The influence of agricultural tillage practices on soil biodiversity: Sequence analysis

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Abstract

Bioinformatic pipleine used to process all sequence reads.

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Protocol

Step 1.
Primer removal: Primers were trimmed from the raw reads using Cutadapt

SOFTWARE PACKAGE (Linux)
cutadapt
Marcel Martin
https://github.com/marcelm/cutadapt/

Step 2.
Read processing: The UPARSE pipeline was chosen to quality filter and cluster all reads

Step 3.
Forward and reverse merge

cmd COMMAND
```
fastq_mergepairs
```

Step 4.
Quality filter

cmd COMMAND
```
fastq_filter -fastq_maxee 1
```
trim all reads to a common length for each gene

Step 5.
Label samples: sample labels were added to each sequence header with -relabel command

Step 6.
Dereplication

cmd COMMAND
```
derep_fulllength -minuniquesize 3
```

Step 7.
ITS2 extraction: For ITS2, the program ITSx was used to extract the ITS2 region from flanking 5.8S

Step 8.
cluster OTUs

cmd COMMAND
```
cluster_otus -otu_radius_pct 0.99
```

Step 9.
OTU table construction: map all sample labelled reads onto OTU file for each gene

Taxonomic Identification

Step 10.
Used BLAST to identify all OTUs against the SILVA_123 (16S, 18S, AMF), UNITE (ITS2) and Genbank (CO1)

Taxonomic Identification

Step 11.
Used MEGAN5 to pick most correct ID using LCA algorith (LCA% 75)

Step 12.
Map tax ids onto OTUS in table format

cmd COMMAND

```
search_exact (UPARSE)
```

Step 13.
Statistical analysis: load OTU table with tax ids into STAMP (program)