Composition of biofilm in filter material of natural pools

A pilot study

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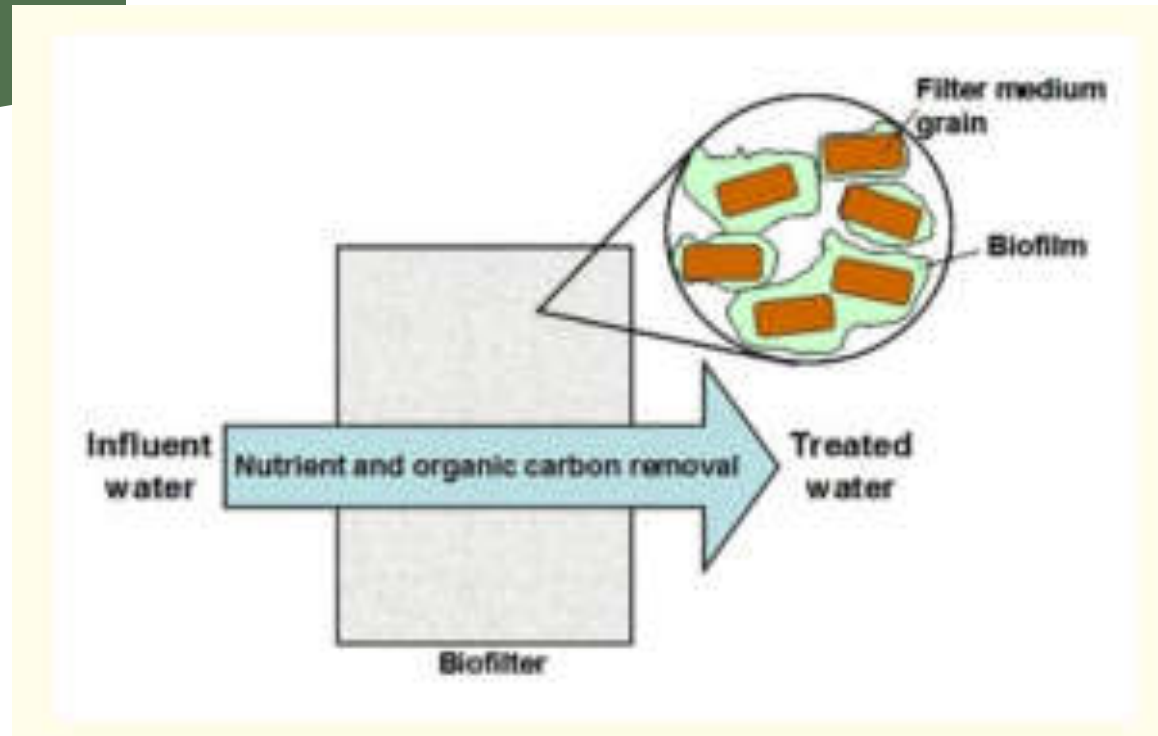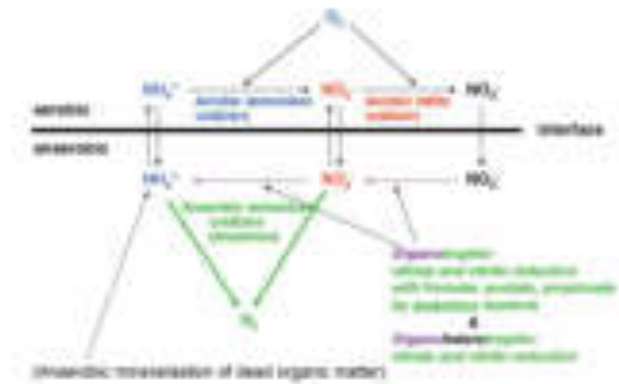
Biofilm is the solution

- Challenge: produce water that is „biologically stable"

- Biofilm = solution

  = biodegrading organic matter
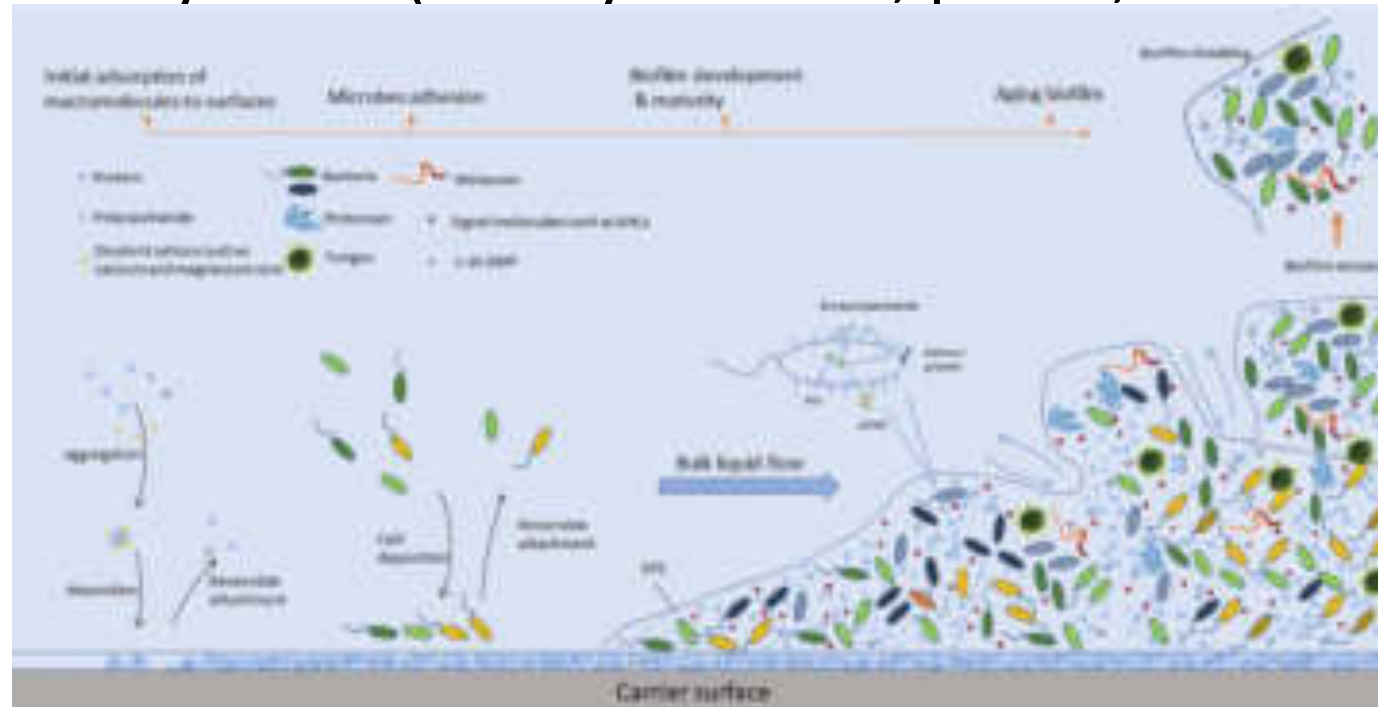
  and ammonium in controlled

  conditions

Bioadsorption, Bioaccumulation and Degradation im Biofilter

Sehar et al., 2015, DOI: 10.5772/63499

If the biofilm is the solution

- What (micro)organisms is the biofilm composed of?
- Pro- and Eukaryotes… (mostly bacteria, plants, animals, protozoa,…)

Composition not known in natural pools !

Biofilm composition:

- Standard microbial analysis – only cover bacteria, and only those that can be **cultured**

- Furthermore: A **dormant status** was described for pathogenic bacteria, such as *Campylobacter* spp., *E. coli*, and *Legionella pneumophila* which makes their isolation and identification problematic
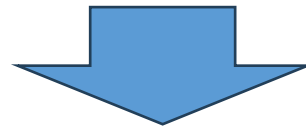
- Therefore, cultivation-independent **molecular-biological methods targeting nucleic acids** are required in addition to biochemico-metabolic analyses

(Moritz et al, 2010).

Aims: Blackbox Biofilm

- Decipher the **blackbox biofilm**: To identify the composition of the biofilm using next-generation sequencing (NGS)

Subaims:

- To figure out the **collection method** needed for analysis of biofilm samples. Therefore local stone collection is compared to complete biofilm removal of the filter by air pressure („blowern").

- Elucidate the differences between **biofilm and water microbiota** composition

METHODS:
sample collection and preparation

Methods:

Amplikon sequencing, metagenomic shot-gun sequencing

Samples: 5 Pools

Biofilm: 5 „stone"samples
3 samples „blowern" (in 2 pools „blowern" was not possible)

Water: 5 „water"samples

Results

Results Amplicon Sequencing:
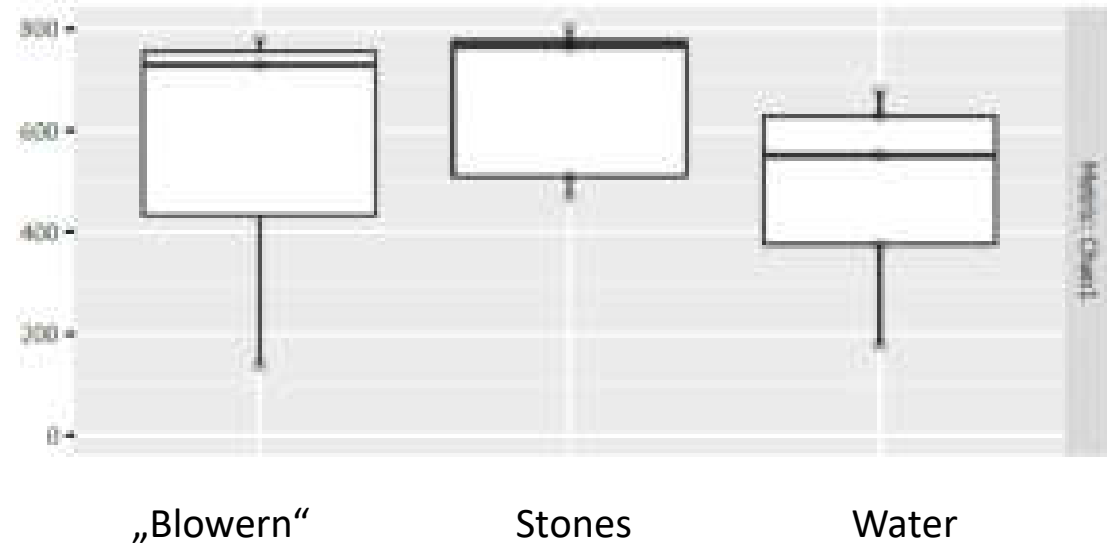
→ Focus on bacteria

Alpha-Diversity:
comparing sample types:

→ Richness

- Bacterial Richness (Chao1 Index) = similar;
- How many bacterial taxa

„Blowern"   Stones   Water

Pairwise Wilcoxon Test:
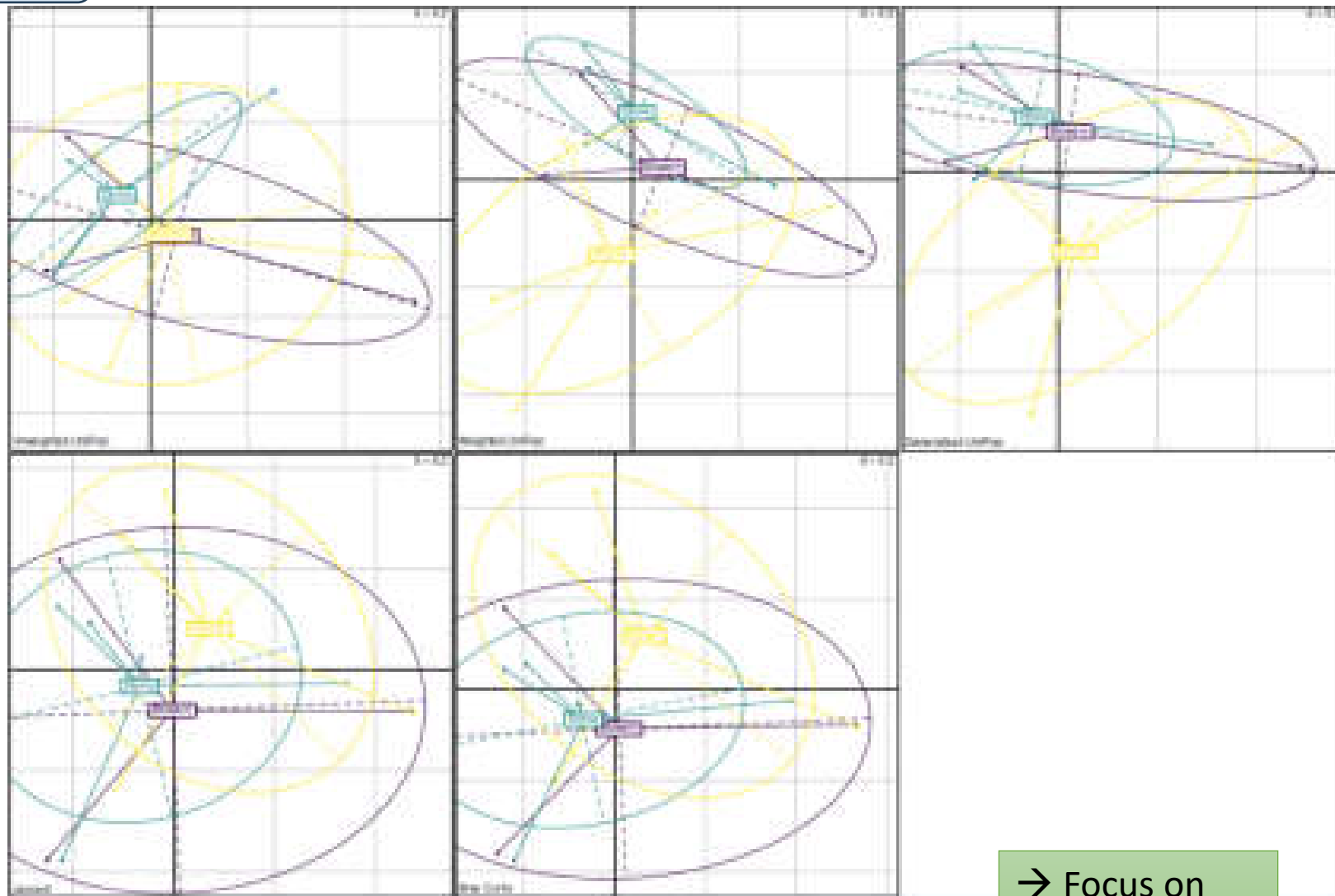
Chao1= n.s.

Beta-Diversity: → Abundance

Difference in relative abundance

PermanovaG with 5 metrics

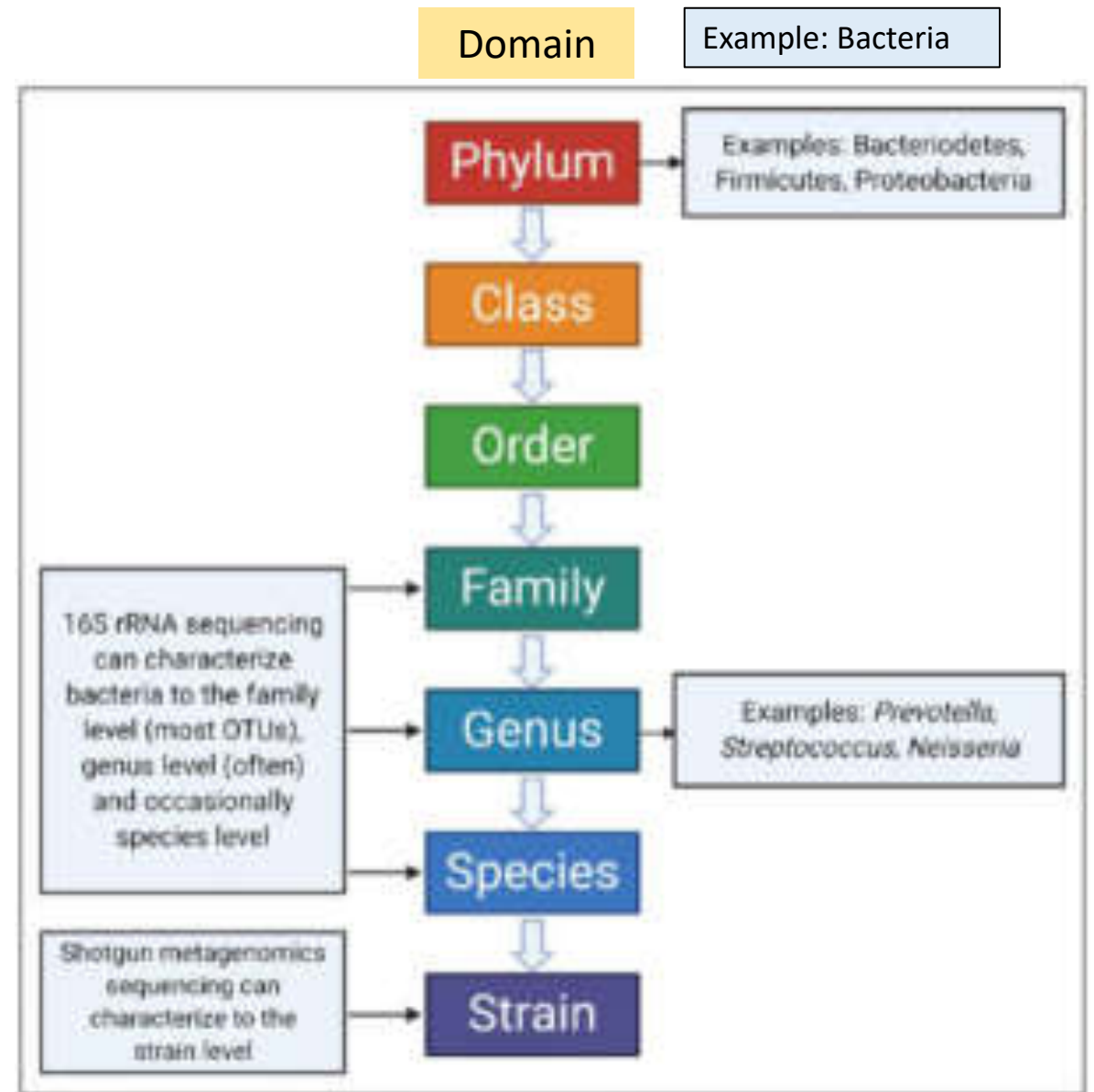Omnibus p-value = n.s.

Ellipses represent a 75% CI around the cluster centroid

→ Focus on stone samples

Taxonomic ranks

Domain   Example: Bacteria

Taxonomic classification of bacteria from phylum to strain level.

Taxonomic composition – Phyla: per sample overview:

Stones, Pools 1-5;

TOP 15 taxa shown (rest in grey);
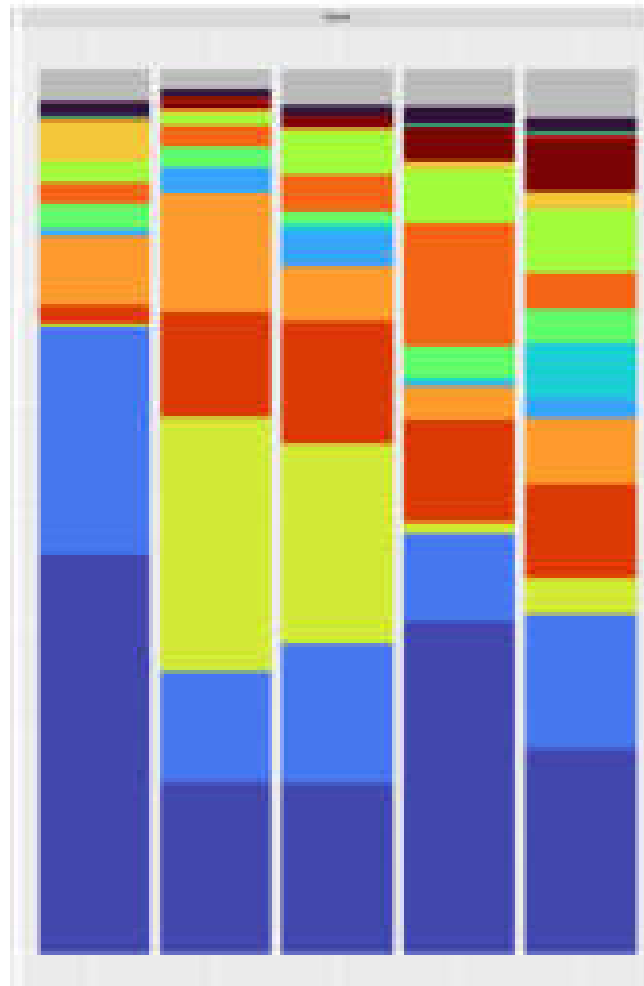
Pools 1-5

Feature %>% fct_rev()

- Others
- Root > Bacteria > Myxococcota
- Root > Bacteria > Dependentiae
- Root > Bacteria > Bdellovibrionota
- Root > Bacteria > Nitrospirota
- Root > Bacteria > Firmicutes
- Root > Bacteria > Chloroflexi
- Root > Bacteria > Acidobacteriota
- Root > Bacteria > Actinobacteriota
- Root > Archaea > Crenarchaeota
- Root > Unclassified > unclassified
- Root > Bacteria > Verrucomicrobiota
- Root > Bacteria > Planctomycetota
- Root > Bacteria > Cyanobacteria
- Root > Bacteria > Bacteroidota
- Root > Bacteria > Proteobacteria

Taxonomic composition – Class: per sample overview:

Stones, Pools 1-5;
TOP 15 taxa shown (rest in grey)

Pools 1-5

→ Summarized to:
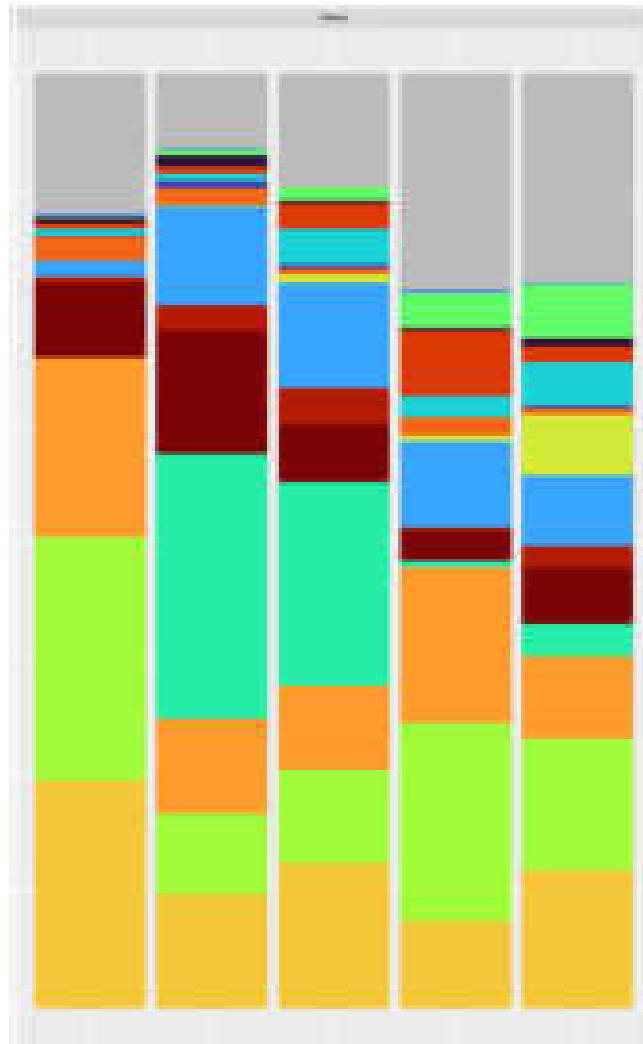
Feature %>% fct_rev()

- Others
- Root > Bacteria > Dependentiae > Babeliae
- Root > Bacteria > Nitrospirota > Nitrospiria
- Root > Bacteria > Acidobacteriota > Acidobacteriae
- Root > Bacteria > Acidobacteriota > Blastocatellia
- Root > Bacteria > Chloroflexi > Anaerolineae
- Root > Bacteria > Proteobacteria > unclassified
- Root > Bacteria > Actinobacteriota > Actinobacteria
- Root > Archaea > Crenarchaeota > Nitrososphaeria
- Root > Bacteria > Planctomycetota > Planctomycetes
- Root > Unclassified > unclassified > unclassified
- Root > Bacteria > Verrucomicrobiota > Verrucomicrobiae
- Root > Bacteria > Cyanobacteria > Cyanobacteria
- Root > Bacteria > Proteobacteria > Alphaproteobacteria
- Root > Bacteria > Proteobacteria > Gammaproteobacteria
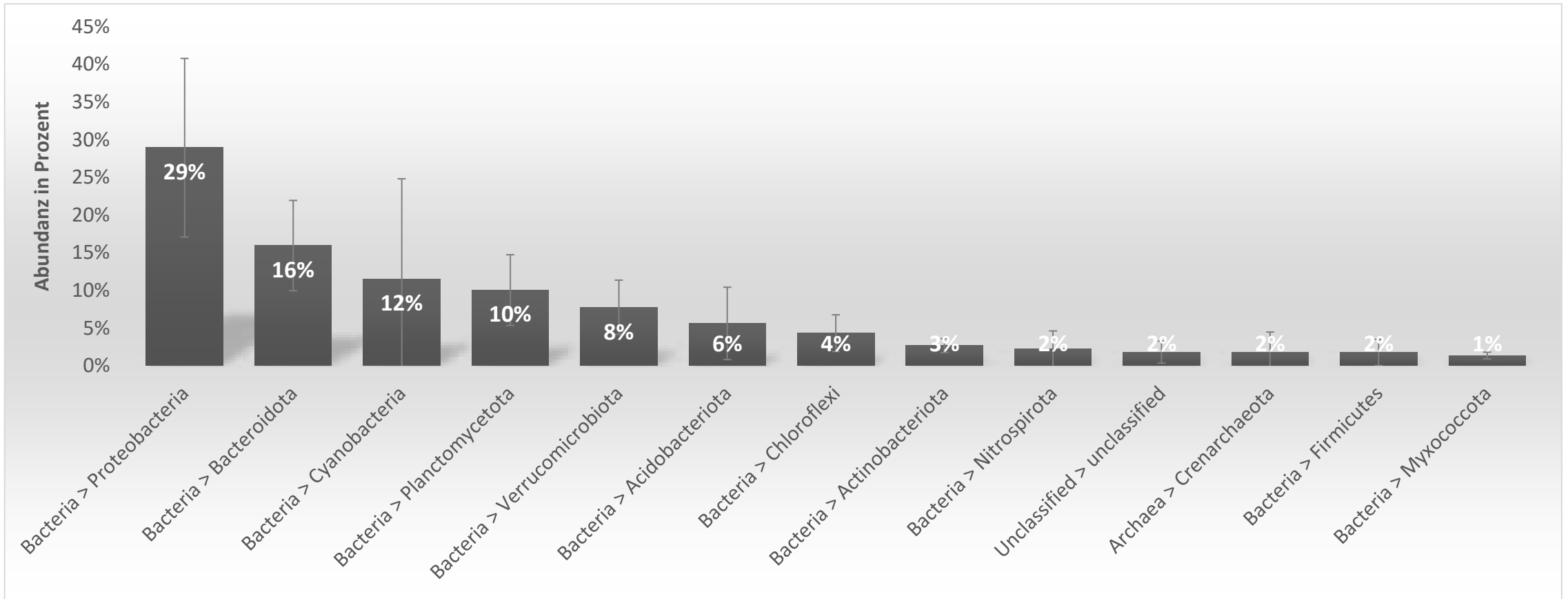- Root > Bacteria > Bacteroidota > Bacteroidia
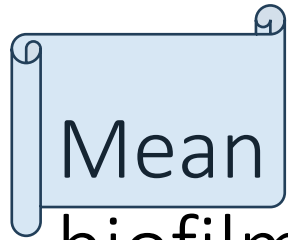
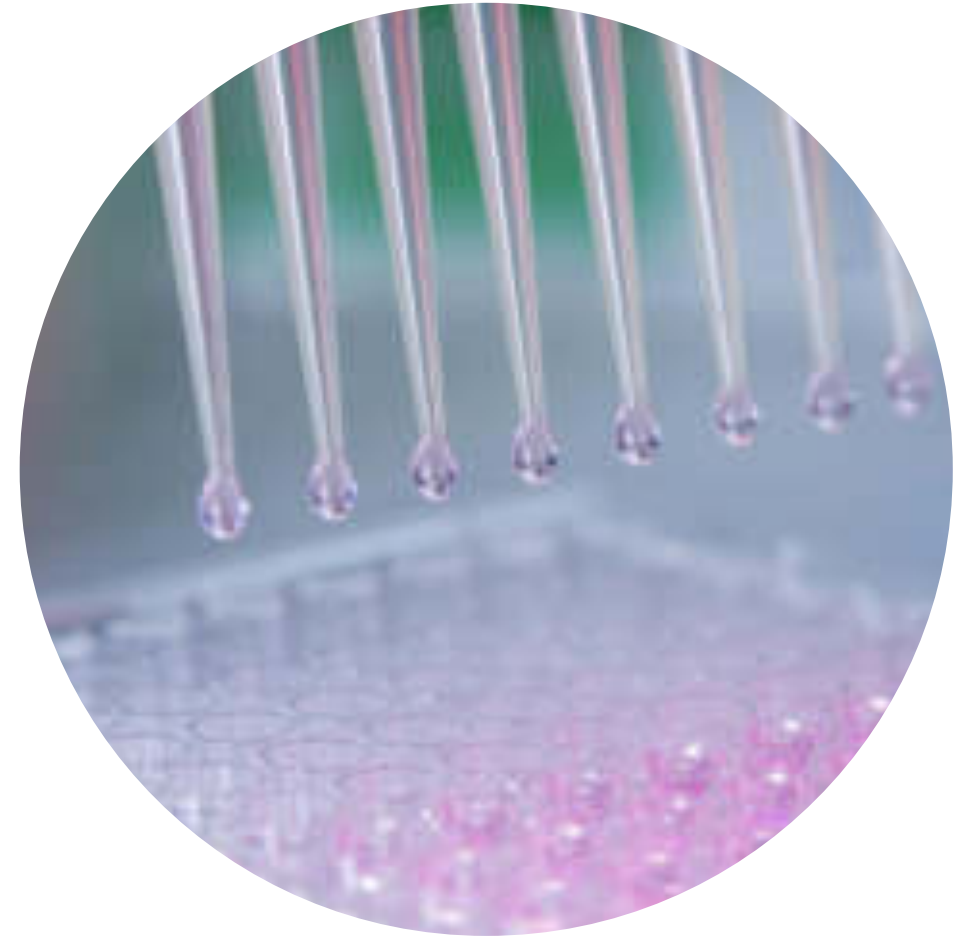# Mean composition (13 most prominent phyla in stone biofilms):
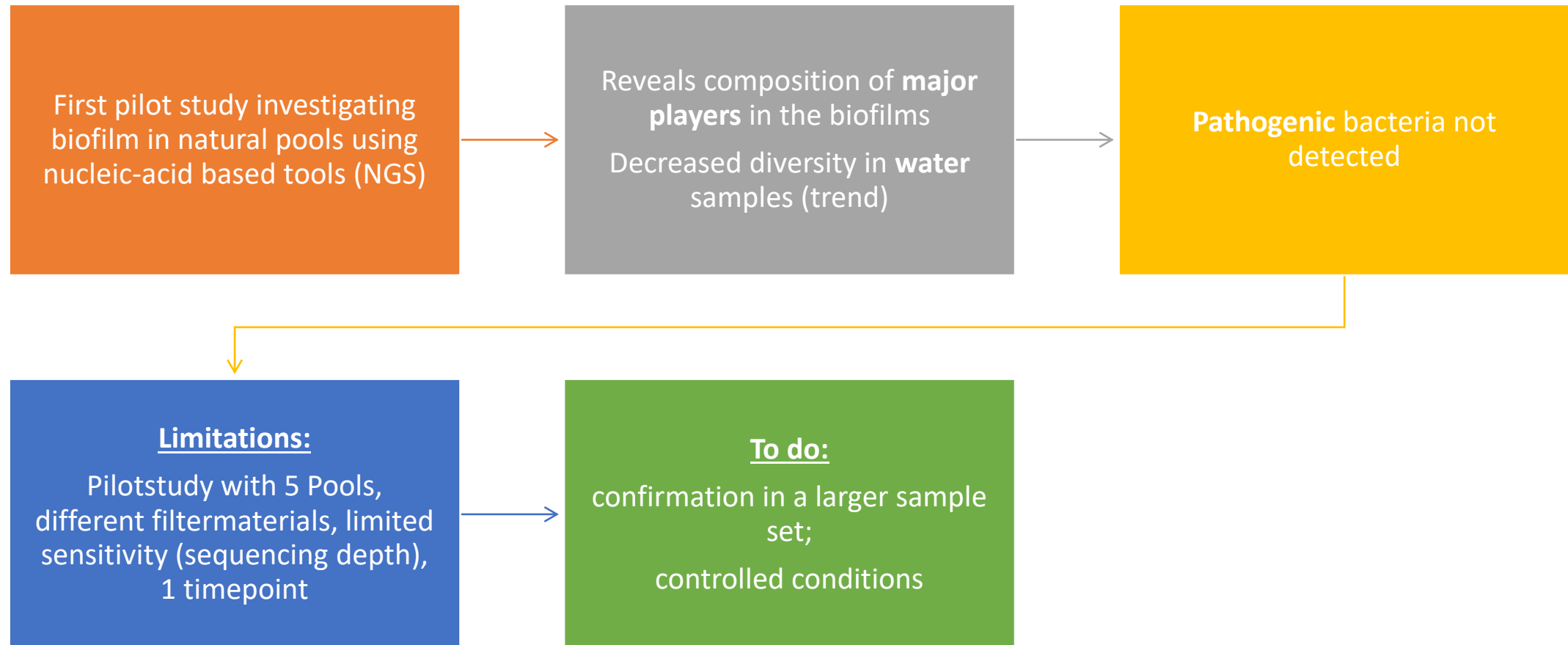
Pathogenic bacteria ?

- No hygienically relevant pathogenic bacteria detected in sequencing results

→E. coli, Enterococcus, Pseudomonas aer.

→ CAVE: Sensitivity !

Discussion:

First pilot study investigating biofilm in natural pools using nucleic-acid based tools (NGS)

Reveals composition of **major players** in the biofilms

Decreased diversity in **water** samples (trend)

Pathogenic bacteria not detected

Limitations:

Pilotstudy with 5 Pools, different filtermaterials, limited sensitivity (sequencing depth), 1 timepoint

To do:

confirmation in a larger sample set;

controlled conditions

Thank you !

Christoph Fink, Garten Hödl

Vergleich mit Biofilmen in der Wasseraufbereitung:

Tabelle 2-3: Klassifikation der Mikroorganismen in biologischen Abwasserreinigungssystemen (nach Rheinheimer et al. 1989)

Organismen-gruppe	C-Quelle	Elektronen-donator	Elektronen-akzeptor	Produkte des Abbaustoffwechsels	Tätigkeitsmerkmale	
Primär-abbauer	org. C (gelöst)	org. C	O_2	CO_2, NH_4	aerobe Bakterien	chemo-organo-heterotrophe Bakterien
			NO_3^-	NO_2^-	Nitratreduzierer	
			NO_3^-, NO_2^-	N_2	Denitrifikanten	
			org. C	org. C	anaerobe Bakterien	
Sekundär-abbauer	organ. C (partikulär)	organ. C	O_2	CO_2, NH_4	Ciliaten, niedere Metazoen	
Nitrifikanten	CO_2	NH_4^+	O_2	NO_2^-	Ammoniakoxidierer	chemo-litho-autotrophe Bakterien
	CO_2	NO_2^-	O_2	NO_3^-	Nitritoxidierer	