

Comparison of sequencing platforms and bioinformatics pipelines for wetland community profiling



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Introduction

Reed beds (also known as constructed wetlands) are used in many industries (whisky, tannery, winery, aquaculture, etc.) to treat wastewater. Bacteria are primarily responsible for biological treatment in these systems. It is crucial to get the most information possible while processing environmental DNA (eDNA) to understand the biotic community in these systems.

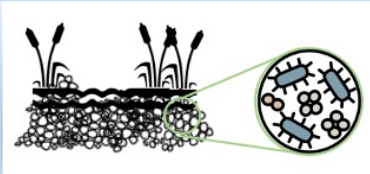


Fig. 1: Bacterial biofilm structures in wetlands

Sampling Site



Fig. 2: Natural wetland sites used for sampling: River Thurso, Saint John's Loch, and Loch More

Aims

- Compare the taxonomy data produced from two popular high-throughput sequencing platforms (Illumina MiSeq and Oxford Nanopore Technologies (ONT) MinION) reads using different databases and pipelines (SILVA v132 ribosomal RNA (rRNA) database on the DADA2 pipeline for MiSeq and the NCBI 16S database on EPI2ME pipeline for MinION)
- Compare the taxonomy data generated from two popular high-throughput sequencing platforms (ONT MinION reads, and Illumina MiSeq reads) using the same (SILVA v132) database

Methods



Fig. 3: Twenty PBS treated gravel biofilms from 3 different wetlands



Fig. 4: PBS filtering and eDNA isolation

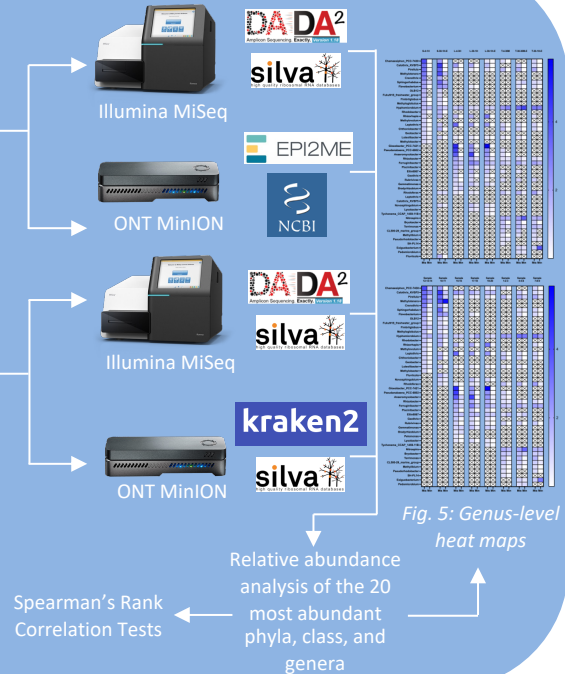


Fig. 5: Genus-level heat maps

Results

- For phylum-level taxonomic classification, MinION sequencing with its EPI2ME pipeline was significantly associated with the Illumina MiSeq and DADA2 pipelines
- A moderate association was seen at the class-level and falling to negligible at the genus level

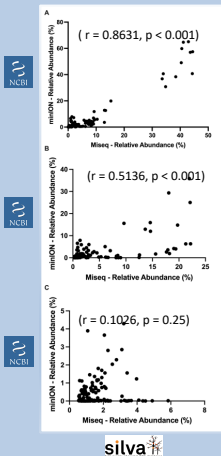


Fig. 6: Spearman's rank correlations for the phylum (A), class (B), and genus (C) level abundance data coming from MiSeq (SILVA Database) and MinION (NCBI Database) analysis – using 8 different sample groups

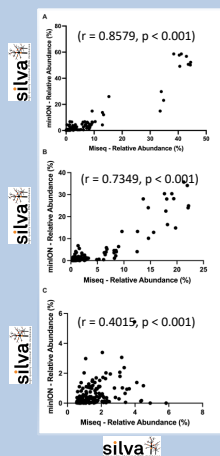


Fig. 7: Spearman's rank correlations for the phylum (A), class (B), and genus (C) level abundance data coming from the MiSeq and MinION (SILVA Database) analysis – using 8 different sample groups

- The very high correlation at the phylum-level persisted while using the same database for each sequencing technology
- The correlation at the class-level improved (to strong)
- Additionally, the correlation at the genus-level improved from negligible to moderate

Future

- Our research indicates that the MinION/EPI2ME workflow is a potentially suitable alternative for bacterial phylum and class-level taxonomic analysis, albeit accepting that some potential differences may emerge
- In future studies, it is necessary to use microbial standards, namely mock communities with known bacterial compositions, and to use these relative abundances as a reference in order to clarify that both platforms give accurate results



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