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Influence of Soil DNA Extraction Procedure to Assess Bacterial Diversity using Pyrosequencing of 16S rDNA

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TITLE

Influence of Soil DNA Extraction Procedure to Assess Bacterial Diversity using Pyrosequencing of 16S rDNA.

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Pipeline; pyrosequencing; soil biodiversity; soil DNA extraction procedure

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During the last two decades, novel molecular and robust methods were developed that were well-suited to characterize soil microbial communities, as they provided access to previously hidden genetic resources. These methods were based essentially on soil DNA characterization and most efforts were devoted to optimize the soil DNA extraction procedure in order to obtain representative and suitable extracts for quantitative and qualitative characterization of microbial communities. Though several studies have evaluated the technical biases of these procedures to compare microbial abundance and community fingerprinting in diverse soils, the recent development of high throughput sequencing technology, which allow the scientific community to get hundreds of thousands of ribosomic sequences from a single metagenomic DNA, need to revisit our evaluation of soil DNA extraction efficiency to recover microbial diversity.

Our study aimed at evaluating three soil DNA extraction procedures (two homemade protocols: Gns-GII and SY3, and one commercial kit: MOBIO) on seven contrasting soils for their efficiency: (i) in recovering soil DNA and, (ii) in the detection of bacterial species richness and evenness estimated with pyrosequencing of 16S rDNA. The seven studied soils originate from the soil library of the French Soil Quality Monitoring Network (RMQS), which contains more than 2,200 soils representative of the pedologic, climatic and land use conditions encountered on the French national territory. Soils were chosen because of their contrasting pH, texture, carbon organic content and land-use characteristics, known to strongly influence microbial abundance and diversity. Significant differences were systematically observed between procedures with, in certain soils, 10 times more DNA recovered than in the other protocols. In the same way, significant differences in the number of taxonomic groups detected (between 2 and 21% at the genus level) were observed between procedures and the lowest recovery systematically recorded for the MOBIO one. These differences were explained by a lower protocol efficiency to recover some major and minor phyla (such as *Firmicutes* or *Crenarchaeota*). Altogether, this study demonstrates the need to revisit the old bias known to evaluate and optimize soil DNA extraction procedure before analysis of microbial diversity by novel massive sequencing technology such as pyrosequencing.

