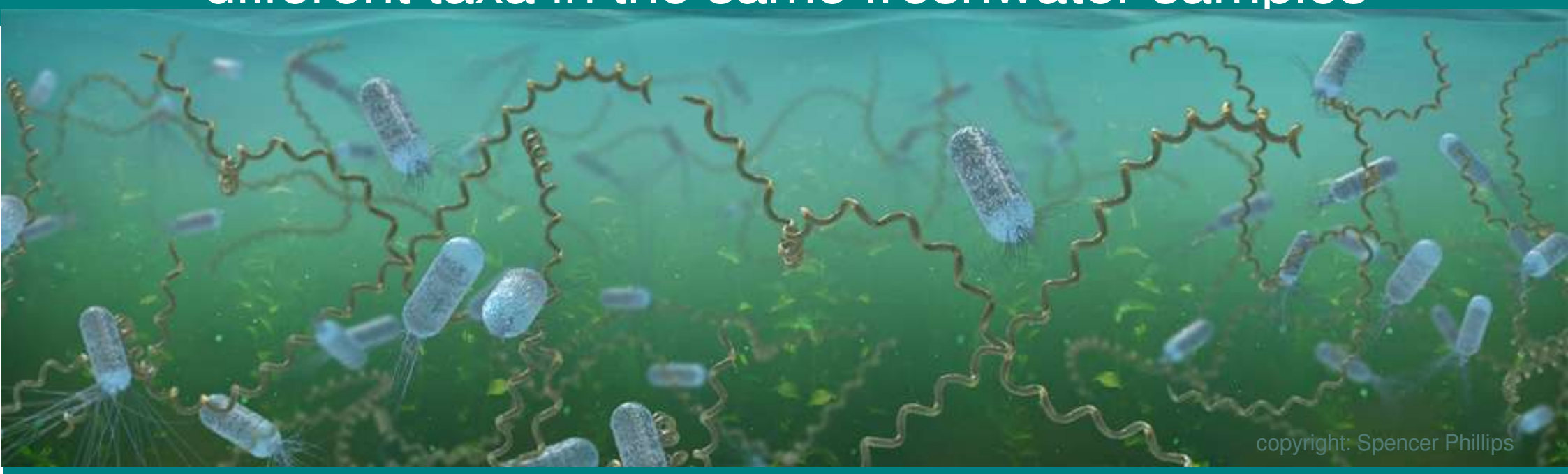
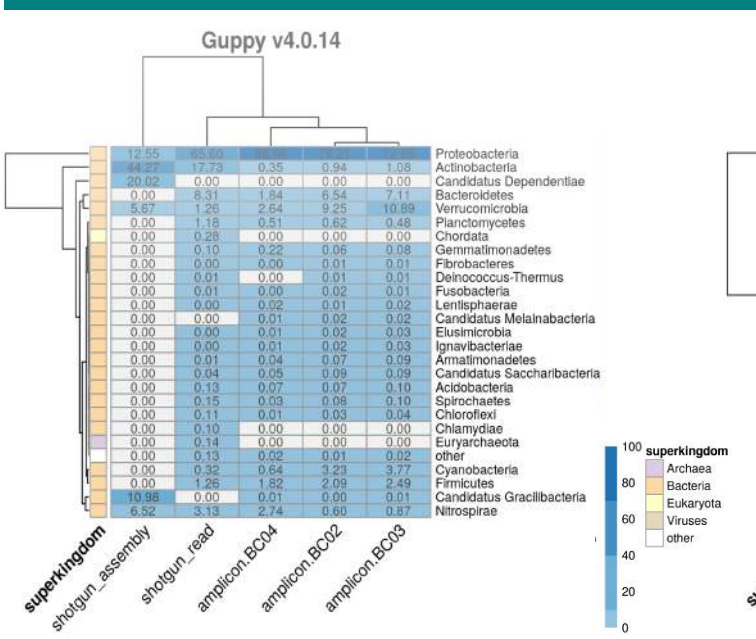
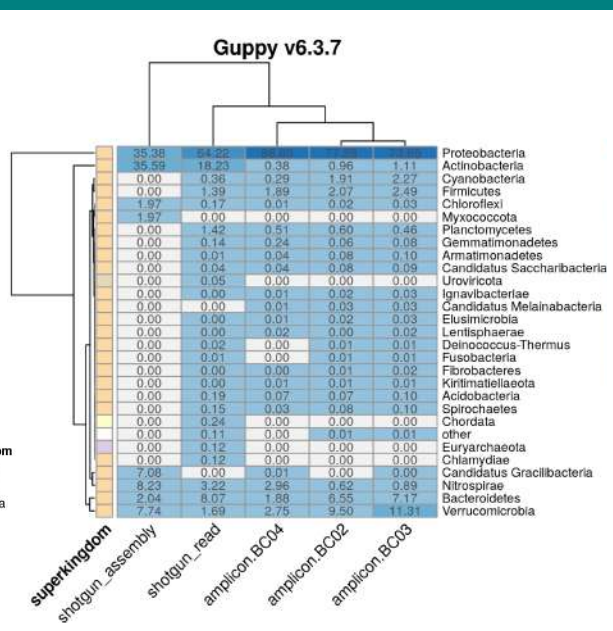
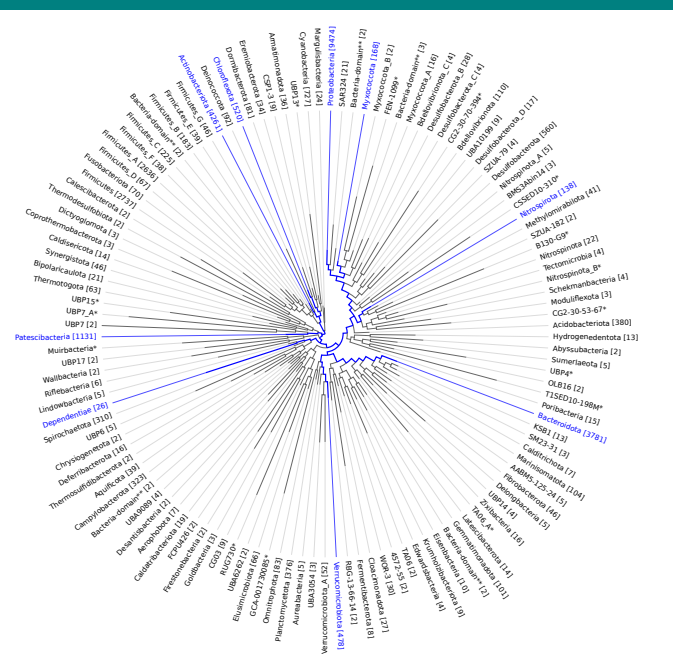
16S vs shotgun nanopore sequencing detected different taxa in the same freshwater samples



Comparison of amplicon and shotgun sequencing for freshwater monitoring







Problem

16S and shotgun assembly- and read -based approaches are widely used for taxonomic profiling. The resulted taxonomic assignments are compared accross studies. We showed that the estimated taxa are biased by the choice of shotgun vs amplicon method.

Methods

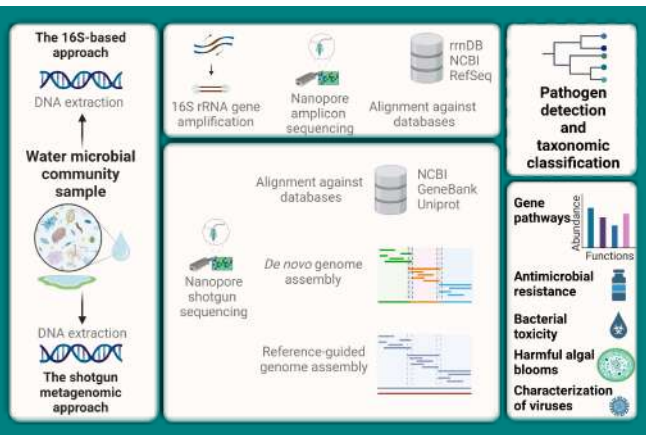
Target database: GTDB Release 207
Shotgun read-based: DIAMOND-Megan-LR
Shotgun assembly-based: MetaFlye; Racon; Medaka; MaxBin; Vamb; Metabat; DasTool
16S amplicon: Emu

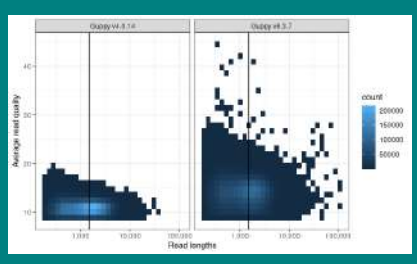
Results

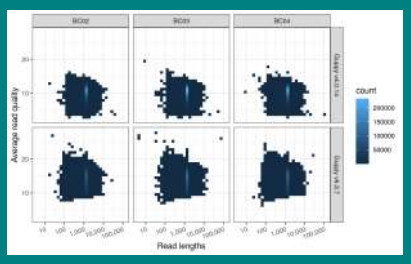
Differences in presence and relative abundance already on phylum level
Discovered diversity: shotgun read-based > 16S > assembly-based approach
High-quality MAGs reflected only the most abundant taxa
Higher basecalling accuracy -> long reads of higher quality -> better alignments -> robust taxonomic profiling for shotgun and amplicon methods

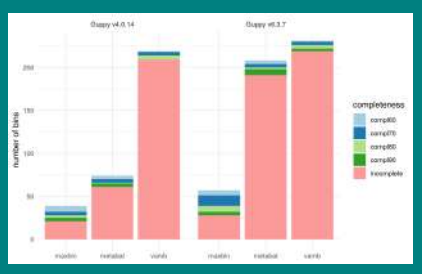
Discussion

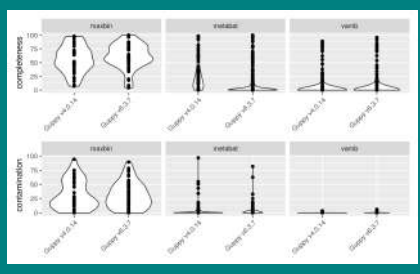
Possible reasons for differences in relative abundance:
high-quality MAGs are not representative for sample composition because many reads discarded since simply not map to bins
amplification bias
differences in genome size
copy-number variation











Anastasia Grekova, Maximilian Stammnitz, Fahad Khokhar, Paul Saary, Andre Holzer, PuntSeq team and Lara Urban
anastasia.grekova@tum.de

References: Urban et al. (2021) Freshwater monitoring by nanopore sequencing eLife 10:e61504; Sereika et al. (2022) Oxford nanopore r10.4 long-read sequencing enables the generation of near-finished bacterial genomes from pure cultures and metagenomes without short-read or reference polishing. Nature Methods, 19:823–826