Root sampling in field

Frozen washed root aliquots

DNA Extraction
DNeasy plant mini kit Qiagen

1. PCR
3 x replicates
Primer RPB1-HSm375-mix7 & RPB1210+linker

Pooling of the 3 PCR replicates
And purification

2. PCR
3x replicates
Primer TitB-adaptor+ RPB1-DR160f-mix10 & Linker+Barcode+TitA-adaptor

Pooling of PCR replicates
Cutting out bands on Gel & recovering of DNA 650 bp -1 Kb

Pooling of sub-samples within plot
Equimolar mixing based on Bioanalyzer results

454 sequencing(GS FLX+ technology)

QIIME
Split library, removal from Primer Linker and barcode

PyNAST
Basic quality control and removal of sequences that fail to align to the reference dataset at 60% similarity.

USEARCH & UCHIME
Initial denoising at 99.7%, de novo chimera detection and removal

MACSE
Denoise against reference dataset

CROP
OTU picking at 99.2% minimum similarity

Normalisation via QIIME - Equalise number of sequences/sample

QIIME
Taxonomic assignment of OTUs

EPA-RAxML
Taxonomic assignment of OTUs

MEGA NJ tree
Taxonomic assignment of OTUs

Combination of taxonomic assignments

QIIME
UniFrac PCoA analysis based on phylogenetic distance and ANOSIM analysis

QIIME
Taxon distribution

R
(Comparison of relative abundance )