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Using metagenomics and metatranscriptomics to study specific bacterial species involved in biological phosphorus removal from wastewater

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AAU

Introduction

Enhanced Biological Phosphorous Removal (EBPR) from wastewater treatment plants is managed by Polyphosphate Accumulating Organisms (PAOs). The model PAO is "*Candidatus Accumulibacter phosphatis*" (Accumulibacter) and despite a large body of knowledge, their detailed physiology remains elusive.

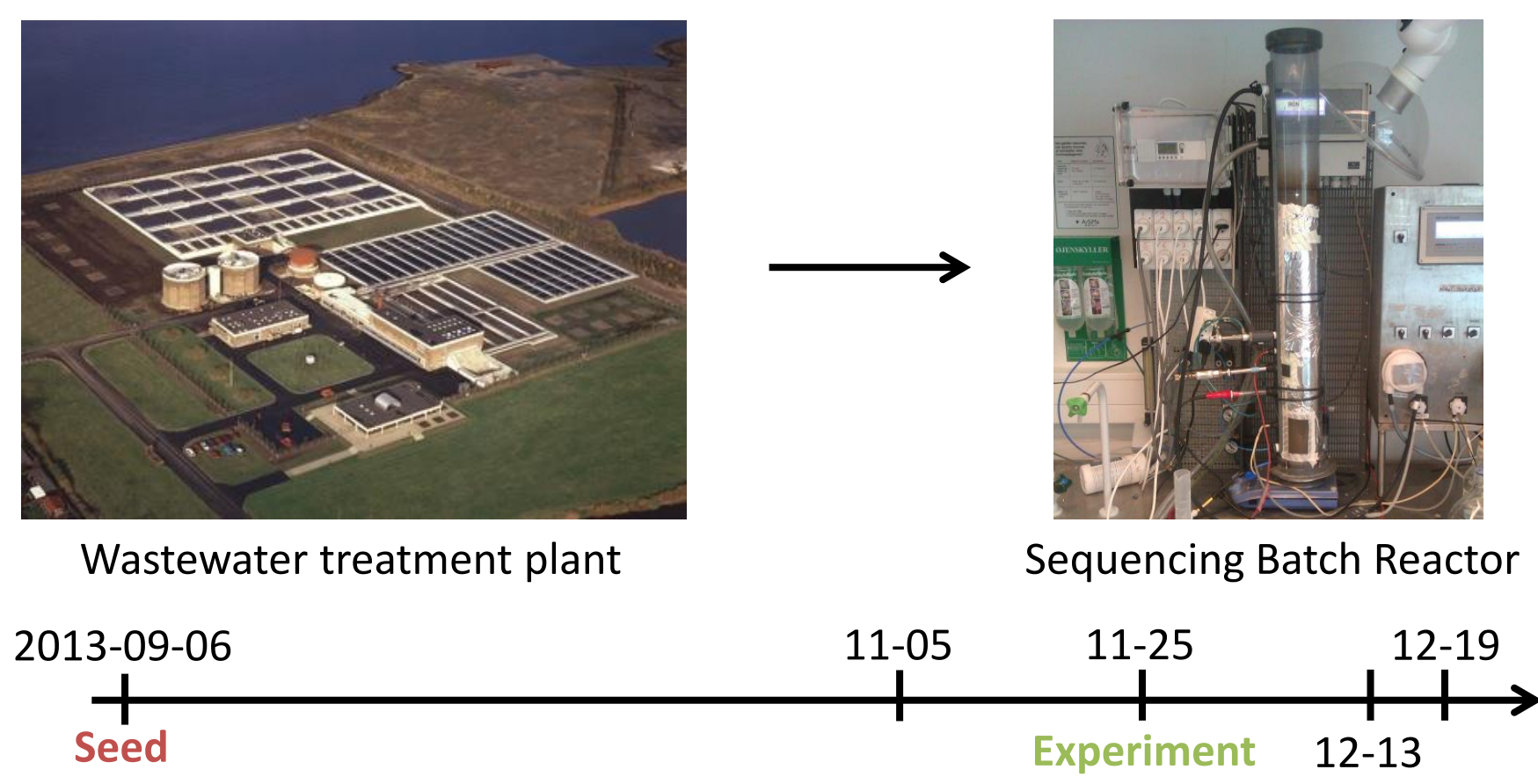
In this study we combined metagenomics, metatranscriptomics and laboratory scale enrichment in order to examine the gene expression of the uncultured Accumulibacter and the co-enriched associated community.

Conclusions

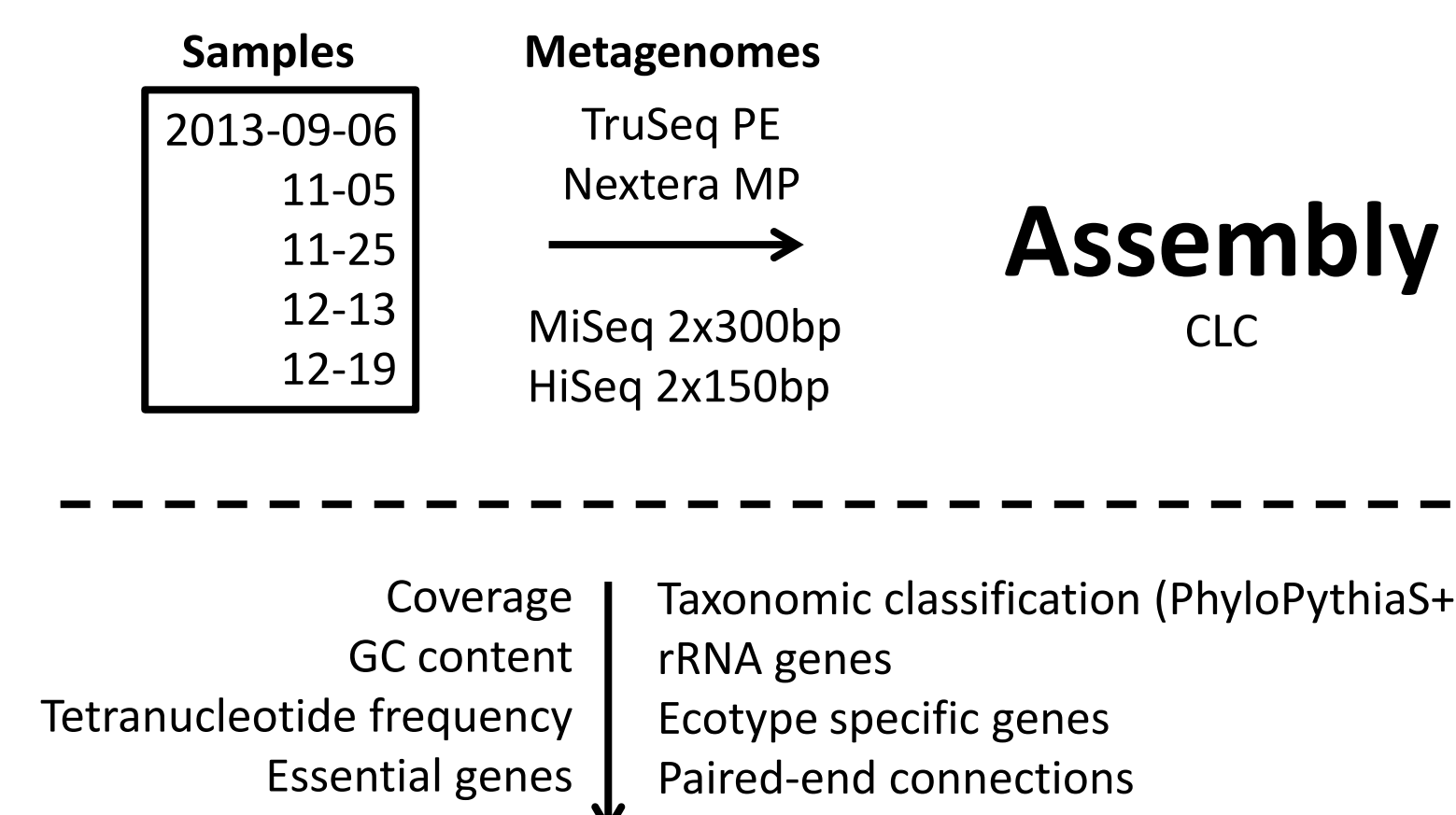
- Combining metagenomics, metatranscriptomics and laboratory scale enrichments enables transcriptome studies of most individual species in the community.
- For the first time we reveal the detailed transcriptome landscape of Accumulibacter during the anaerobic feast and aerobic famine conditions of the EBPR process.
- A new GAO (competitor to PAOs with a negative impact on EBPR) was discovered. Propionivibrio is closely related to Accumulibacter and hit by the current FISH probes used to define PAOs.

Methods

Enrichment



Genome recovery



mmgenome Binning

A toolbox for reproducible genome extraction from metagenomes

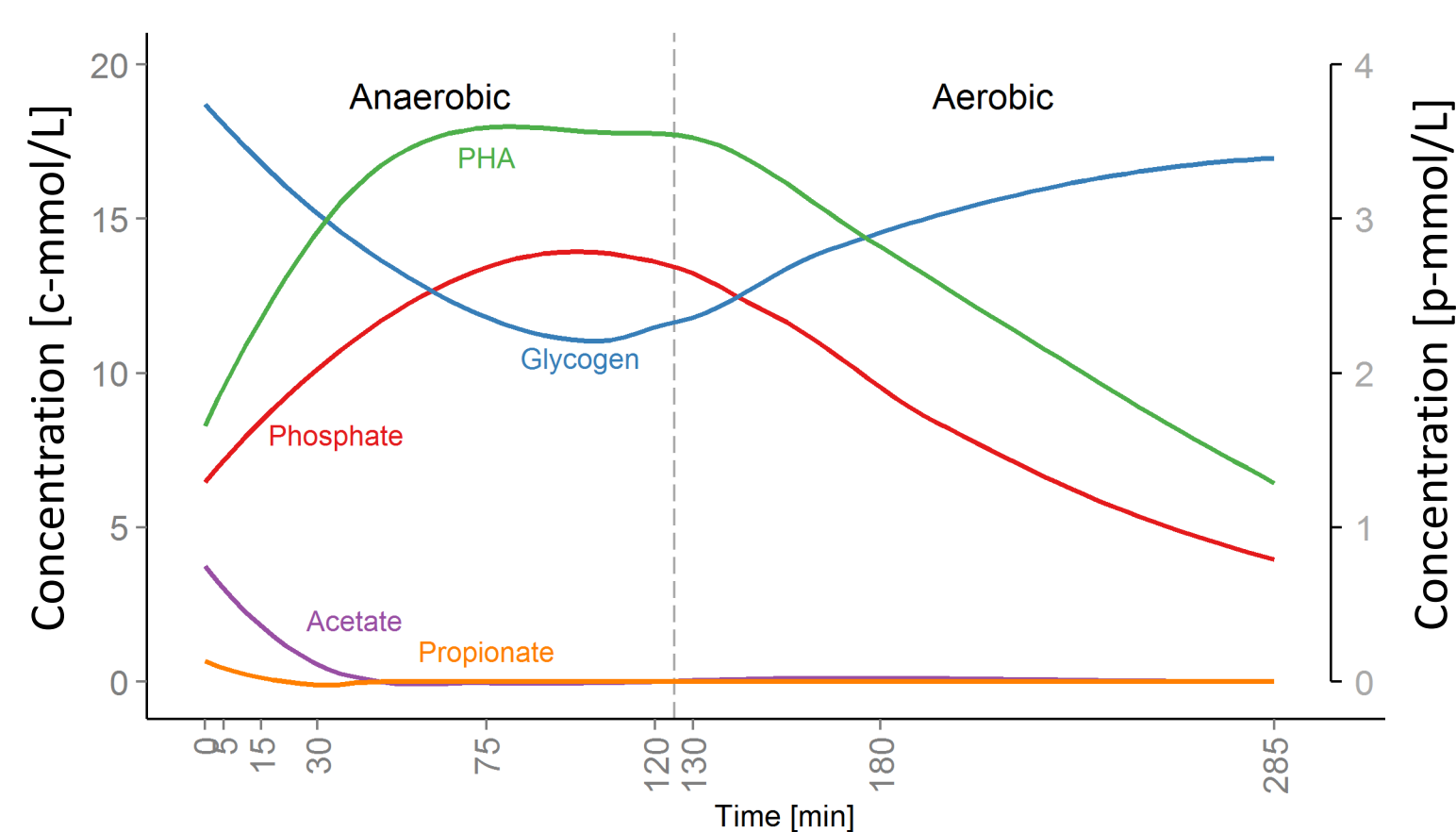


MadsAlbertsen/mmgenome

Example



Transcriptomics

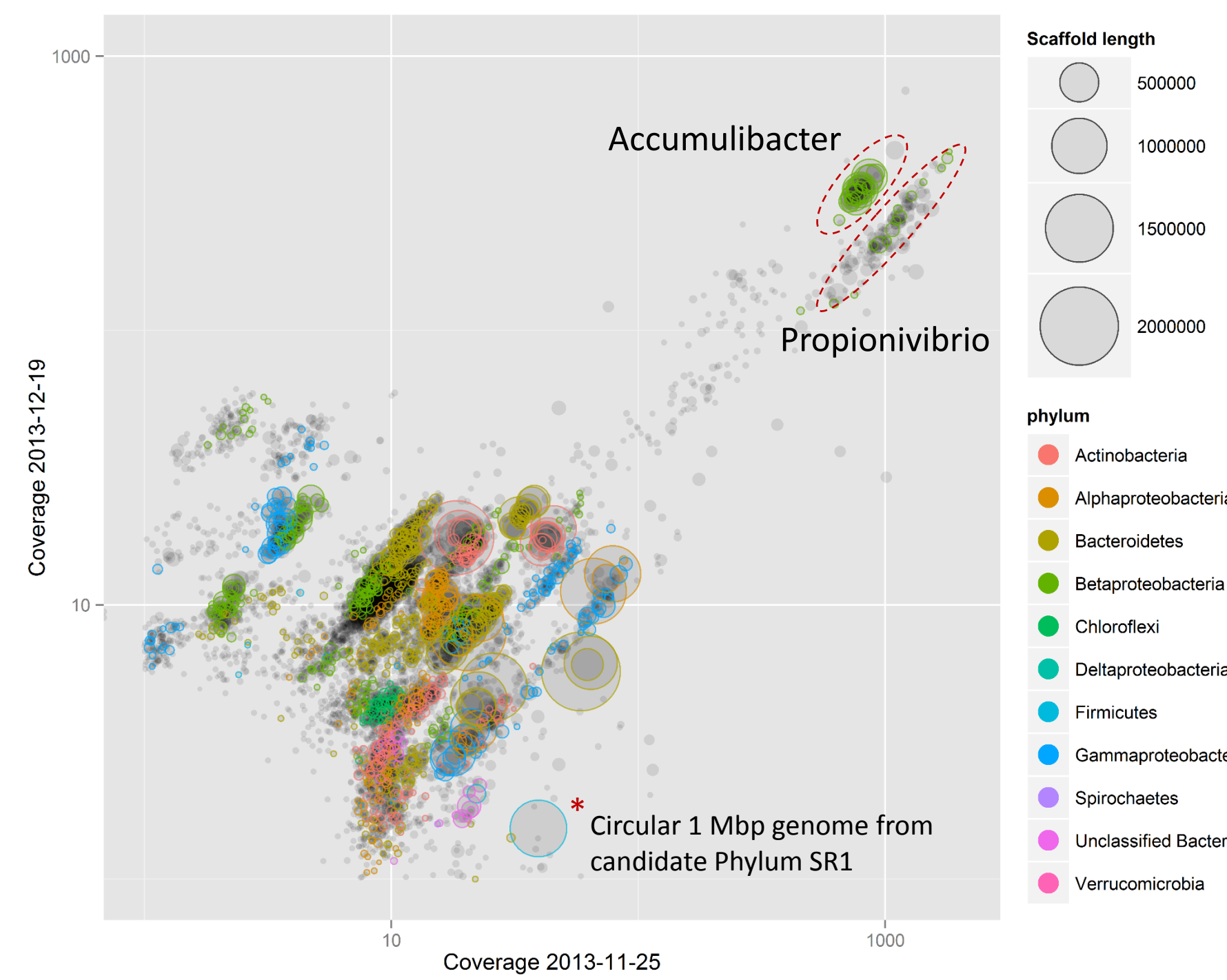


9 timepoints
27 samples
400 million reads

rRNA removal (Ribo-Zero Bacteria)
mRNA seq (TruSeq Stranded)
HiSeq 1x50 bp

Mapping to the metagenome bins enables transcriptomics of the individual species in the community.

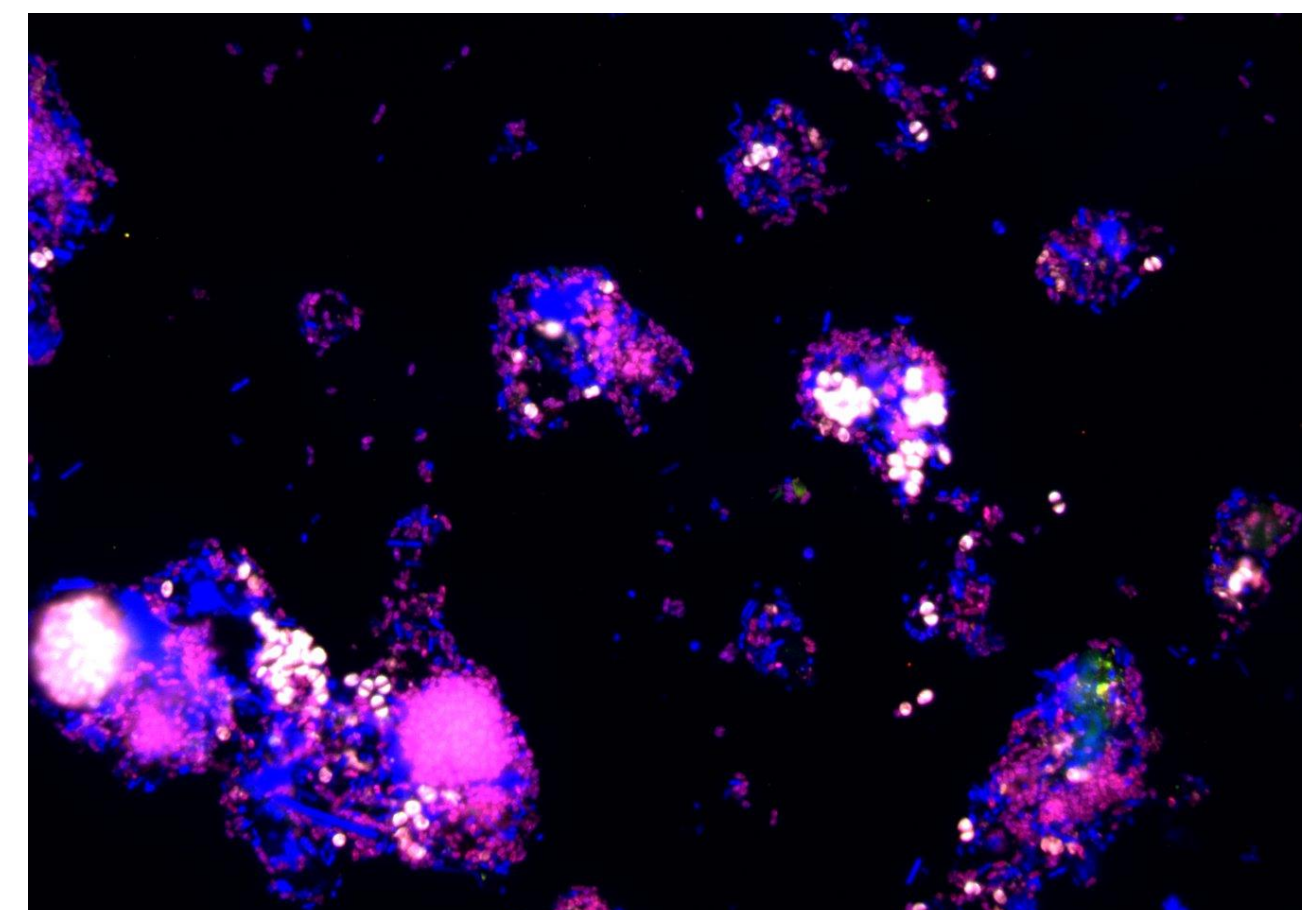
Results



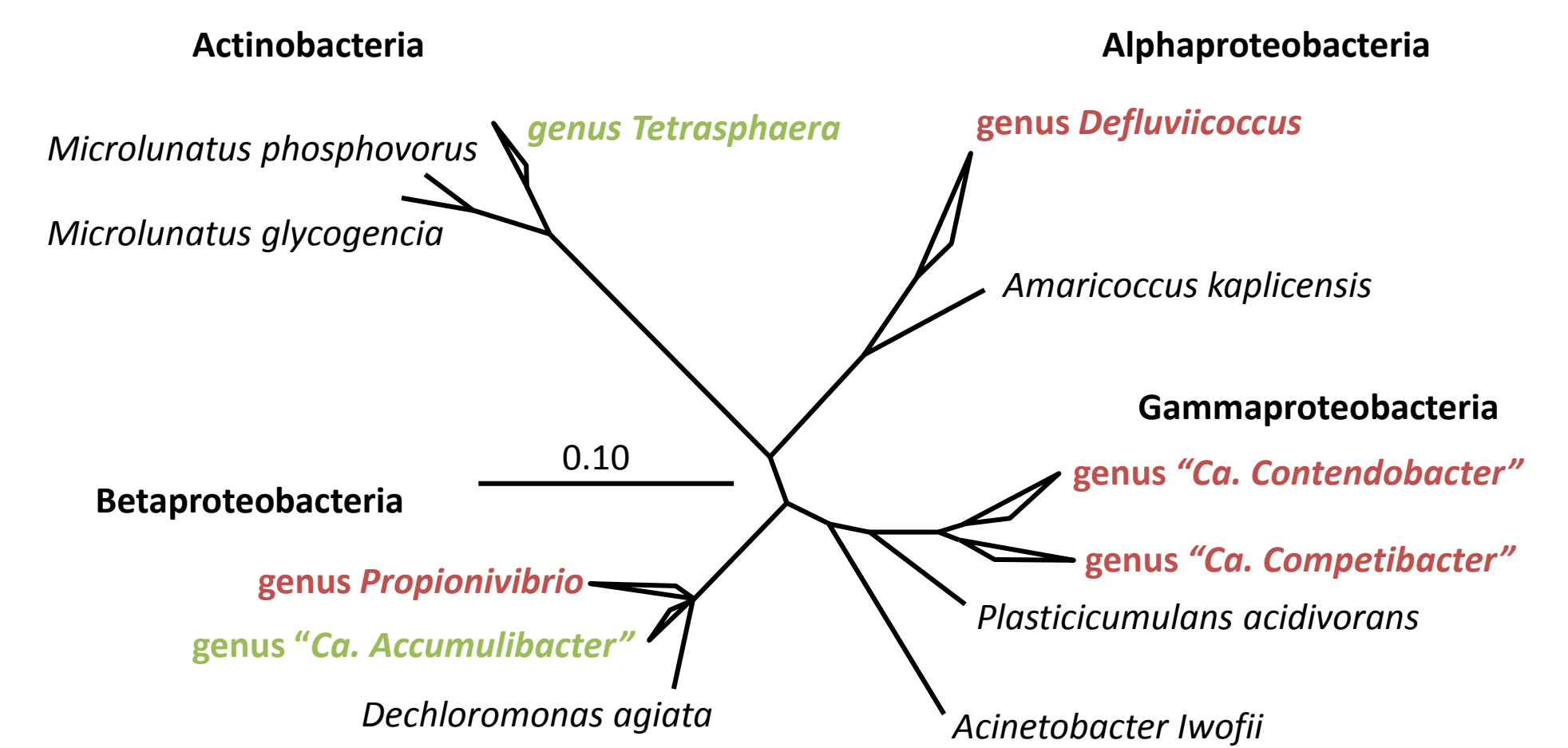
Genome extraction from metagenomes. Differential coverage binning is used as the first step in extracting individual genomes from the metagenomes. Each point represents a scaffold, colored by essential single copy genes and scaled by scaffold length. The plot was made using the mmgenome R package. Surprisingly a bacteria from the genus *Propionivibrio* was enriched along with Accumulibacter.

	Accumulibacter	Propionivibrio
Genome size	4.7 Mbp	3.8 Mbp
Scaffolds	30	88
Completeness	100 %	> 95 %
Strains	1	2-3
Plasmids	1	1-2
Non-rRNA transcriptome reads	70 million	159 million
Abundance: Experiment	15 %	32 %
Abundance: Seed WWTP	0.2 %	0.2 %
Abundance: Other WWTPs*	< 0.1 - 0.2 %	0.1 - 0.8 %

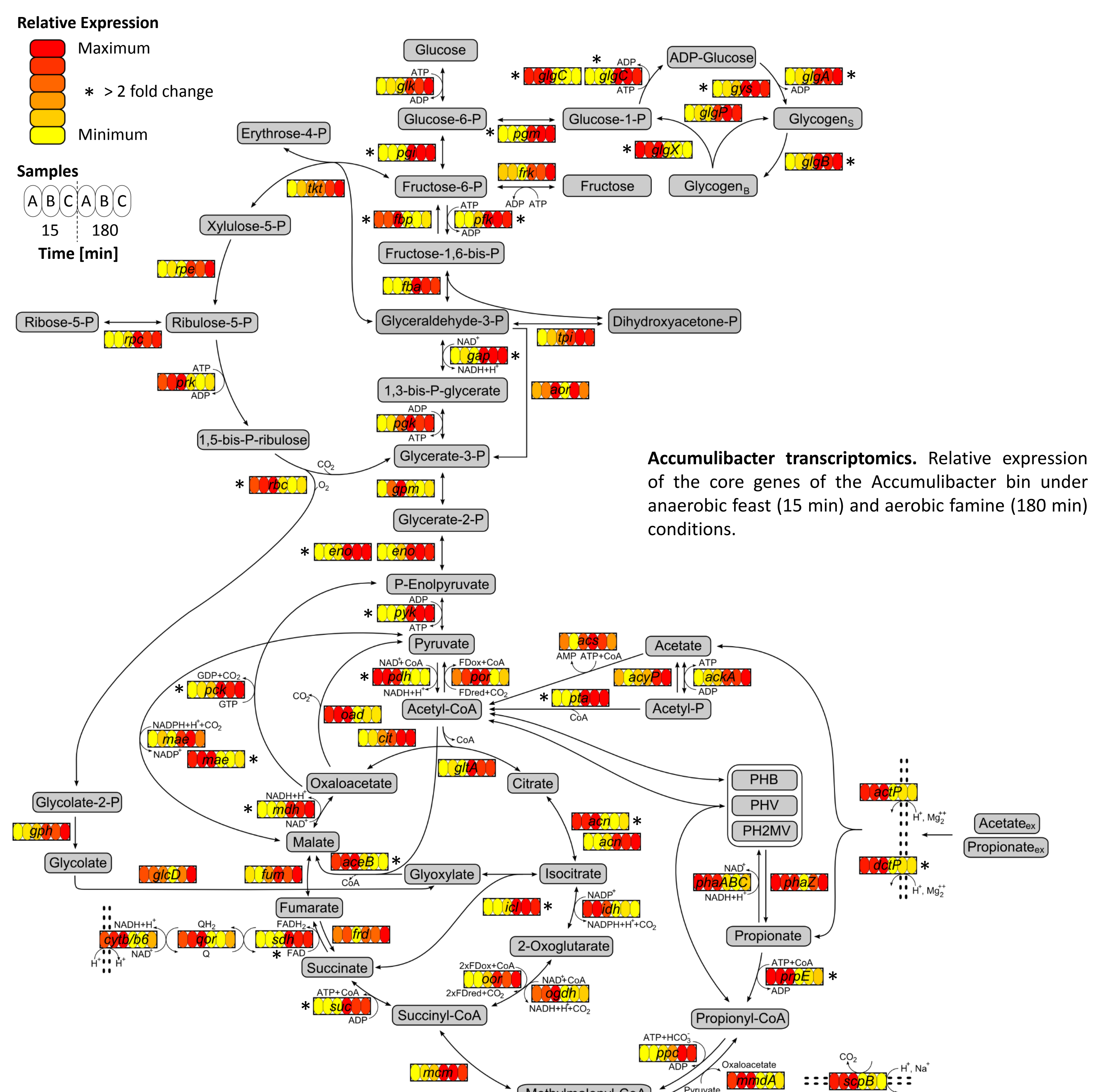
*Estimated from 15 full scale WWTP metagenomes (2.3 bn reads).



A GAO disguised as a PAO. The putative GAO *Propionivibrio* (magenta) is hit by the probes used to quantify the PAO *Accumulibacter* (white). However, it does not accumulate excessive amounts of polyphosphate. Probes: PAO651 (FLUOS, *Accumulibacter*); PAO462 (Cy3, both); PAO846 (Cy3, both); EUBmix (Cy5, all bacteria).



Phylogenetic tree (16S rRNA) of known PAOs and GAOs. Until now the PAOs and GAOs have been separated into distant phylogenetic lineages. The discovery of a GAO from the genus *Propionivibrio* makes interpretations more difficult. The binned *Accumulibacter* genome was closely related to *Accumulibacter* clade IIC based on phylogenetic analysis of the *ppk1* gene.



Accumulibacter transcriptomics. Relative expression of the core genes of the Accumulibacter bin under anaerobic feast (15 min) and aerobic famine (180 min) conditions.