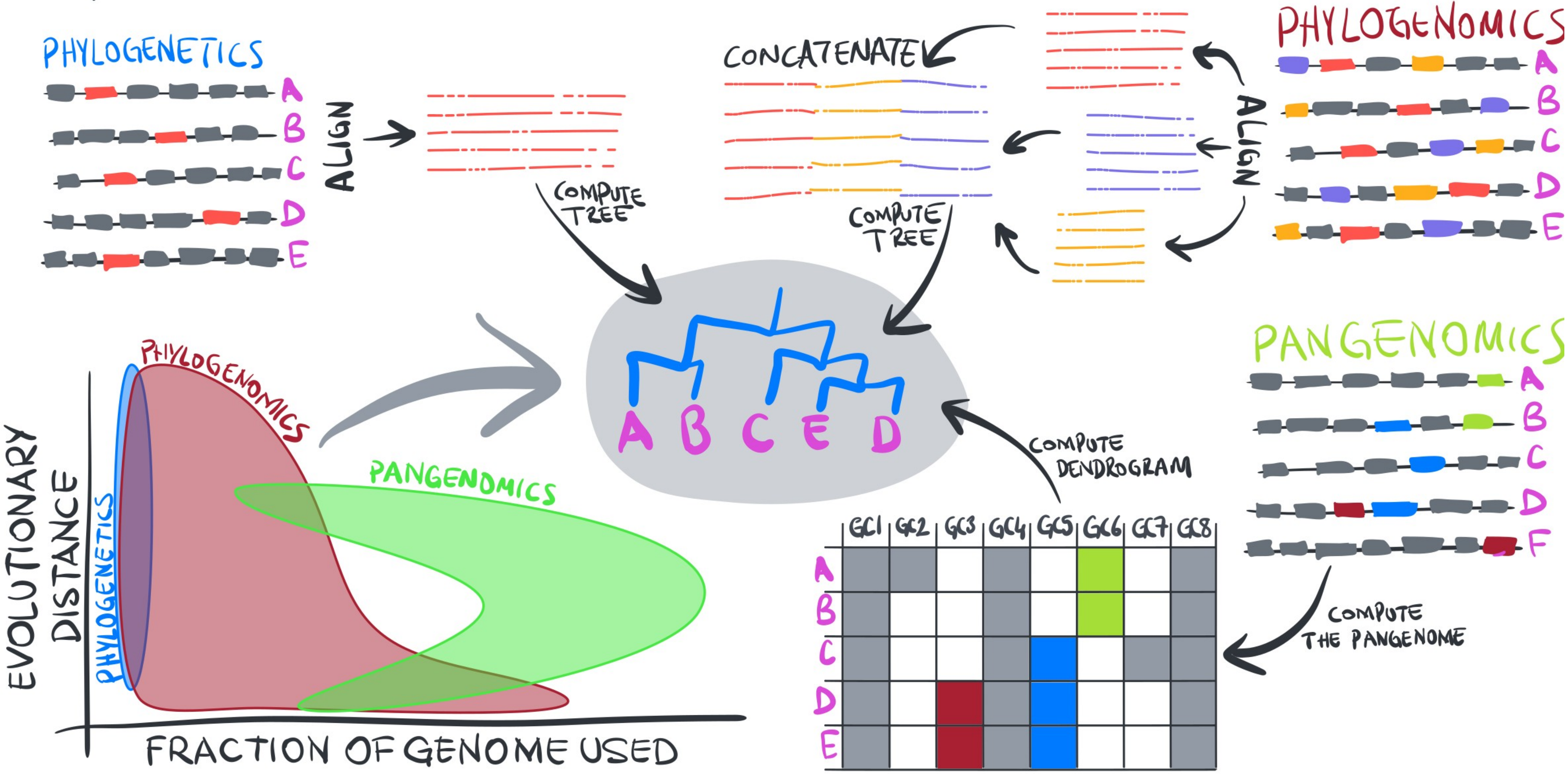
Functional genes

Metabolic pathways

We increasingly focus on functions rather than taxonomy - who is there matters less than what they are doing.



PHYLOGENETICS vs. PHYLOGENOMICS vs. PANGENOMICS (IN PRACTICE)

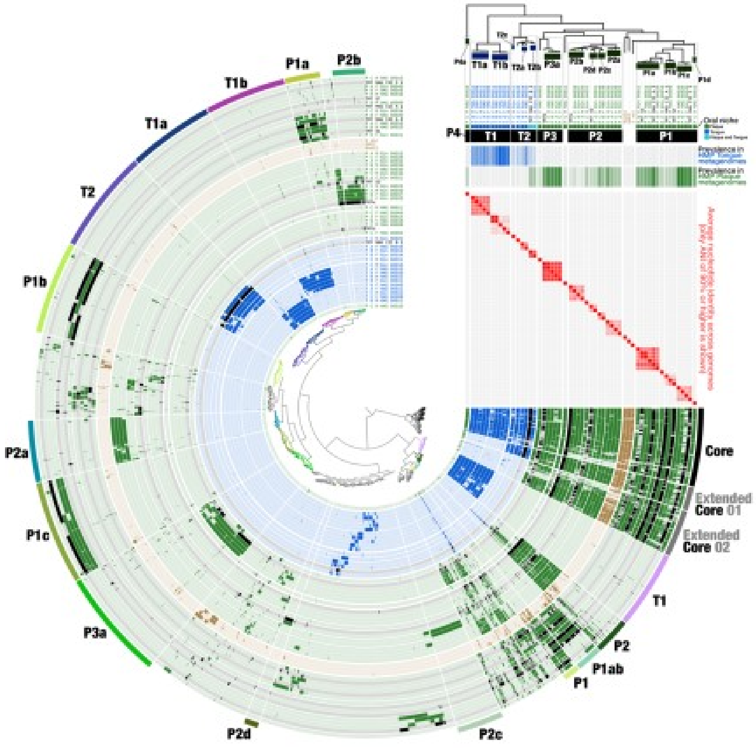


	GC1	GC2	GC3	GC4	GC5	GC6	GC7	GC8
A	⬛	⬛		⬛		🟩		⬛
B	⬛			⬛		🟩		⬛
C	⬛			⬛	🟦		⬛	⬛
D	⬛		🟥	⬛	🟦			⬛
E	⬛		🟥	⬛	🟦			⬛

Functional and genetic markers of niche partitioning among enigmatic members of the human oral microbiome

Shaiber A, Willis AD, Delmont TO, Roux S, Chen L, **Schmid AC**, **Yousef M**, **Watson AR**, **Lolans K**, **Esen ÖC**, **Lee STM**, Downey N, Morrison HG, Dewhirst FE, Mark Welch JL‡, **Eren AM**‡

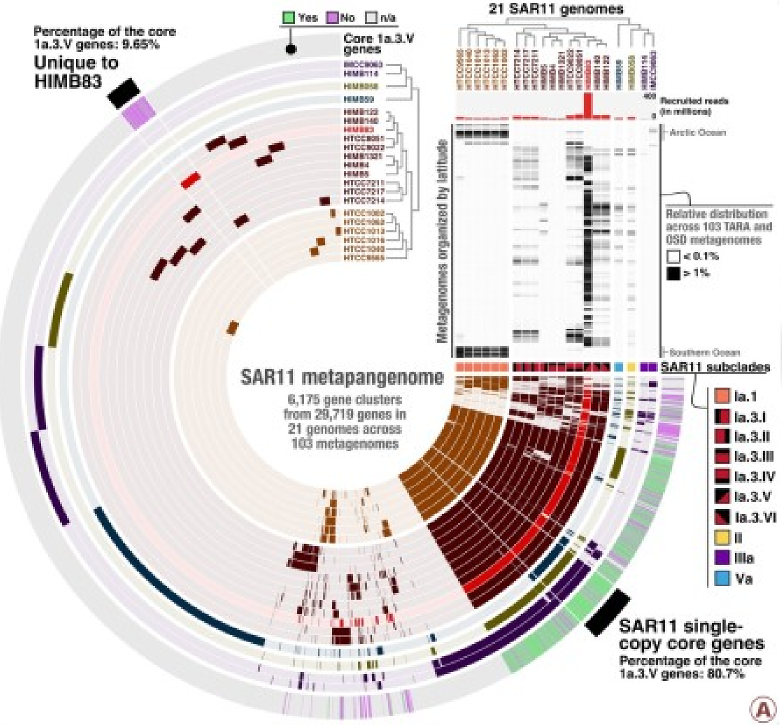
‡Co-senior authors



Single-amino acid variants reveal evolutionary processes that shape the biogeography of a global SAR11 subclade

Delmont TO◒, **Kiefl E**◒, Kilinc O, **Esen ÖC**, Uysal I, Rappé MS, Giovannoni S, **Eren AM**

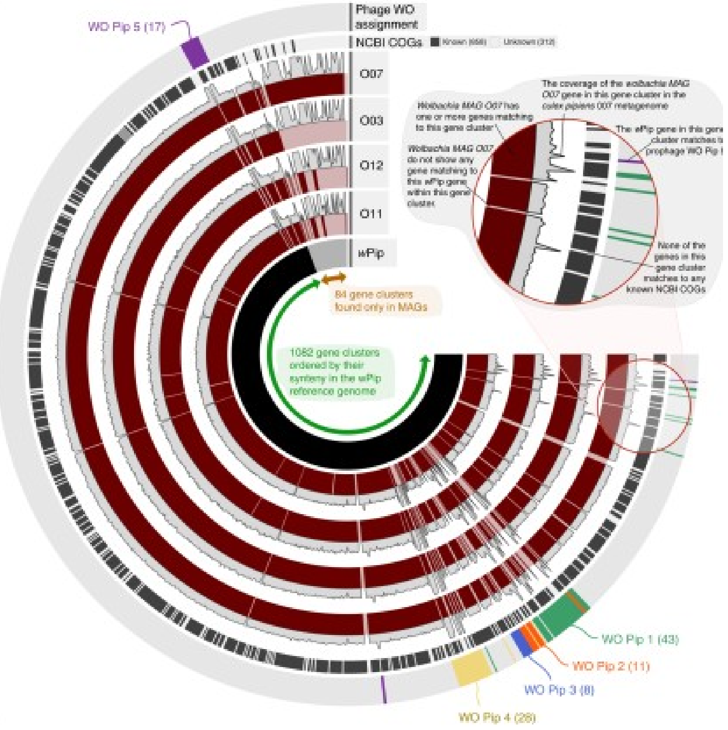
◒Co-first authors

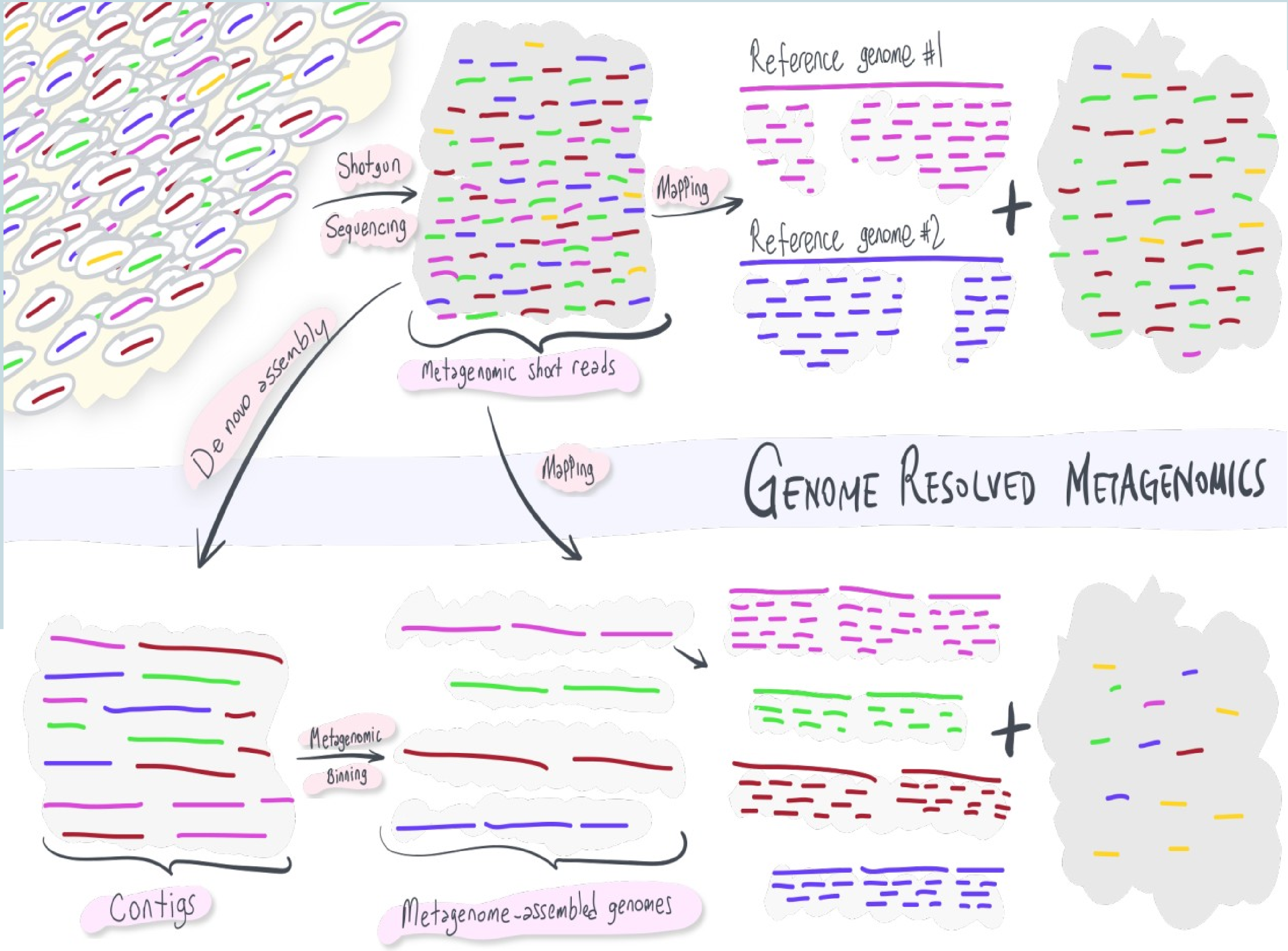


The Wolbachia mobilome in Culex pipiens includes a putative plasmid

Reveillaud J◒, Bordenstein SR◒, Cruaud C, **Shaiber A**, **Esen ÖC**, Weill M, Makoundou P, **Lolans K**, **Watson AR**, Rakotoarivony I, Bordenstein S, **Eren AM**

◒Co-first authors




Shotgun
Sequencing
Metagenomic short reads
Mapping
Reference genome #1
Reference genome #2
+
De novo assembly
Mapping
GENOME RESOLVED METAGENOMICS
Contigs
Metagenomic
Binning
Metagenome-assembled genomes
+

Assembly & Binning
MAGs: Metagenome-assembled genomes

→ fragmented reads → contigs → genomes

What is Metagenome Assembly?

- Assembly reconstructs longer genomic sequences (contigs) from millions of short sequencing reads obtained from environmental samples
- Enables recovery of genes, metabolic pathways, and draft genomes directly from microbial communities without cultivation.
- “microbial dark matter”
- host–microbe interaction studies

Assembly Strategies

Two main approaches are used:

- De novo assembly: reconstructs genomes without reference
- Reference-guided assembly: aligns reads to known genomes

Most microbiome studies rely on de novo assembly due to unknown diversity.

Technical Methods

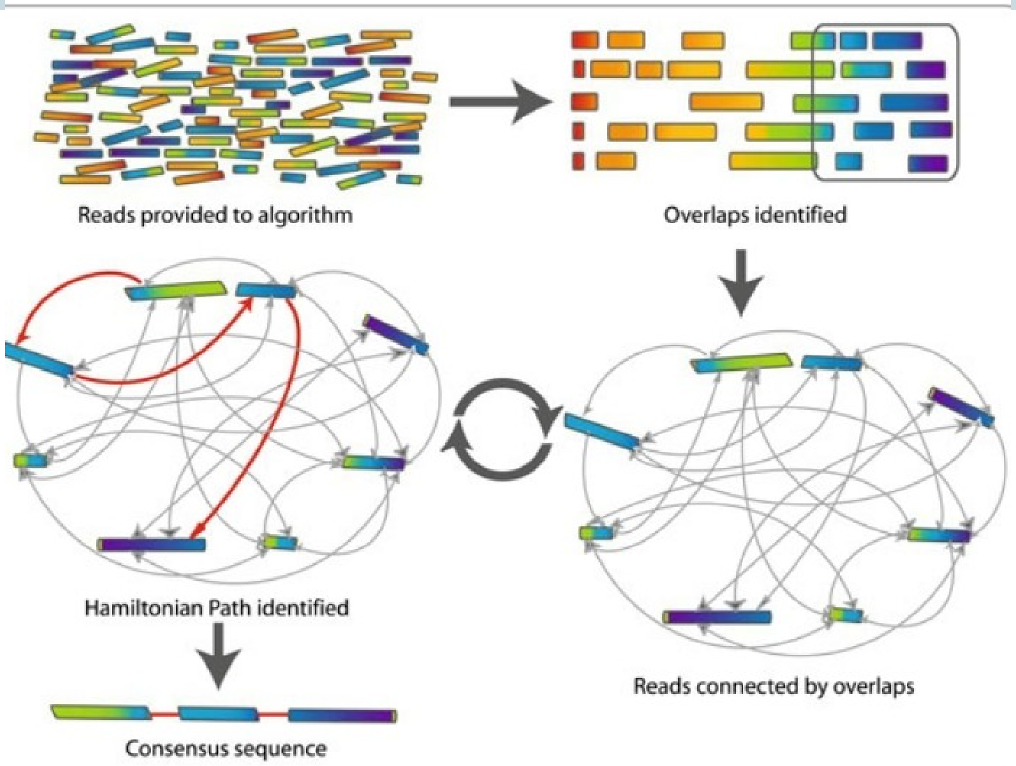
- **De Bruijn graph assemblers (using K-mers)**
- **Overlap-layout-consensus (long reads)**

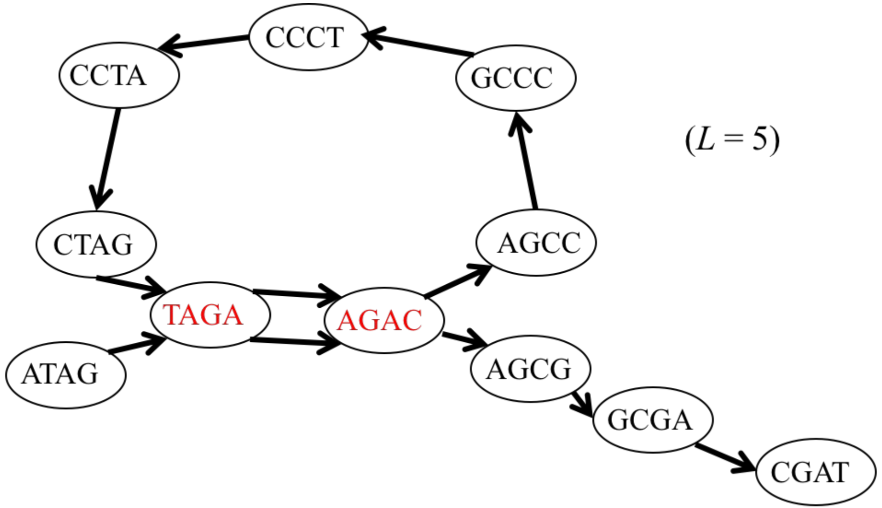
Pros & Cons

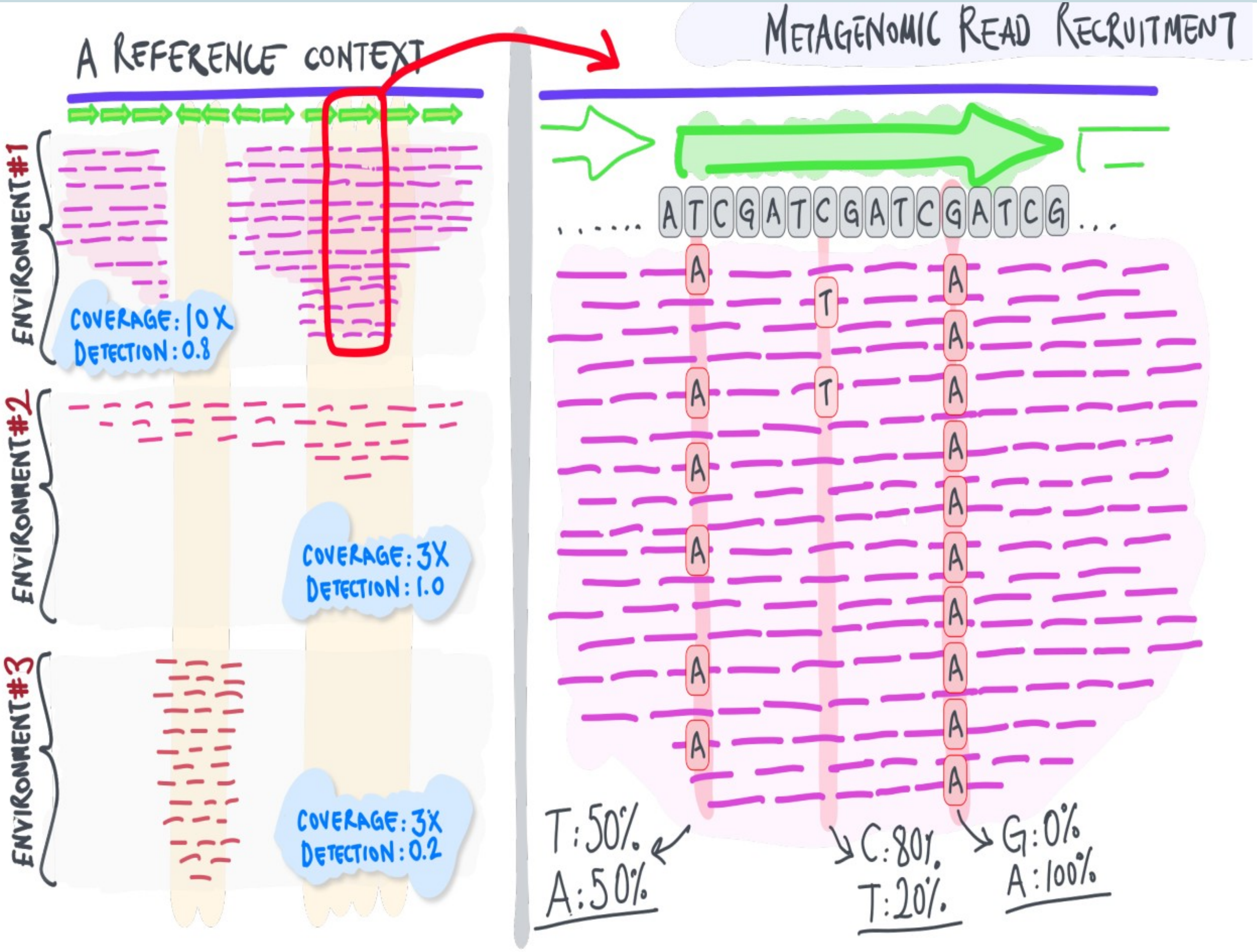
✓ **Captures novel taxa**

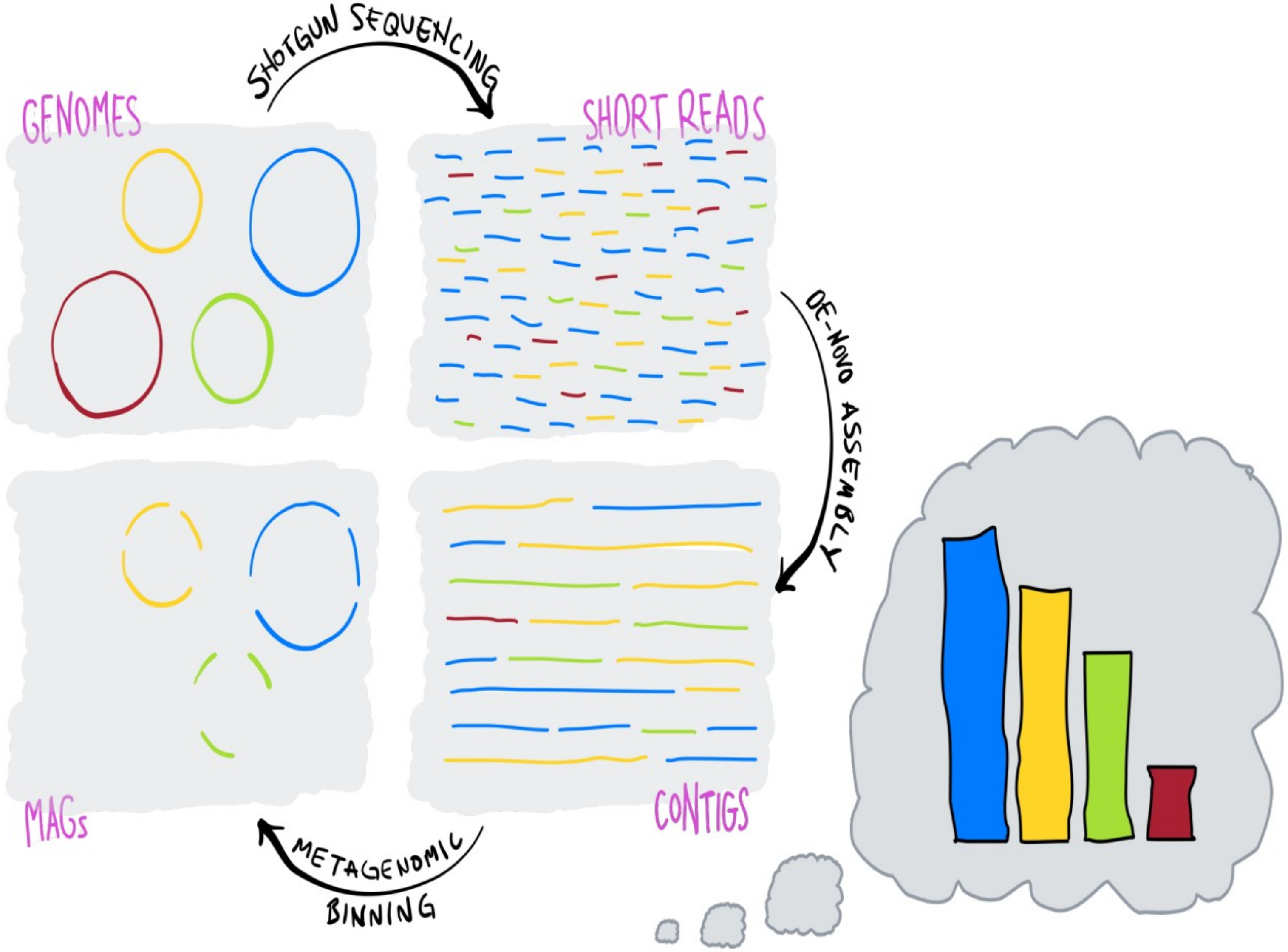
✖ **Computationally demanding**

✖ **Fragmentation in complex communities**






A REFERENCE CONTEXT
METAGENOMIC READ RECRUITMENT
ENVIRONMENT #1
COVERAGE: 10X
DETECTION: 0.8
ENVIRONMENT #2
COVERAGE: 3X
DETECTION: 1.0
ENVIRONMENT #3
COVERAGE: 3X
DETECTION: 0.2
...... A T C G A T C G A T C G A T C G ...
A
T
A
T: 50%
A: 50%
C: 80%
T: 20%
G: 0%
A: 100%


SHOTGUN SEQUENCING
GENOMES
SHORT READS
DE-NOVO ASSEMBLY
CONTIGS
METAGENOMIC BINNING
MAGs

Challenges in Metagenome Assembly

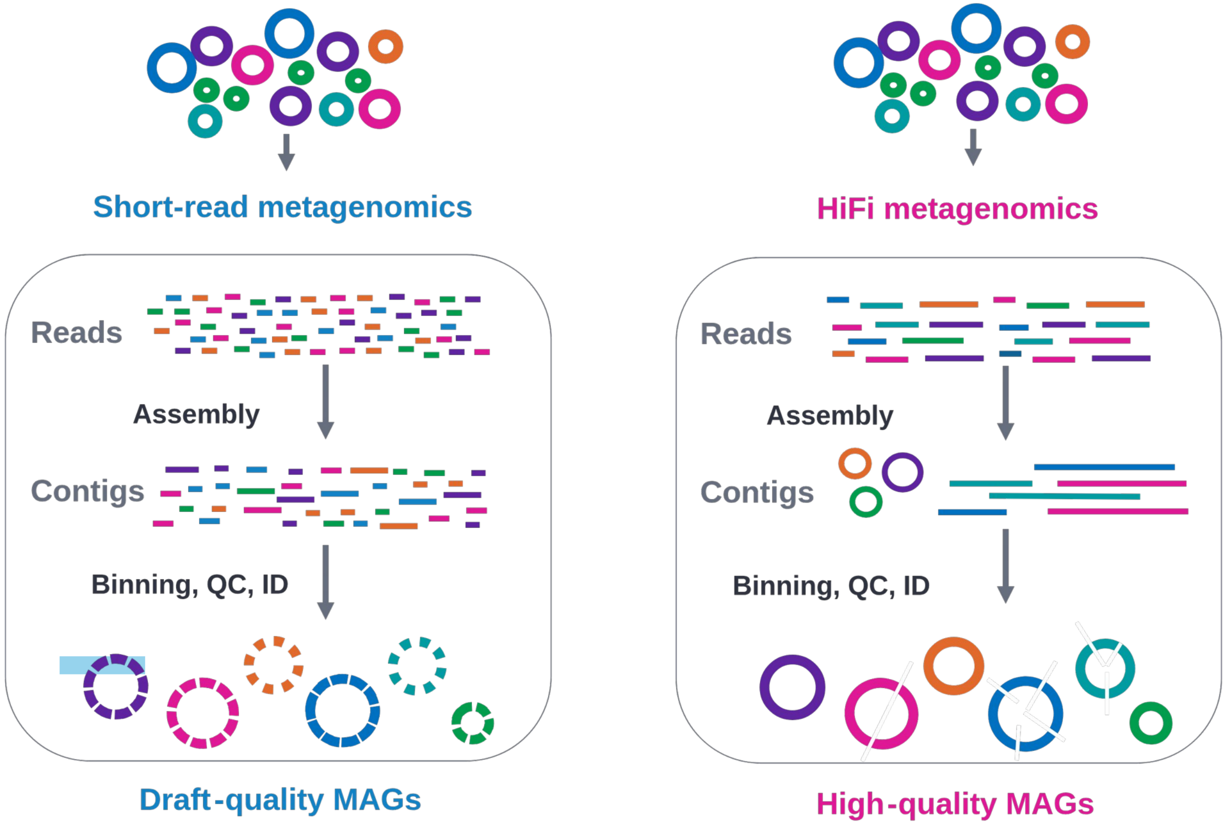
Assembly is difficult because environmental samples contain:
- **Many species with uneven abundance**
- **Closely related strains**
- **Repetitive sequences**

Key Issues

- Low-abundance genomes poorly assembled
- Chimeric contigs
- Assembly bias toward dominant taxa

LONG READS as new tool

- **Nanopore**
- **PacBio**



Assembly & Binning

MAGs: Metagenome-assembled genomesFrom Contigs to Genomes: Binning

Binning groups assembled contigs into Metagenome-Assembl Genomes (MAGs) representing individual organisms.

Approaches

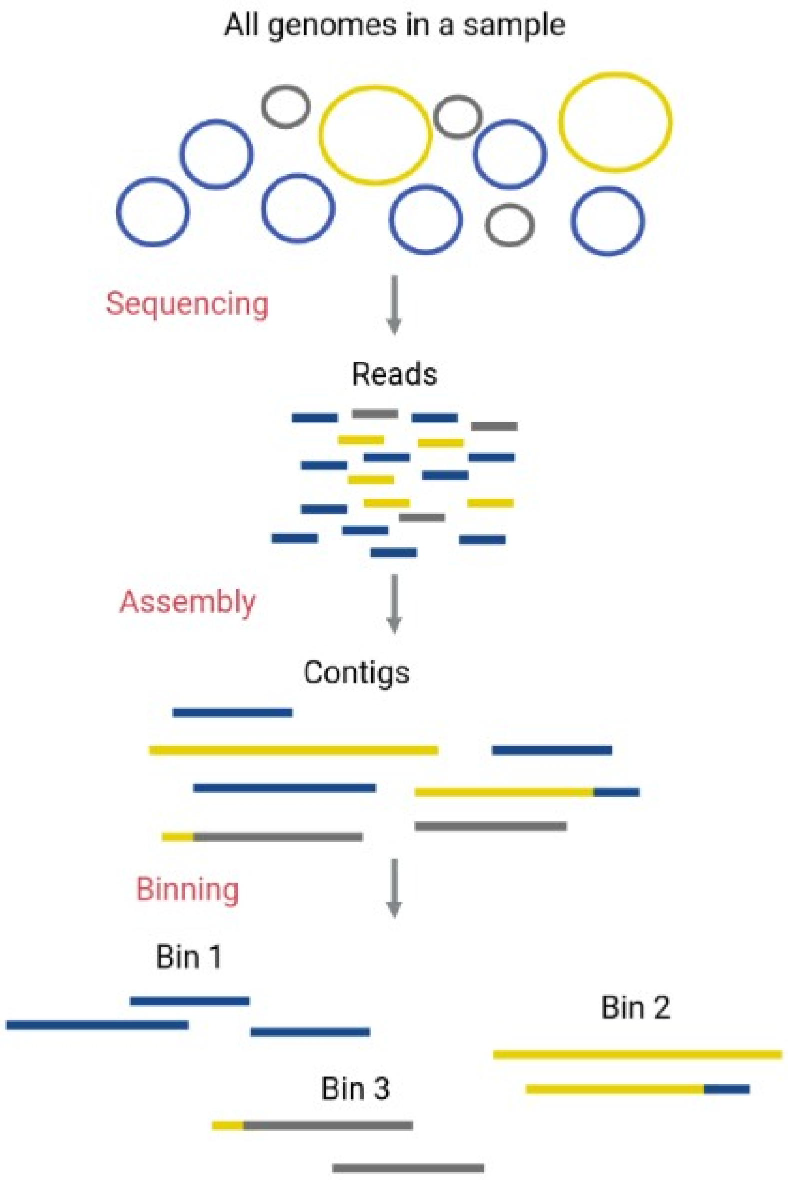
- Composition-based (GC content, k-mers)
- Coverage-based (abundance across samples)
- Hybrid methods

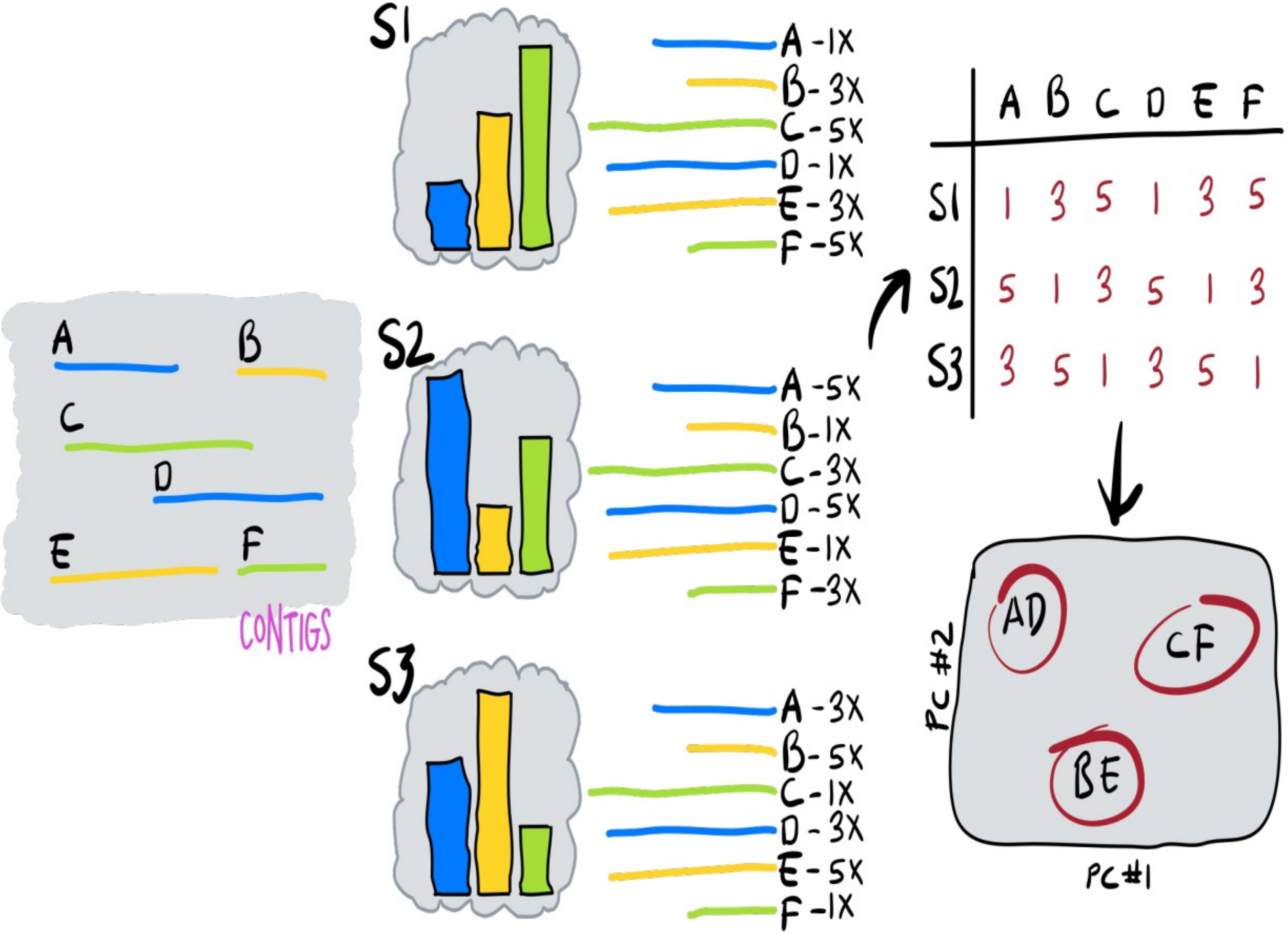
Outcome

- **Draft genomes of uncultured microbes.**

Metagenomics Challenges

- Host DNA contamination
- Computational demands
- Cost
- Reference database gaps





	A	B	C	D	E	F
S1	1	3	5	1	3	5
S2	5	1	3	5	1	3
S3	3	5	1	3	5	1

Quality Assessment of MAGs

MAG quality must be evaluated to ensure biological reliability.

Key metrics

Completeness: measures the percentage of a microbial genome recovered from environmental DNA, often assessed via universal **single-copy genes**

Contamination: refers to the presence of DNA sequences from organisms other than the target organism

Strain heterogeneity

Standards

High-quality MAGs:

✓ **>90% completeness**

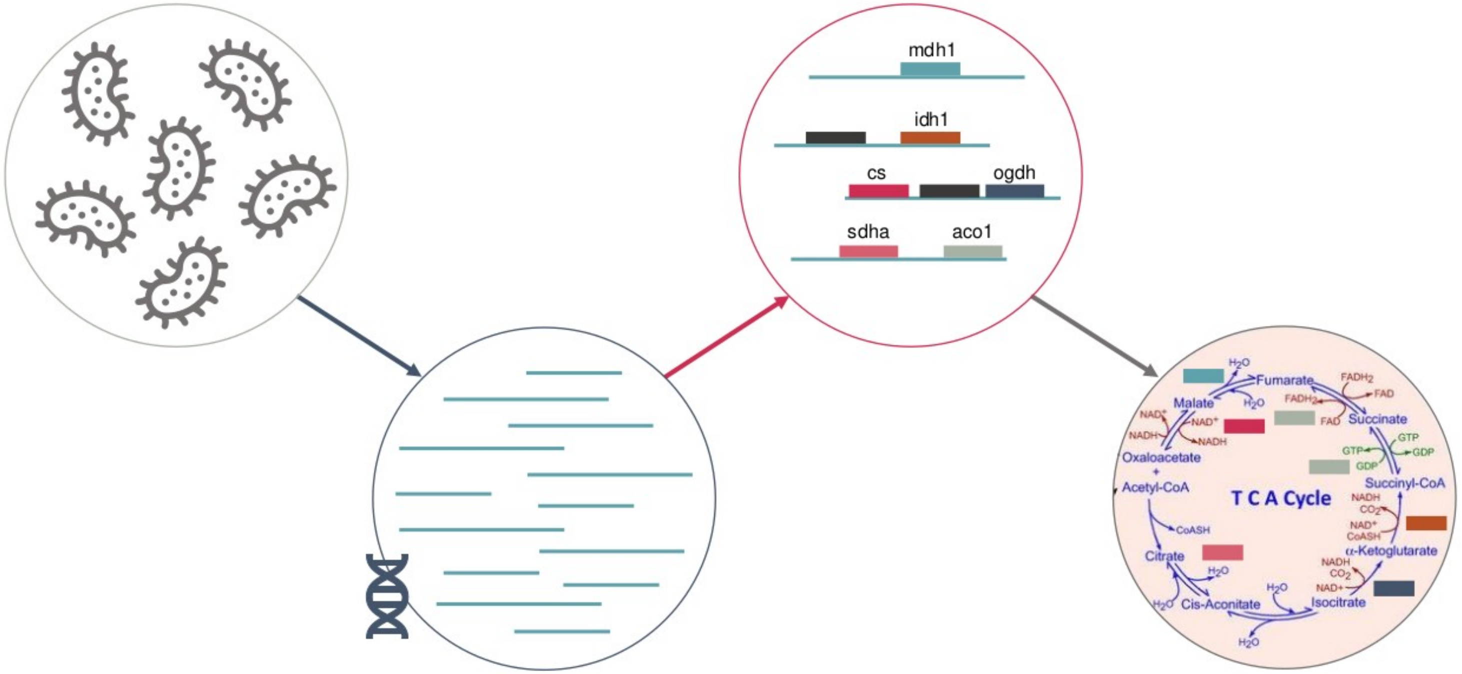
✓ **<5% contamination**

Applications in microbiome research

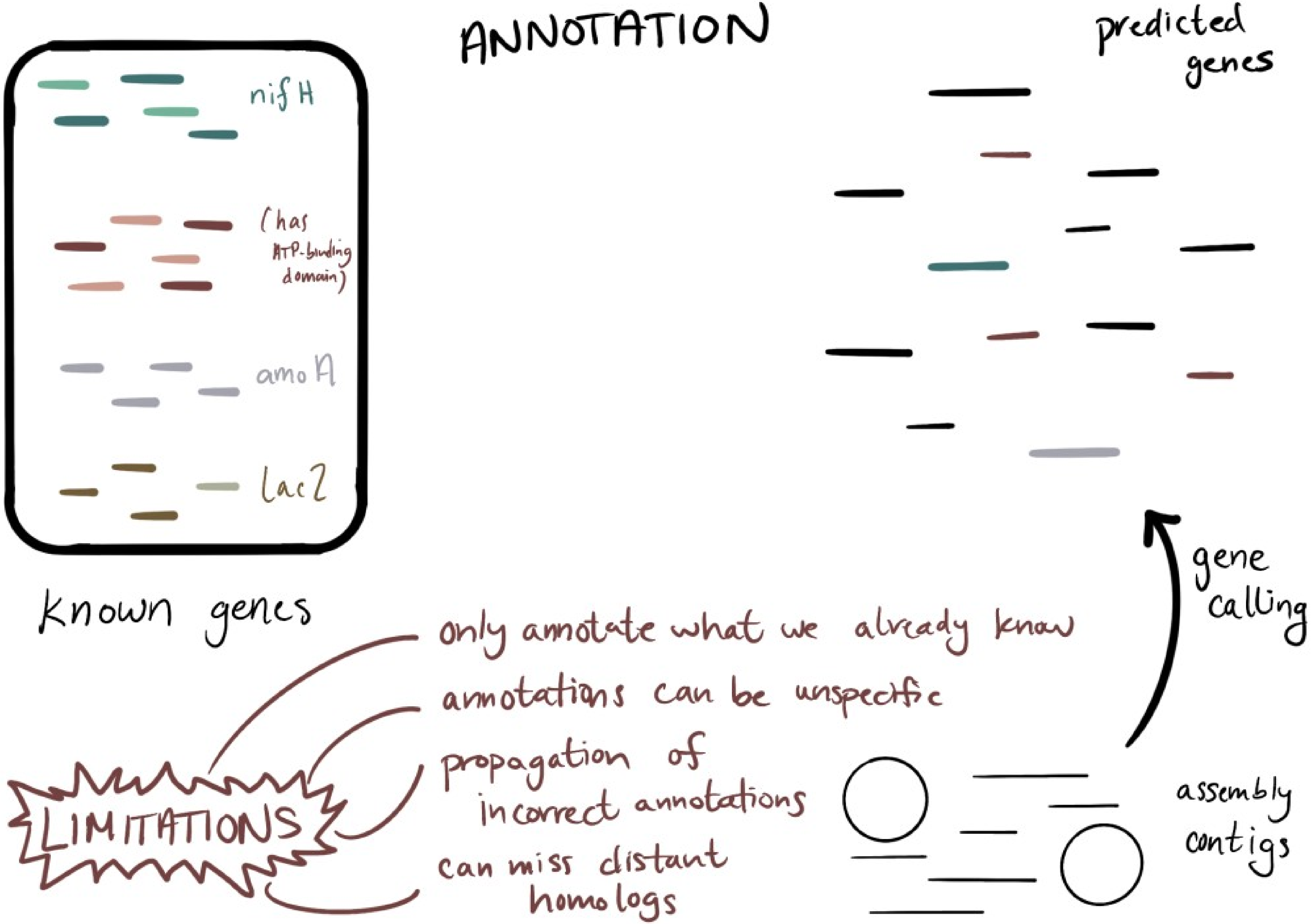
- Reconstruction of metabolic pathways
- Identification of antibiotic resistance genes or other target genes
- Discovery of probiotic candidates
- Study of host–microbe interactions

In Aquaculture:

- Pathogen surveillance
- Microbiome engineering
- Functional profiling of gut communities



ANNOTATION



- only annotate what we already know
- annotations can be unspecific
- propagation of incorrect annotations
- can miss distant homologs

Single-Cell Genomics Integration

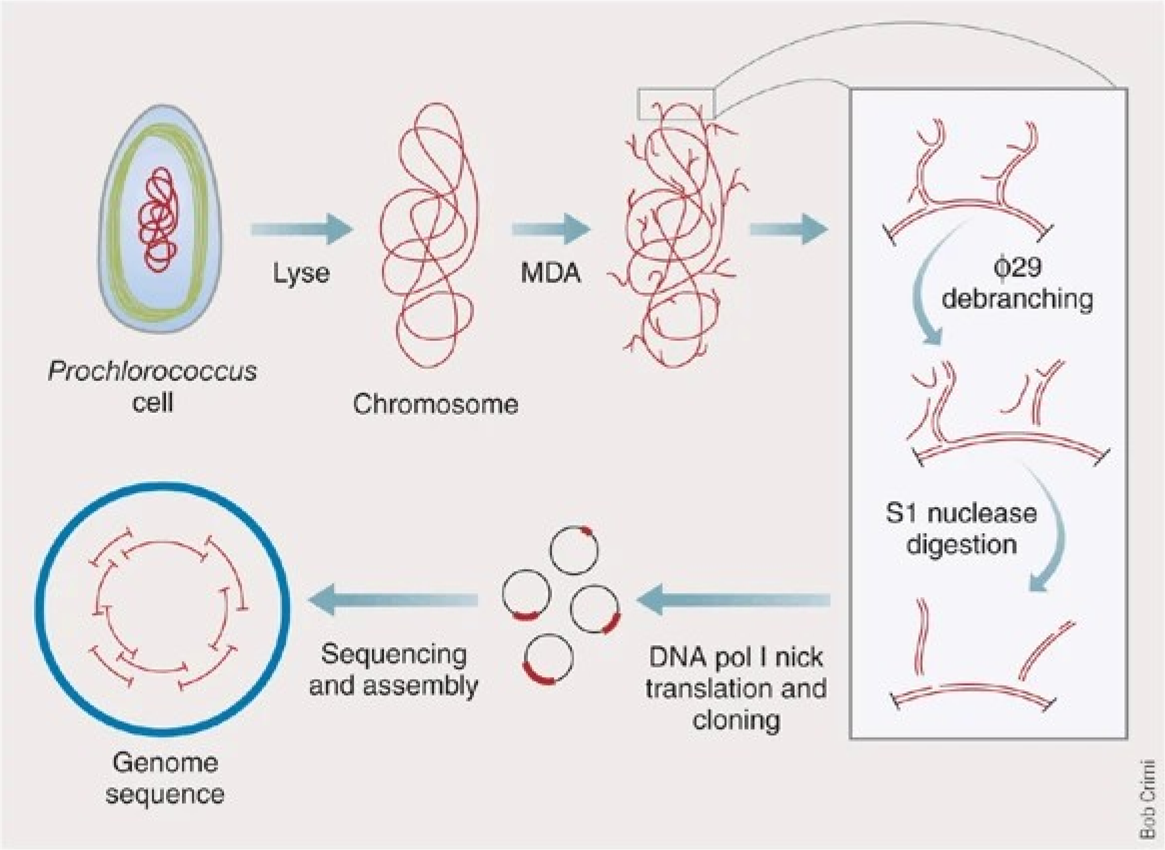
Single-cell genomics complements metagenomics by sequencing genomes from individual cells.

Advantages

- Resolves rare taxa
- Avoids assembly complexity
- Links genotype to phenotype

Limitations

- Amplification bias
- Incomplete genomes



Assembly for Eukaryotic genomes

Eukaryotic genomes present additional challenges:

- Larger genome size
- Introns and repeats
- Multiple chromosomes

Tools & Strategies

Hybrid assembly (short + long reads)

Hi-C scaffolding

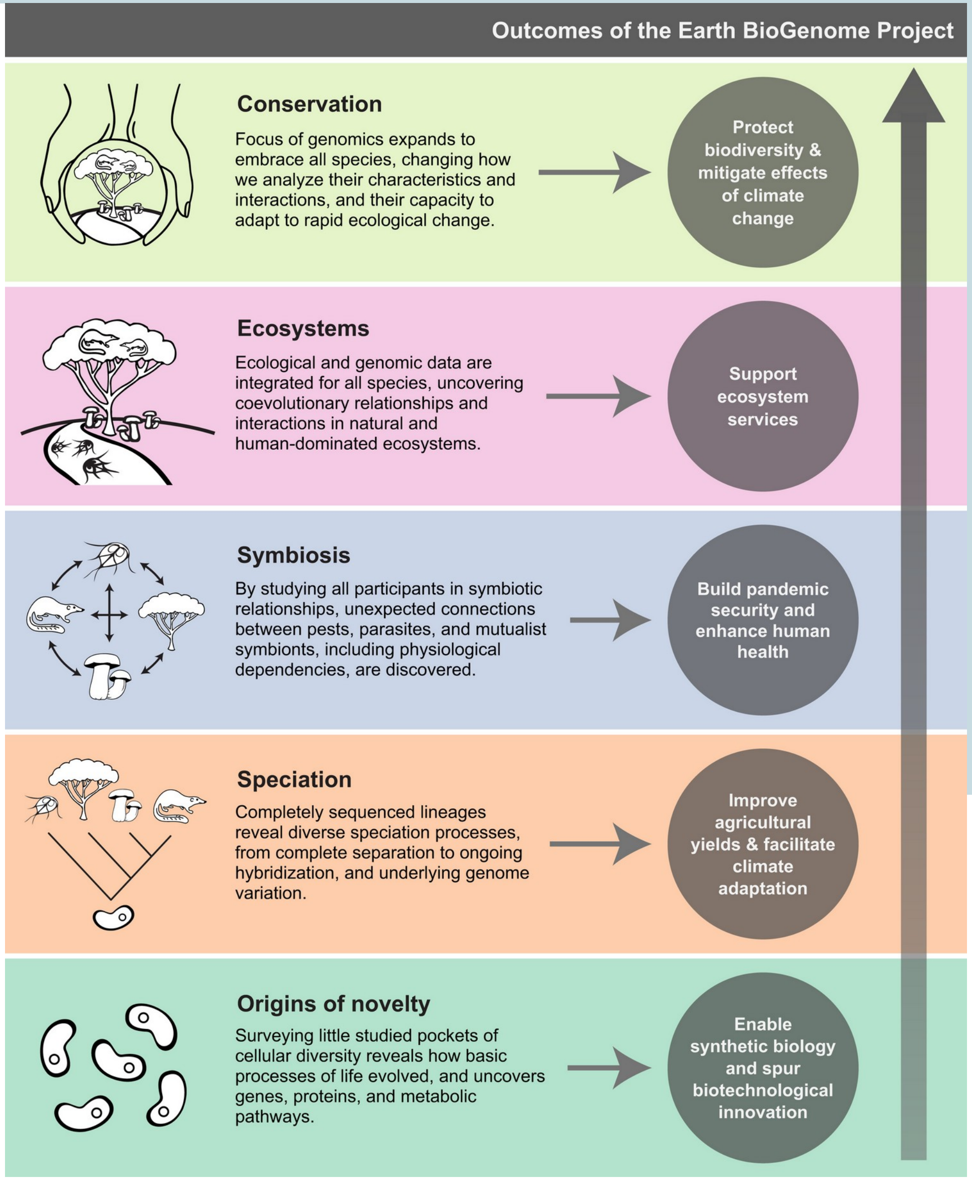
Targeted enrichment

Applications

Microbial eukaryotes - protist

Parasites

Microbiome-associated fungi



Outcomes of the Earth BioGenome Project

Conservation

Focus of genomics expands to embrace all species, changing how we analyze their characteristics and interactions, and their capacity to adapt to rapid ecological change. → Protect biodiversity & mitigate effects of climate change

Ecosystems

Ecological and genomic data are integrated for all species, uncovering coevolutionary relationships and interactions in natural and human-dominated ecosystems. → Support ecosystem services

Symbiosis

By studying all participants in symbiotic relationships, unexpected connections between pests, parasites, and mutualist symbionts, including physiological dependencies, are discovered. → Build pandemic security and enhance human health

Speciation

Completely sequenced lineages reveal diverse speciation processes, from complete separation to ongoing hybridization, and underlying genome variation. → Improve agricultural yields & facilitate climate adaptation

Origins of novelty

Surveying little studied pockets of cellular diversity reveals how basic processes of life evolved, and uncovers genes, proteins, and metabolic pathways. → Enable synthetic biology and spur biotechnological innovation

Future Directions & Curiosities

Best Practices

- Combine short and long reads
- Use multiple binning tools + refinement
- Validate with complementary approaches

Future Trends

- **Hologenomic studies**
- **AI-driven genome reconstruction**
- **Real-time environmental monitoring**

Most microbial diversity on Earth is known only from MAGs — not cultured organisms.

Metatranscriptomics - Active genes (RNA)

Metatranscriptomics analyzes the total RNA to profile gene expression

From Potential (DNA) to Activity (RNA)

Metagenomics reveals the genetic potential of microbial communities, while metatranscriptomics identifies which genes are actively expressed at a given time.

They distinguish between “who is present” and “what they are doing”.

DNA = functional potential
RNA = real-time activity

Essential for understanding microbial responses to environmental changes
Critical for host–microbe interaction studies

Many abundant microbes are metabolically inactive — metatranscriptomics reveals the truly active minority.

Technical Challenges of RNA-Based Studies

RNA is highly unstable and degrades rapidly, making sampling and preservation crucial.

Challenges
- Rapid RNase activity
- Need for immediate stabilization
- Host RNA contamination (in host-associated microbiomes)

Best Practices
- Standardize sampling time
- Include replicates
- Combine with metagenomics

Future Trends
- Real-time transcriptomics
- Single-cell transcriptomics
- Environmental monitoring systems

Flash-freezing samples or using RNA stabilization buffers is essential.

Applications in Microbiome Research

Reveals active processes such as:

- Nutrient metabolism
- Virulence factor expression
- Stress responses
- Host immune modulation

Hologenome Concept: Host + microbiome = unit of selection.

Simultaneous analysis of host and microbial transcripts allows investigation of cross-talk mechanisms.

- Captures host response
- Captures microbial activity
- Reve mechanisms of symbiosis or disease

Metagenomics vs Metatranscriptomics

Combining both approaches provides a comprehensive view of structure and function in microbial ecosystems.

Multi-omics Approach

Metaproteomics analyzes the complete set of proteins produced by a microbial community, revealing which genes are actually translated into functional enzymes and structural molecules at a given time. It provides direct evidence of active biological processes and host–microbe interactions.

Metabolomics examines the small molecules and metabolic products (e.g., amino acids, lipids, organic acids) present in a sample, offering a snapshot of the biochemical activities and physiological state of the microbiome and its environment.

Environmental DNA (eDNA)

eDNA refers to genetic material released by organisms into the environment (water, sediment, soil) via cells, mucus, feces, or tissue.

Used primarily to detect species presence, biodiversity, and community composition without direct sampling of organisms.

Methods:
Water/sediment filtration → DNA extraction
Marker amplification (e.g., 12S, COI, 18S)
Metabarcoding or targeted qPCR assays

Widely applied in conservation biology, invasive species detection, and ecosystem m

Limitations of eDNA

- DNA persistence may detect species no longer present (false positives).
- Limited functional information (presence ≠ activity).
- Sensitivity to environmental degradation and transport.

Complementary Applications

- **Integrated monitoring of aquatic ecosystems:**
    - **eDNA → fish and macrofauna biodiversity**
    - **Microbiome → ecosystem health and functional status**
- **Aquaculture management:**
    - **eDNA for pathogen detection**
    - **Microbiome for water quality and fish health assessment**
- **Early warning systems for environmental change and disease outbreaks.**

Combining eDNA and microbiome approaches provides a multiscale view of biodiversity, ecosystem function, and environmental quality, essential for future ecological research and sustainable management.

Methodological Challenges

- **Sampling Bias**
- **Spatial variability**
- **Temporal variability**
- **Handling effects**

DNA Extraction Bias

- **Different protocols → different results.**
- **Bioinformatics Challenges**

Database incompleteness

- **Pipeline variability**
- **Reproducibility issues**

Why Learn These Tools?

Future researchers must:

- **Integrate multi-omics approach**
- **Link microbes to ecosystem processes**
- **Translate data into management tools**

Thanks for the attention!!

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MARINE

Marco Basili
Microbial ecology Lab.
marco.basili@irbim.cnr.it