

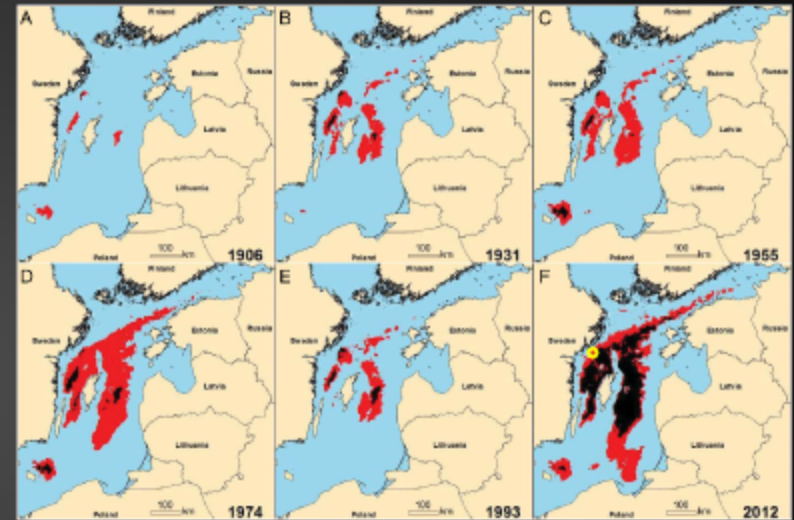
The living and the dead

What are photic and aerobic bacteria doing in an anaerobic sediment at 460 m depth?

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Metagenome discoveries

- Landsort Deep
 - 460 m deep
 - Stably anoxic
- Sediment metagenome:
 - Lots of anaerobes (sulfate and nitrate respirers, methanogens etc.)
 - **Cyanobacteria**: Photic
 - **Mycobacteria**: Strictly aerobic(?)

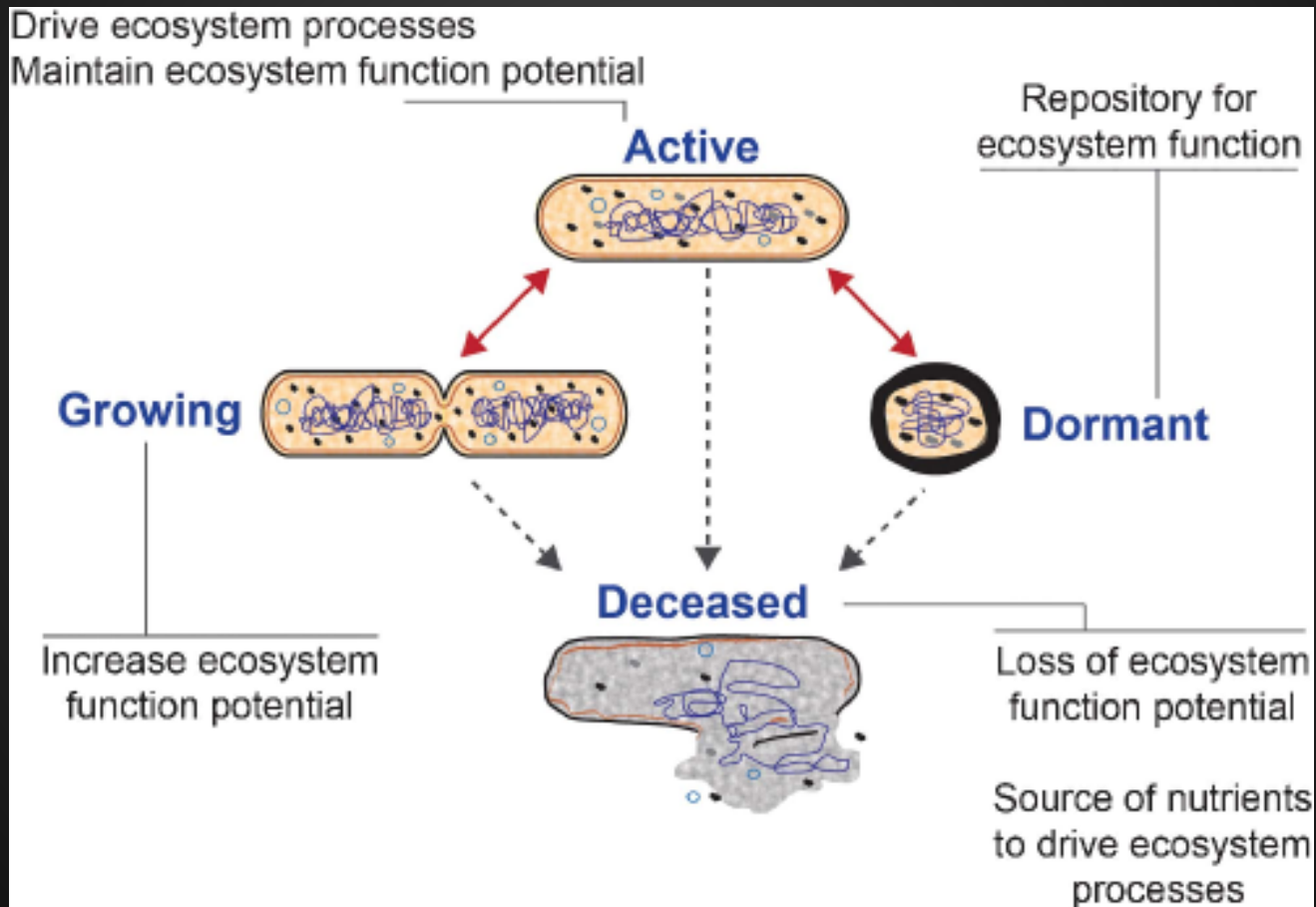


Thureborn et al. 2013

Fossil DNA, dormant, active?

- Sediment receives “snow”: Dead cells from water column
- Mycobacteria have sturdy cells – could their DNA survive?
- Cyanobacteria – sinking to collect P?
- Dormancy?

When do bacteria die and how do we know?



Activity is not so easy...

- Ribosomes ↔ potential for protein synthesis
- rRNA content very taxon specific
- Dormant cells contain ribosomes
 - Long- or short-term preparation for protein synthesis

How do we address this – three sequence types

- Total RNA: containing much rRNA
- Total DNA: containing some rDNA
- V3V4 16S amplicons

Analysis

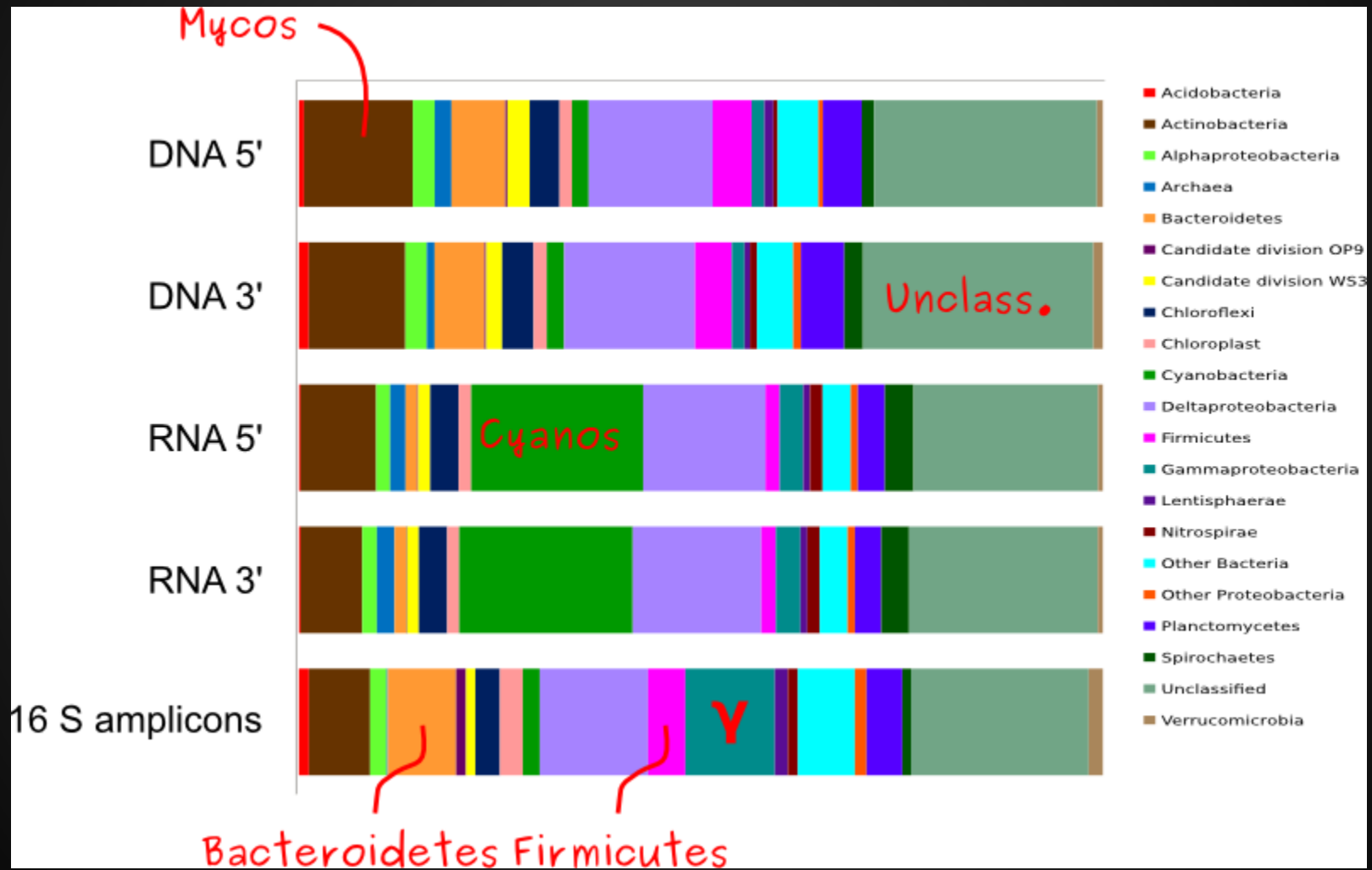
1. Amplicons clustered into 8800 OTUs (1.0% radius)
2. OTU's taxonomy established (Silva)
3. Total RNA and DNA mapped against OTUs, 5' and 3' read separately

Total DNA	4791	4570
Total RNA	2.7 million	2.4 million
Amplicons	83988	

Differences between rRNA and amplicons

- More amplicon than rRNA sequences ↔ Dead, surviving DNA
- Equal proportions ↔ Alive/active/dormant
- More rRNA than amplicons ↔ Highly active
(?)

Taxon distributions



Most phyla are equal in RNA and DNA

	% amplicons	% rRNA	Ratio	N. OTUs
Actinobacteria	7.59	8.54	0.89	397
Chloroflexi	3.05	3.52	0.87	300
Nitrospirae	1.24	1.55	0.80	84
Planctomycetes	4.45	3.31	1.34	385
Alphaproteobacteria	2.06	1.78	1.16	227
Deltaproteobacteria	13.47	15.61	0.86	943
Unclassified	22.09	23.20	0.95	3049

Some contain many aerobes...

	% amplicons	% rRNA	Ratio	N. OTUs
Mycobacterium spp.	2.06	2.13	0.97	56

- *M. tuberculosis* survives anaerobically, but does not divide (Watanabe et al. 2011)
- Sturdy cells
- Common pathogens
- Possible “snow” contribution, but not certain

Low RNA content

	% amplicons	% rRNA	Ratio	N. OTUs
Bacteroidetes	8.49	1.44	5.90	804
Firmicutes	4.63	1.79	2.59	322
Gammaproteobacteria	11.13	2.99	3.72	514
Verrucomicrobia	1.76	0.61	2.89	100

Bacteroidetes

	% amplicons	% rRNA	Ratio	N. OTUs
BD2-2	2.11	0.40	5.28	176
Flavobacteriaceae	2.69	0.30	8.97	247
vadinHA17	1.24	0.13	9.54	117

- At least Flavos common heterotrophs in surface water

Gammaproteobacteria

	% amplicons	% rRNA	Ratio	N. OTUs
Alteromonadales	0.49	0.82	0.60	57
Escherichia-Shigella	8.80	0.03	293	206

- Host-associated, some pathogens
- Facultative anaerobe
- Not doing much here...

Verrucomicrobia

	% amplicons	% rRNA	Ratio	N. OTUs
Spartobacteria	1.51	0.08	18.9	63

- Common heterotroph in Baltic Sea
- Complex sugar degrader (Herlemann et al. 2013)
- Obligate aerobe (?)

High rRNA content

	% amplicons	% rRNA	Ratio	N. OTUs
Cyanos, excl. chloroplasts	2.07	21.47	0.10	98
Subsection I (Synechococcus)	1.65	17.64	0.09	60
Subsection IV (Anabaena)	0.41	3.84	0.11	38
Spirochaetes	1.08	3.51	0.31	130

- Cyanos: 10-fold higher in rRNA than amplicons!
 - Both small (Synechococcus) and filamentous (Anabaena)
- Spirochaetes common pathogens

Cyanobacteria deep below photic zone

- Sampling was done in late april, before Cyanos appear in surface water
- Sinking to collect P?
- Dormant during winter?

Conclusions

- Equal proportion rDNA/rRNA common
- Cyanos are exceptional with 10-fold more rRNA
- Spirochaetes and Mycobacteria surprisingly high in RNA – survival?
- Many taxa seem dormant or dead:
 - Escherichia, Verruco, Flavo