



Metagenomics Assessment of BIOMETORE samples

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Outline of this task

Who is there? (*ecology*)

deep sequencing of 16S + 18S RNA genes to determine taxonomic composition of samples

What is there? (*application*)

deep sequencing all all genes to investigate which metabolic pathways characterise each sample, discover new enzymes, industrially useful organisms etc.

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all results today are thus restricted to

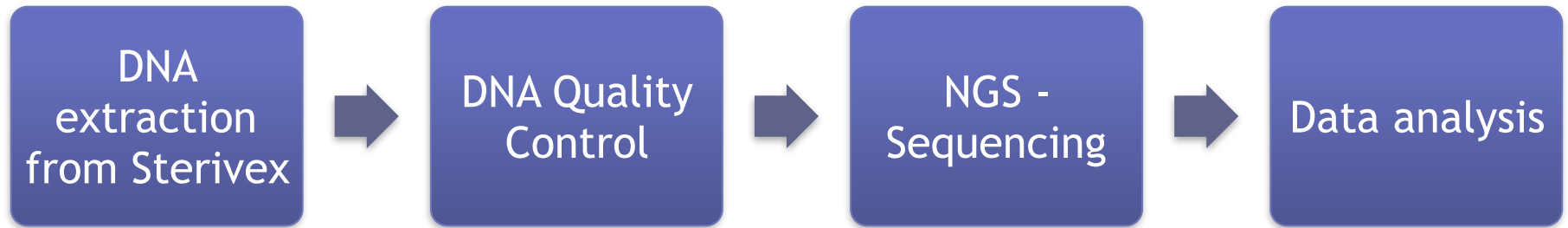
- prokaryotes
- photosynthetic organisms (chloroplast genome)

What is there? (*application*)

deep sequencing all all genes to investigate which metabolic pathways characterise each sample, discover new enzymes, industrially useful organisms etc.

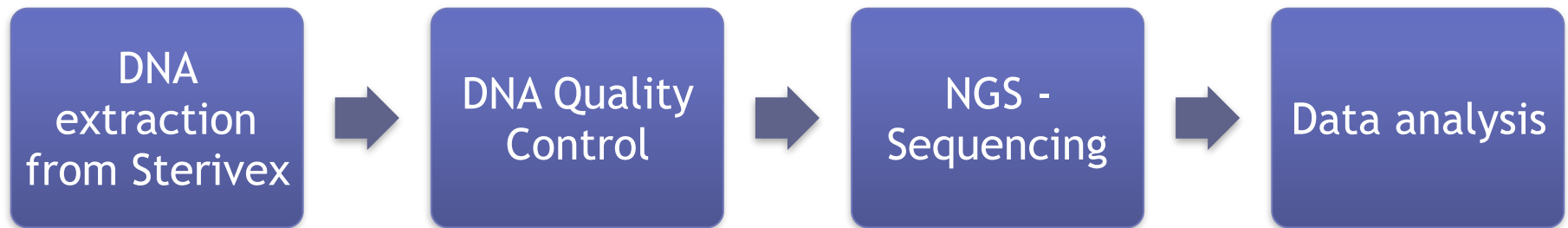
Approach

Initial plan : Process **125** samples for 16S and 18S rRNA gene metagenomics from Azores campaign



Approach

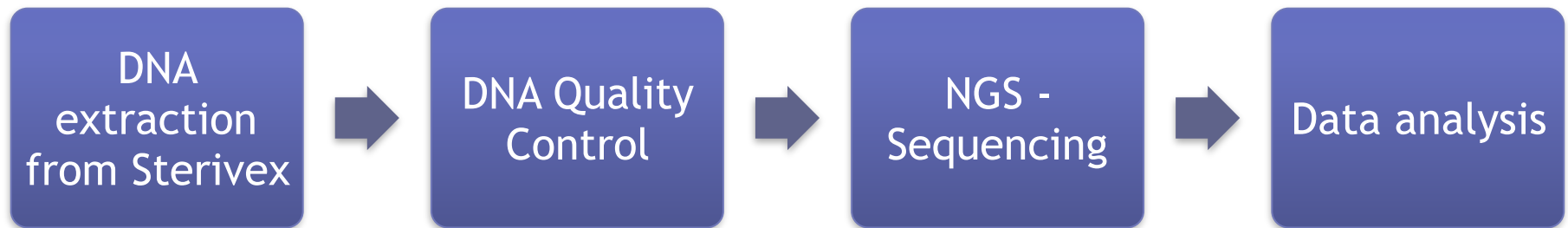
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Water samples collected
using sterivex tubes during the campaigns : **535**

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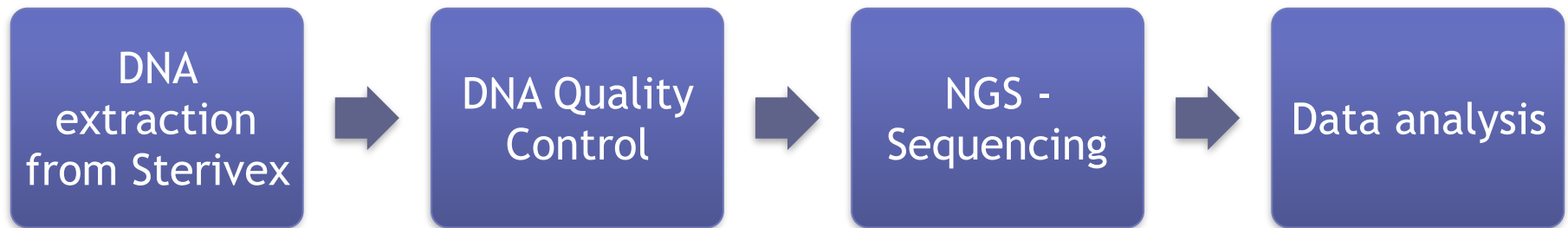


Water samples collected
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380 samples out of **535** were processed.

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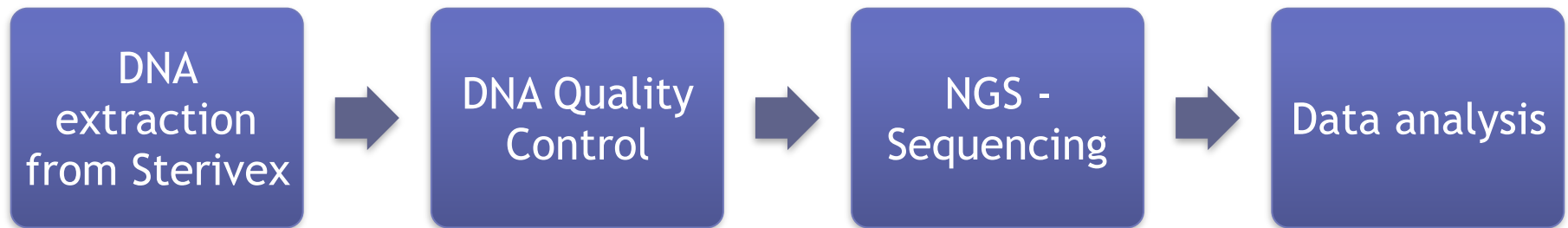
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330 passed quality filtering

Problems: Low DNA content or presence of PCR inhibitors

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**16S RNA
sequenced**

Visual Protocol -1



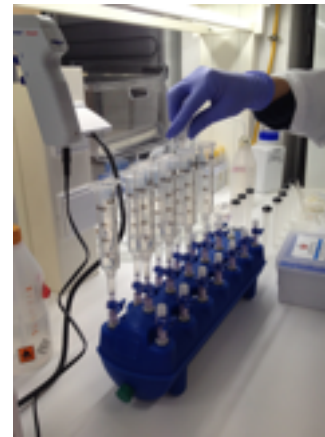
Samples were collected on board, filtered and stored in LN_2



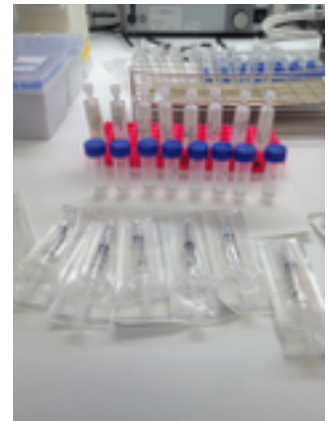
Upon arrival samples were transferred to -80°C after label checking



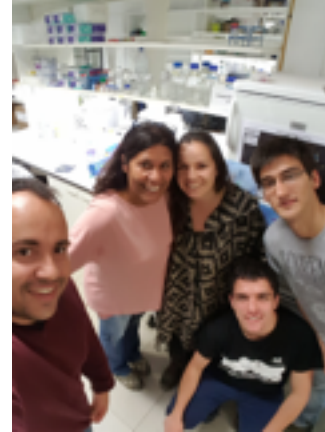
All the first extractions steps are conducted inside the filter



After retrieving DNA from the filter it is filtered to concentrate it and clean it.



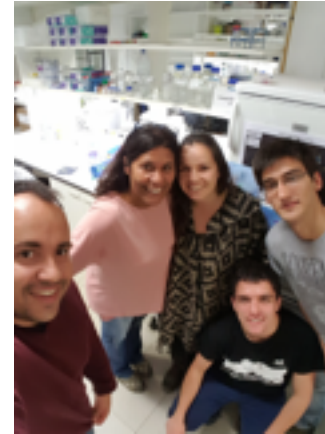
Visual Protocol -2



All the samples were measured
twice using nanodrop and Qubit
before sequencing

Happiness upon completion

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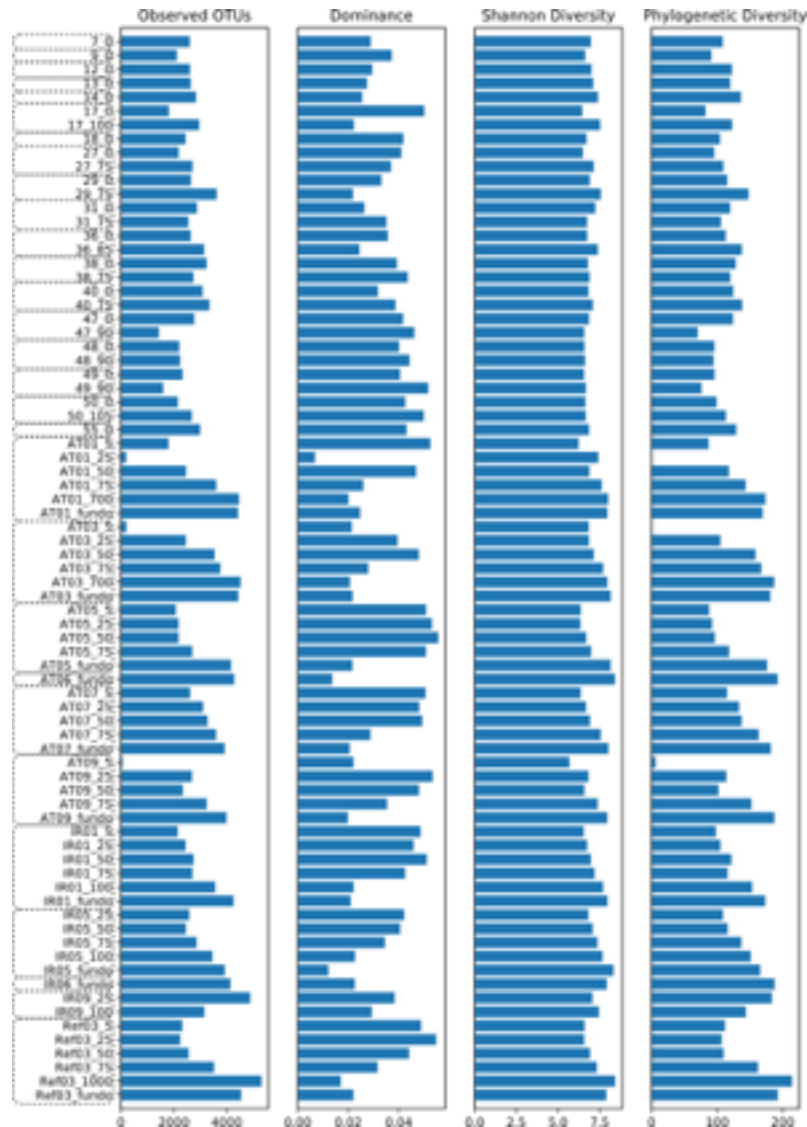
António Sousa
Catarina Magalhães



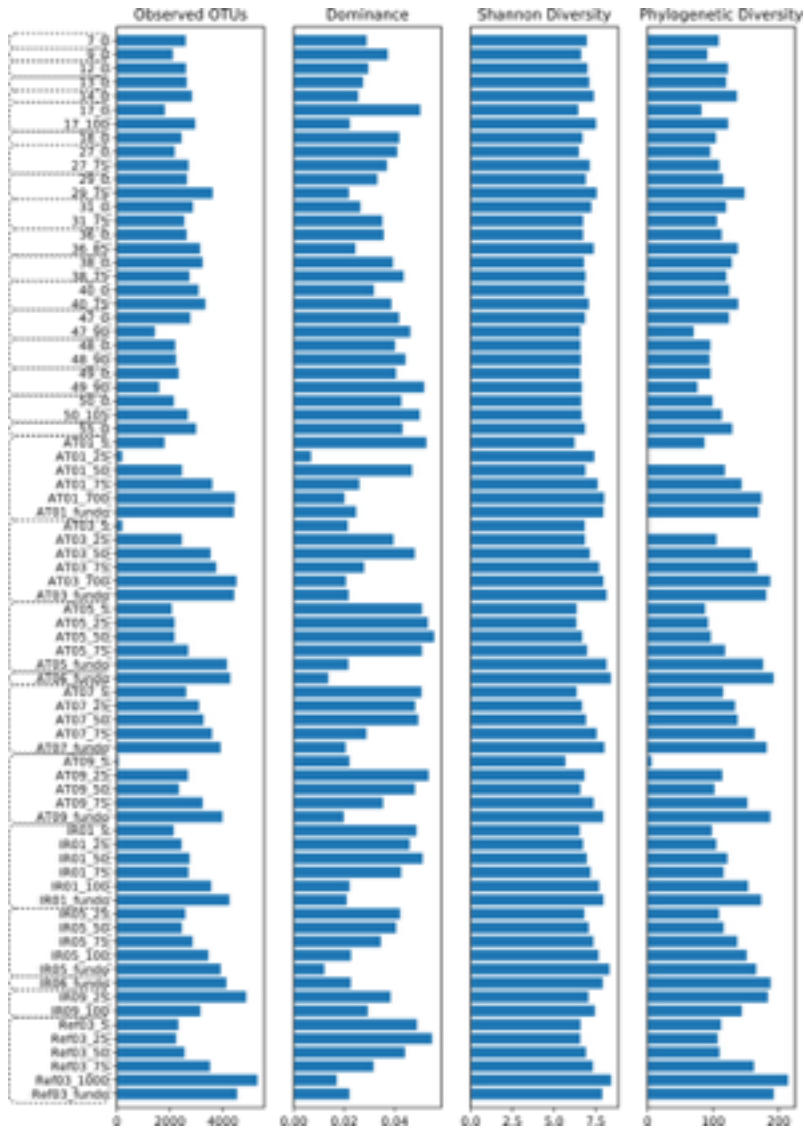
ILLUSTRATIVE RESULTS

TYPES OF RESULTS, NOT VERY INTERESTING YET

Quantifying Biodiversity



Quantifying Biodiversity

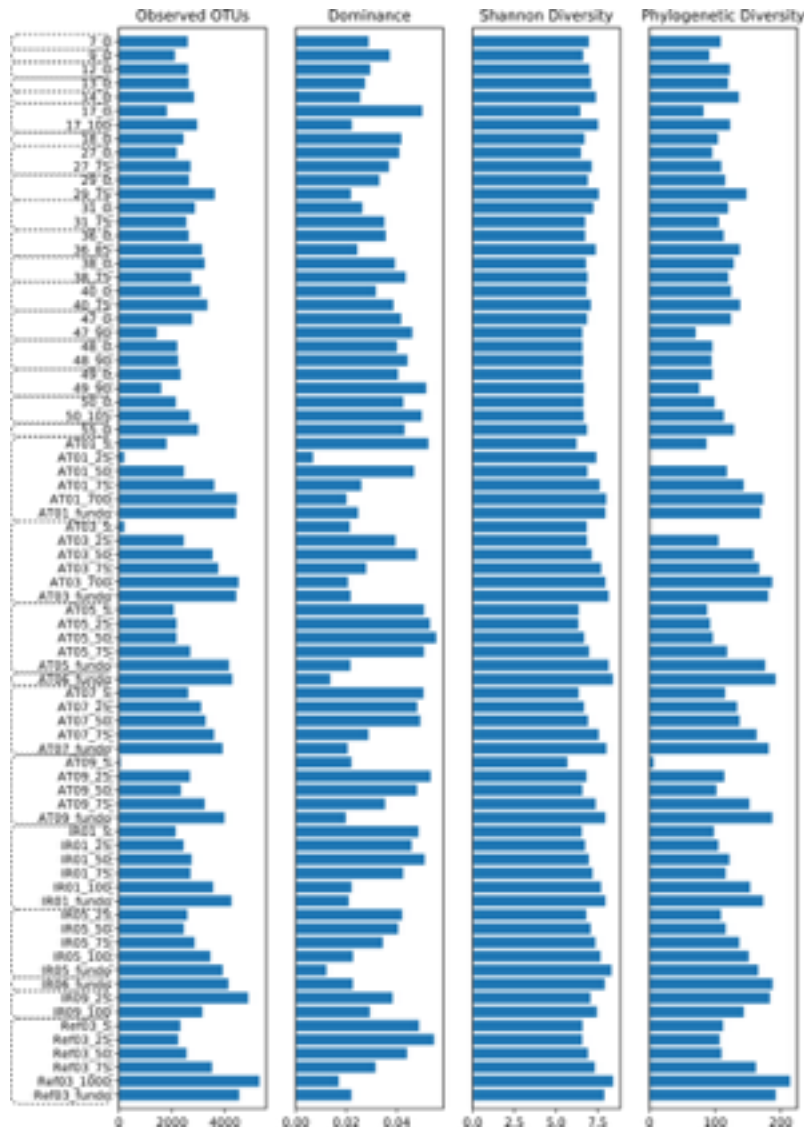


Diversity increases with sampling depth

sediments?



Quantifying Biodiversity



Diversity increases with sampling depth
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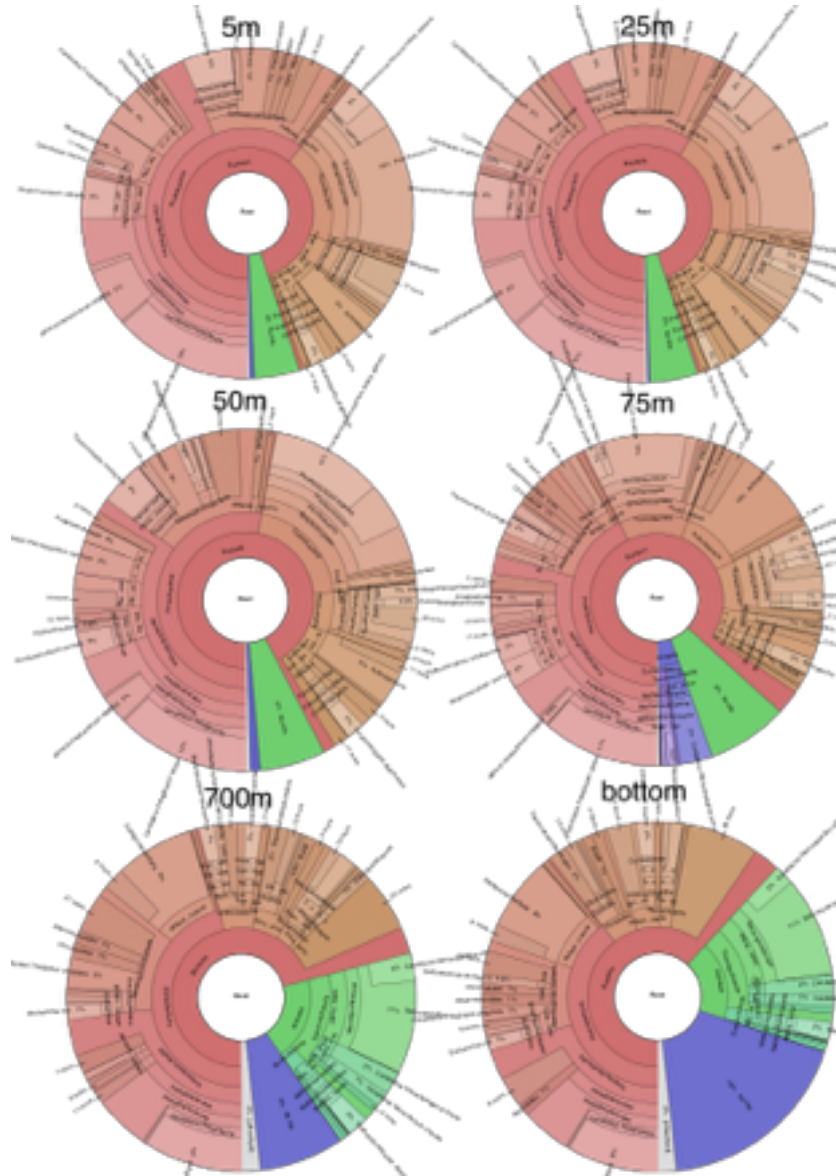
Really interesting questions

*where is the higher diversity
of archaea?*

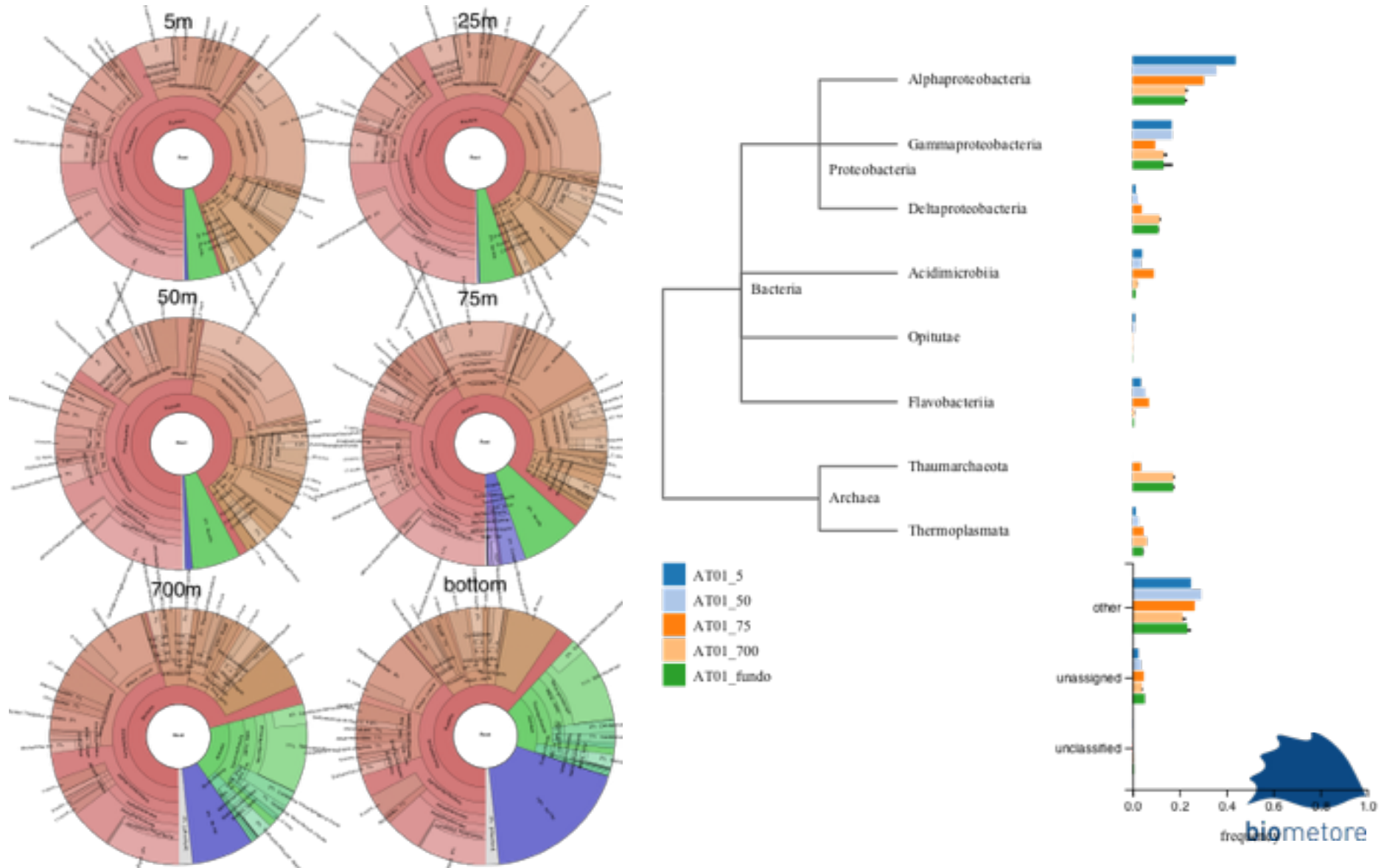
*what biotic/abiotic factors
determine this diversity?*

your question here!

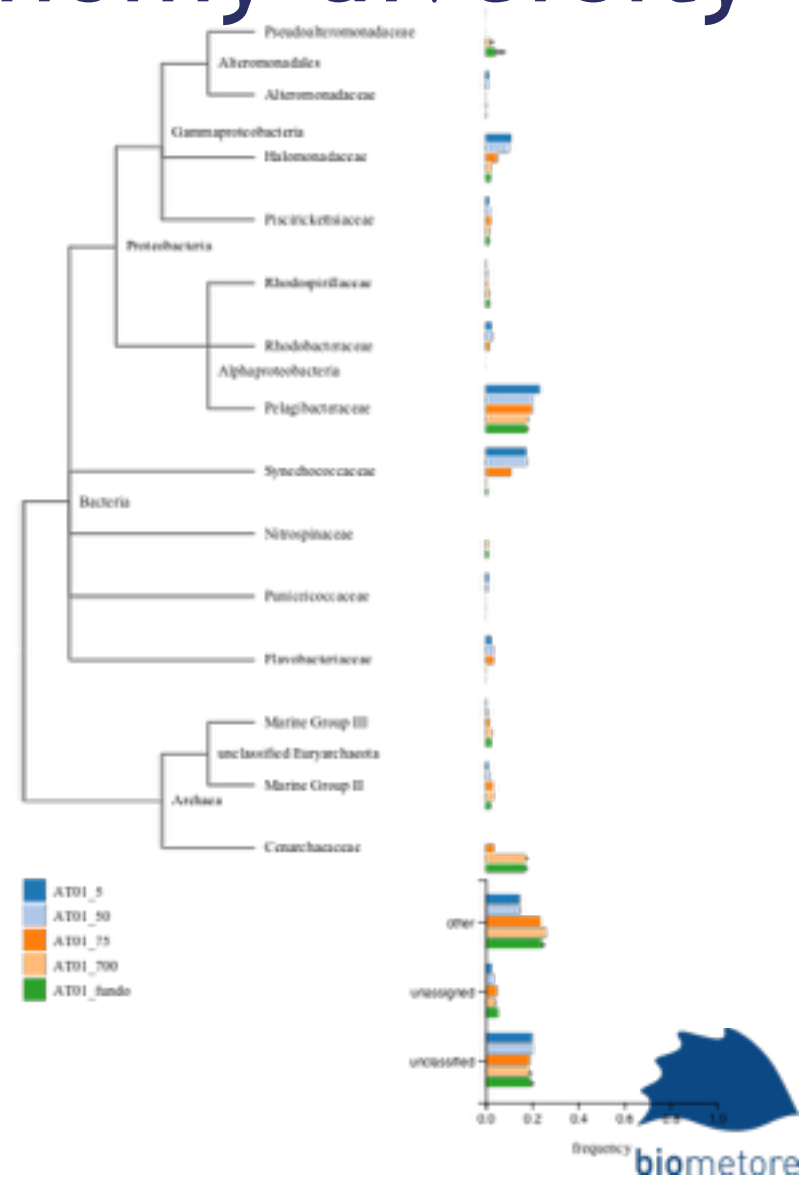
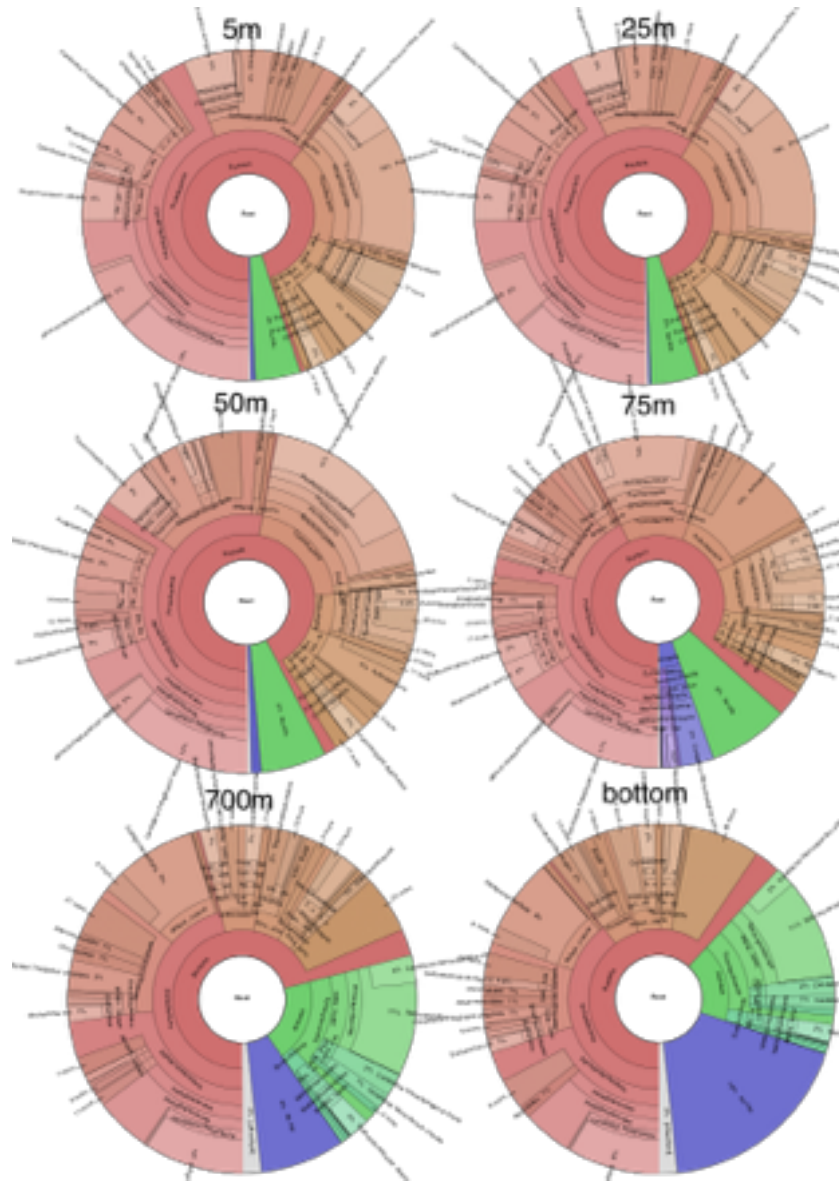
Representing taxonomy diversity



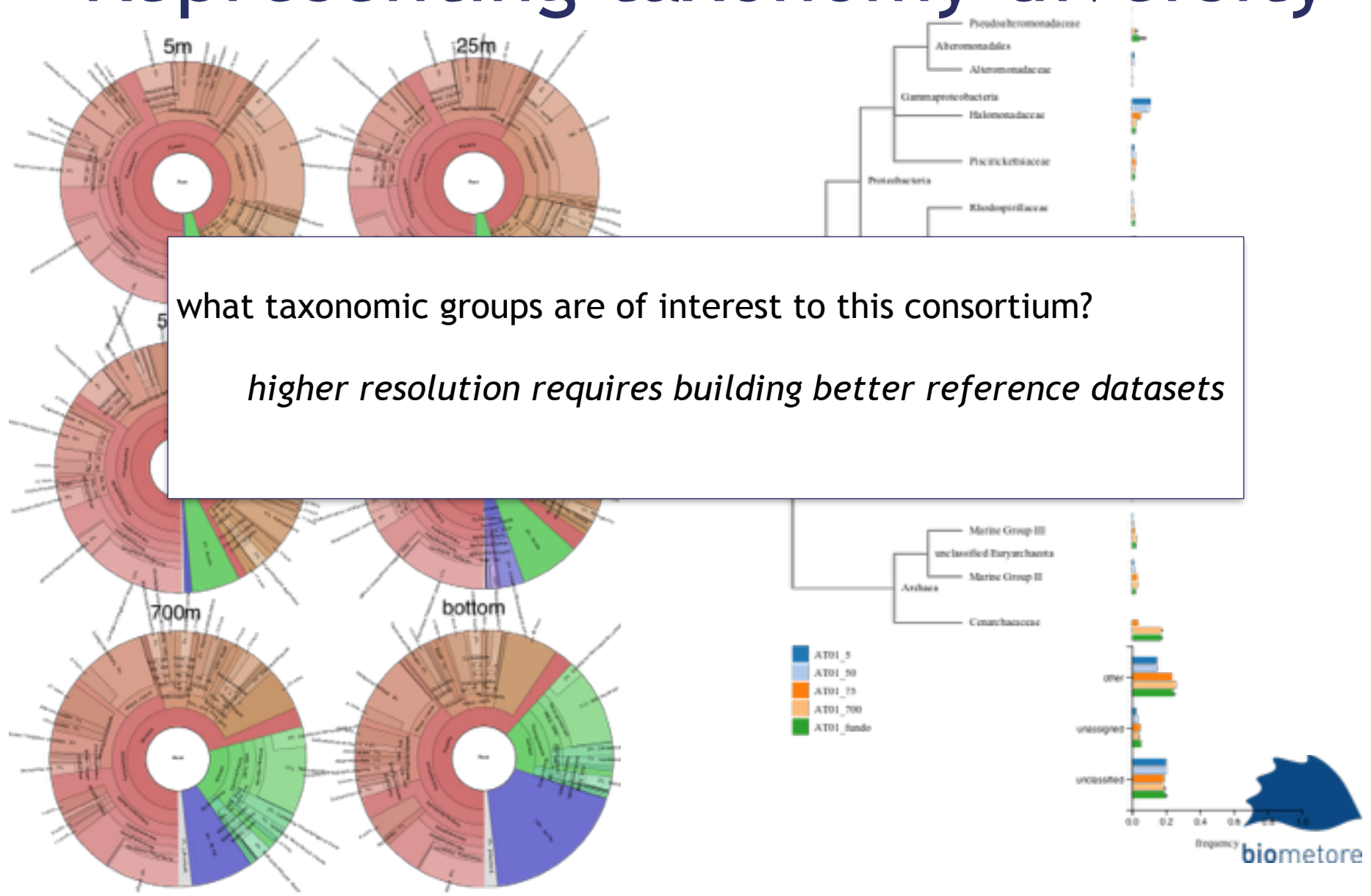
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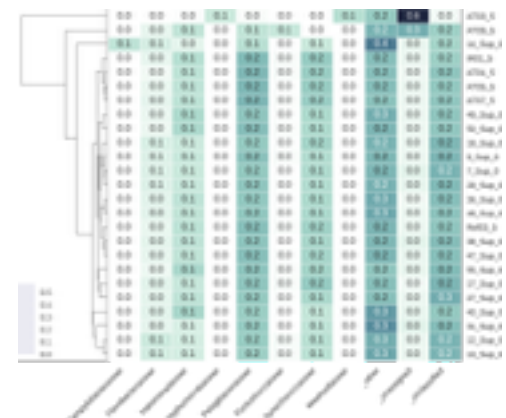
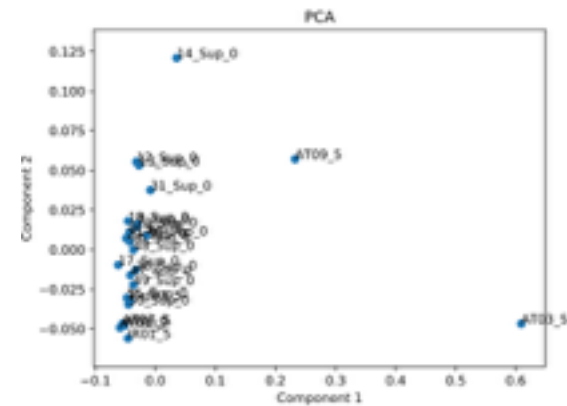
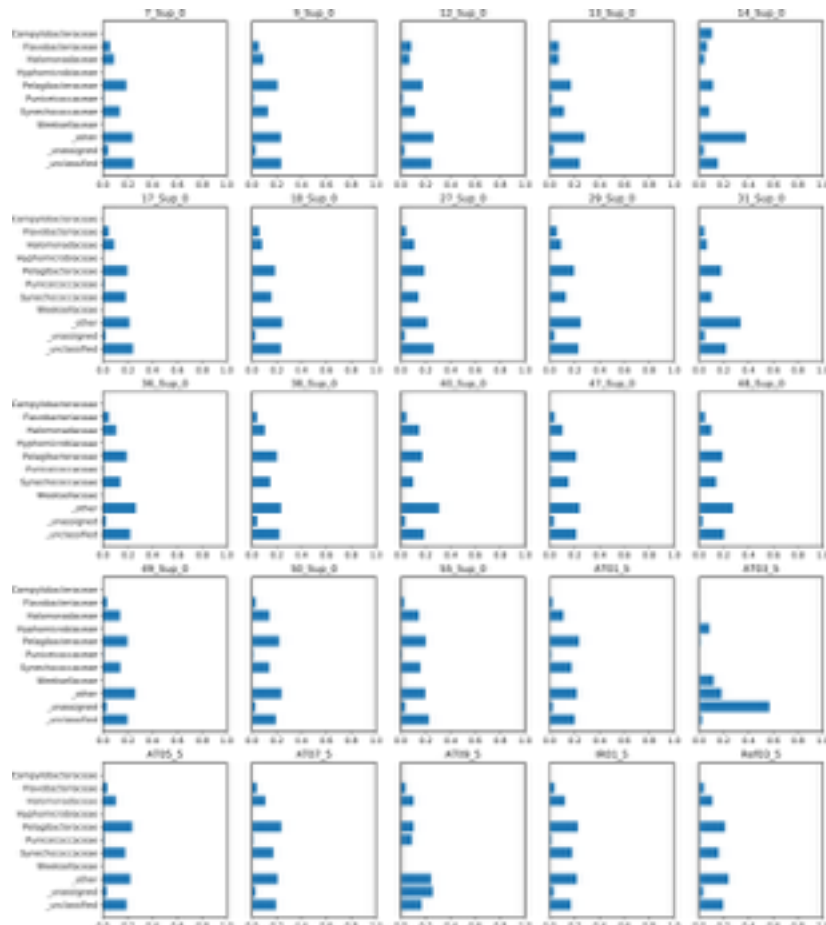


Taxonomic diversity across surface samples



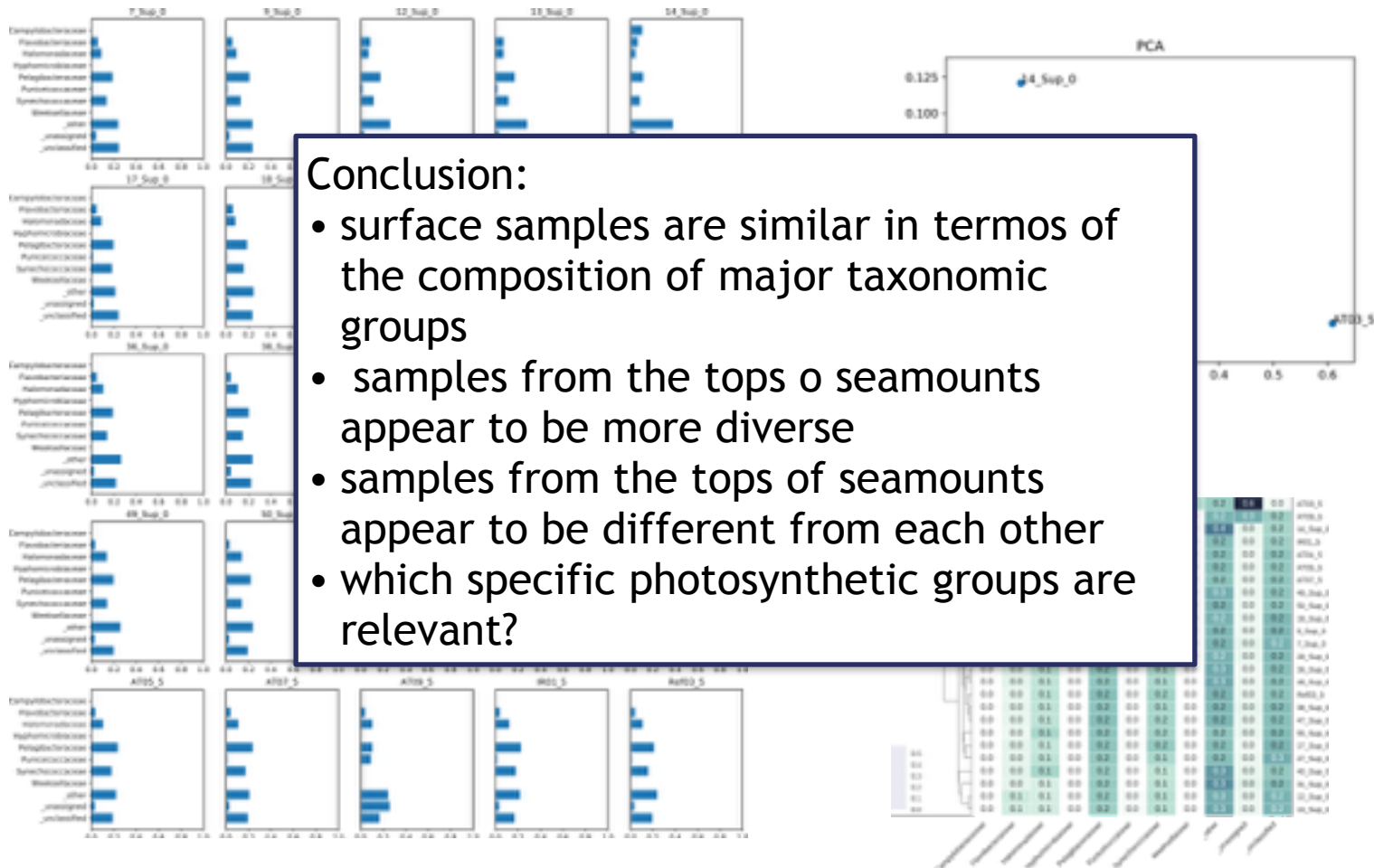
Taxonomic diversity across surface samples

examples of analytics



Taxonomic diversity across surface samples

examples of analytics



next

Raw data available as per consortium decision -(available to all?)

we have our own database with web interfaces for data visualisation

We will provide any data in any format desired by consortium members

e.g. upload to consortium database

We will prepare raw data for submission to EBI - SRA archive

We will work with individual groups to ask specific questions about the data to answer scientific questions

Further sequencing?

16S ; 18S; full metagenomes

sediments; macro-species; gut contents?

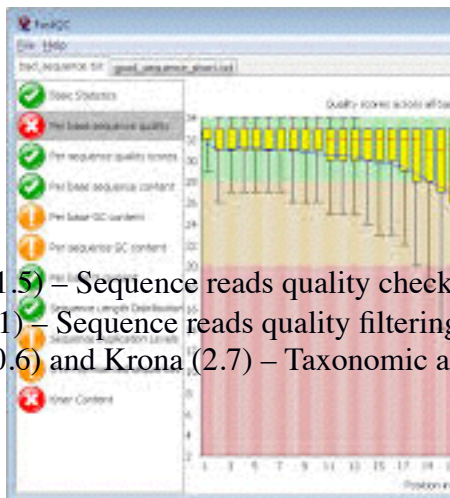
Collection of DNA extracted from samples?



Thank You



- FastQC (0.11.5) – Sequence reads quality check.
- QIIME (1.9.1) – Sequence reads quality filtering, OTU prediction, taxonomic assignment, and taxonomic diversity estimation.
- Kraken (0.10.6) and Krona (2.7) – Taxonomic assignment and visualization.



Kraken Metagenomics

