Microbial communities through the lens of high throughput sequencing, data integration and metabolic networks analysis

developing computational approaches to better understand microbial assemblages

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PhD candidate

Microbial ecology & biogeochemical cycles

a corner-stone for life on earth

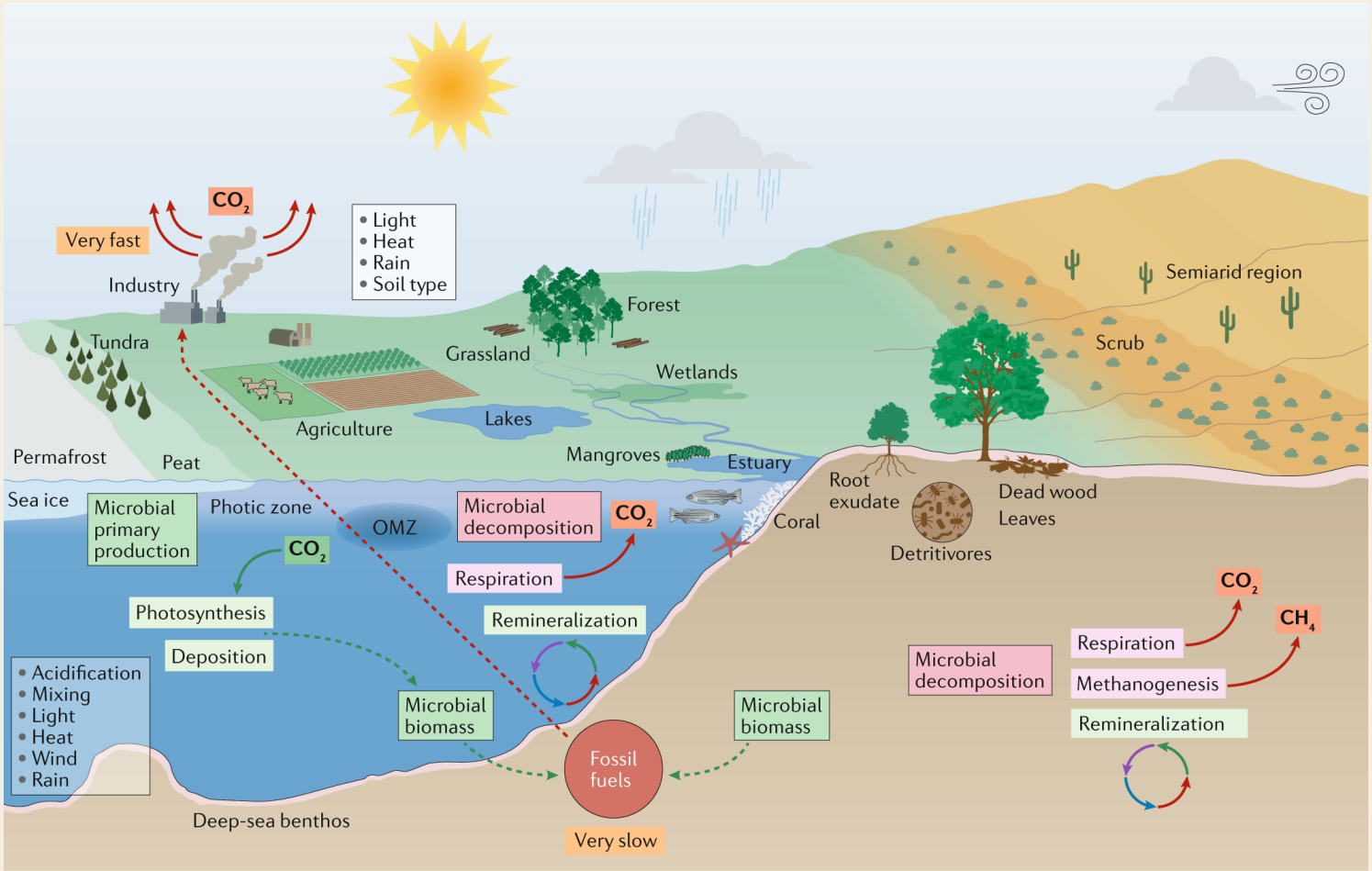


Figure from: Nature Reviews Microbiology 17.9 (2019): 569-586.

Main questions regarding a microbial community

for a deeper understanding of such assemblages

community structure
who

taxa, abundance

ecosystem type
where

habitats

functional potential
what

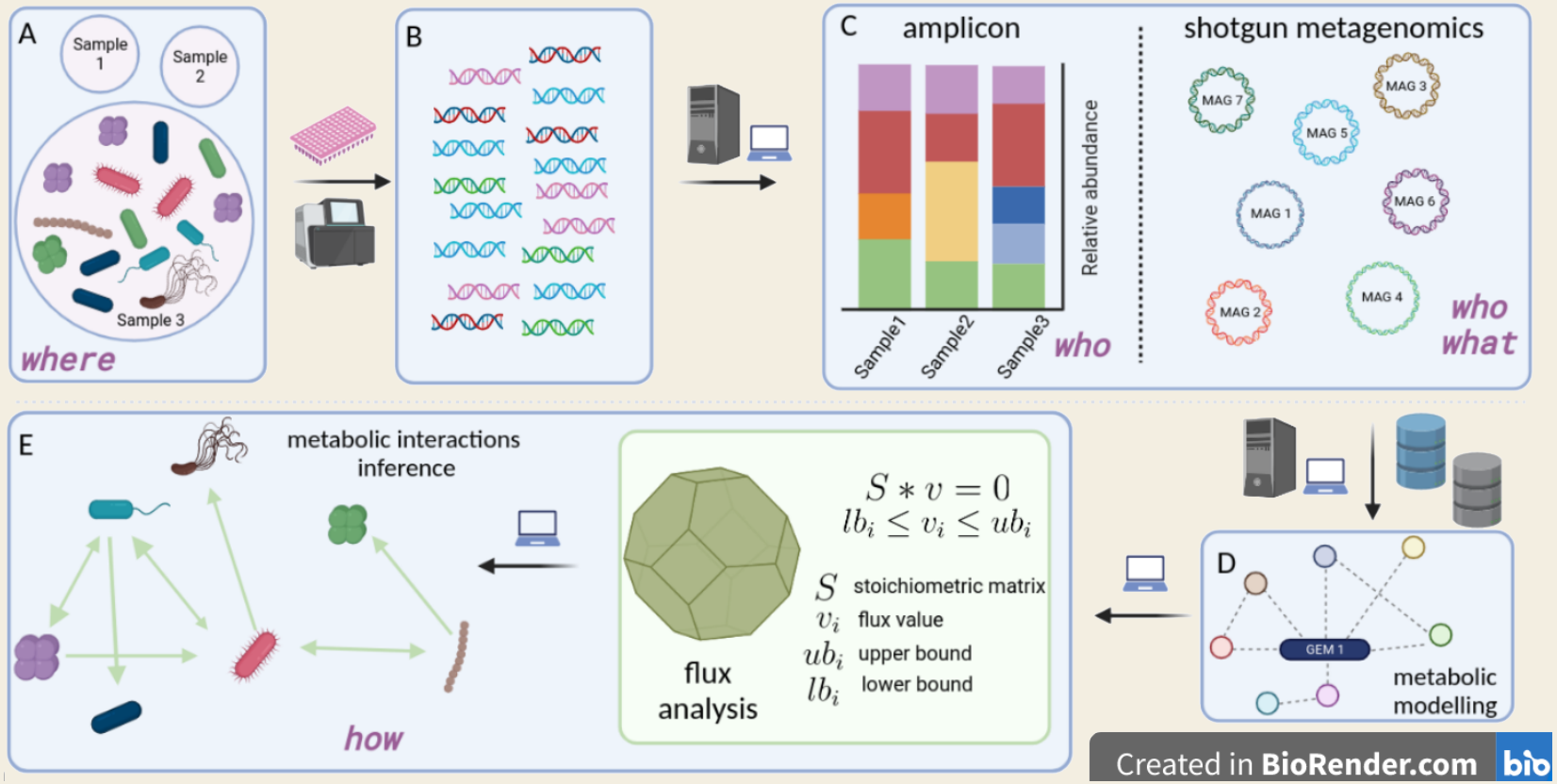
processes

underlying mechanisms
how

intreactions, fluxes

Reverse ecology

transforming ecology into a high-throughput field



From raw reads to community analysis

not a straight-forward way

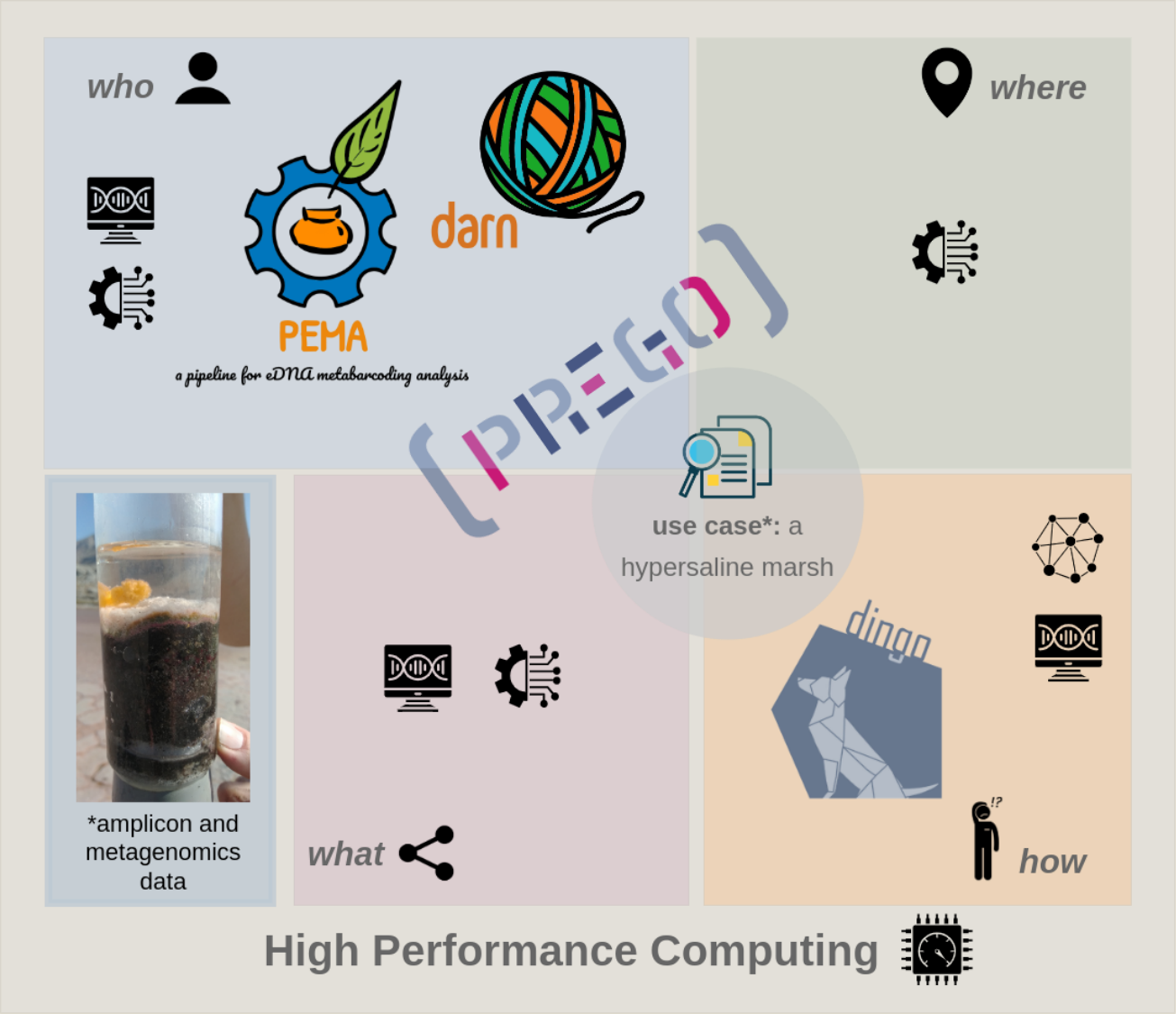
- **computing requirements**
- **multi-step analyses**
- **reproducibility**
- **eDNA and PCR limitations**
- **algorithmic - oriented**
- **data integration applications**

Aims and objectives

- build algorithms and software to address on-going challenges in microbiome data analysis

- identify taxa & functions with a key role in microbial community assemblages in hypersaline sediments

Graphical abstract of this PhD thesis

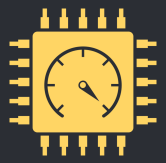


Legend

- data integration
- HTS
- metabolic modelling
- HPC

0s and 1s in marine molecular research

a regional HPC



challenge category: compuring requirements

aim & contribution: a retrospective analysis of resource allocation and computational methods supported by a regional HPC facility

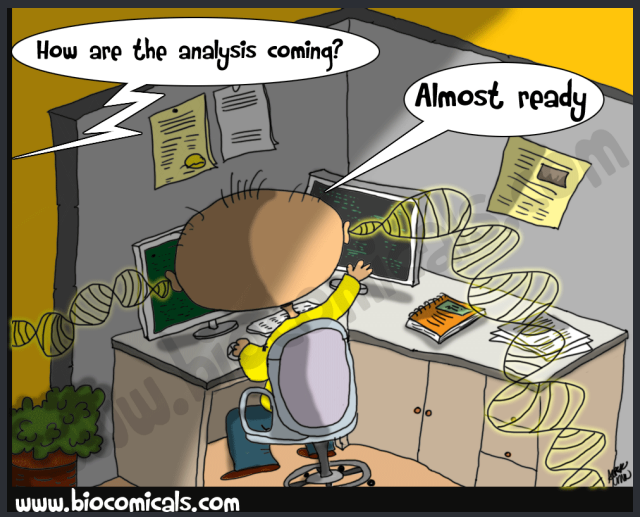
Computational requirements

for trivial bioinformatic tasks

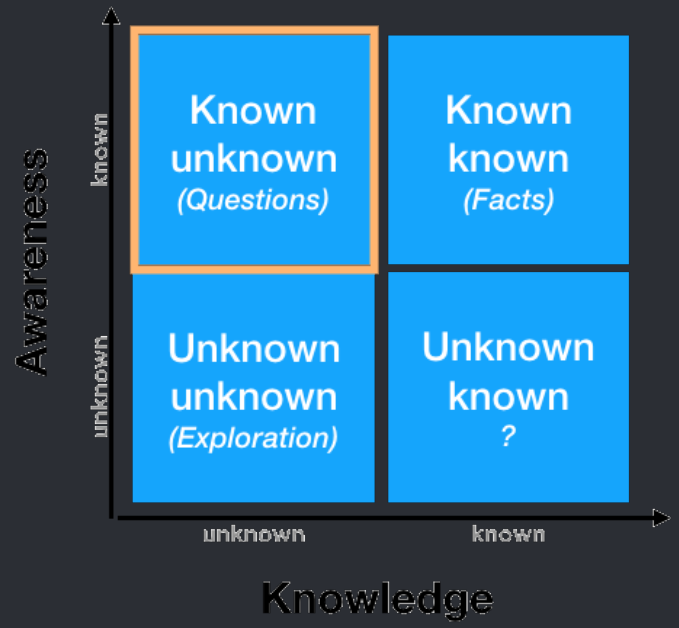
Red bars denote published research with high resource requirements
of the various computational methods employed at the IMBBC HPC facility

Bioinformatics challenges

for the analysis and the interpretation of amplicon data



- multiple steps
- several tools & databases
- computing power
- scalability & reproducibilty



OTUs/ASVs with no taxonomy assignment:
novel or non-target taxa

PEMA

a flexible Pipeline for Environmental DNA Metabarcoding Analysis

challenge category: complex bioinformatics analysis, analysis reproducibility

aim & contribution

To build an open source pipeline that bundles state-of-the-art bioinformatics tools for amplicon analysis that is:

- a one-stop-shop for several marker genes & approaches
- easy-to-set & easy-to-use
- scalable & flexible
- reproducible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a pipeline for eDNA metabarcoding analysis
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dingo
*amplicon and metagenomics data
what
how
High Performance Computing
Legend
data integration
HTS
metabolic modelling
HPC

Methods / Implementation

PEMA coding insights

```
for(int i : range(1,
    in  := "in_$i.tx
    sys date > $in

    out := "out_$i.t
    task( out <- in
        sys echo Tas
    }
}
```

Big-DataScript programming language

Containerization

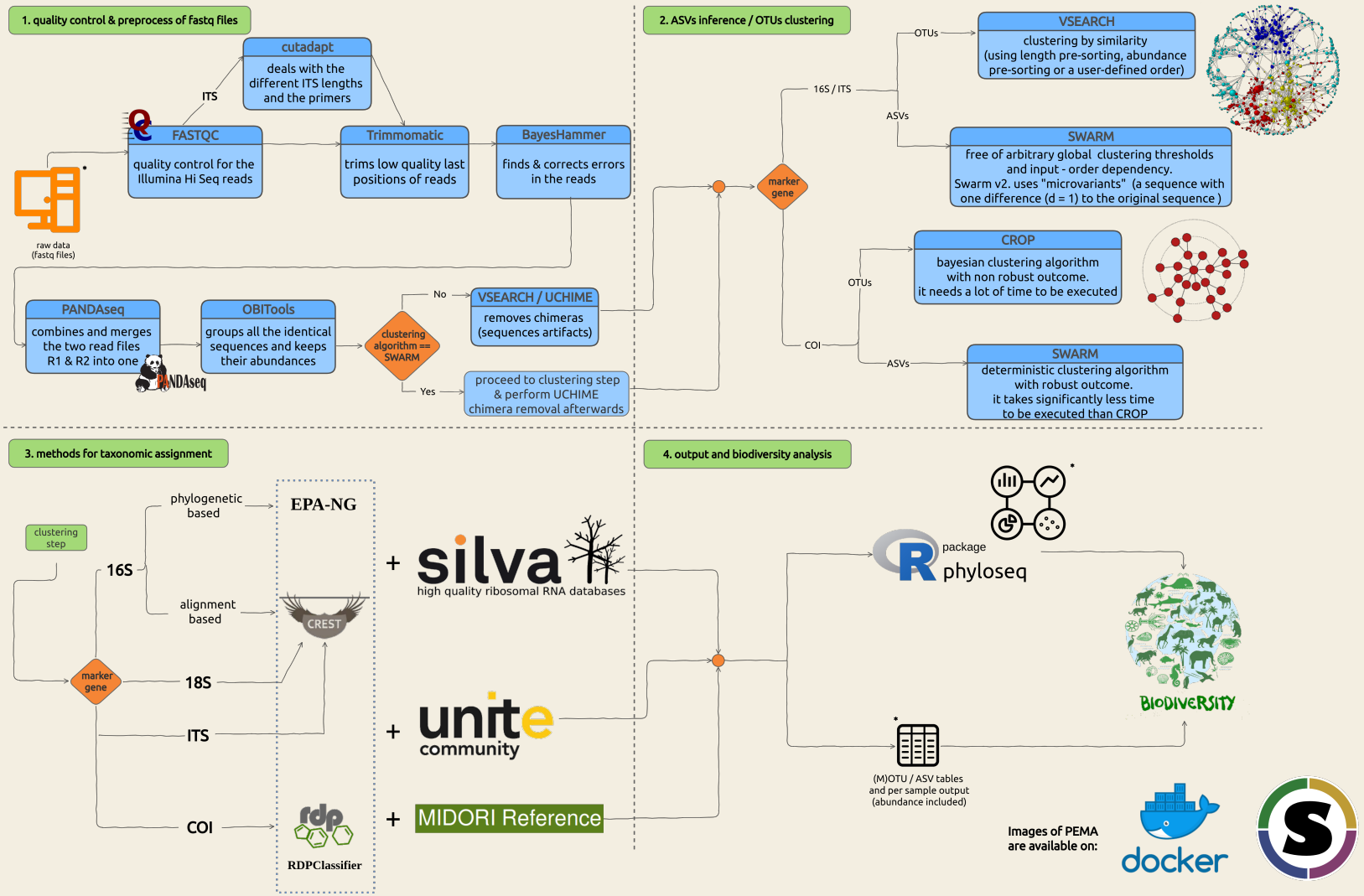
High performance computing

PEMA features

an overview

PEMA

PEMA in a nutshell



* Illustrations with asterisk are from the Noun Project

Results: tuning effects

in mock communities

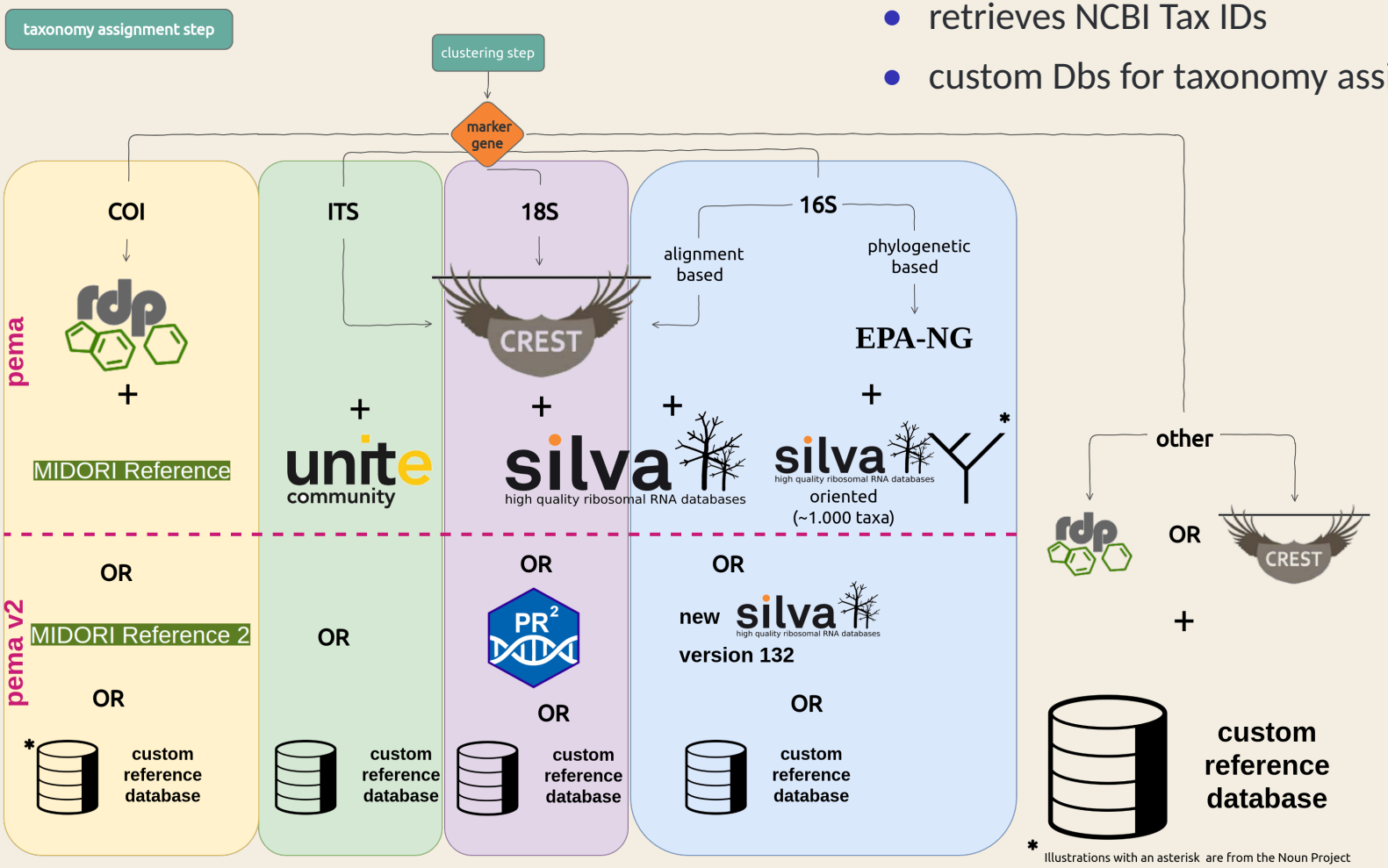
Mock communities using the 16S rRNA gene and multiple parameter sets (identification at the genus level; part of the initial table):

mock community Gohl et al. (2016)	**Swarm (d = 1 strict = 0.8 no singletons)**	**Swarm (d = 3 strictness = 0.6 no singletons)**	**Swarm (d = 3 strictness = 0.8 with singletons)**	**Swarm (d = 10 strictness = 0.8 with singletons)**	**Swarm (d = 10 strictness = 0.8 no singletons)**	**Swarm (d = 25 strictness = 0.6)**	**Swarm (d = 25 strictntess = 0.8)**	**Swarm (d = 30 strictness = 0.6)**
TP	12	15	18	18	15	17	17	17
FP	2	2	21	11	6	5	5	4
FN	8	5	2	2	5	3	3	3
PREC (TP / TP+FP)	0.86	0.88	0.46	0.62	0.71	0.77	0.77	0.81
REC (TP / TP+FN)	0.6	0.75	0.9	0.9	0.75	0.85	0.85	0.85
F1 (2 * (PREC * REC) / (PREC+REC)	0.71	**0.81**	0.61	0.73	0.73	0.81	0.81	0.83

PEMA v.2

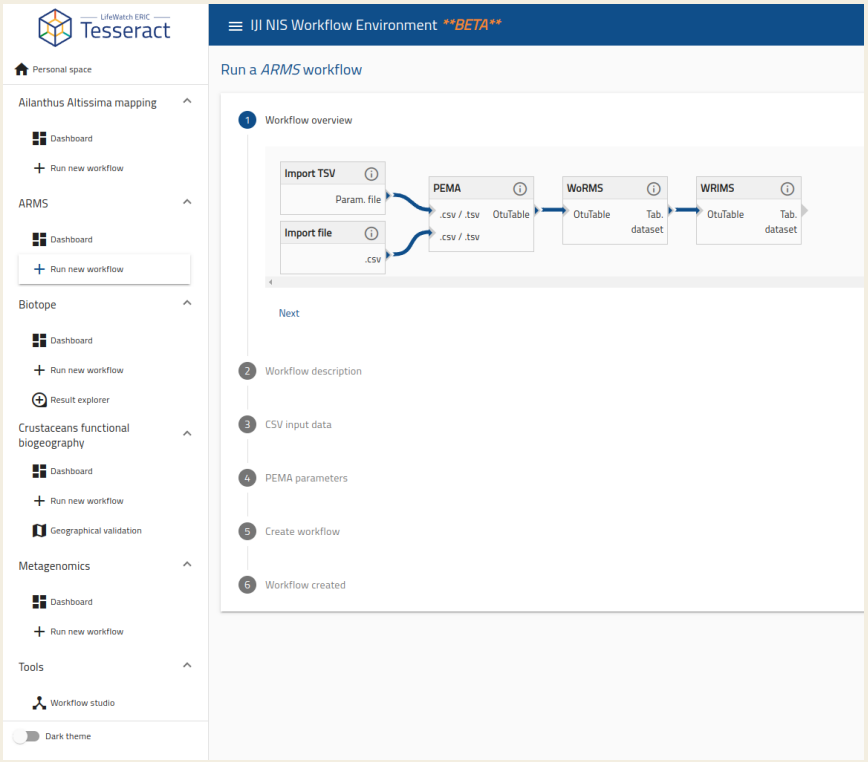
addressing some of the challenges

- new code architecture
- 12S rRNA now supported
- retrieves NCBI Tax IDs
- custom Dbs for taxonomy assignment



Moving at the large scale

PEMA @ infrustructures



1. web - interface make analysis even easier
2. researchers without access to HPC/clouds are now able to run big scale analyses
3. combine with other tools

LifeWatch ERIC:
www.lifewatch.eu/
Tesseract VRE Development Portal:
www.lifewatch.dev/dashboard

Elixir Greece:
https://elixir-greece.org/
Hypatia cloud infrastructure:
https://hypatia.athenarc.gr/

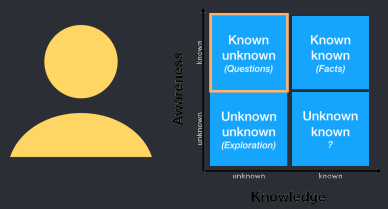
Conclusions

on PEMA and eDNA metabarcoding

- **parameters tuning** is essential in metabarcoding analyses; **mock communities** among samples under study benefit to that end
- workflow managers & containers enable complex and reproducible workflows
- **e-infrustuctures** benefit studies with great number of samples and CLI non-familiar users

DARN

dark matter invesigator tool



challenge category: eDNA - oriented issues

aim & contribution:

extract "dark matter" from COI amplicon data

i.e. non-target, unassigned or assigned with low confidence sequences.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Building a COI-oriented tree of life

sequences retrieved

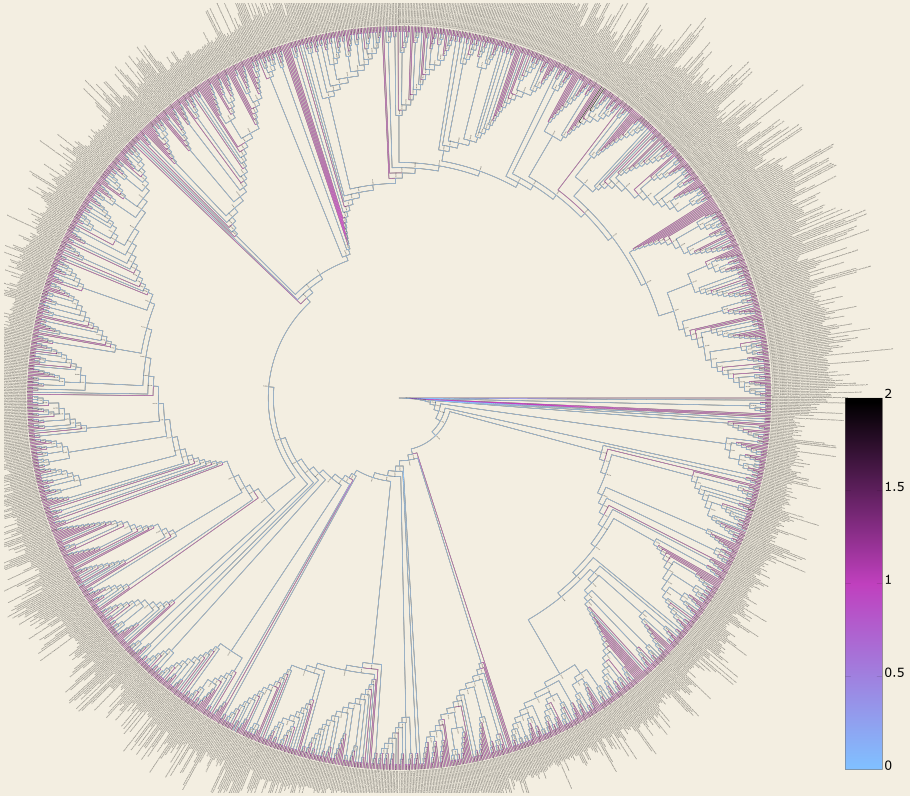
Resources	bacteria		archaea		eukaryotes	
	# of sequences	# of strains	# of sequences	# of strains	# of sequences	# of species
BOLD	3,917	2,267	117	117		
PFam-oriented	9,154	4,532	217	115		
Midori 2					1,315,378	183,330
Total unique entries	**11,421**	**6,798**	**334**	**201**	**1,315,378**	**183,330**

↓

	bacteria	archaea	eukaryotes
consensus sequences (tree branches)	463	25	1,109

Reference phylogenetic tree

of the COI consensus sequences retrieved

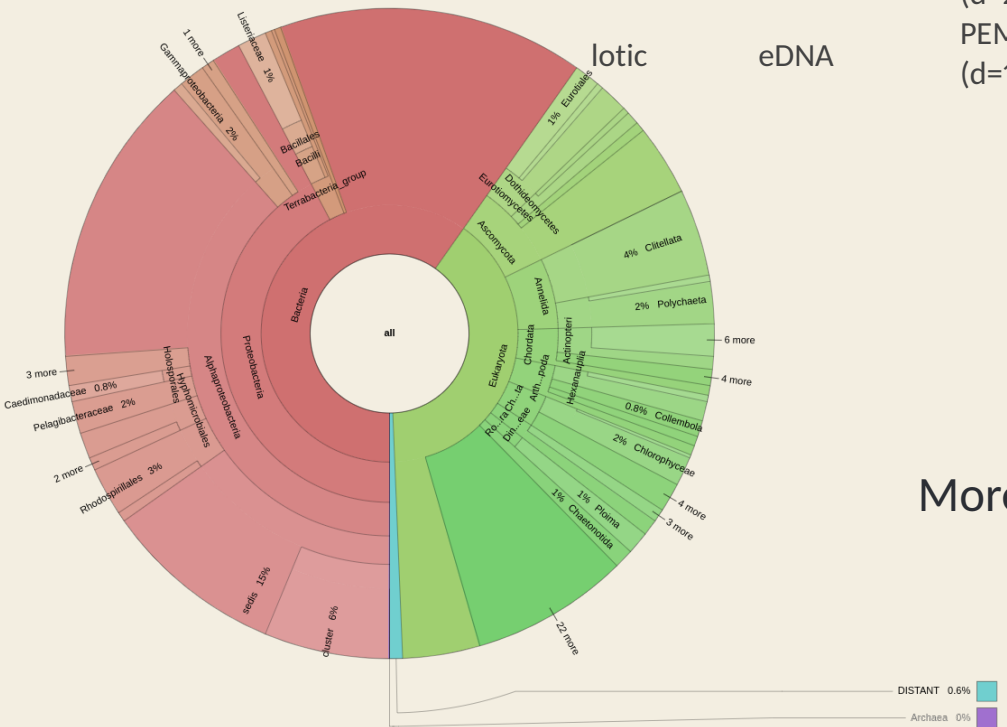


The consensus sequences have been placed in their corresponding taxonomic branches, proving the tree valid.

DARN using real-world data

with multiple sample types, primers, PCR protocols and bioinformatics pipelines

Envo type	Sample type	Bioinfo pipeline(s)	# of ASVs	~% of sequence assignments per domain		
				eukaryotes	bacteria	archaea
marine	eDNA	QIIME2 - Dada2	13,376	**11**	88	**0.02**
		PEMA (d=10)	39,454	**25**	75	**0.1**
marine	bulk	PEMA (d=2)	193	**99**	**1**	-
marine	bulk	PEMA (d=2)	74	**97**	0	-
lotic	eDNA	PEMA (d=10)	1,940	**64**	34	**2**



More results at: https://hariszaf.github.io/darn/

Conclusions

on DARN and COI amplicon studies

- dark matter is widely common in eDNA samples compared to bulk ones
- bacteria make up a significant proportion of sequences generated in COI based eDNA metabarcoding datasets
- including non-eukaryotic COI sequences in reference databases could benefit the method

dingo

a new MCMC algorithm for sampling the flux Space of metabolic networks

challenge category: algorithm-oriented

aim & contribution: support flux sampling at high dimensional polytopes such as those of complex organisms and multispecies and/or host-microbe communities

Definitions

- *flux*: the rate of turnover of molecules through a reaction
- *polytope*: a bounded polyhedron
- *flux sampling*: calculation of a sufficiently large number of uniformly distributed points in the polytope derived from a metabolic model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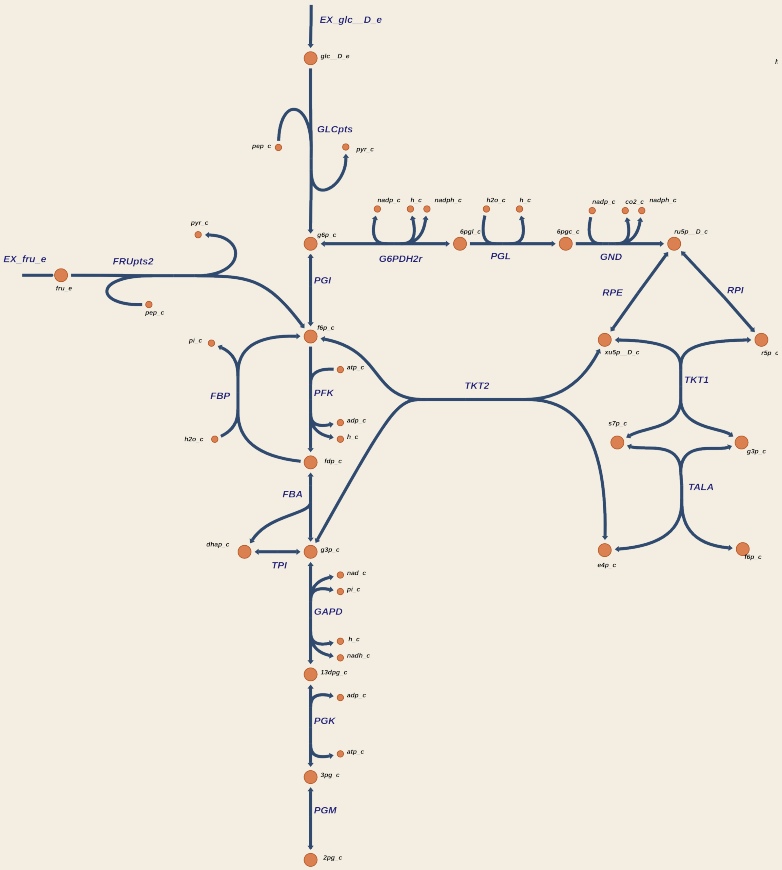
Metabolic modelling

and the biomass function



Metabolic models allow us

to move from a metabolic map to

mathematical structures

the study of which may provide

fundamental biological insight.

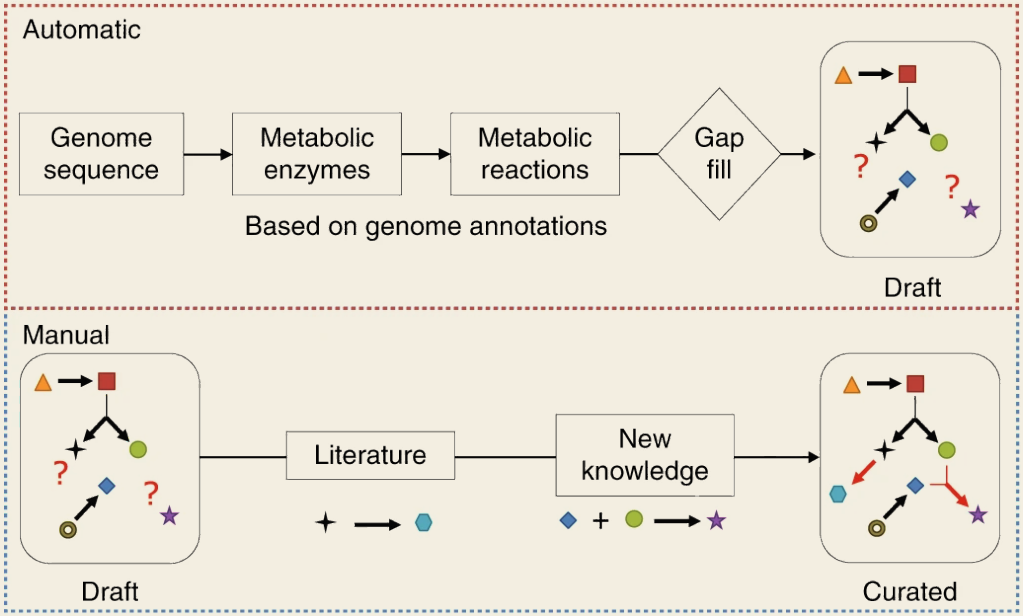
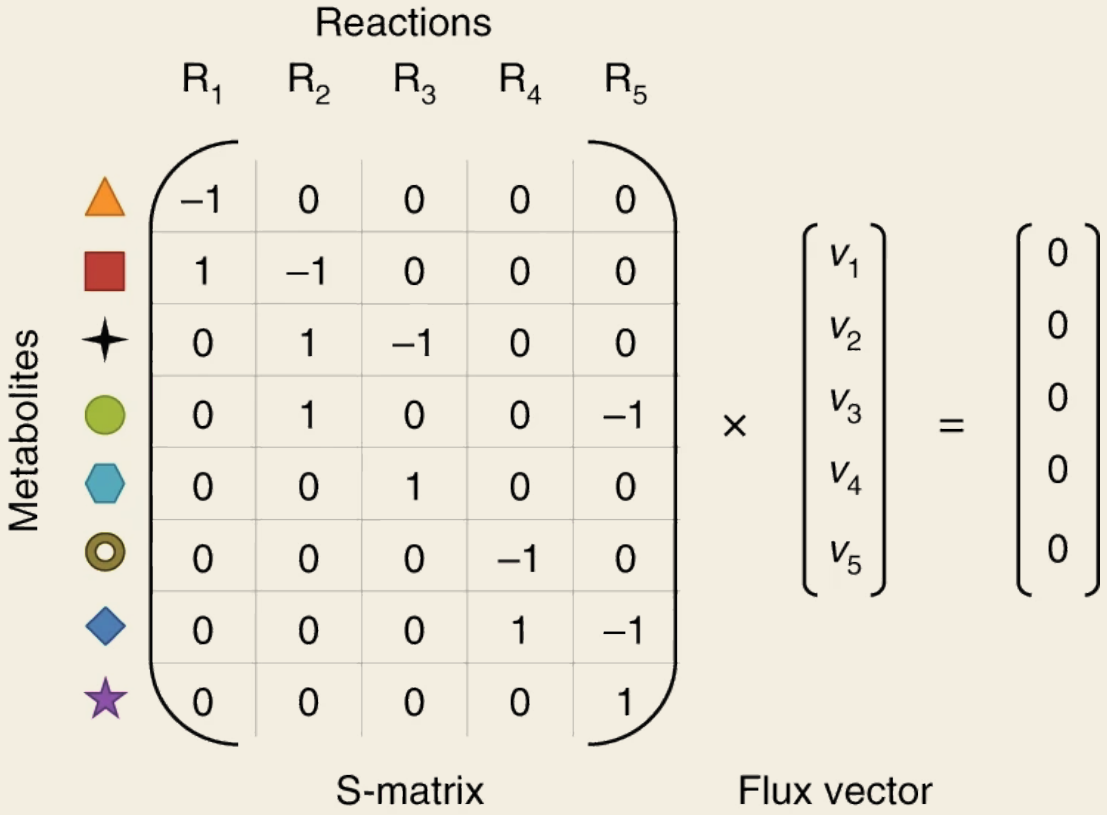


Figure from: Heirendt et al. Nature protocols 14.3 (2019): 639-702.

From a stoichiometric matrix

to a constraint-based model



In a **steady state**
the production rate
of each metabolite
equals its consumption rate.

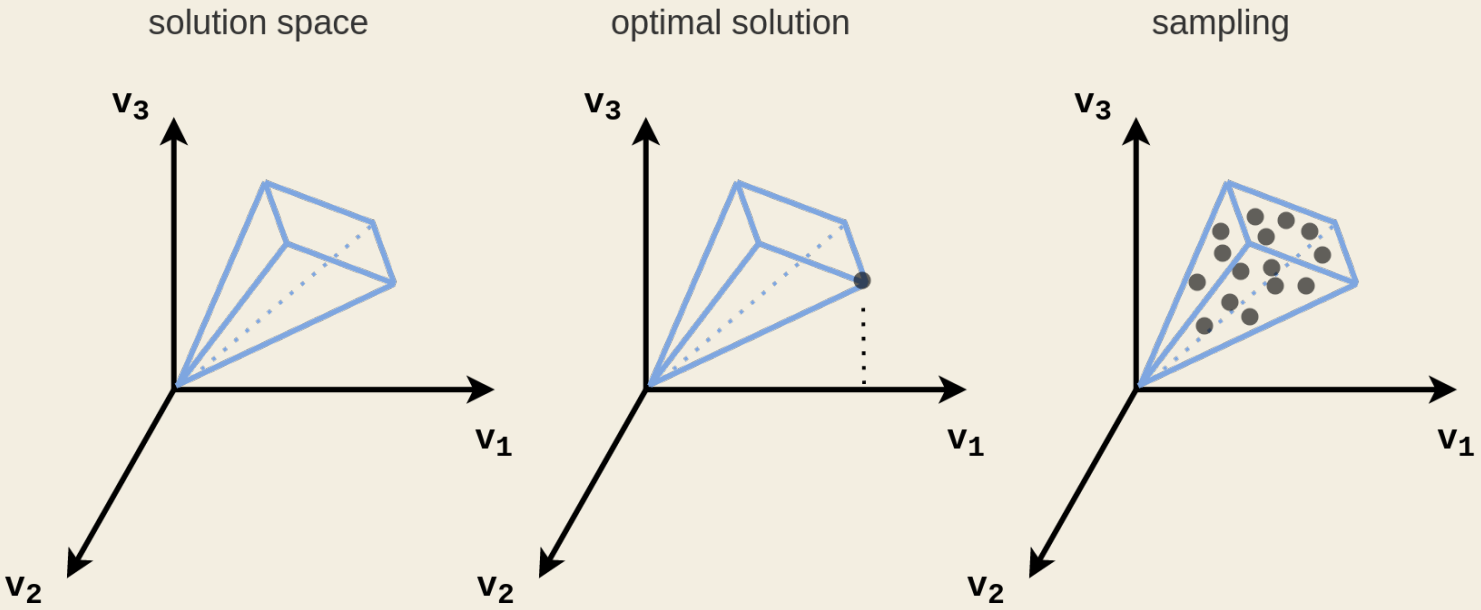
The **flux vector** is a vector with
the value of
each reaction flux
in a certain steady
state.

The steady state assumption
is ensured by
the **zero-vector**.

Figure from: Heirendt et al. Nature protocols 14.3 (2019): 639-702.

Flux sampling

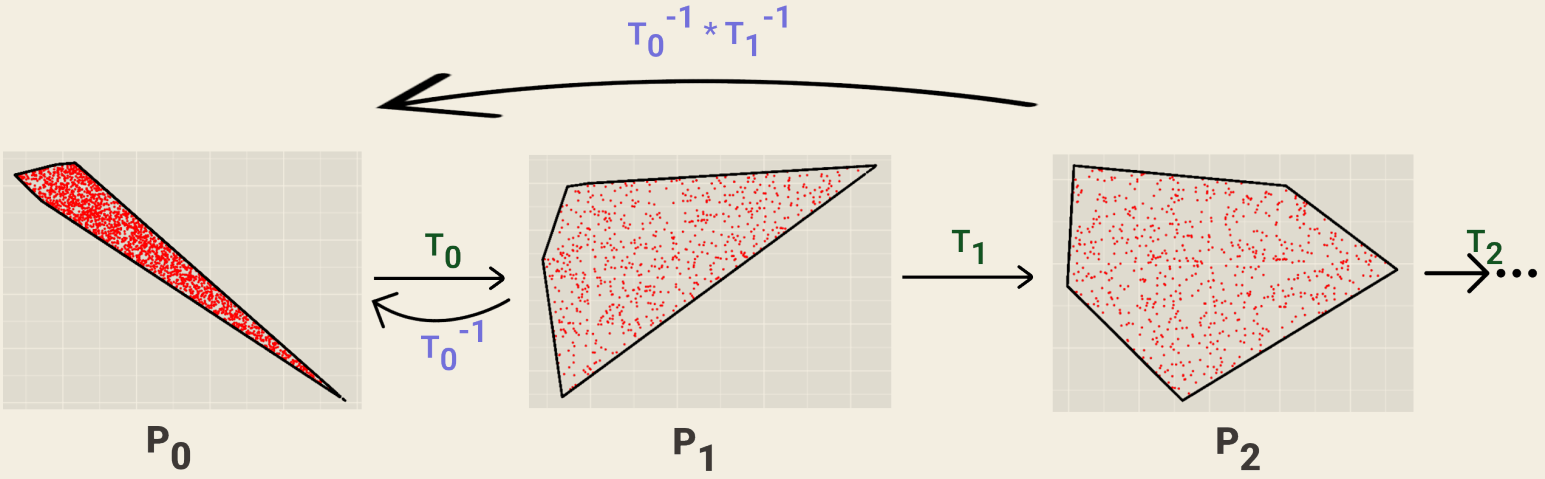
an alternative approach



- enables the analysis of GEMs without the need of an objective function
- determines the feasible solution spaces for fluxes in a network based on a set of conditions as well as the probability of obtaining a solution

A new Markov Chain Monte Carlo algorithm

for flux sampling

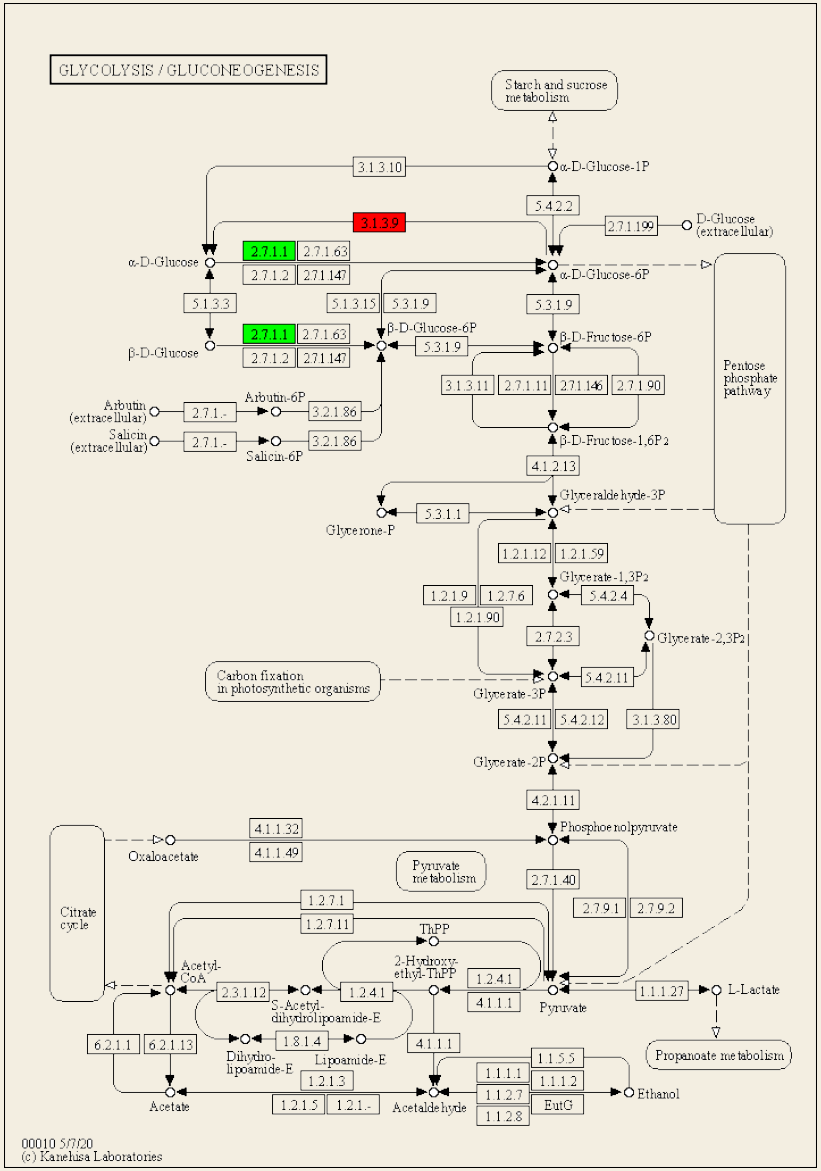


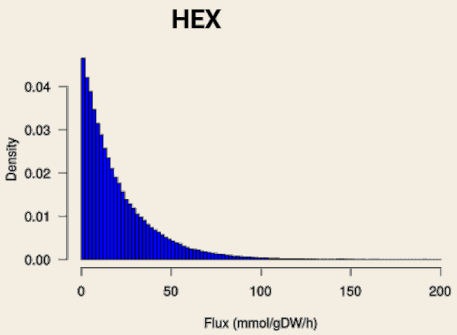
Steps of an MMCS phase

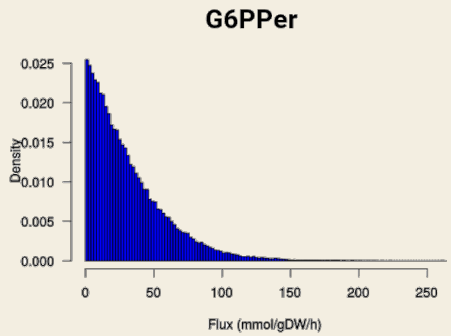
- **sampling step:** using a variant of the **Billiard walk**
- **rounding step:** calculate a linear transformation T_i that puts the sample into isotropic position and then apply it on P_i to obtain the polytope of the next phase
- check several statistic tests

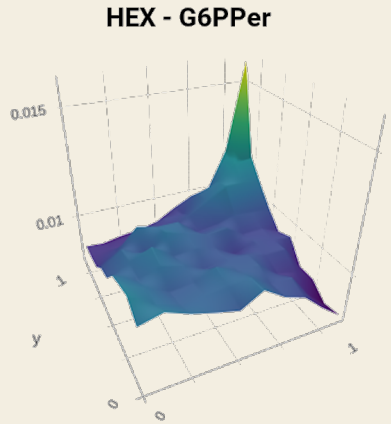
Flux sampling output

marginal distributions and copulas









HEX:
atp_c + glc__D_c → adp_c + g6p_c + h_c :

G6PPer:
g6p_r + h2o_r → pi_r + glc__D_r

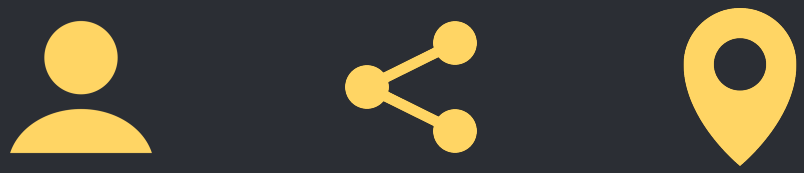
Conclusions

on sampling the flux space of metabolic models

- flux sampling provides essential insight (knock-out genes, host-microbe interactions etc)
- manual curation of the models is essential
- our multiphase MCMC algorithm enables sampling on the so-far largest metabolic model (13543 reactions; $d = 5335$)
- sampling the flux space of community metabolic models is now possible

PREGO

a literature- and data-mining resource to associate microorganisms, biological processes & environment types

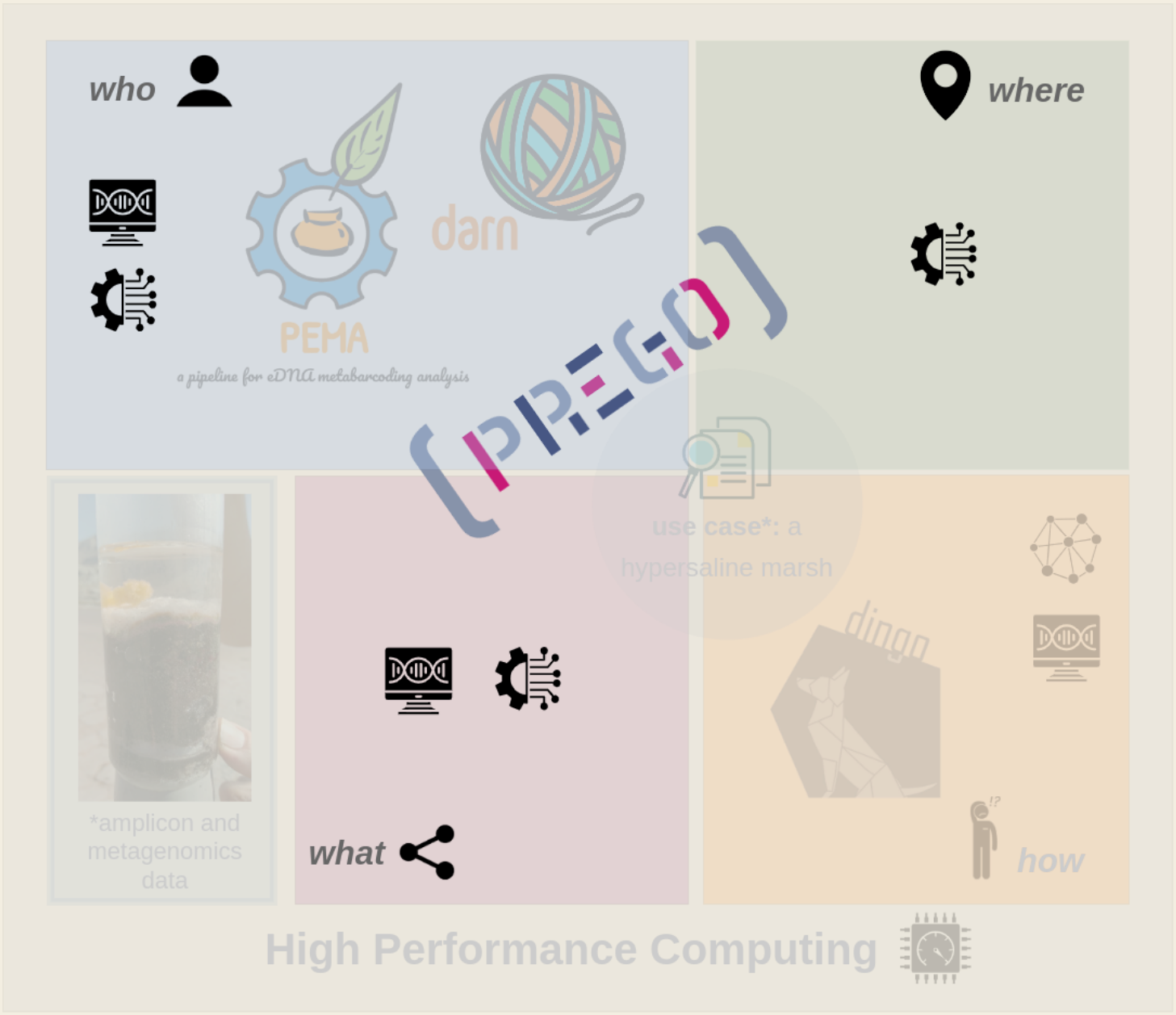


challenge category: data integration applications

aim & contribution:

To build a hypothesis generation resource based on associations between:

- *organisms* and the *environments* they inhabit
- *organisms* and the biological *processes* they are involved with
- *processes* and the *environments* where they occur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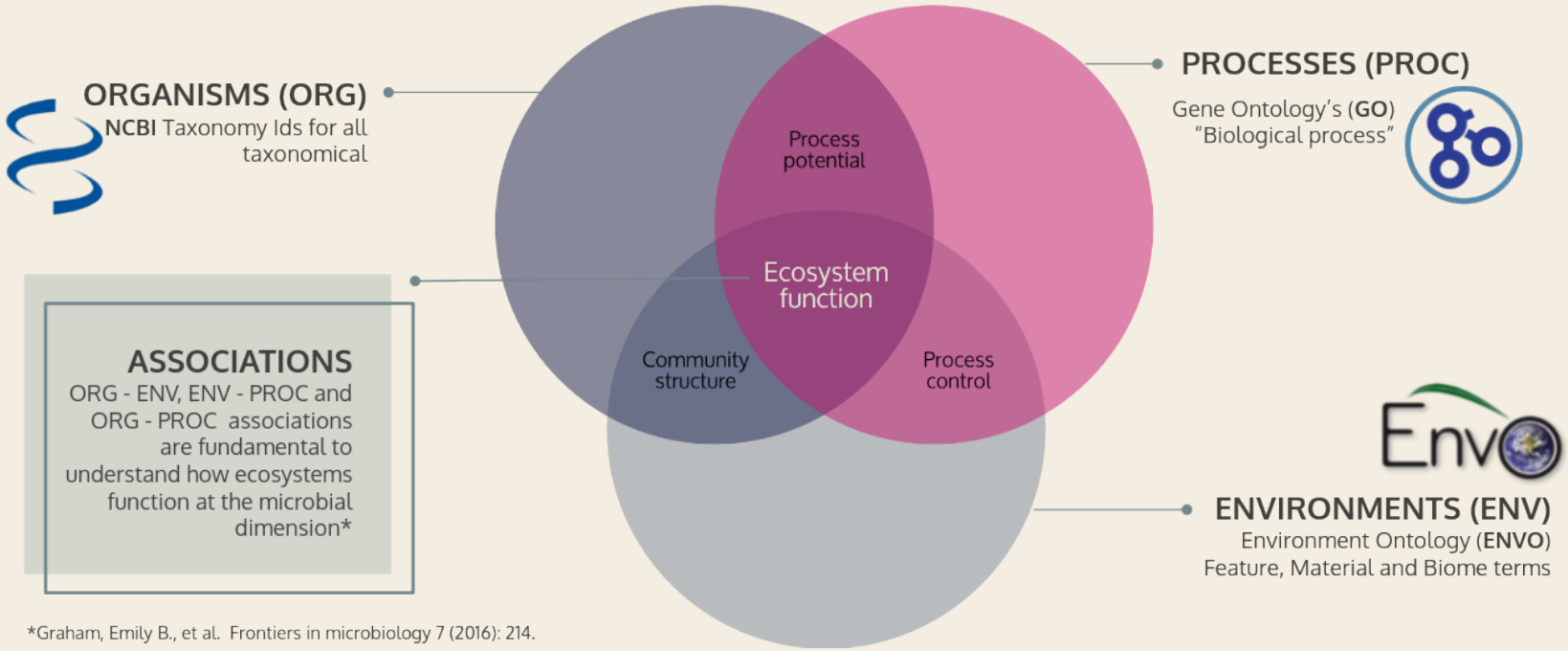
PREGO and its referring terms

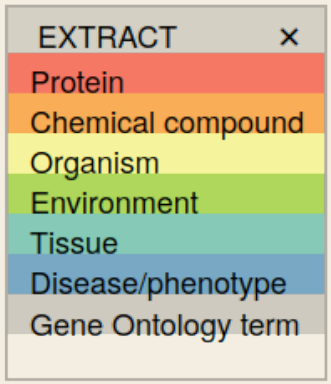
the fundamental role of ontologies



*Graham, Emily B., et al. Frontiers in microbiology 7 (2016): 214.

Named Entity Recognition

counting co-occurences to export associations



Identification of potentially important pathways missing from the model

From the metagenomic bins, we were able to identify two metabolic processes that were not previously included in the model. A number of MAGs (bin.59, bin.15, bin.73) clustered to the KEGG genomes of freshwater sulfur-oxidizing autotrophs capable of denitrification, *Sulfuritalea hydrogenivorans* [41], and *Sulfuricella denitrificans* [42]. These MAGs contained the diagnostic genes for carbon fixation (*rbcLS*), sulfur cycling (*dsrAB*), and denitrification (*nosZ*). One MAG (bin.59) also clustered with iron oxidizing autotroph *Sideroxydans lithotrophicus* ES-1. Bin.59 is the most relatively abundant bin from 17 to 21 m depth. Thus, if this MAG is associated with iron oxidation, it also contains sulfur-cycling genes that add to metabolic flexibility, which was previously observed [40]. The model did not include sulfide oxidation with nitrate, so it is unclear from the current model predictions where this process is expected to occur within the water column to compare to the MAG distributions.

Example text from Arora-Williams et al. Microbiome 6.1 (2018): 1-16.

Publicly available omics datasets

and the role of metadata

Sample metadata [-]

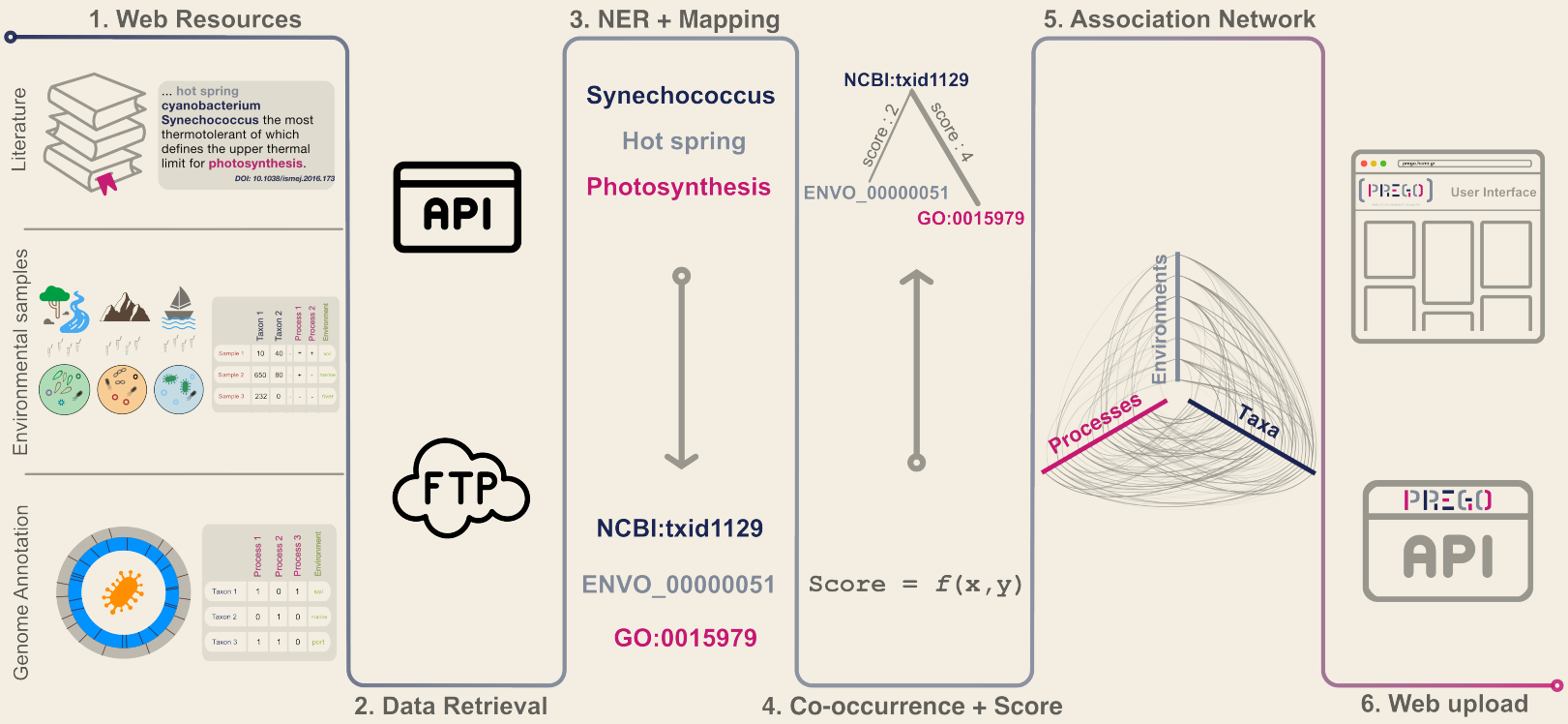
Collection date:	11/1/11
Elevation:	200
Environment (biome):	soil
Environment (feature):	nosZ
Environment (material):	soil DNA
Environmental package:	MIGS/MIMS/MIMARKS.soil
Geographic location (depth):	15-20cm
Instrument model:	454 GS FLX Titanium
Investigation type:	miens-survey
NCBI sample classification:	410658
Project name:	EcoFINDERS

Project Information	
Cultured	No
Ecosystem	Environmental
Ecosystem Category	Aquatic
Ecosystem Subtype	Oceanic
Ecosystem Type	Marine

MG-RAST ID	name	biome	feature	material	sample	library	location	country	coordinates	download
mgm4702467.3	06032015b_S2_L001_R2_001	Large lake biome	lake	water	mgs485560	mgl485562	Cincinnati	USA	39.11, -84.5	metadata submitted
mgm4702469.3	06052015a_S3_L001_R2_001	Large lake biome	lake	water	mgs485566	mgl485568	Cincinnati	USA	39.11, -84.5	metadata submitted
mgm4702471.3	06032015a_S1_L001_R2_001	Large lake biome	lake	water	mgs485554	mgl485556	Cincinnati	USA	39.11, -84.5	metadata submitted

PREGO methodology

3 channels of information - 1 framework



Source databases integrated in PREGO

and the number of items retrieved

Source	# Items	Data Type	Metadata	License
MEDLINE and PubMed	33 million	abstracts (text)	no	NLM Copyright
PubMed Central OA Subset	2.7 million	full article (text)	no	CC for Commercial, non-commercial
JGI IMG	9,644	Isolates Annotated genomes	yes	JGI Data Policy
Struo	21,276	Annotated genomes	no	MIT, CC BY-SA 4.0
BioProject	18,752	Annotated genomes with abstracts (text)	yes	INSDC policy
MG-RAST	16,096	marker gene samples	yes	CC0
	7,965	metagenomic samples	yes	CC0
MGnify	10,500	marker gene samples	yes	CC-BY, CC0

PREGO in action

with a taxon as an entry point

Desulfatiglans anilini DSM 4660 [1121399]

Synonyms: Desulfatiglans anilini DSM 4660, D. anilini DSM 4660, D anilini DSM 4660, Desulfatiglans DSM 4660, Desulfatiglans str. DSM 4660 ...

Environments | Biological Processes | Molecular Function | Documents | Downloads

Literature

Search:

Name	Z-score	Confidence
Oil seep	3.0	★★☆☆☆
Marine mud	3.0	★★☆☆☆
Marine sediment	2.8	★★☆☆☆
Brackish water	2.4	★★☆☆☆
Oil reservoir	2.2	★★☆☆☆
Anaerobic sediment	2.0	★☆☆☆☆
Cold seep	1.8	★☆☆☆☆
Contaminated sediment	1.7	★☆☆☆☆
Neritic sub-littoral zone	1.4	★☆☆☆☆
Oil spill	1.4	★☆☆☆☆
Petroleum	1.1	★☆☆☆☆
Sea floor	1.1	★☆☆☆☆

Showing 1 to 12 of 12 entries

Environments | Biological Processes | Molecular Function | Documents | Downloads

Literature

Search:

Name	Z-score	Confidence
benzoyl-CoA catabolic process	4.3	★★★☆☆
Benzene catabolic process	3.6	★★☆☆☆
Acetone metabolic process	3.5	★★☆☆☆
Phenanthrene catabolic process	3.5	★★☆☆☆
Sulfate reduction	3.3	★★☆☆☆
Ketone body catabolic process	3.2	★★☆☆☆
Naphthalene catabolic process	3.2	★★☆☆☆
Alkane catabolic process	3.1	★★☆☆☆
Benzoate catabolic process	3.0	★★☆☆☆
Denitrification pathway	2.8	★★☆☆☆
Sulfide ion homeostasis	2.6	★★☆☆☆
Ketone catabolic process	2.6	★★☆☆☆
Methanogenesis	1.8	★☆☆☆☆
Electron transport chain	1.0	★☆☆☆☆

Showing 1 to 14 of 14 entries

The PREGO knowledge-base is available at http://prego.hcmr.gr/.

Conclusions

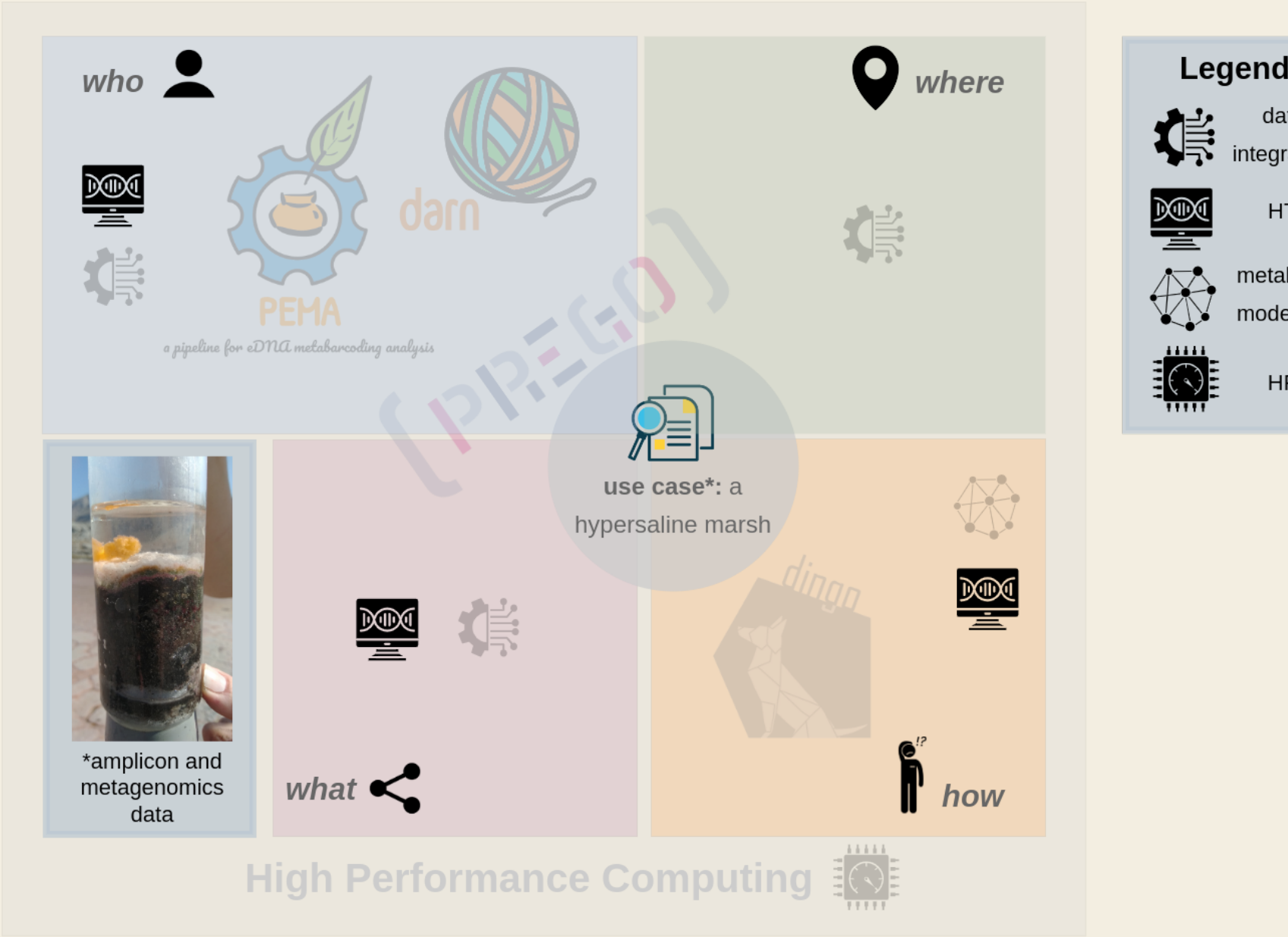
on PREGO and its associations

- metadata can give substantial added value in experimental data
- data integration methods may lead to associations not mentioned in the literature
- more and more valid associations as omics' dataset keep increasing exponentially and metadata standards gain space

Deciphering the functional potential of a hypersaline marsh microbial mat community

Aim of the study and contribution

To exploit state-of-the-art methods to identify taxa and functions that play a key part in microbial community assemblages in hypersaline sediments.

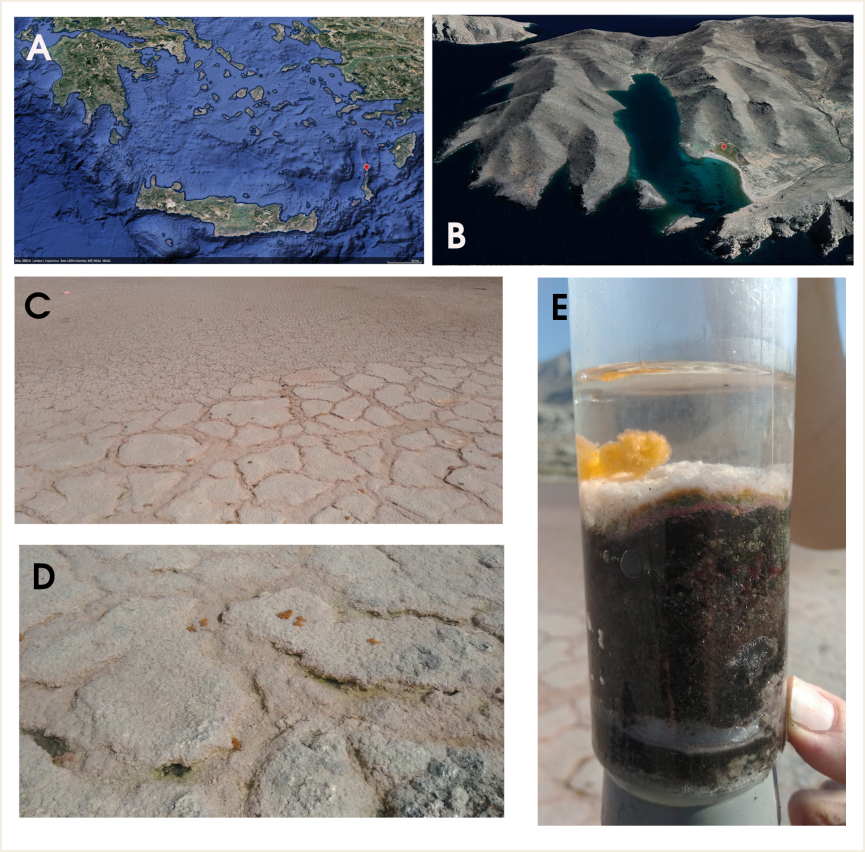

who
where
darn
PEMA
a pipeline for eDNA metabarcoding analysis
(PREGO)
use case*: a hypersaline marsh
dingo
*amplicon and metagenomics data
what
how
!?
High Performance Computing
Legend
data integration
HTS
metabolic modelling
HPC

Tristomo swamp in Karpathos

a seasonal brackish water marsh

Sample	Type	Season
Elos01	top	summer
Elos02	bottom	summer
Elos03	orange aggregate	summer
Elos07	pink aggregate	summer
Elos10	combined*	winter
Elos12	combined	winter

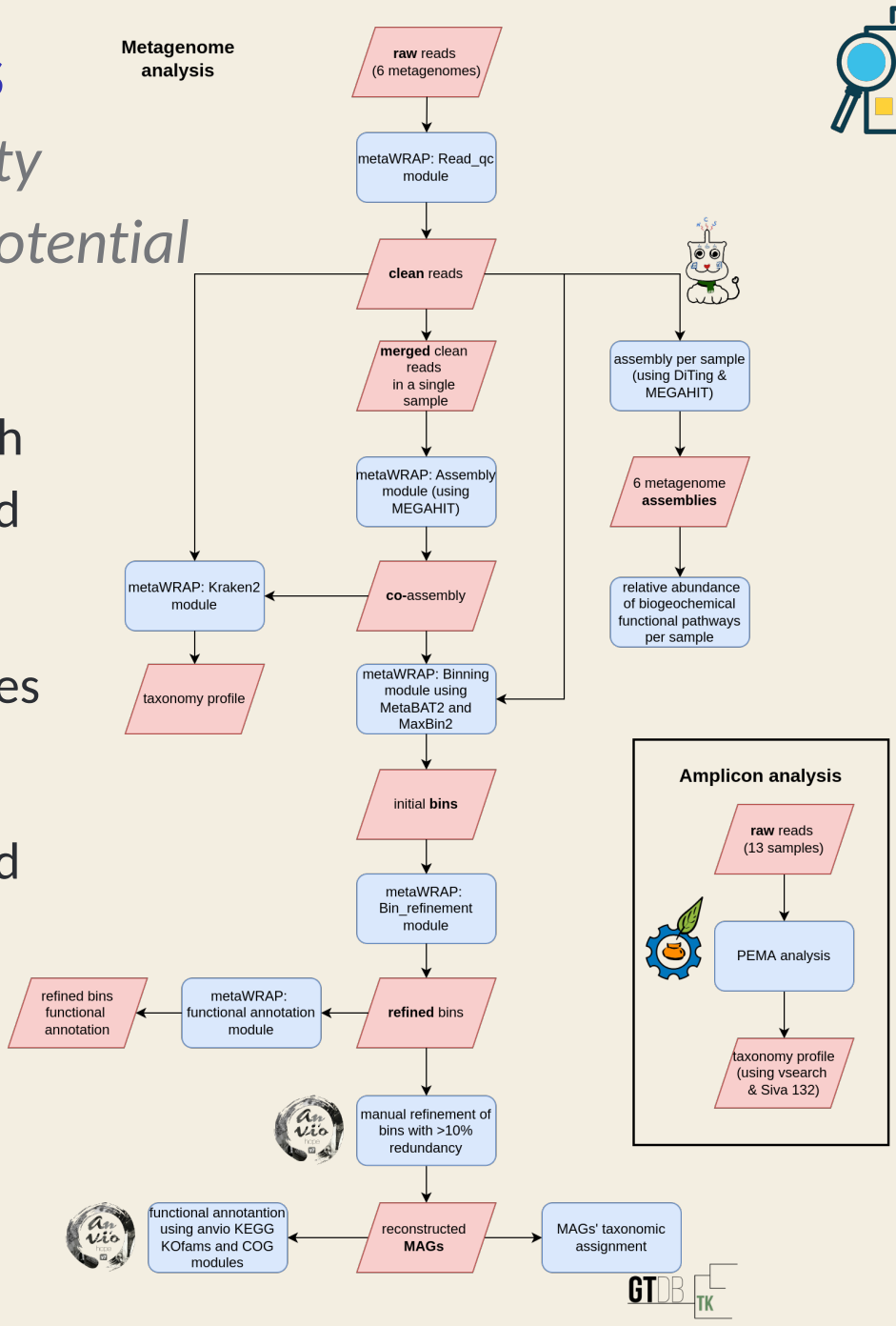
*combined: samples with no slicing



Bioinformatics analysis

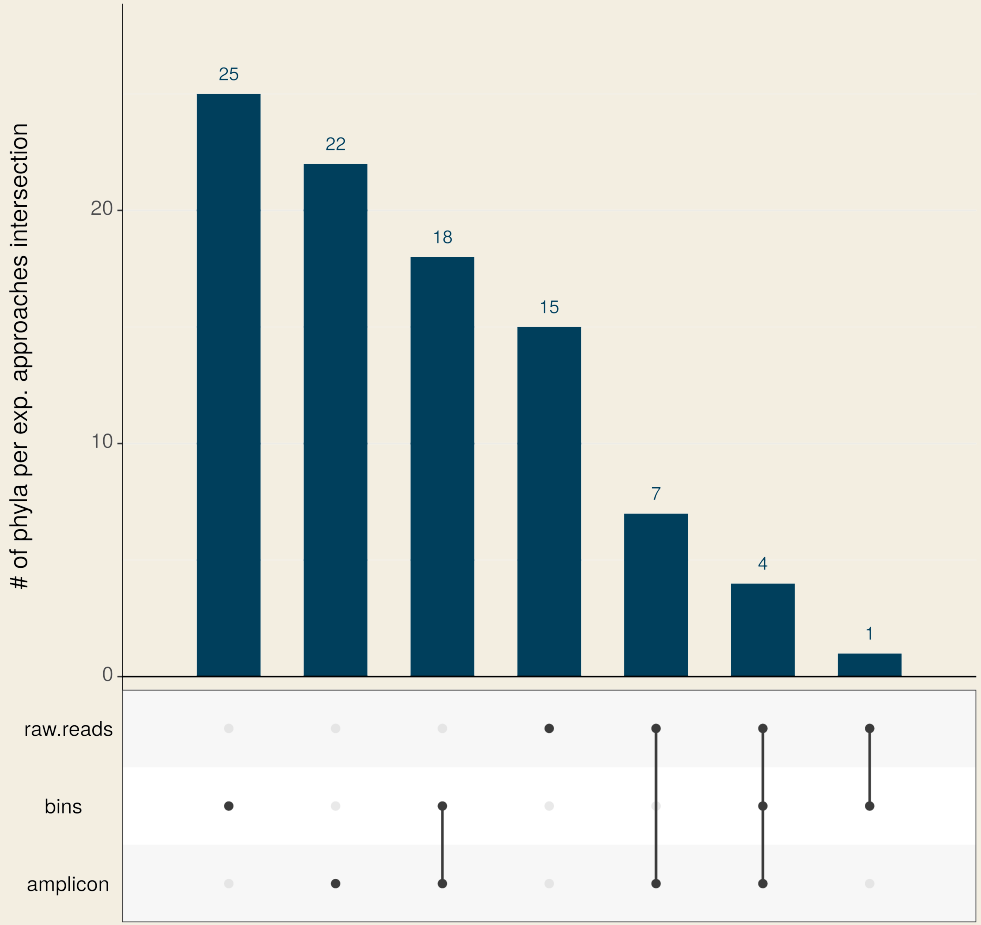
from raw data to community composition & functional potential

- metagenomic reads were both co - assembled and assembled at the sample level
- taxonomic & functional profiles per sample were retrieved
- MAGs were reconstructed and annotated



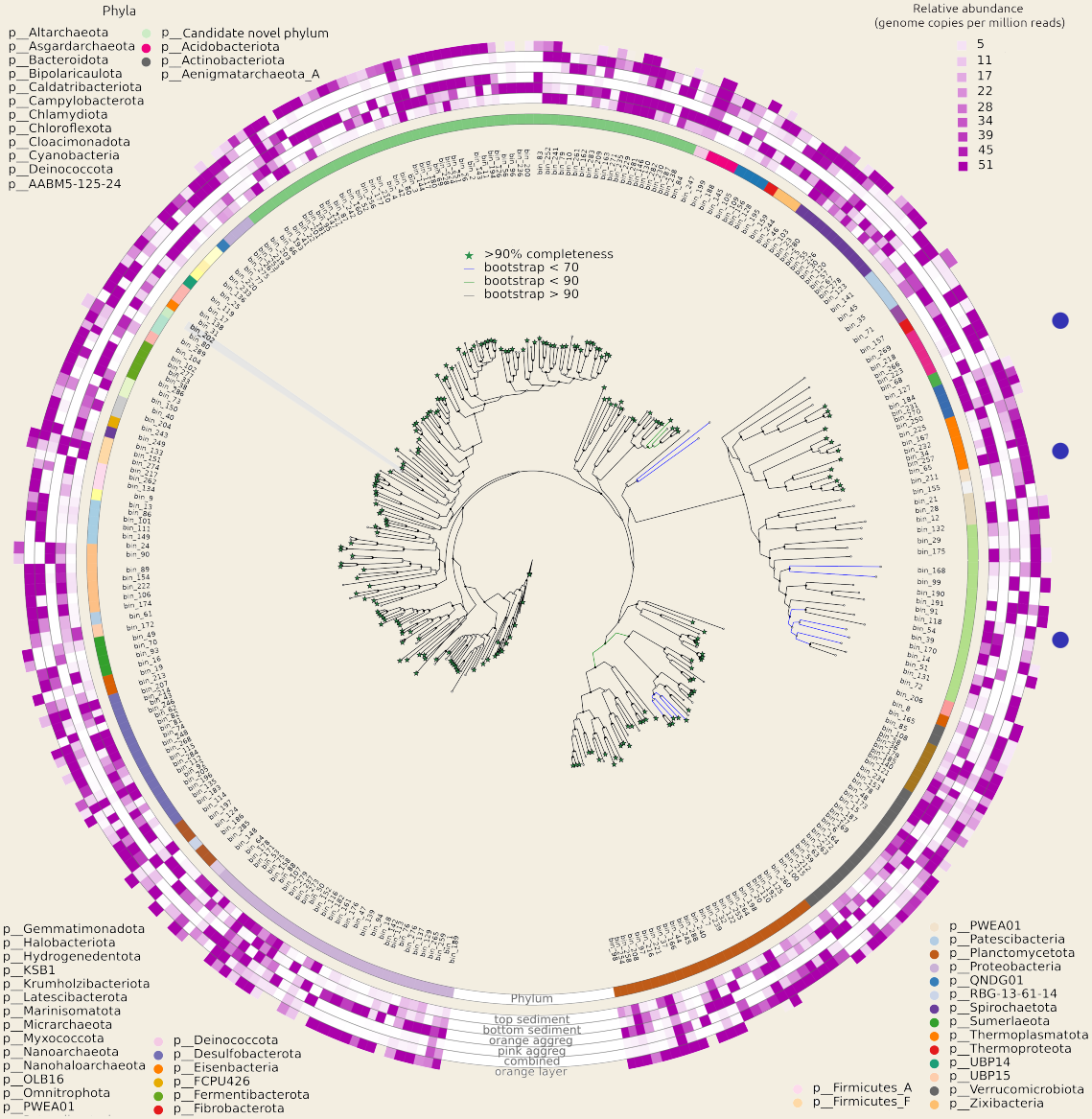
Amplicon vs metagenomics

number of phyla retrieved per method



MAGs phylogeny

based on 25 single-copy genes



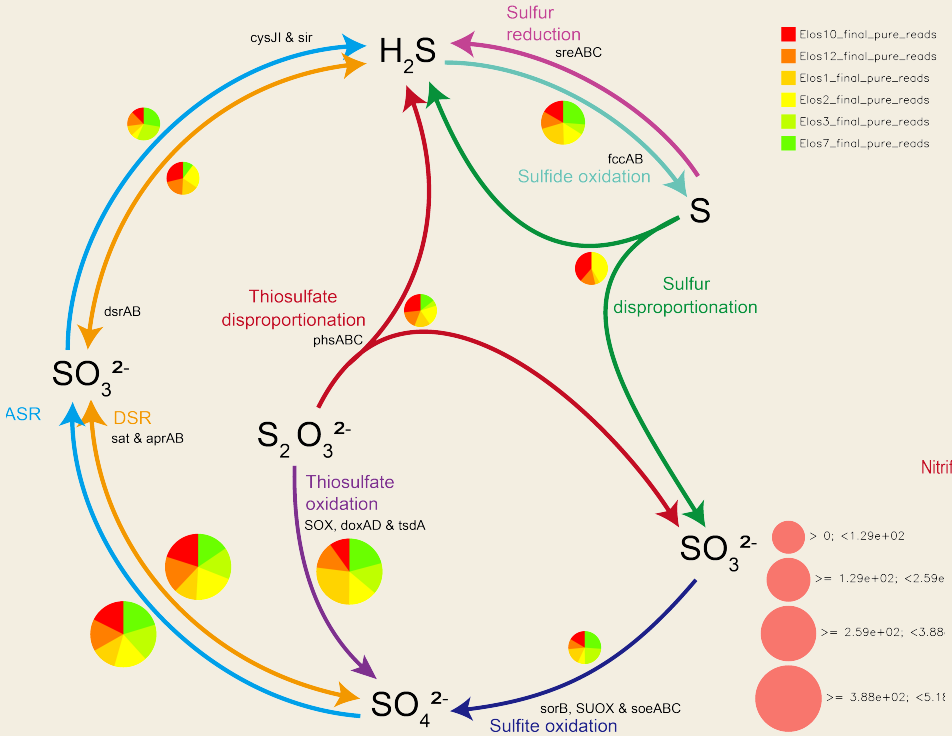
- **Bacteroidota** & **Proteobacteria**: most abundant
- **Myxococcota**: only in aggregates

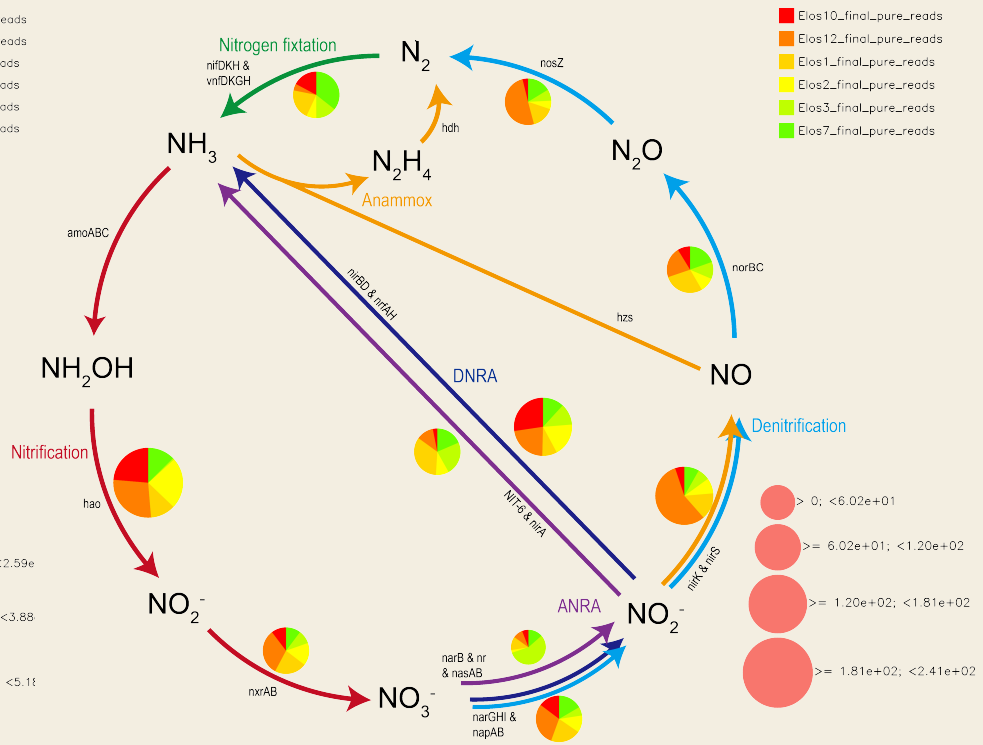
- **Nanoarchaeota**: mostly in top layer
- **Asgardarchaeota** & **Thermoplasmatota**: absent from aggregates
- **Halobacteriota**: absent from the winter samples

The S and the N cycle

using KEGG annotation terms

Sample	Type	Season
Elos01	top	summer
Elos02	bottom	summer
Elos03	orange aggregate	summer
Elos07	pink aggregate	summer
Elos10	combined	winter
Elos12	combined	winter





Conclusions

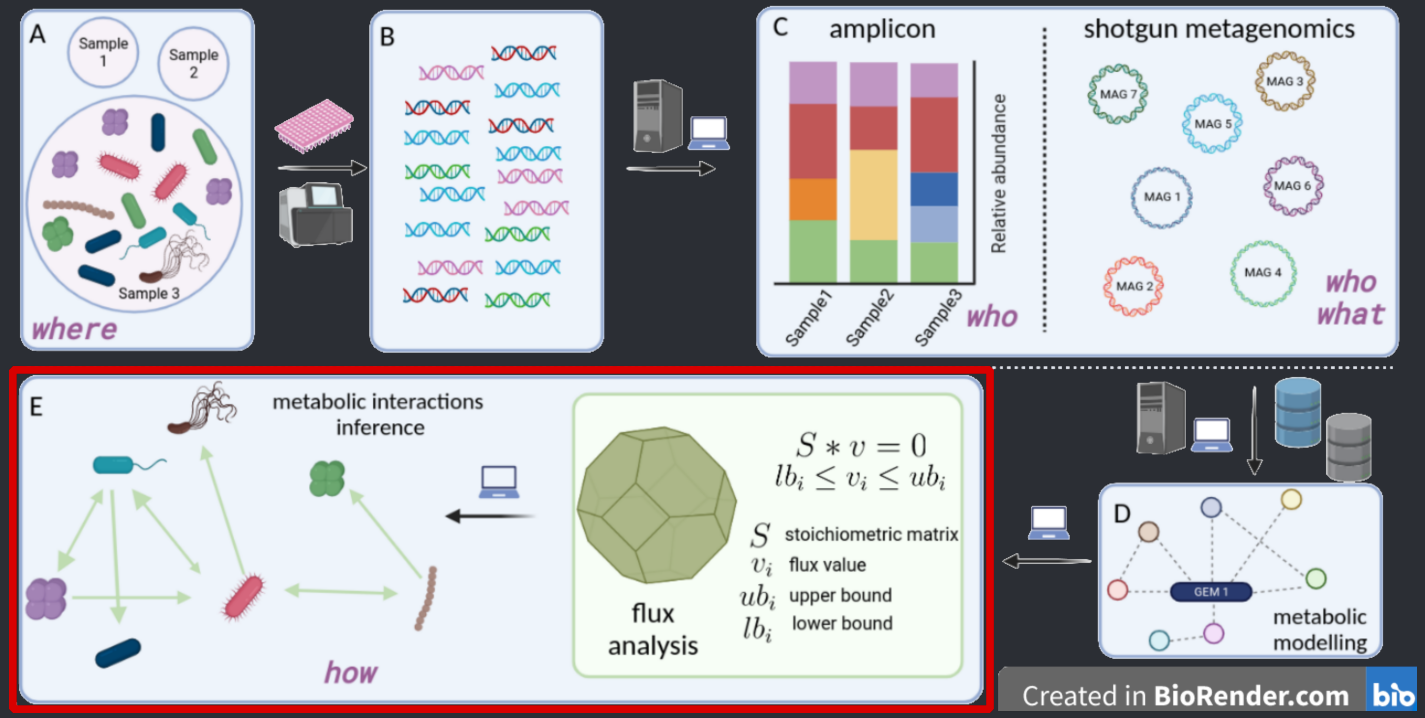
based on metagenomics analysis in the Tristomo marsh

- shotgun metagenomics are a poweful tool in the discovery of novel taxa
- seasonality plays a key-role for the mats under study, ensuring oxygenic photosynthesis
- anaplerotic reactions may play a key-role in replenishing the intermediates of the TCA cycle
- metabolic modelling can shed further light on the mechanisms governing the mat communities

General conclusions

- Bioinformatics approaches enhance microbial diversity assesment based on HTS data.
- Containerization technologies and e-infrastructures provide the means for computational capacity and reproducibility.
- High quality metadata enable efficient exploitation of sequencing data in a meta-analysis level.
- Markov Chain Monte Carlo approaches enable flux sampling in high-dimensional polytopes.
- Hypersaline mats host a great range of novel taxa & their functioning might be subject to anaplerotic reactions.

Future perspectives

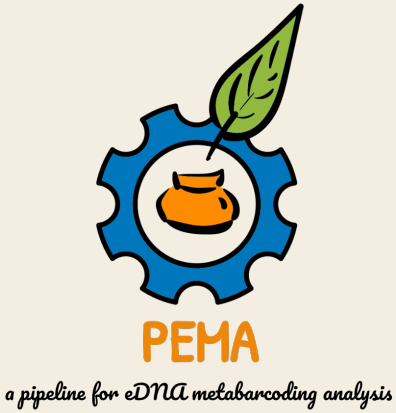


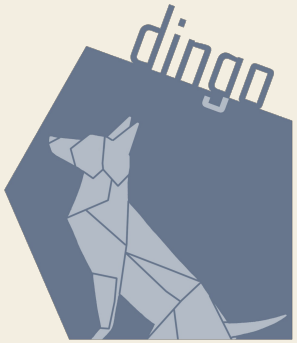
A (bit) more holistic framework

"a combination of quantitative high - throughput experiments and predictive metabolic models can elucidate the genotype - phenotype map of microbial metabolic strategies" - Bajic and Sanchez (2020)

Wrap-up

software





github.com/hariszaf/pema

github.com/hariszaf/darn

github.com/lab42open-team/ the `prego*` repositories

github.com/GeomScale/dingo

Wrap-up

publications

[1] **Zafeiropoulos, H.**, Paragkamian, S., Ninidakis, S., Pavlopoulos, G.A., Jensen, L.J. & Pafilis, E. (2022). PREGO: a literature- and data-mining resource to associate microorganisms, biological processes, and environment types. Microorganisms 10(2), 293.

[2] **Zafeiropoulos, H.**, Gargan, L., Hintikka, S., Pavloudi, C. & Carlsson, J. (2021). The Dark mAtteR iNvestigator (DARN) tool: getting to know the known unknowns in COI amplicon data. Metabarcoding and Metagenomics, 5, e69657.

[3] Polymenakou, P.N., Nomikou, P., **Zafeiropoulos, H.**, ..., Kyrpides, N.C., Kotoulas, G. & Magoulas, A. (2021). The santorini volcanic complex as a valuable source of enzymes for bioenergy. Energies, 14(5), p.1414.

[4] Chalkis, A., Fisikopoulos, V., Tsigaridas, E. & **Zafeiropoulos, H.** (2021). Geometric algorithms for sampling the flux space of metabolic networks, 37th International Symposium on Computational Geometry (SoCG 2021).

[5] **Zafeiropoulos, H.**, Gioti, A., Ninidakis, S., Potirakis, A., Paragkamian, S., ... & Pafilis, E. (2021). 0s and 1s in marine molecular research: a regional HPC perspective. GigaScience, 10(8), giab053.

[6] **Zafeiropoulos, H.**, Viet, H. Q., Vasileiadou, K., Potirakis, A., Arvanitidis, C., Topalis, P., Pavloudi, C. & Pafilis, E. (2020). PEMA: a flexible Pipeline for Environmental DNA Metabarcoding Analysis of the 16S/18S ribosomal RNA, ITS, and COI marker genes. GigaScience, 9(3), giaa022.

[7] Paragkamian, S., Sarafidou, G., ..., **Zafeiropoulos, H.**, Arvanitidis, C., Pafilis, E. & Gerovasileiou, V. Automating the curation process of historical literature on marine biodiversity using text mining: the DECO workflow ***(accepted in Frontiers in Marine Science)***

[8] Pavloudi, C. & **Zafeiropoulos, H.** (2022) Deciphering the community structure and the functional potential of a hypersaline marsh microbial mat community ***(under review at FEMS Microbiology Ecology)***

[9] Garza, D.R., Gonze, D., **Zafeiropoulos, H.**, Liu, B. & Faust, K., (2022) Metabolic models of human gut microbiota: advances and challenges ***(under review at Cell systems)***

[10] Chalkis, A., Fisikopoulos, V., Tsigaridas, E. & **Zafeiropoulos, H.** dingo: a Python library for metabolic networks analysis ***(under preparation)***

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Prof. Jens Carlsson

Prof. Karoline Faust

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Dr. Laura Gargan

Dr. Sanni Hintikka

Mr. Stelios Ninidakis

Mr. Antonis Potirakis

Dr. Apostolos Chalkis

Dr. Vissarion Fisikopoulos

Prof. Elias Tsigaridas

My mojo:

Dr. Christina Pavloudi

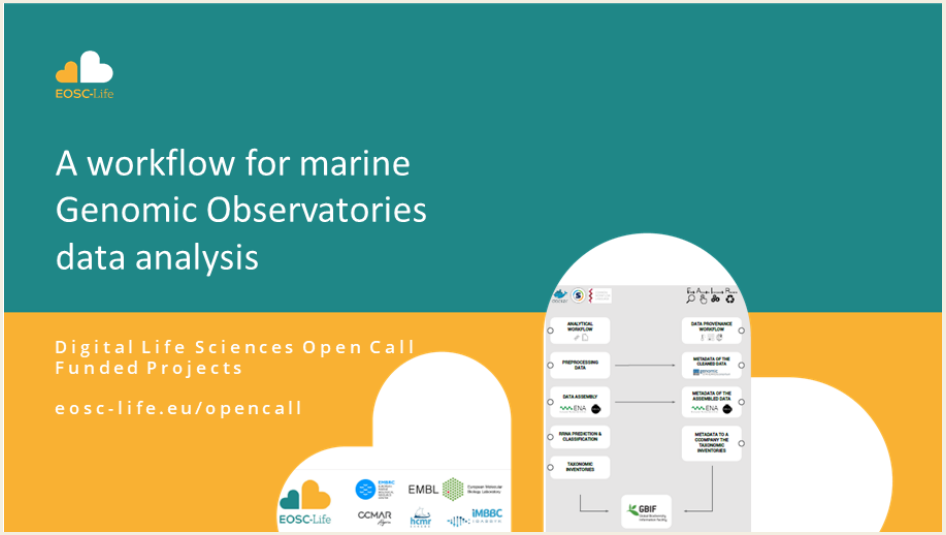
My corner:

Would not be here

if it was not with you.

Computing infrastructures

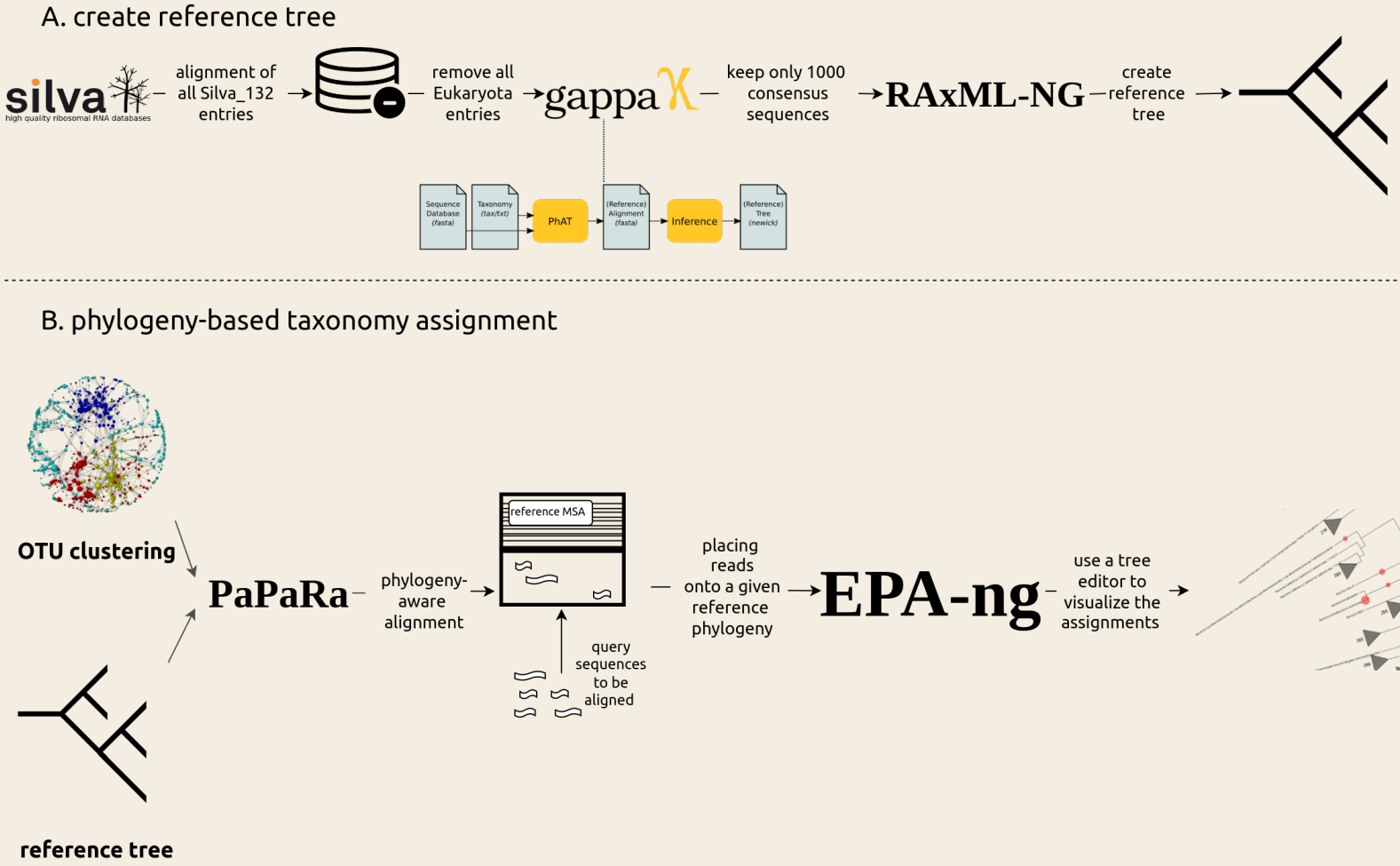
an alternative for the most!



We will develop a workflow for the analysis of Genomic Observatories data that will allow researchers to deal better with the increasing amount of data.

PEMA features

phylogeny-based taxonomy assigment for the case of 16S rRNA gene



A real case hypothesis generation scenario

Posidonia and its microbiome

What about ***Posidonia*** ?
Literature suggests that

Planctomycetes
and especially *Blastopirellula*
and *Rhodopirellula*
are commonly
found in its microbiome.
Why so ?
Let us have a look here!

chapter 3

Co-mentioning and scoring scheme

which are the most worthy and relevant associations

- genome annotation oriented associations: fixed scores
- associations in the *Environmental Samples* channel are scored based on the number of samples in which they co-occur
- similarly, in the *Literature* channel, based on the number of publications

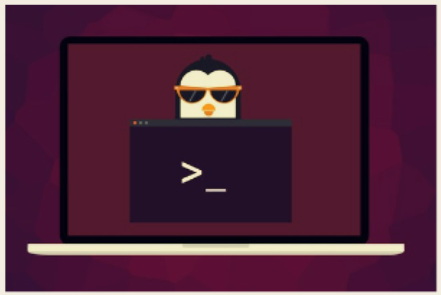
		Y = y		
		Yes	No	Total
X = x	Yes	$c_{x,y}$	$c_{x,0}$	$c_{x,.}$
	No	$c_{0,y}$	$c_{0,0}$	$c_{0,.}$
	Total	$c_{.,y}$	$c_{.,0}$	$c_{.,.}$

Environmental samples score:

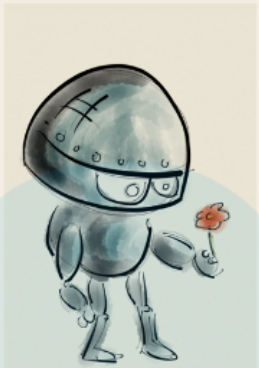
$$score_{x,y} = 2.0 * \sqrt{\frac{c_{x,y}}{c_{.,y}}}^{a} \quad (1)$$

Building a knowledge-base

development and information technology operations

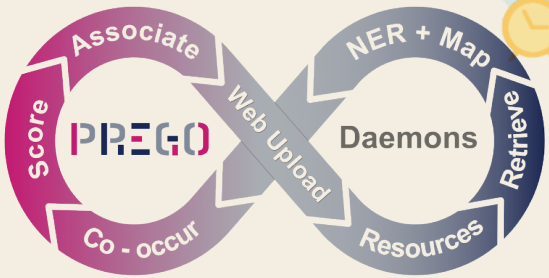


frequently updates;
`robots` allow to keep up with
literature and metagenomic data



building a
knowledge base (KB)

server dedicated
for building and
accessing the KB



From concentrations to fluxes

to study changing environments

chapter 4

We can describe the mass balance of a chemical compound as the difference between the sum of the fluxes of all the reactions that form it and the sum of all that degrade it.

$$\frac{d\omega_i}{dt} = \sum_k s_{ik} v_k = \langle s_i, v \rangle$$

and thus:

$$\frac{d\omega}{dt} = Sv$$

The region of steady states

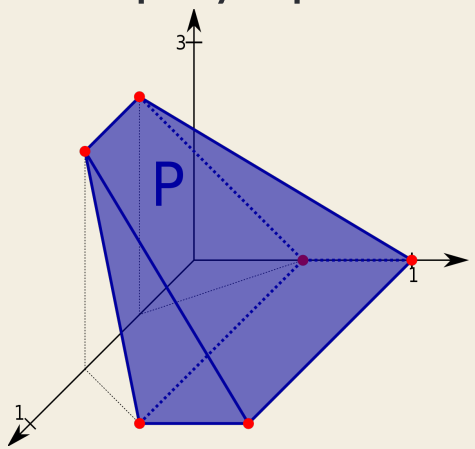
moving to full dimensional polytope

The *constraints* on the reactions fluxes.

$$\begin{aligned} Sv &= 0, \\ v_{lb} \leq v &\leq v_{ub} \end{aligned} \quad (2) \quad v = Nx$$

$S \in \mathbb{R}^{m \times n}, v \in \mathbb{R}^n$

As a *full dimensional* polytope



$$P := \{x \in \mathbb{R}^d | Ax \leq b\}$$

$N \in \mathbb{R}^{n \times d}$ denotes the matrix of the null space of S, i.e. $SN = 0_{m \times d}$.

By replacing v with Nx in Equation 2, we get the full dimensional polytope P, where $A = \begin{pmatrix} I_n N \\ -I_n N \end{pmatrix}$ and $b = \begin{pmatrix} v_{ub} \\ v_{lb} \end{pmatrix} N$, (in $\mathbb{R}^d$).

Computational geometry basics

A powerful approach to obtain well **roundness** is to put P in *near isotropic position*. In general, $K \subset \mathbb{R}^d$ is in isotropic position if the uniform distribution over K is in isotropic position, that is $\mathbb{E}_{X \sim K}[X] = 0$ and $\mathbb{E}_{X \sim K}[X^T X] = I_d$, where I_d is the $d \times d$ identity matrix.

Thus, to put a polytope P into isotropic position one has to generate a set of uniform points in its interior and apply to P the transformation that maps the point-set to isotropic position; then iterate this procedure until P is in c-isotropic position for a constant c.

Random walk performance metrics

- **cost per iteration:** the number of operations the algorithm needs to sample a single point
- **mixing time:** the number of samples the algorithm needs to burn in order to lose dependency from previous iterations (i.i.d)
- **cost per sample:** the total number of operations the algorithm needs to sample an i.i.d point

MCMC convergence diagnostics:

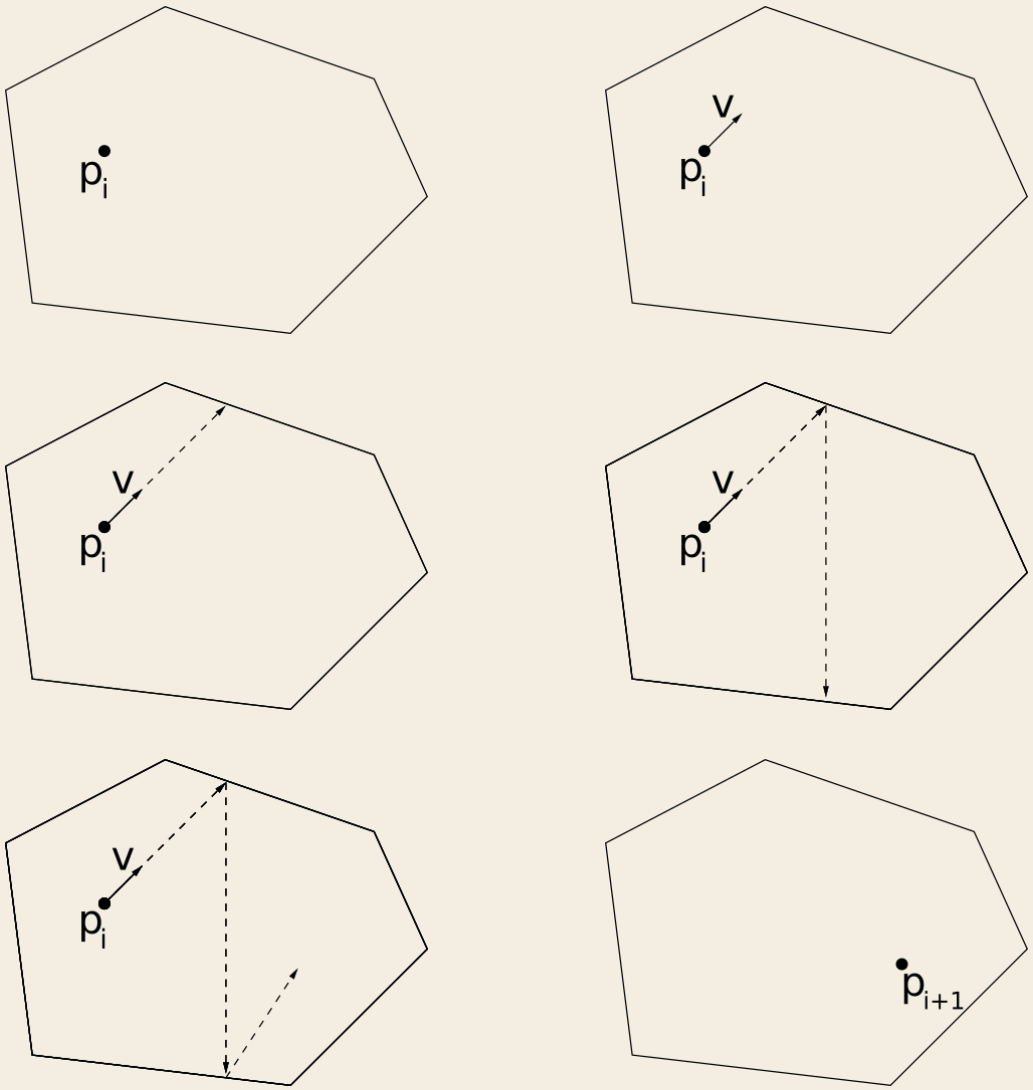
- **Effective Sample Size (ESS):** the number of effectively independent draws from the target distribution that the Markov chain is equivalent to

  a function proportional to the ratio between the variance of the ideal Monte Carlo estimator over the variance of the estimator obtained by MCMC using the same number of samples

- **Potential Scale Reduction Factor (PSRF)** statistical test to evaluate the quality of a generated sample

Billiard walk

for random sampling



Generate the length of the trajectory $L \sim D$.

Pick a uniform direction v to define the trajectory.

The trajectory reflects on the boundary if necessary.

Return the end of the trajectory as $p_i + 1$.

Find possible targets against SARS-CoV-2

a flux sampling application

Bioinformatics, 36(26), 2020, i813–i821
doi: 10.1093/bioinformatics/btaa813
ECCB2020

OXFORD

Systems

FBA reveals guanylate kinase as a potential target for antiviral therapies against SARS-CoV-2

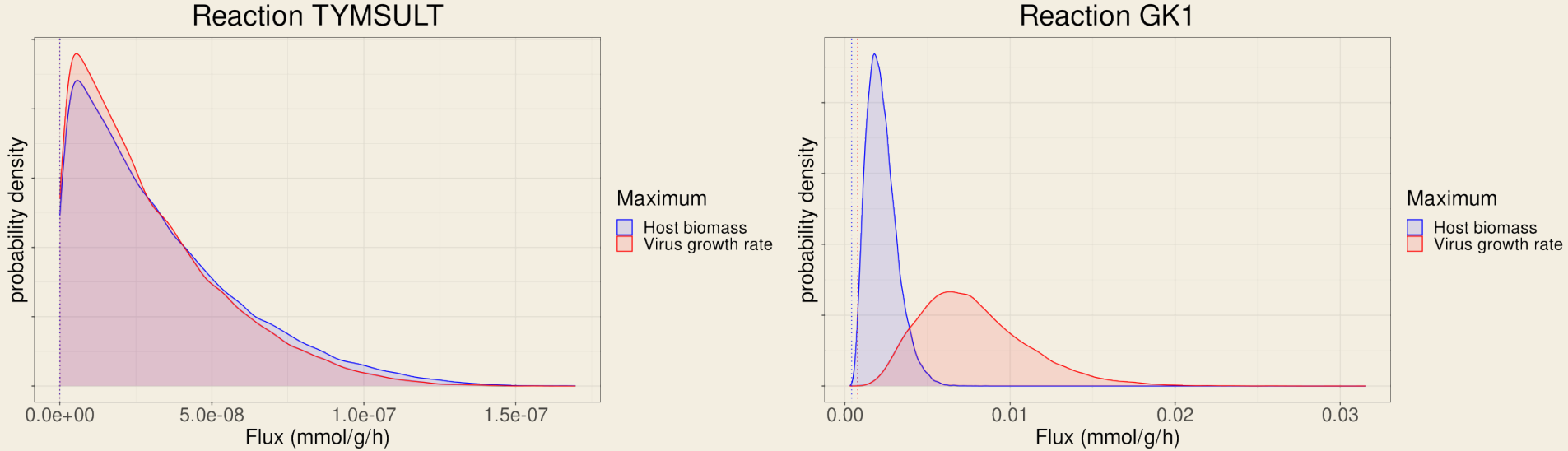
Alina Renz[1,2,*], Lina Widerspick[1] and Andreas Dräger[1,2,3,*]

[1]Computational Systems Biology of Infections and Antimicrobial-Resistant Pathogens, Institute for Bioinformatics and Medical Informatics (IBMI) and [2]Department of Computer Science, University of Tübingen, Tübingen 72076, Germany and [3]German Center for Infection Research (DZIF), partner site Tübingen, Germany

- Renz *et al.* 2020 built the biomass function of SARS-Cov-2 to build a host - virus network
- Using FBA they computed an optimal steady state using
  (i) human biomass maintenance
  (ii) virus growth rate
- They found reaction GK1 as a possible anti-viral target

Find possible targets against SARS-CoV-2

a flux sampling application



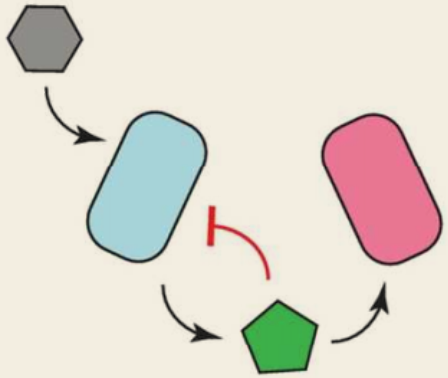
- Check if the flux distribution of a reaction changes.
- Find possible anti-viral targets and study further.

For more about this example case, you may check this blog-post.

Further applications

of metabolic flux sampling

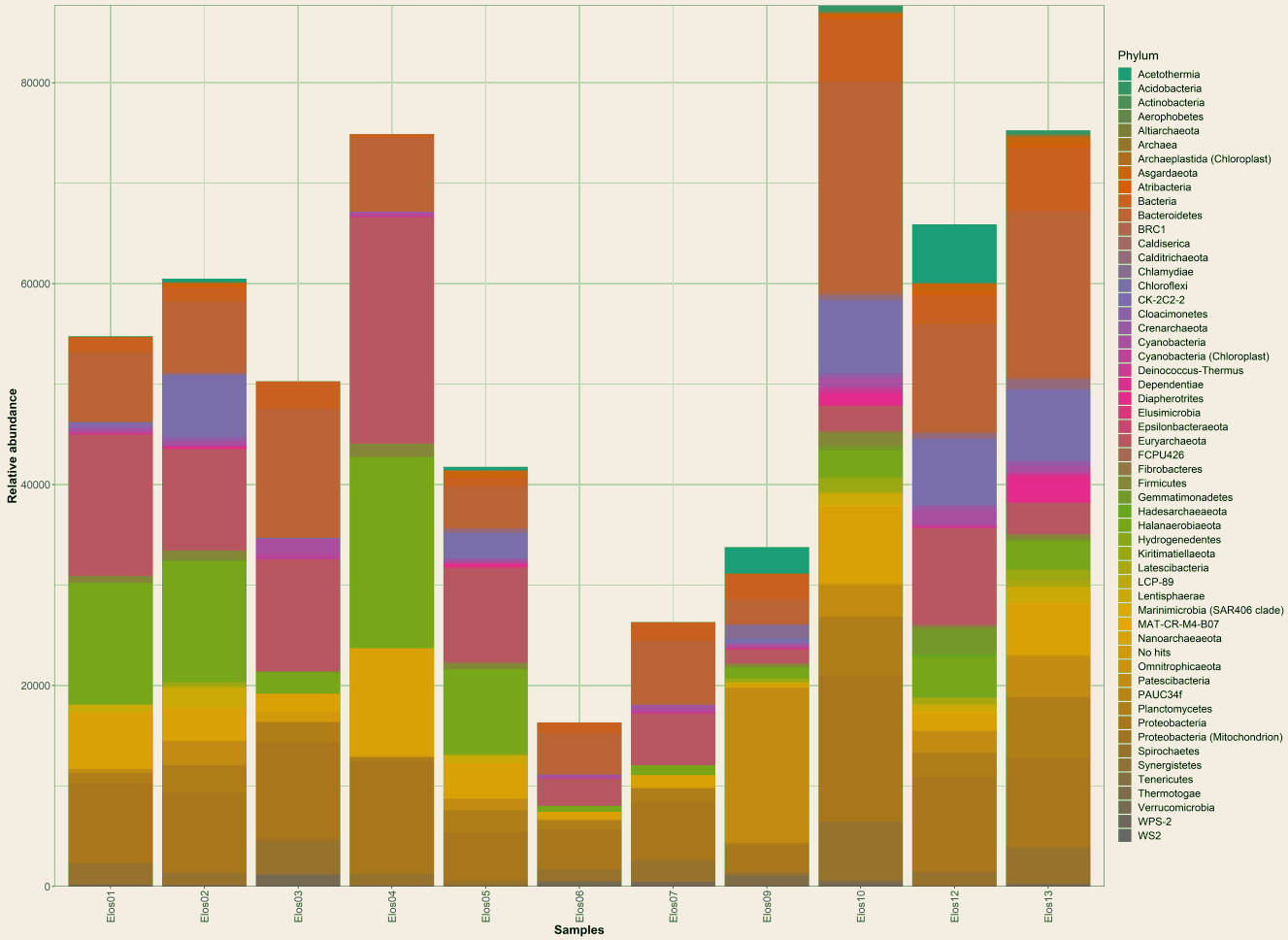
Scott, William T., *et al.* "Metabolic flux sampling predicts strain-dependent differences related to aroma production among commercial wine yeasts." Microbial cell factories 20.1 (2021): 1-15.



What about microbial interactions ?

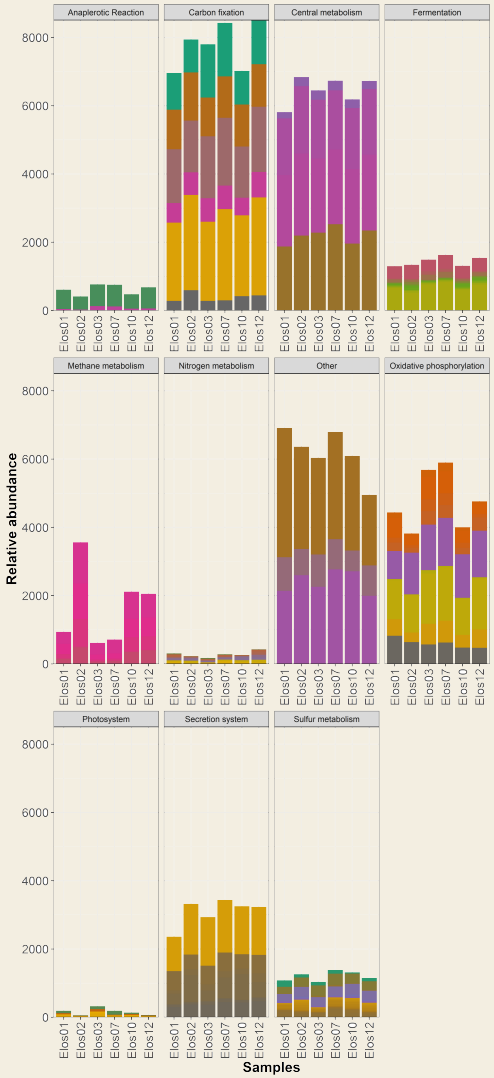
Abundances of the main microbial taxa, at the phylum level

based on 16S rRNA amplicon data



Metabolic pathways per biogeochemical cycle

and their relative abundance at each sample



Pathway

- 3-hydroxypropionate bicycle
- Alternative thiosulfate oxidation (doxAD)
- Alternative thiosulfate oxidation (tsdA)
- Anaplerotic genes
- Anoxygenic photosystem II (pufML)
- Assimilatory nitrate reduction. nitrate -> nitrite (narB or NR or nasAB)
- Assimilatory nitrate reduction. nitrite -> ammonia (NIT-6 or nirA)
- Assimilatory sulfate reduction. sulfate -> sulfite
- Assimilatory sulfate reduction. sulfite -> sulfide (cysJI or sir)
- Bacterial chemotaxis
- Calvin-Benson-Bassham cycle
- Cytochrome b6/f complex (petABCDGLMN)
- Cytochrome bc1 complex
- Cytochrome bd ubiquinol oxidase
- Cytochrome c oxidase. cbb3-type
- Cytochrome c oxidase. prokaryotes
- Cytochrome o ubiquinol oxidase
- Denitrification. nitric oxide -> nitrous oxide (norBC)
- Denitrification. nitrite -> nitric oxide (nirK or nirS)
- Denitrification. nitrous oxide -> nitrogen (nosZ)
- Dicarboxylate-hydroxybutyrate cycle
- Dissimilatory arsenic reduction (arsRBC or arsRDABC)
- Dissimilatory nitrate reduction. nitrate -> nitrite (narGHI or napAB)
- Dissimilatory nitrate reduction. nitrite -> ammonia (nirBD or nrfAH)
- Dissimilatory sulfate reduction. sulfate -> sulfite (reversible) (sat and aprAB)
- Dissimilatory sulfate reduction. sulfite -> sulfide (reversible) (dsrAB)
- DMS oxidation. DMS -> DMSO (ddhABC or tmm)
- DMSO reduction. DMSO -> DMS (dms or dorA)
- Entner-Doudoroff pathway. glucose-6P => glyceraldehyde-3P + pyruvate
- F-type ATPase
- Flagellar assembly
- Gluconeogenesis. oxaloacetate => fructose-6P
- Glycolysis (Embden-Meyerhof pathway). glucose => pyruvate
- Glyoxylate shunt
- Hydroxypropionate-hydroxybutylate cycle
- Methane oxidation. methanol -> Formaldehyde
- Methanogenesis. acetate => methane
- Methanogenesis. CO2 => methane
- Methanogenesis. dimethylamine -> methane
- Methanogenesis. methanol => methane
- Methanogenesis. methylamine -> methane
- Methanogenesis. trimethylamine -> methane
- Mixed acid. acetate
- Mixed acid. ethanol. acetate to acetylaldehyde
- Mixed acid. ethanol. Acetyl-CoA to acetylaldehyde (reversible)
- Mixed acid. ethanol. acetylaldehyde to ethanol
- Mixed acid. formate
- Mixed acid. formate to CO2 & H10
- Mixed acid. formate to CO2 & H2
- Mixed acid. formate to CO2 & H3
- Mixed acid. formate to CO2 & H4
- Mixed acid. formate to CO2 & H5
- Mixed acid. formate to CO2 & H6
- Mixed acid. formate to CO2 & H7
- Mixed acid. formate to CO2 & H8
- Mixed acid. formate to CO2 & H9
- Mixed acid. lactate (Pyruvate to Lactate)
- Mixed acid. succinate (phosphoenolpyruvate to succinate via oxaloacetate. malate & fumarate)
- NAD(P)H-quinone oxidoreductase
- NADH-quinone oxidoreductase
- Nitrification. hydroxylamine -> nitrite (hao)
- Nitrification. nitrite -> nitrate (nxrAB)
- Nitrogen fixation. nitrogen -> ammonia (nifKDH or vnfDKGH)
- Photosystem I (psaABCDEF)
- Photosystem II (psbABCDEF)
- Reductive citrate cycle
- Sec-SRP
- Succinate dehydrogenase (ubiquinone)
- Sulfhydrogenase. (sulfide)n -> (sulfide)n-1
- Sulfhydrogenase. (sulfide)n -> (sulfide)n-2
- Sulfhydrogenase. (sulfide)n -> (sulfide)n-3
- Sulfhydrogenase. (sulfide)n -> (sulfide)n-4
- Sulfide oxidation. sulfide -> sulfur (fccAB)
- Sulfite oxidation. sulfite -> sulfate (sorB. SUOX. soeABC)
- Sulfoquinovose degradation (SFT pathway)
- Sulfoquinovose degradation (sulfo-EMP pathway) (yihSVTU)
- Sulfur dioxygenase
- Sulfur disproportionation. sulfur -> sulfide & sulfite
- TCA cycle
- Thiosulfate disproportionation. thiosulfate -> sulfide & sulfite (phsABC)
- Thiosulfate oxidation by SOX complex. thiosulfate -> sulfate
- Twin arginine targeting
- Type I Secretion
- Type II Secretion
- Type III Secretion
- Type IV Secretion
- Type VI Secretion
- V/A-type ATPase
- Wood-Ljungdahl pathway