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Poulsen, Pernille Hasse Busk; Abu Al-Soud, Waleed; Hansen, Lars H.; Sørensen, Søren Johannes

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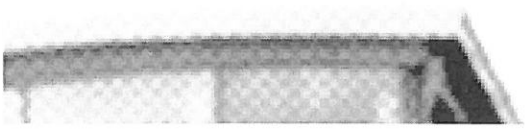

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INVESTIGATION OF PROKARYOTIC DIVERSITY IN AN URBAN WASTE FIELD TRIAL BY HIGH THROUGHPUT SEQUENCING

Pernille H. B. Poulsen, Waleed Abu Al-Soud, Lars H. Hansen and Søren J. Sørensen
Molecular Microbial Ecology Group, University of Copenhagen, Sølvgade 83H, DK-1307K Copenhagen, Denmark

In this study, the impact of different fertilizer treatments on prokaryotic diversity in a Danish long-term urban waste field trial was investigated using high throughput sequencing. The field trial was established in 2002 to investigate possibilities of recycling of urban organic waste in agriculture (organic fertilizers) and to discern the effects on soil quality. The soils have been treated annually with different urban organic fertilizers and reference fertilizers at two levels. GS FLX sequencing from Roche is a high throughput, no cloning bias, technique that produce sequences long enough to investigate diversity and functional analysis of complex microbial communities such as soil. The aim of the study was to investigate changes in prokaryotic diversity in soil treated with different fertilizer, which contain a lot of nutrients but possibly also unwanted toxic compounds that influence microbial flora in the soil. Three soils treated with accelerated levels of different fertilizers where included in this study (composted household waste, sewage sludge, cattle manure), two soils with normal level treatment (human urine, NPK inorganic fertilizer) and one control soil (unfertilized). Each treatment was represented by 3 field replicates. Total DNA was extracted from the soil samples using FastDNA® SPIN for Soil Kit (Qbiogene) and the primers 530F and 1061R were used to amplify a 562 bp fragment of 16S rDNA flanking the V7 and V8 regions. The PCR products of each soil sample were tagged using custom primers, quantified using Qubit fluorometer (Invitrogen), mixed in approximately equal amounts and sequenced using a modified version of the GS FLX amplicon sequencing protocol (Roche). The number of sequences (>150bp) obtained from each of the 18 samples ranged from 8,000 to 80,000. Sequences were sorted, trimmed and classified using the RDP database (http://rdp.cme.msu.edu/). Five to ten percent of the sequences in each sample were classified as Archaea and the rest as Bacteria. The major classified phyla of Archaea were the Crenarchaeota. No major changes in the community composition due to different fertilizer treatments were found demonstrating a high robustness of the soil microbiota. However, some differences between the treatments were observed at the phylum level e.g. Cyanobacteria were more abundant in the unfertilized soil compared to the soils treated with high nitrogen content fertilizers. The GS FLX sequencing is an excellent tool for investigating microbial diversity in soil and to provide a massive amount of data in a short time.