Marine microbial community taxonomic and functional structure and its association with oil exposure and oil biodegradation activity

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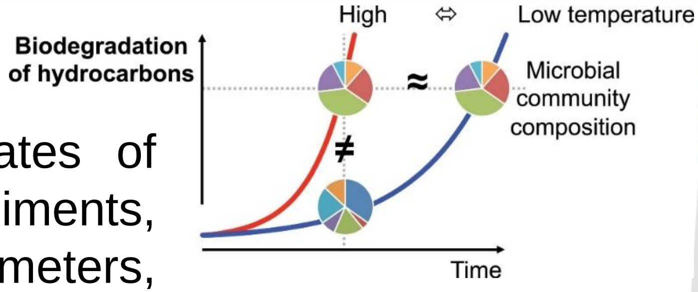
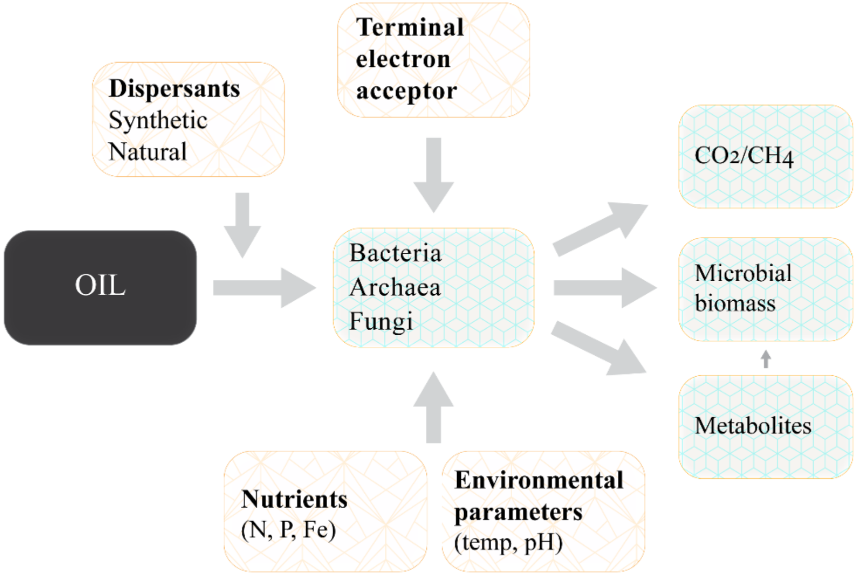
WP2 Oil biodegradation and bioremediation



1. Assessment of natural degradation rates of different oil fractions in seawater and sediments, and taking into account environmental parameters, dispersants application and electro-kinetic treatment.
2. To determine key bacterial species and metabolic pathways responsible for the degradation of different oil fractions in different compartments (aerobic and anaerobic water and sediments) of the Baltic Sea and the Northern Atlantic.
3. Information about microbial community taxonomic composition and metabolic markers will be related to oil biodegradation kinetic parameters and oil remediation strategies using different modelling approaches.

The environmental factors affecting oil biodegradation in the marine environment



OIL

Dispersants
Synthetic
Natural

Terminal electron acceptor

Bacteria
Archaea
Fungi

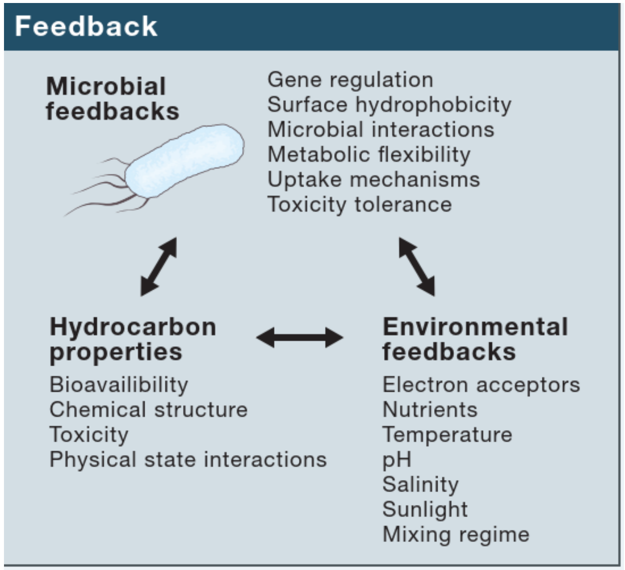
Nutrients
(N, P, Fe)

Environmental parameters
(temp, pH)

CO_2/CH_4

Microbial biomass

Metabolites



Feedback

Microbial feedbacks

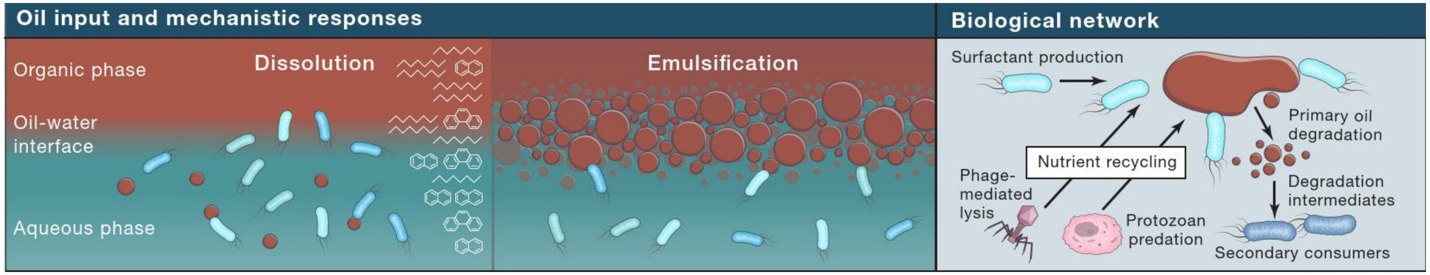
Gene regulation
Surface hydrophobicity
Microbial interactions
Metabolic flexibility
Uptake mechanisms
Toxicity tolerance

Hydrocarbon properties

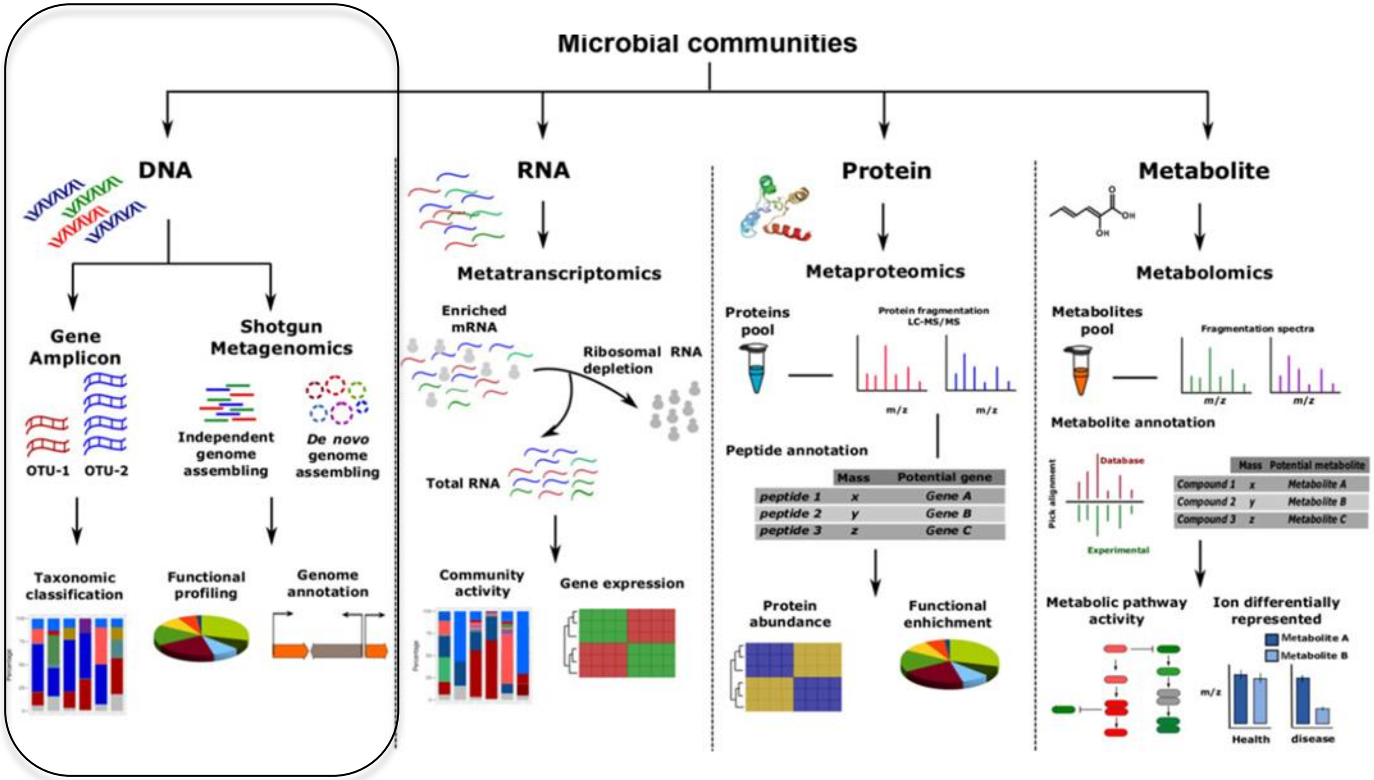
Bioavailibility
Chemical structure
Toxicity
Physical state interactions

Environmental feedbacks

Electron acceptors
Nutrients
Temperature
pH
Salinity
Sunlight
Mixing regime



Different omic techniques for studying marine microbes



	Mass	Potential gene
peptide 1	*x*	*Gene A*
peptide 2	*y*	*Gene B*
peptide 3	*z*	*Gene C*

	Mass	Potential metabolite
Compound 1	*x*	*Metabolite A*
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Microbial Biotechnology (2017) 10(6), 1500–1522, doi:10.1111/1751-7915.12855

Example of the workflow

Locations of collecting the seawater in Svalbard

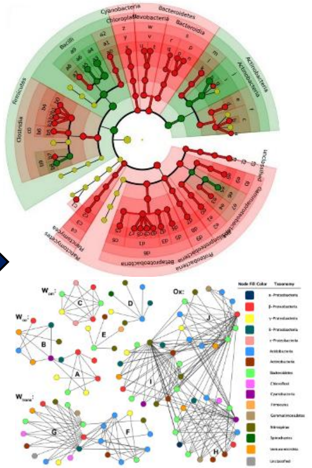
Mesocosm tank

Oil encapsulated in the ice

Experiments at Norut (Narvik)

Mock community

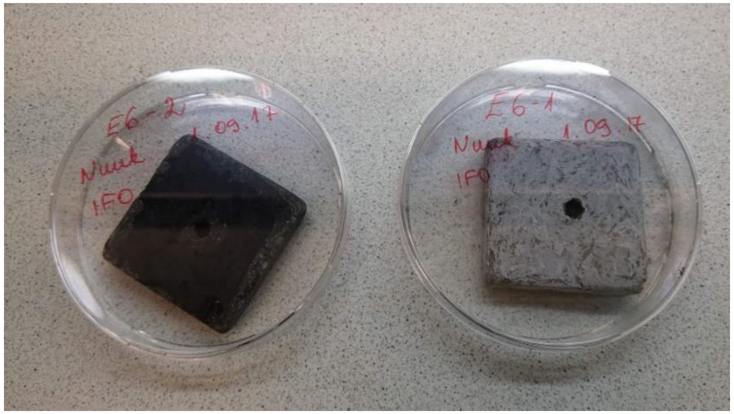
Microbial DNA sequencing / qPCR



Bioinformatic and statistical analysis

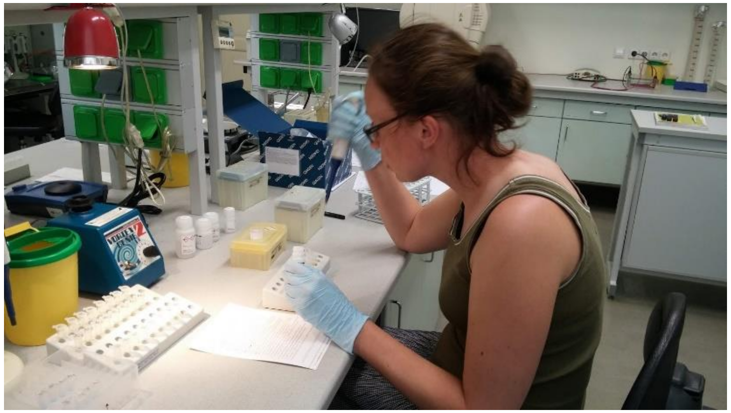
Natural removal of stranded oil in Arctic

Photo: Kim Gustavson & Susse Wegeberg



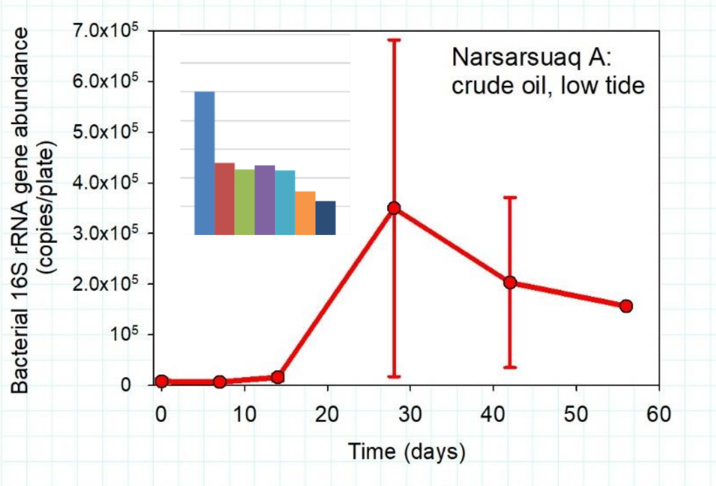
Removal of biofilm from tiles

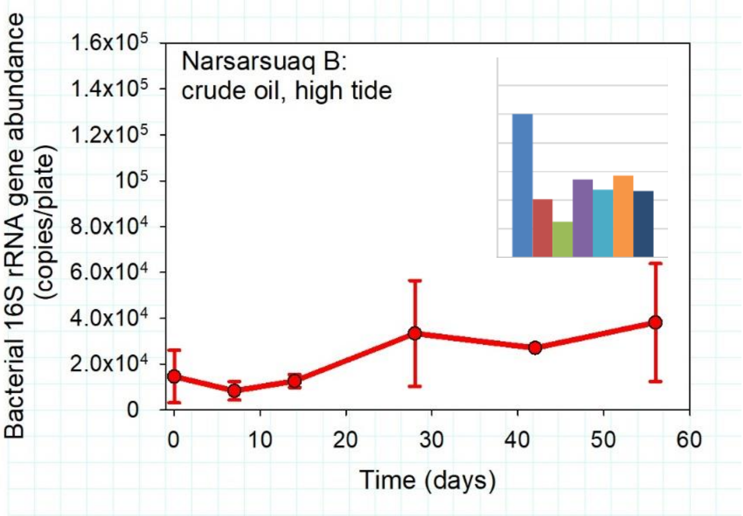
Removal of biofilm from tiles

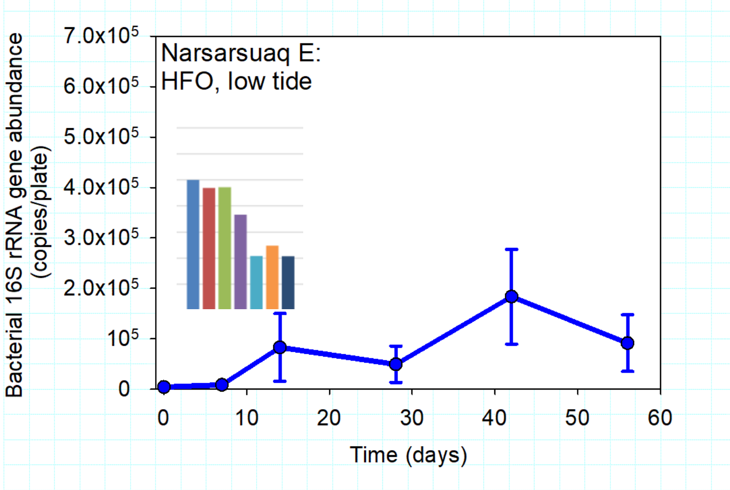


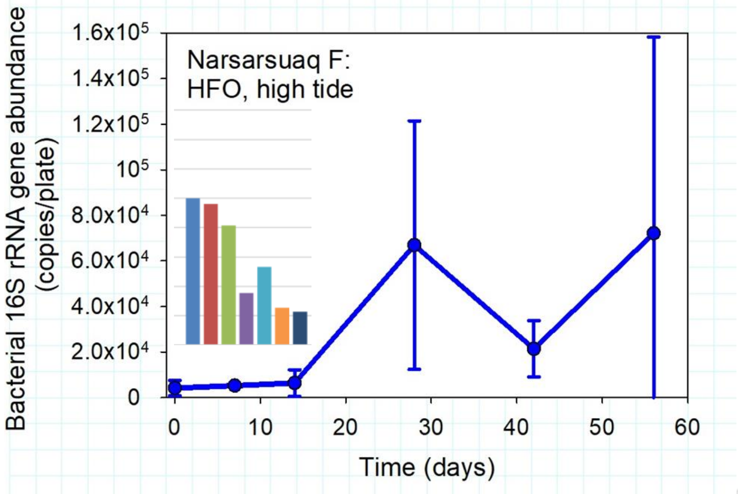
DNA isolation from biofilm

Dynamics of bacterial community abundance









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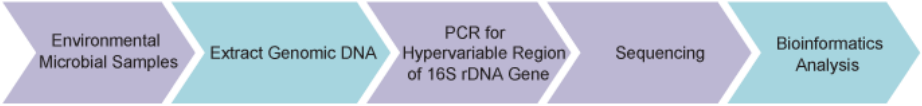
Task 2.5 Integrative knowledge discovery from multiple omics sources

- Information about microbial community taxonomic composition and metabolic markers will be related to oil biodegradation kinetic parameters and oil remediation strategies using different modelling approaches.

- Results of meta-analysis of obtained omics data sets that describe specific microbial community response to accidental release of oil into the marine ecosystem and the impact of different treatment strategies including dispersant application.

16S rDNA Metagenomics Sequencing



Whole Genome Metagenomics Sequencing

Relationships between different tasks in WP2

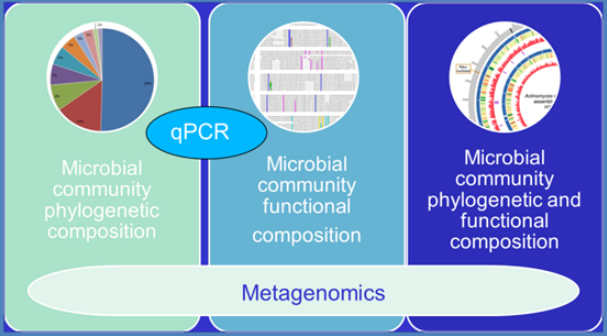
Task 2.1 Oil biodegradation in seawater and impact of dispersants on oil biodegradation characteristics

Task 2.2 Oil biodegradation is sea water-ice interface

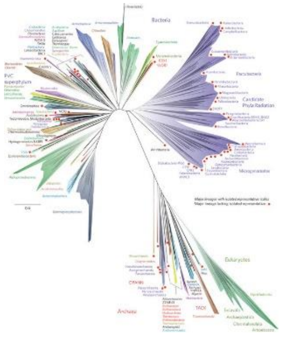
Task 2.3 Remediation of oil contaminated sediments using electrokinetic treatment

Task 2.4. Effect-based assessment of biodegradation and remediation success

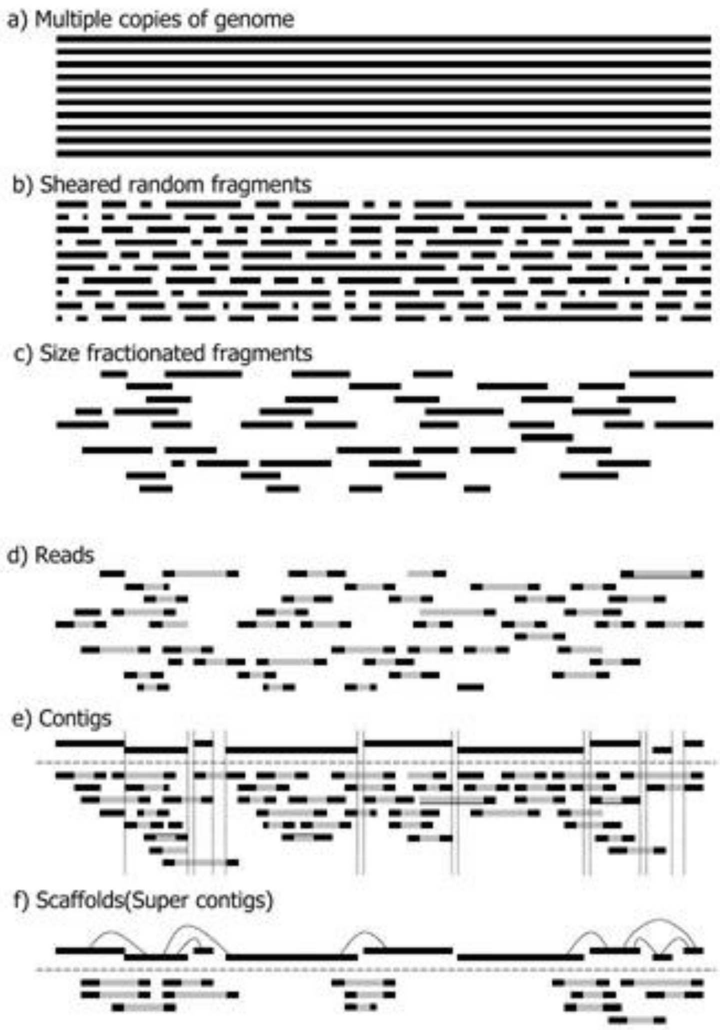
Public domain omics data



Task 2.5 Omics data integration and meta-analysis



Shotgun metagenomics



Pieces of genomic information from many different organisms mixed together.

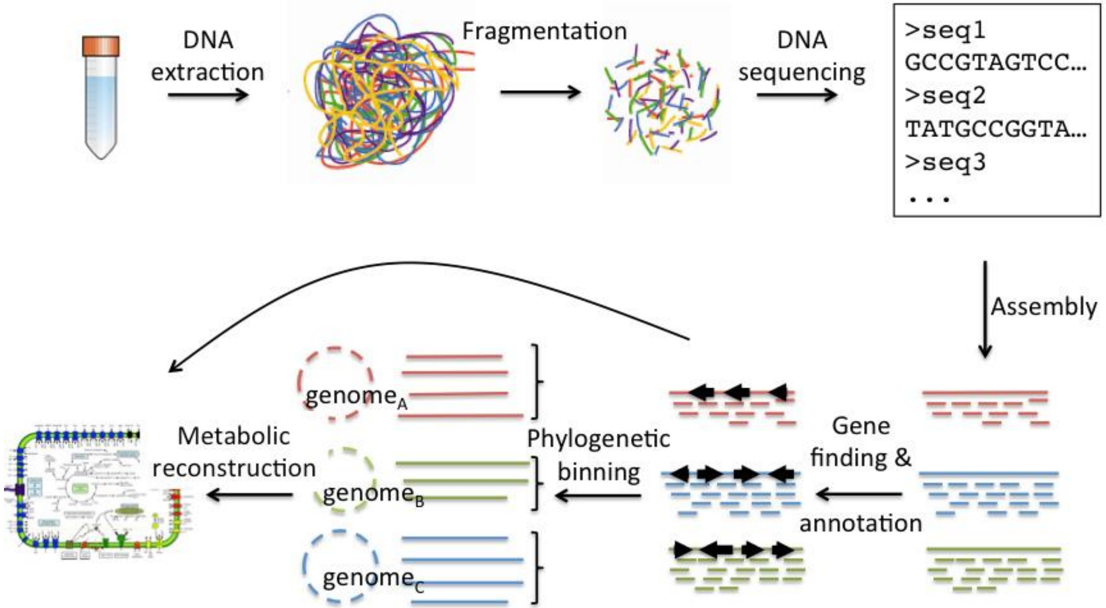
In theory possible to assemble complete bacterial genomes from community genomic material

The difficulty of assembly directly correlates with diversity of species in the given metagenome.

Metagenomics studies the collective genomes of all the microorganisms of a water/sediment sample by sequencing the genetic material directly recovered from the sample.

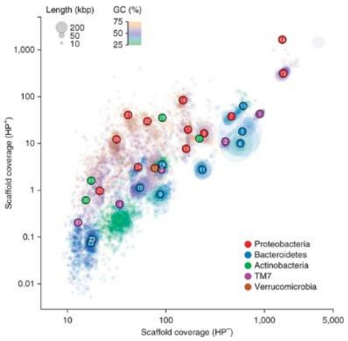
Data analysis steps in shotgun metagenomics

- Annotation of metagenomic sequences
- Assembly of shotgun metagenomics data
- Binning tools for metagenomes
- Data management, storage, and sharing

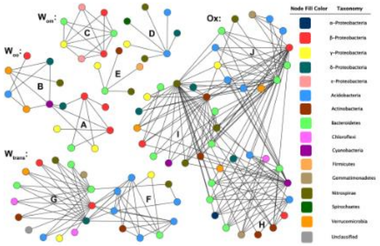


Data integration - outcome

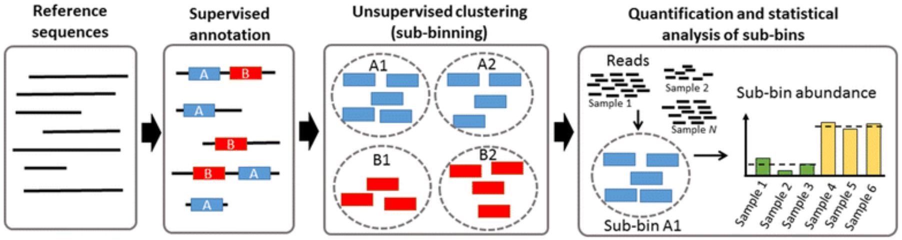
- Recovery of individual genomes from obtained metagenomics datasets using binning of assembled contigs to species-level groups both from single metagenomes and related multiple metagenomes. *This approach enables to better understand the role of uncultivated microbial species in oil biodegradation.*



- Information about microbial community taxonomic composition and metabolic markers together with abiotic factors will be related to oil biodegradation kinetic parameters and oil remediation strategies using different modelling approaches (*structural equation modelling, network analysis, random forest, in silico biodegradation network reconstruction*).



- Creation of metagenomic prediction platform for inferring oil biodegradation activity.



Annotation of sequences

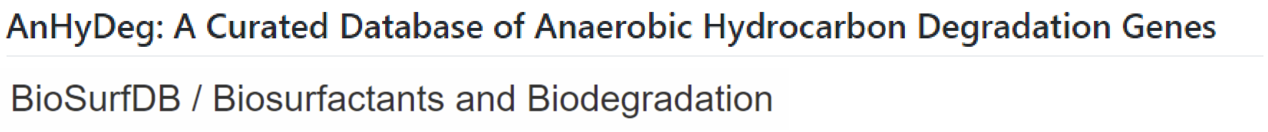
Aim is to

1) Identify taxonomic composition of microbial community
2) Identify specific genes of metabolic pathways of oil degradation

Reference data bases for identification of genes involved in oil biodegradation

Available databases:

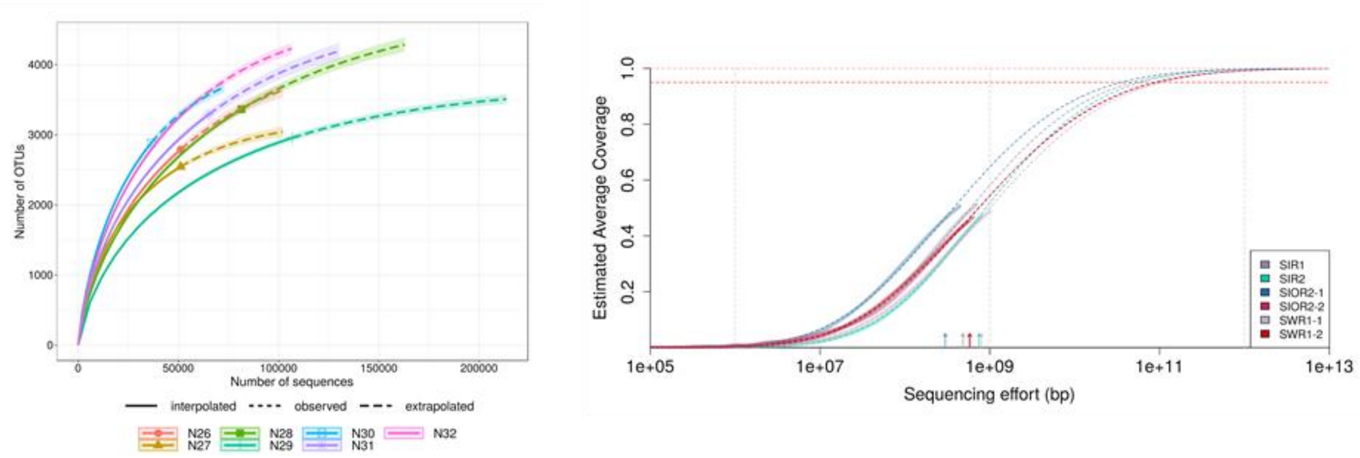
AromaDeg
aromatic hydrocarbon degradation database



AnHyDeg: A Curated Database of Anaerobic Hydrocarbon Degradation Genes

BioSurfDB / Biosurfactants and Biodegradation

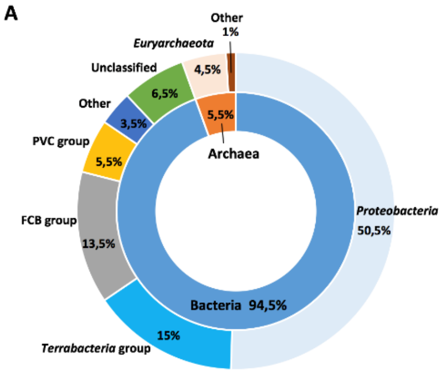
Sequencing coverage estimation for samples



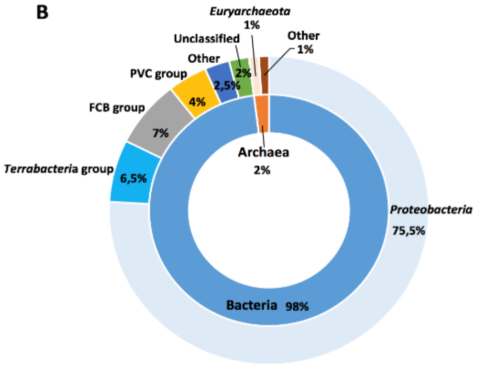
A. Bacterial community richness estimation using iNEXT software (http://chao.stat.nthu.edu.tw/wordpress/software_download/inext-online/). Estimated coverage values are in the range 0.97 - 0.99.
B. Metaganome sequencing coverage estimation using Nonpareil software. Estimated coverage values are in the range 0.45 - 0.51.

Sample code abbreviations: Plot A – samples from 8 month long mesocosm experiment: N26, N27, N28 – seawater control; N29, N30 – seawater with oil; N31, N32 - seawater with oil with NPK amendment; Plot B: SWR1-1 and SWR1-2 – collected seawater, SIR1 and SIR2 sea-ice control in mesocosms, SIOR1-1 and SIOR1-2 – sea-ice with oil in mesocosms.

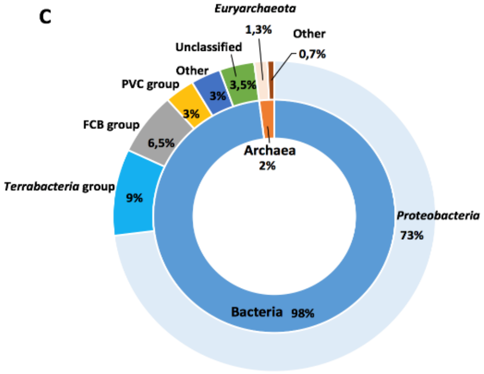
Microbial community composition



Sea water



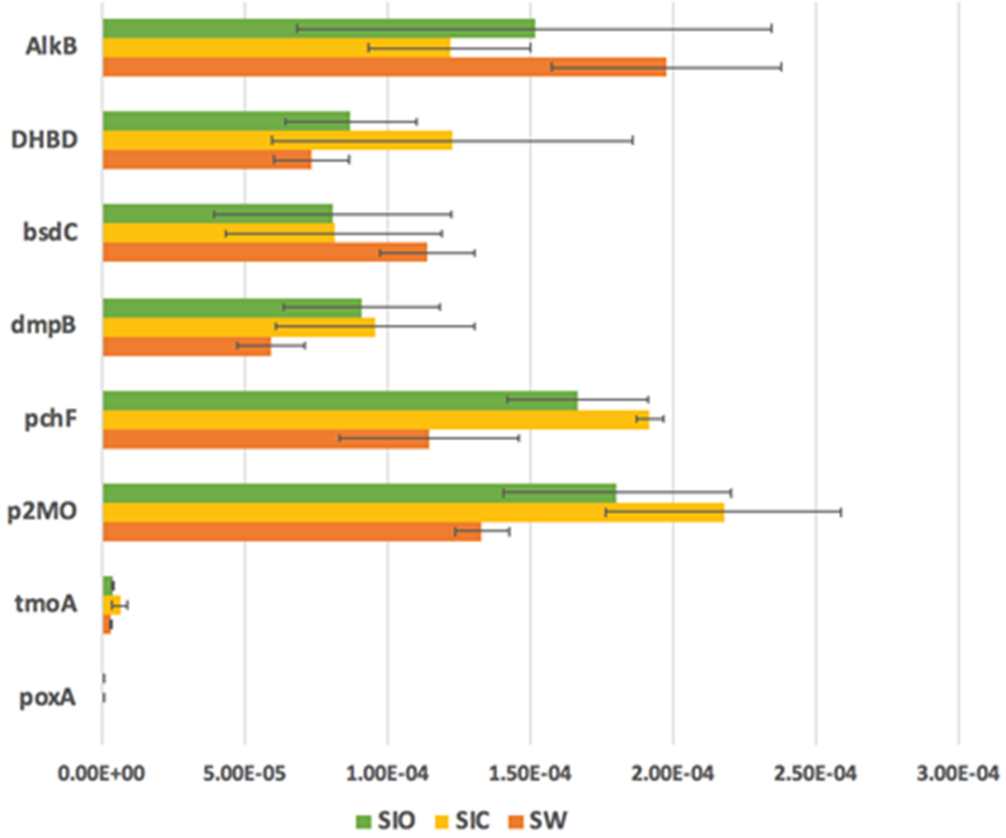
Sea ice



Sea ice with crude oil

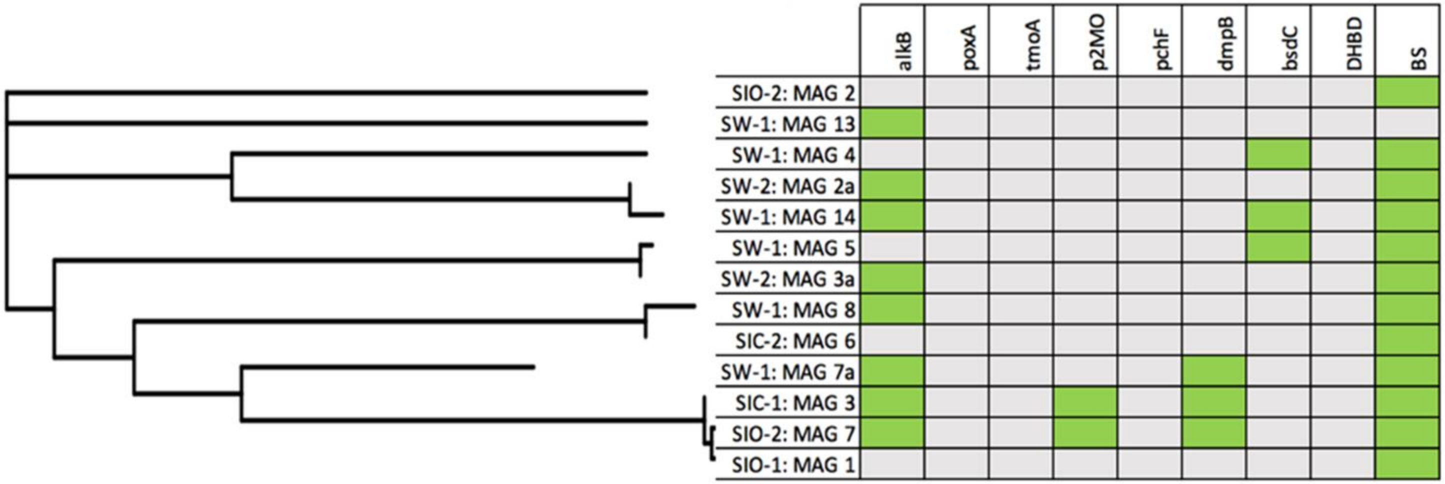
Bacterial community of sea-ice with oil was dominated by *Pelagibacteriales, Rhodobacteriales* and *Altermonadales*

Microbial community functional properties



AlkB – alkane 1-monooxygenase
DHBD – 2,3-dihydroxybenzoate decarboxylase
bsdC – 4-hydroxybenzoate decarboxylase subunit C
dmpB – catehol 2,3-dioxyganase
pchF – p-cresol dehydroxygenase
p2MO – phenol 2-monooxygenase
tmoA – toluen monooxygenase system protein A
poxA – phenol hydrolase P0 protein

Metagenome assembled genomes



	alkB	poxA	tmoA	p2MO	pchF	dmpB	bsdC	DHBD	BS
SIO-2: MAG 2									🟩
SW-1: MAG 13	🟩								
SW-1: MAG 4							🟩		🟩
SW-2: MAG 2a	🟩								🟩
SW-1: MAG 14	🟩						🟩		🟩
SW-1: MAG 5							🟩		🟩
SW-2: MAG 3a	🟩								🟩
SW-1: MAG 8	🟩								🟩
SIC-2: MAG 6									🟩
SW-1: MAG 7a	🟩					🟩			🟩
SIC-1: MAG 3	🟩			🟩		🟩			🟩
SIO-2: MAG 7	🟩			🟩		🟩			🟩
SIO-1: MAG 1									🟩

Similarity of obtained metagenome assembled genomes and their functional properties

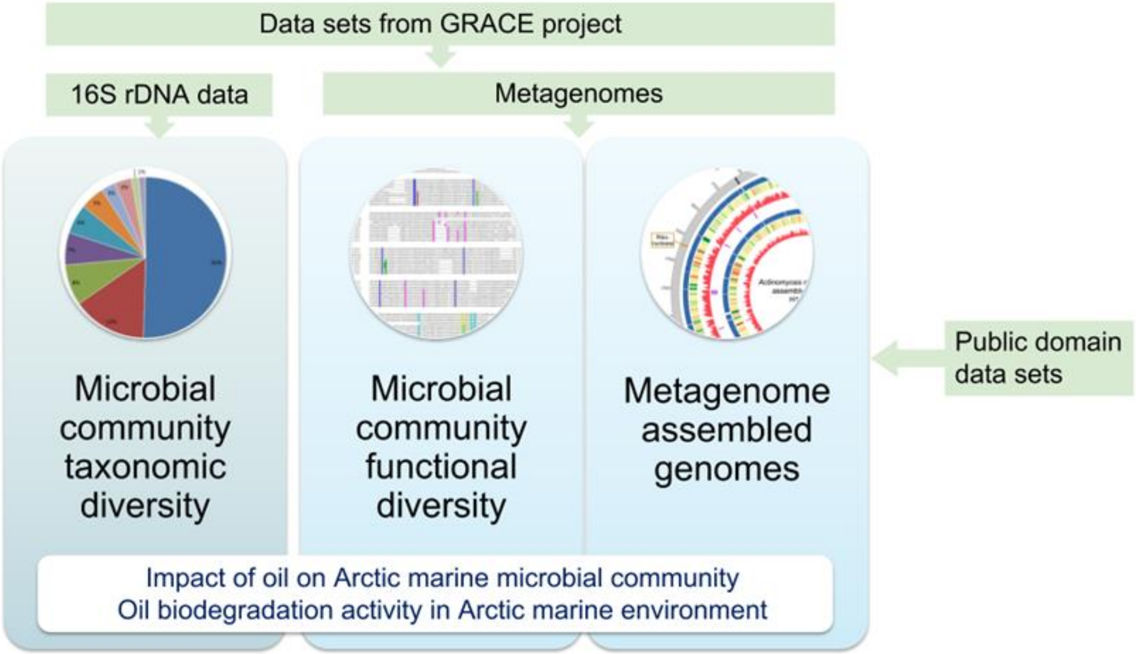
○ Ten and three MAGs were recovered from control and oil-treated mesocosms, respectively.

○ MAGs from oil treated mesocosms belonged to the genera *Glaciecola, Sulfitobacter* and *Ascidiaceihabitans*.

○ These MAGs possessed pathways for degradation of aromatic compounds, alkanes and synthesis of biosurfactants.

○ Additional analysis revealed that two oil-degrading bacterial lineages, *Roseobacter* and *Colwellia* were abundant in obtained metagenomes but were not recovered during the binning process.

Using public domain data



Data sets from GRACE project

16S rDNA data

Metagenomes

Microbial community taxonomic diversity

Microbial community functional diversity

Metagenome assembled genomes

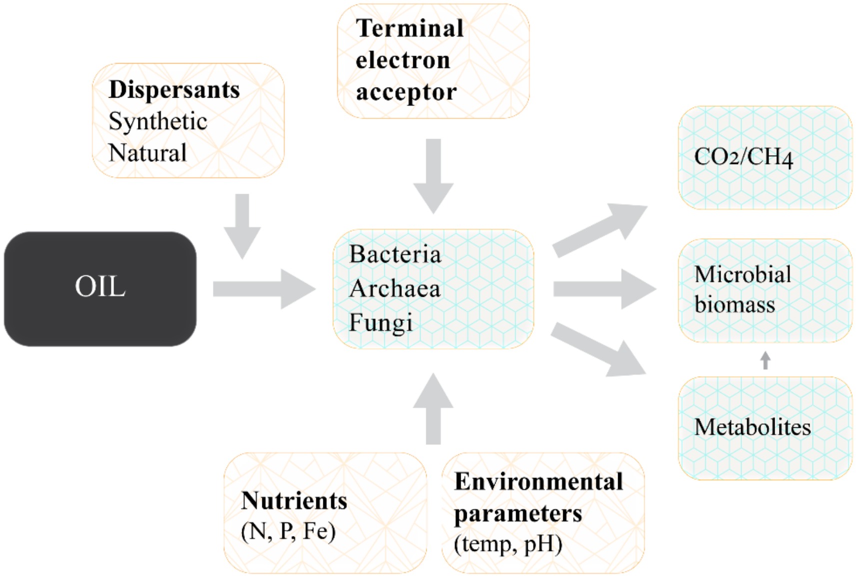
Public domain data sets

Impact of oil on Arctic marine microbial community
Oil biodegradation activity in Arctic marine environment

Problems:

published articles do not provide accession numbers for deposited data sets,
quality of the deposited data sets
metadata

Knowledge gaps and further studies



Dispersants
Synthetic
Natural

Terminal electron acceptor

OIL

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Archaea
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CO2/CH4

Microbial biomass

Metabolites

Nutrients
(N, P, Fe)

Environmental parameters
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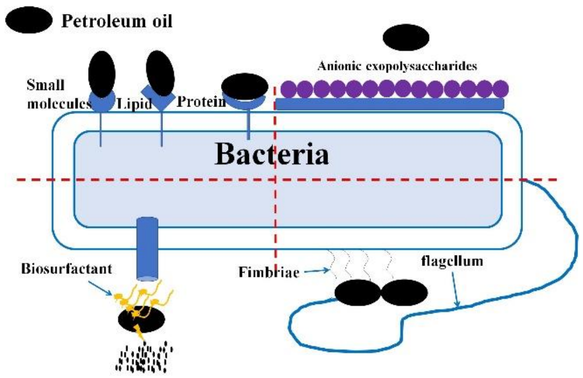
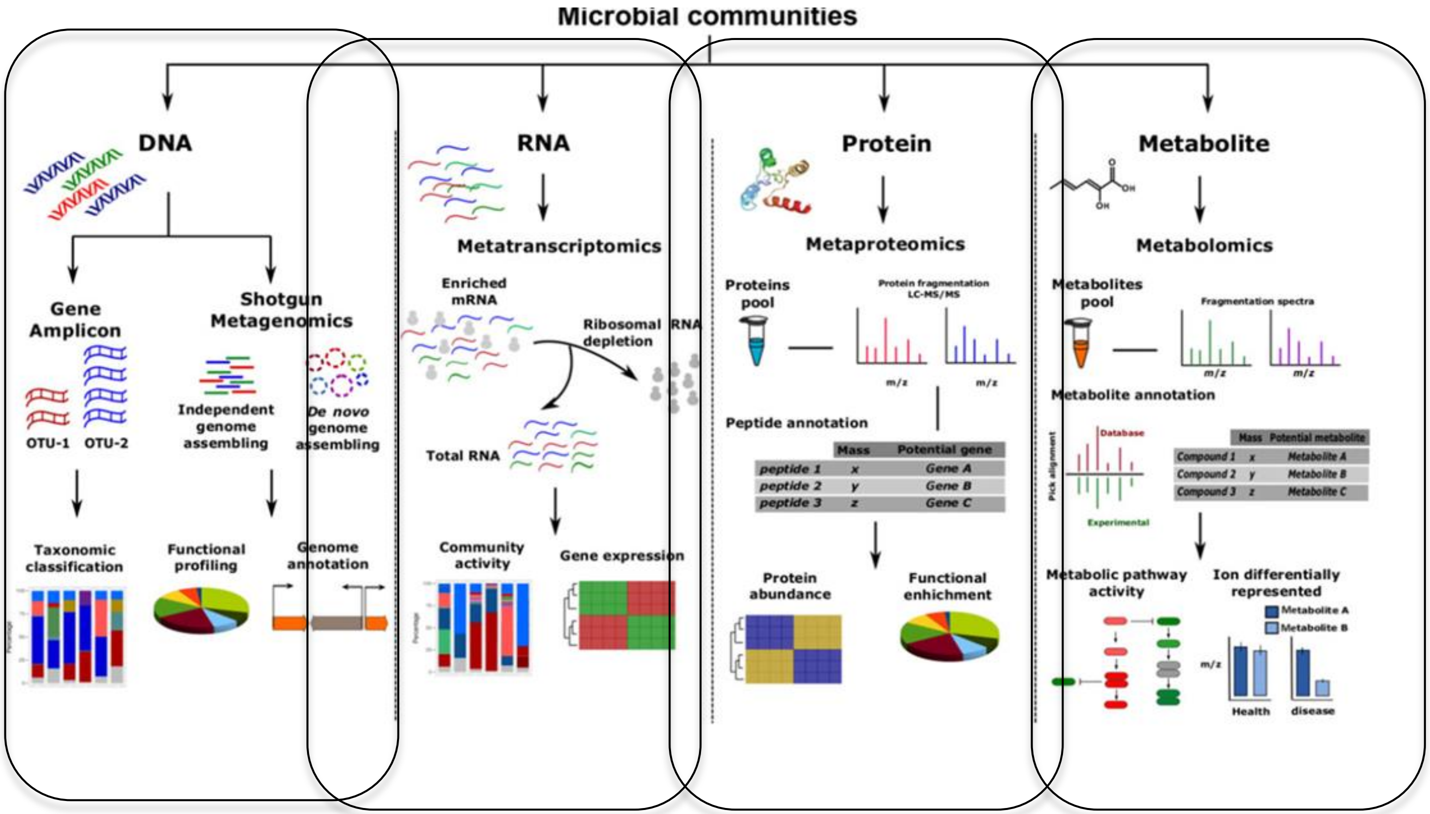


- **Fimbriae or flagellum of bacteria attach to petroleum oil.**
- **Biosurfactants secreted by bacteria emulsify petroleum oil.**
- **Proteins , lipids and other small molecules on bacterial surface for adhering petroleum oil.**
- **Some anionic exopolysaccharides on bacterial surface prevent bacteria from attaching to petroleum oil.**

- Impact of different types of dispersants on marine (oil-degrading) microbial community (incl Baltic Sea)
- Microbial communities of oil-degrading biofilms (stranded oil)
- Oil-degrading microbial community interactions (for prospect of designing microbial inocula for removal of oil in Arctic)
- Treatment of oil polluted marine sediments (bioelectrochemical systems)

Different omic techniques for studying marine microbes



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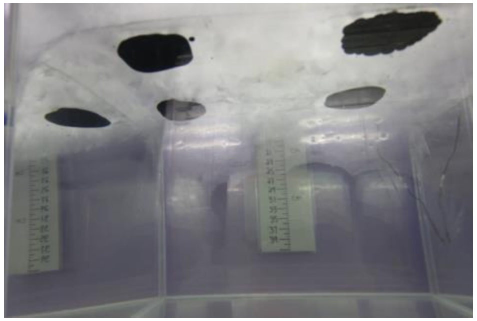
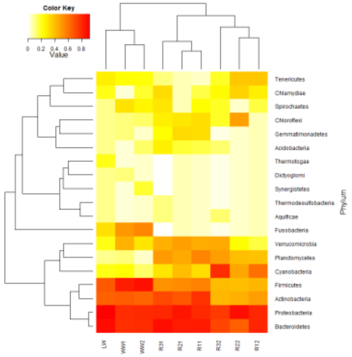
WP2 meeting in Tartu, Feb 2017

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Value
Tenericutes
Chlamydiae
Spirochaetes
Chloroflexi
Gemmatimonadetes
Acidobacteria
Thermotogae
Dictyoglomi
Synergistetes
Thermodesulfobacteria
Aquificae
Fusobacteria
Verrucomicrobia
Planctomycetes
Cyanobacteria
Firmicutes
Actinobacteria
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Phylum
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WW1
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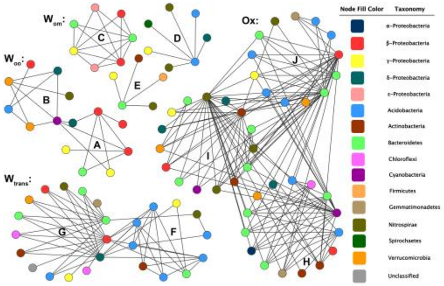
R11
R32
R22
R12

Thank you!


W_{om}:
W_{oo}:
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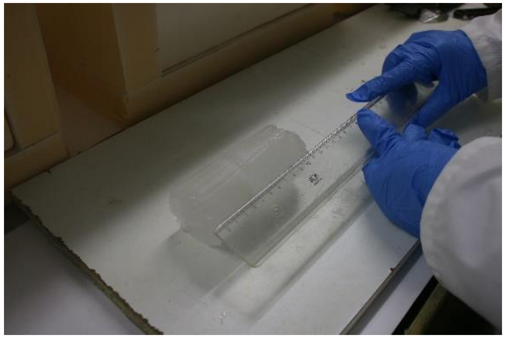
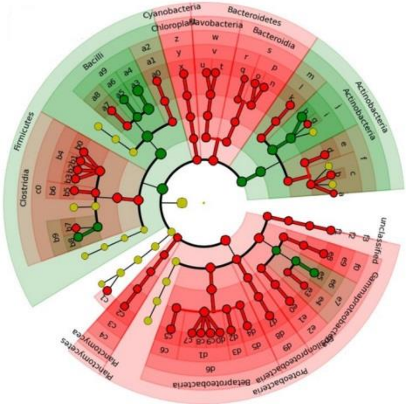
Node Fill Color
Taxonomy
α-Proteobacteria
β-Proteobacteria
γ-Proteobacteria
δ-Proteobacteria
ε-Proteobacteria
Acidobacteria
Actinobacteria
Bacteroidetes
Chloroflexi
Cyanobacteria
Firmicutes
Gemmatimonadetes
Nitrospirae
Spirochaetes
Verrucomicrobia
Unclassified




Cyanobacteria
Chloroplast
Flavobacteria
Bacteroidetes
Bacteroidia
Bacilli
Actinobacteria
Actinobacteria
Firmicutes
Clostridia
Unclassified
Gammaproteobacteria
Epsilonproteobacteria
Proteobacteria
Betaproteobacteria
Planctomycetes
Planctomycea