

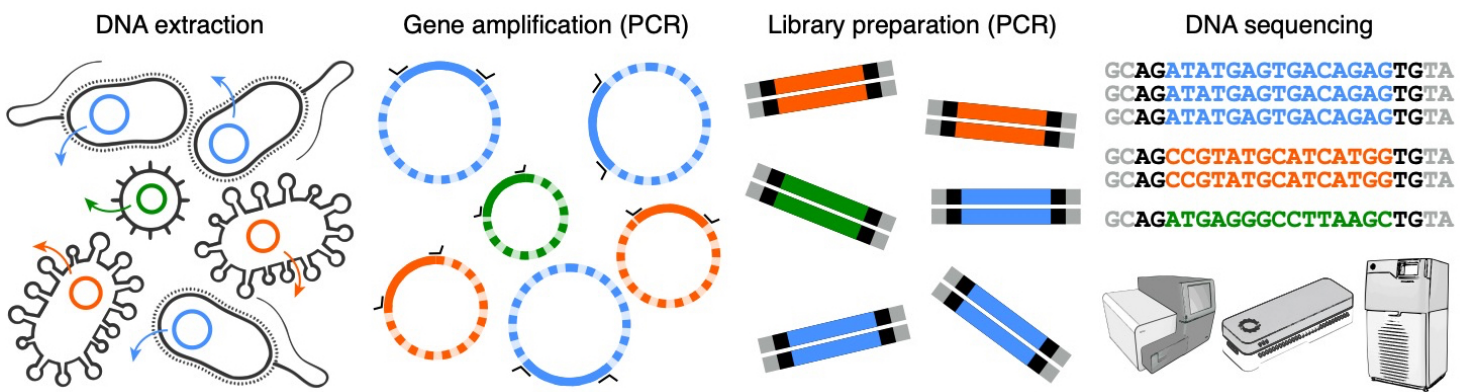
Microbial community analysis



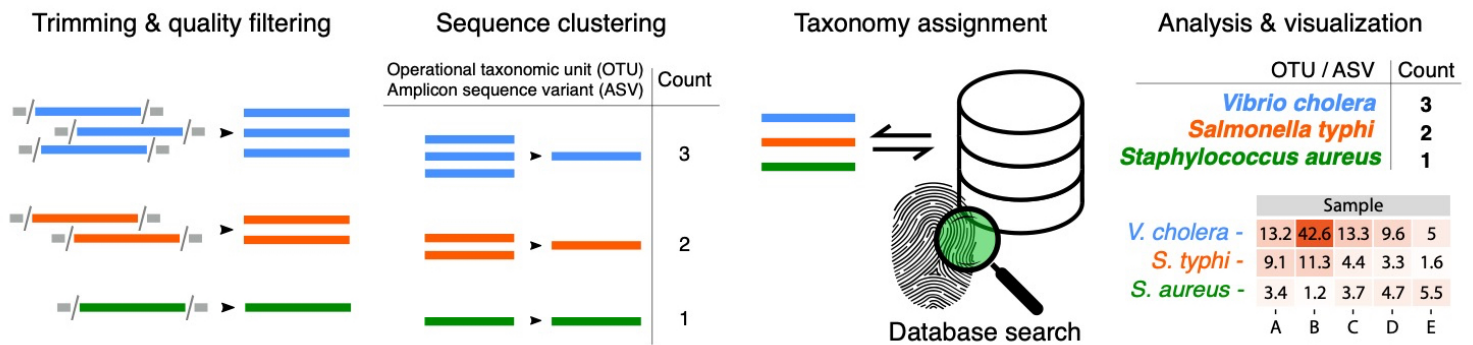
- Microbial communities affect all aspects of human activity, and understanding their significance requires high resolution community analysis
- Analysing the structure and species abundances of microbial communities is possible by sequencing taxonomic marker genes (e.g. 16S rRNA and ITS genes)

DNA Sense provides sample-to-answer services for gene sequencing and microbial community analysis

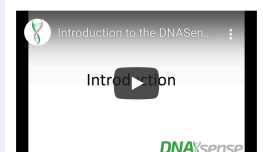
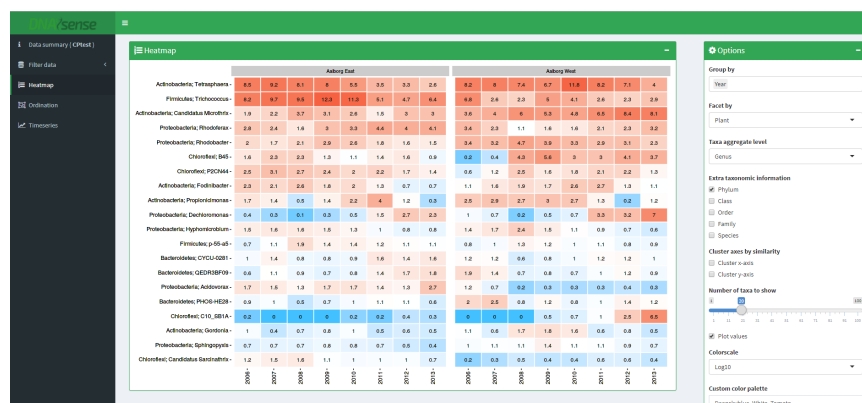
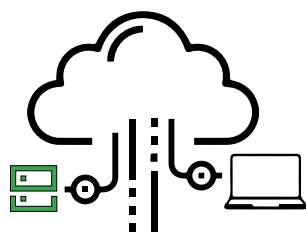
DNA extraction and sequencing



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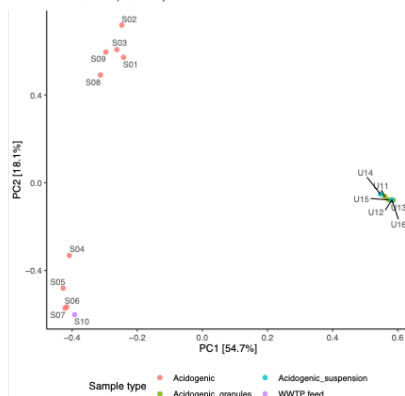
3.2 Table 2: The 25 most abundant genera in samples S01-S10

The most abundant genera across all samples. If no genus level classification could be obtained, the lowest assigned taxonomic classification is given. In addition, the phylum level classification is given (Proteobacteria at class level).

	Reactor S									
Actinobacteria; Candidatus Microthrix	0	0	0	0	0	0	0	0	0	0
Firmicutes; Peptostreptococcus	1.2	0.8	1.9	0.2	0.1	0	0	0	0	0
Firmicutes; Veillonella	0.9	0.6	0.9	0.2	0.1	0.1	0.1	0.1	0.1	0.1
Firmicutes; Clostridium sensu stricto 1	0.1	0.1	0.4	0.3	0.1	0.2	0.2	0.2	0.2	0.2
Actinobacteria; Tetrasphaera	0.4	1.2	0.7	0.3	0.3	0.3	0.1	1.5	0.8	0.7
Firmicutes; Clostridium sensu stricto 12	0	0.2	0.3	0.2	0.7	1.1	0.8	0.7	0	0
Actinobacteria; T_PaM15_OTU_45	1.5	0.3	1.4	1.3	0.8	1.9	1.2	1	1.1	1.9
Firmicutes; Tepidimicrobium	0	0.3	0.2	0	0	0	0	0	0.2	0
Firmicutes; Terrisporobacter	1.2	0.2	1.4	0.3	0.2	0.1	0.1	0.3	0.6	0.1
Chloroflexi; T78	0.8	0	0	0.2	0.3	0.1	0.1	0.2	0.1	0.1
k__Unassigned_OTU_55; k__Unassigned_OTU_55	0	0	0.1	0	0	0.2	0.5	0.5	0.1	0
Chloroflexi; Candidatus Deftuvillum	0.6	0.1	0.1	1.1	1.4	1.6	1.7	0.2	0.4	1.7
Actinobacteria; Onchinosarcus	0.1	0	0.1	1.6	1.6	1.5	1.6	0.3	0.3	1.5
Firmicutes; Turicibacter	1.4	0.3	1.2	0.7	0.4	0.3	0.2	0.3	0.5	0.1
Betaproteobacteria; Dechloromonas	0.3	0.1	0	0.4	1.7	0.8	0.3	0	0	0
Alphaproteobacteria; MNG7	0.4	0.1	0.6	0.6	1.2	0.9	1.1	0.8	0.5	1.5
Actinobacteria; Fodinicola	0.5	0.1	0.3	0.5	1.1	1	1	0.6	0.3	1.2
Gammaproteobacteria; QCEB116B10	0.1	0	0.1	0.7	1.2	1	1.2	0.4	0.4	1.1
Actinobacteria; T_PaM15_OTU_2355	1.8	0.3	1.2	0.2	0.1	0.2	0.2	0.4	1	0.3
Bacteroidetes; vadinBC27 wastewater-sludge group	0	0	0.1	0.2	0.4	0.1	0.1	0	0	0
Alphaproteobacteria; Rhodobacter	0.1	0.1	0.1	1	1.2	0.6	0.8	0.4	0.2	1.1
Bacteroidetes; MK04	0.1	0	0.1	0.2	1	1	1.3	0	0	1.2
Actinobacteria; S01-K2-141	0.2	0	0.2	0.4	0.7	0.7	1.1	0.8	0.3	1.1
Betaproteobacteria; Simplicicoccus	0	0	0	0.7	1.3	0.6	1.4	0.1	0	1.2
Actinobacteria; K2-78	0	0	0.1	0.3	0.7	0.7	1.4	0.6	0.2	1.1
S01	S02	S03	S04	S05	S06	S07	S08	S09	S10	

3.4 Figure 2: Principal component analysis

Identification of samples with similar microbial communities using multivariate statistics (PCA). Each point represent the microbial community in a specific sample. Distance between the sample dots signifies similarity; the closer the samples are, the more similar microbial composition they have.



4 Materials and methods

4.1 DNA extraction

4.1.1 FastDNA SPIN kit for Soil

DNA extraction was performed using the standard protocol for FastDNA Spin kit for Soil (MP Biomedicals, USA) with the following exceptions. 500 μ L of sample, 480 μ L Sodium Phosphate Buffer and 120 μ L MT Buffer were added to a Lysing Matrix E tube. Bead beating was performed at 6 m/s for 4x40s (Abertsen et al., 2015). Gel electrophoresis using TapeStation 2200 and Genomic DNA screentapes (Agilent, USA) was used to validate product size and purity of a subset of DNA extracts. DNA concentration was measured using Qubit dsDNA HS/BR Assay kit (Thermo Fisher Scientific, USA).

4.2 Bacterial community analysis targeting 16S V1-3 rRNA

4.2.1 Library preparation

Bacteria 16S V1-3 rRNA gene sequencing libraries were prepared by a custom protocol based on Caporaso et al. (2012). Up to 10 ng of extracted DNA was used as template for PCR amplification of the bacteria 16S V1-3 rRNA gene amplicons. Each PCR reaction (25 μ L) contained dNTPs (100 μ M of each), MgSO₄ (1.5 mM), Platinum Taq DNA polymerase HF (0.5 U/reaction), Platinum High Fidelity buffer (1X) (Thermo Fisher Scientific, USA) and barcoded library adaptors (400 nM of each forward and reverse). PCR was conducted with the following program: Initial denaturation at 95 °C for 2 min, 30 cycles of amplification (95 °C for 20 s, 56 °C for 30 s, 72 °C for 60 s) and a final elongation at 72 °C for 5 min. Duplicate PCR reactions were performed for each sample and the duplicates were pooled after PCR. The adaptors contain 16S V1-3 specific primers: [27F] AGAGT TTGATCCTGGCTCAAG and [54R] ATTACCGCGGCTGCTGG (Ward et al., 2012). The resulting amplicon libraries were purified using the standard protocol for Agencourt Ampure XP Beads (Beckman Coulter, USA) with a bead to sample ratio of 4:5. DNA was eluted in 25 μ L of nuclease free water (Qiagen, Germany). DNA concentration was measured using Qubit dsDNA HS Assay kit (Thermo Fisher Scientific, USA). Gel electrophoresis using TapeStation 2200 and D1000/High sensitivity D1000 screentapes (Agilent, USA) was used to validate product size and purity of a subset of sequencing libraries.

4.2.2 DNA sequencing

The purified sequencing libraries were pooled in equimolar concentrations and diluted to 6 nM. The samples were paired-end sequenced (2x300 bp) on a MiSeq (Illumina, USA) using a MiSeq Reagent kit v3 (Illumina, USA) following the standard guidelines for preparing and loading samples on the MiSeq. >10% PhiX control library was spiked in to overcome low complexity issues often observed with amplicon samples.

4.2.3 Bioinformatic processing

Forward and reverse reads were trimmed for quality using Trimmomatic v. 0.32 (Bolger et al., 2014) with the settings SLID-INGWINDOW:5:3 and MINLEN:275. The trimmed forward and reverse reads were merged using FLASH v. 1.2.7 (Magoc and Szabó, 2011) with the settings -m 10 -M 200. The trimmed reads were dereplicated and formatted for use in the UPARSE workflow (Edgar, 2013). The dereplicated reads were clustered, using the usearch v. 7.0.1090 -cluster_otus command with default settings. OTU abundances were estimated using the usearch v. 7.0.1090 -usearch_global command with -id 0.97 -maxaccepts 0 -maxrejects 0. Taxonomy was assigned using the RDP classifier (Wang et al., 2007)

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