SPP2322: Comparisons of microbial communities between long-term field trials

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1. Summary

Analyses to quantify the size, diversity and composition of microbial communities within the five SPP2322 soils was performed. In terms of size, Reckenholz and QualiAgro had more Bacteria, Archaea and Fungi than Dikopshof and Thyrow. In terms of alpha-diversity, all soils were similar with the exception of unfertilised Thyrow (high) and unfertilised Reckenholz (low). Worthy of note is that, while unfertilised Dikopshof 2 was not significantly less diverse than the other Dikopshof soils, it was dissimilar. However, it is difficult to predict whether this would translate to dissimilarities in observed thermodynamics between Dikopshof 1 and 2. In terms of beta-diversity, Reckenholz and Thyrow both had highly unique community compositions. These differences were driven by enrichment of Bacteroidota and oligotrophic Verrucomicrobia, and depletion of Actinobacteria in Reckenholz *versus* enrichment of Chloroflexi and potentially copiotrophic Firmicutes in Thyrow. The QualiAgro and Dikopshof communities were similar to each other, despite Dikopshof favouring Archaeal ammonia oxidising Crenarchaeota *versus* QualiAgro favouring the highly diverse Phylum Proteobacteria. Fertilisation had no consistent effect on overall community composition, although as expected, Firmicute populations were larger here. In conclusion, community driven differences in thermodynamics are likely to be greatest between relatively copiotrophic Thyrow and oligotrophic Reckenholz, while differences between Dikopshof and QualiAgro may be more dependent on interactions between diverse carbon and nitrogen cyclers of the Proteobacteria and Crenarchaeota.

2. Results

Quantitative PCR of universal marker genes indicated that Bacterial populations were consistently between 1 x 10^9 to 1 x 10^{10} 16S rRNA gene copies g^{-1} dry soil, with Archaea between 1 x 10^7 to 1 x 10^8 16S rRNA gene copies g^{-1} dry soil (Figure 1). The unfertilised Thyrow soil was an exception, with unusually low Archaea. Fungal ITS copies were similar to Archaea at 1 x 10^7 to 1 x 10^8 copies g^{-1} dry soil, excepting a slight enrichment in the QualiAgro and fertilised Reckenholz soils.

Illumina MiSeq was also performed on the V4 region of the universal prokaryote 16S rRNA gene. Prokaryote alpha-diversity was calculated as Shannon Index (H') determined based on discrete counts of amplicon sequence variants (ASVs) identified in three technical replicates per soil. All soils were similar, as determined by Fisher's Least Significant Difference *post hoc* test (Figure 1) with the exception of higher alpha-diversity in the unfertilised Thyrow soil (a), and low in the unfertilised Reckenholz soil (d). While not significantly less diverse ($p > 0.05$), the unfertilised Dikopshof 2 soil was dissimilar to the other three Dikopshof soils.

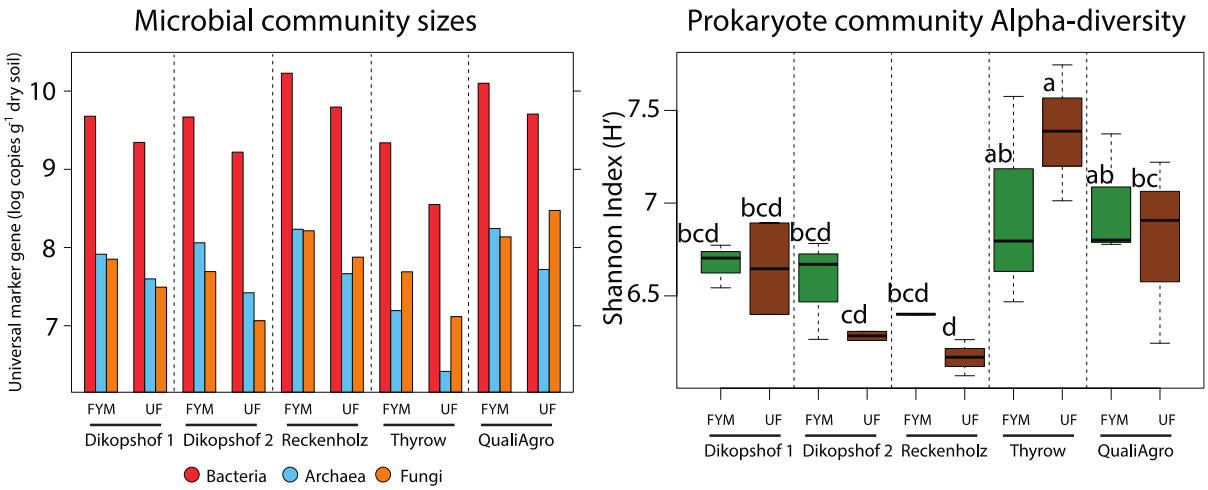

Figure 1: Quantitative PCR and Prokaryote alpha-diversity comparisons between fertilised (FYM) and unfertilised (UF) soils of the five SPP2322 long-term field trials. *Post hoc* results are shown as letters above H' measurements.

Prokaryote beta-diversity was visualised with Principle Components Analysis of Bray-Curtis transformed ASVs (Figure 2). Communities primarily differed between sites (ANOSIM R = 0.7, $p < 0.001$) with no consistent effect of fertilisation (ANOSIM R = 0.05, $p > 0.05$). Community composition of Dikopshof and QualiAgro were highly similar (notable as overlap of grey dotted lines showing 95% confidence intervals) while Reckenholz and Thyrow each had unique, dissimilar communities.

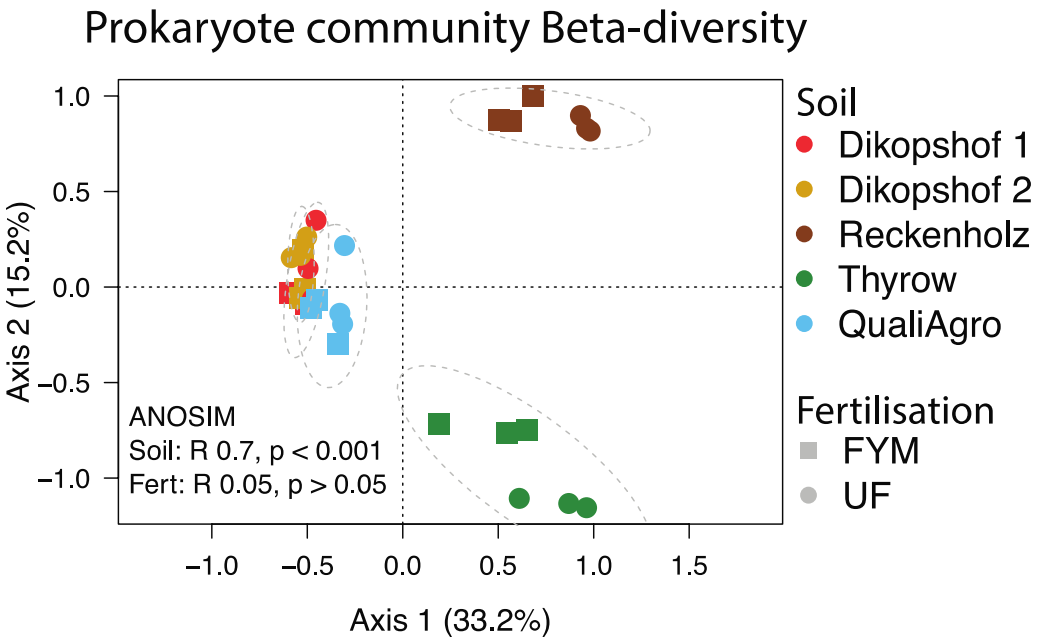

Figure 2: Principle Components Analysis of prokaryote communities within the five SPP2322 long-term field trials. Percent variation explained by the first two axes are noted. Grey dotted lines indicate 95% confidence intervals of each site. Results of Analysis of Similarity (ANOSIM) hypothesis testing for differences between soil and fertilisation are also noted.

Finally, a summary of the major Prokaryote Phyla that represent greater than 5% of the total community is shown as Figure 3. Phyla that differed between soils (S) and/or fertilisation (F), as determined by

general linear modelling, are also noted. General trends were: a) Dikopshof was enriched in the Candidate phylum Methylomirabilota, Myxococcota and Desulfobacterota (both formerly of the Deltaproteobacteria), and Archaeal ammonia oxidisers of the Crenarchaeota. Note that Crenarchaeota are depleted in unfertilised Dikopshof 2 relative to other Dikopshof soils; b) Reckenholz was enriched in oligotrophic Verrucomicrobia and Planctomycetota, Bacteroidota and Latescibacterota while also depleted in Actinobacteria; c) Thyrow was enriched in Chloroflexi, potentially copiotrophic Firmicutes and oligotrophic Planctomycetota; and d) QualiAgro was enriched in Proteobacteria and Bacteroidota. Fertilisation tended to enrich Firmicutes whilst also depleting Armatimonadota.

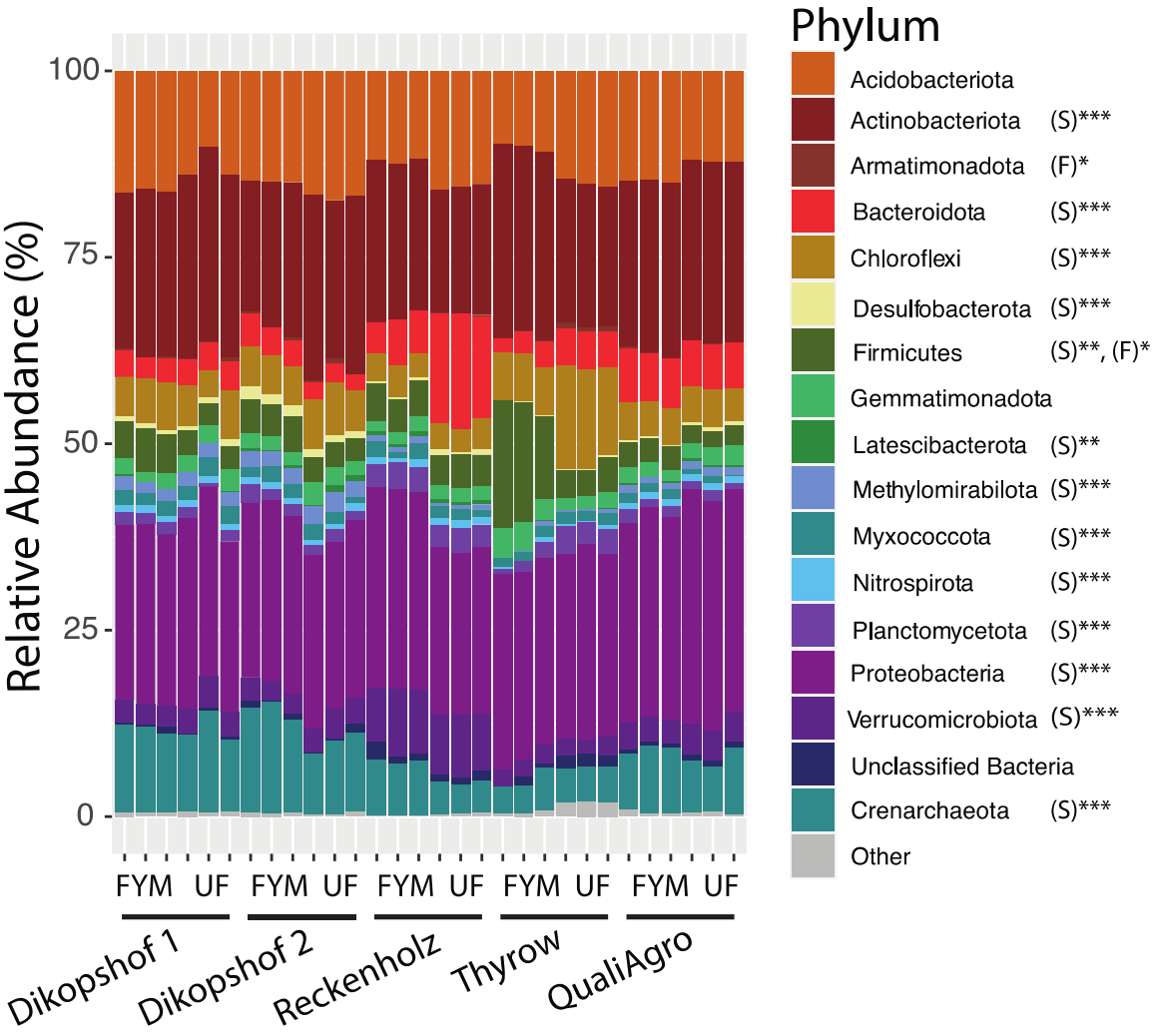

Figure 3: Stacked bar chart of the relative abundances of dominant prokaryote Phyla in fertilised (FYM) and unfertilised (UF) SPP2322 soils. General linear model results of soil (S) and fertilisation (F) effects are noted for each Phylum as: $p < 0.001$ (***), $p = 0.001$ (**), $p < 0.05$ (*). Phyla that comprise less than 5% of the total community are consolidated as the category 'Other'.