



Temporal dynamics of soil microbial communities under different moisture regimes: high-throughput sequencing and bioinformatics analysis

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Recent climate scenarios predict not only continued global warming but also an increased frequency and intensity of extreme climatic events such as strong changes in temperature and precipitation regimes. Microorganisms are well known to be more sensitive to changes in environmental conditions than to other soil chemical and physical parameters. In this study, we determined the shifts in soil microbial community structure as well as indicative taxa in soils under three moisture regimes using high-throughput Illumina sequencing and range of bioinformatics approaches for the assessment of sequence data.

Incubation experiments were performed in soil-filled (Greyic Phaeozems Albic) rhizoboxes with maize and without plants. Three contrasting moisture regimes were being simulated: 1) optimal wetting (OW), a watering 2-3 times per week to maintain soil moisture of 20-25% by weight; 2) periodic wetting (PW), with alternating periods of wetting and drought; and 3) constant insufficient wetting (IW), while soil moisture of 12% by weight was permanently maintained.

Sampled fresh soils were homogenized, and the total DNA of three replicates was extracted using the FastDNA[®] SPIN kit for Soil. DNA replicates were combined in a pooled sample and the DNA was used for PCR with specific primers for the 16S V3 and V4 regions. In order to compare variability between different samples and replicates within a single sample, some DNA replicates treated separately. The products were purified and submitted to Illumina MiSeq sequencing. Sequence data were evaluated by alpha-diversity (Chao1 and Shannon H' diversity indexes), beta-diversity (UniFrac and Bray-Curtis dissimilarity), heatmap, tagcloud, and plot-bar analyses using the MiSeq Reporter Metagenomics Workflow and R packages (phyloseq, vegan, tagcloud).

Shannon index varied in a rather narrow range (4.4-4.9) with the lowest values for microbial communities under PW treatment. Chao1 index varied from 385 to 480, being a more flexible indicator than Shannon index. Chao1 had similar values for OW and IW communities, but alpha-diversity of microbial communities has sharply decreased under PW treatment. There was no visible difference in beta-diversity depending on sampling date and wetting regime, however, it could be possible to distinguish microbial communities in soils with maize and without plants. The presence of maize was acting as scattering agent, making microbial communities more distinguished.

In all studied samples, the most dominant phyla were Proteobacteria, Firmicutes, Verrucomicrobia, Actinobacteria, and Acidobacteria. Chthoniobacter, Bacillus, Alicyclobacillus, Rhodoplanes, Cohnella, Kaistobacter, and Solibacter were the most abundant genera. Moreover, these genera were found as the most reactive and variable taxa in microbial community.

Thus, DNA high-throughput sequencing revealed no dramatic shifts in bacterial community structure in soils under different moisture regimes. However, this technique allowed us to determine the effect of wetting regime and the presence of plants on soil microbial community which were adaptable to insufficient wetting, but lost diversity under periodic wetting. Furthermore, we detected the indicative taxa which dominate in microbial communities and at the same time strongly react to environmental changes.