



## Molecular and biochemical characterization of soils from semi-arid Mediterranean ecosystems

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Microorganisms play an essential role in the maintenance of ecosystems - whether directly, in biogeochemical cycles, or indirectly, by participating in the processes of degradation and transformation of soil organic matter. The environmental functions of microorganisms in soil have been analyzed by biochemical properties, which are considered useful indicators of soil quality because of their high sensitivity to human and environmental perturbations. Nevertheless, molecular and physiological knowledge about microorganisms in natural environments is relatively scarce, mainly because most soil microorganisms cannot be cultured. Advances in molecular biology have made it possible to study these microorganisms, although until now the relationships between the structure of the microbial communities and their environmental functions have not been established.

The main objective of the present study was to compare the information provided by molecular (16S RNA-based phylogenetic analysis) and biochemical (microbial biomass carbon, soil basal respiration, nitrogen mineralization and enzymatic activities) methods, in order to obtain a better understanding of the relationships between the structure of microbial communities and the functions of these microorganisms in soil. An attempt was also made to discover whether these molecular properties can be used to assess human impact in semiarid Mediterranean soils located at different altitudes and under different types of land use and vegetation (natural pine forests, evergreen oak forests, juniper shrubs, scrub, reforested pine stands and almond tree crops).

Analysis of the biochemical parameters microbial biomass carbon, soil basal respiration, qCO<sub>2</sub> and enzymatic activities enabled differentiation of soils according to vegetation/land use (forest soils and degraded or cultivated soils). Moreover, analysis of nitrogen mineralization enabled differentiation of soils according to altitude. The study of microbial structure by PCR-TGGE with specific primers (alpha and beta-proteobacteria) also revealed that some subpopulations of microbes behaved differently from others, depending on environmental variables (vegetation/land use and altitude). Beta-proteobacteria profiles grouped the samples according to land use and vegetation, whereas alpha-proteobacteria profiles clearly grouped the samples according to altitude. Sequencing of 16S rDNA amplicon libraries generated from pine samples at different altitudes showed proteobacteria to be the most abundant microbial group (50% of sequenced clones), followed by chloroflexi and acidobacteria. The bacterial groups involved in nitrogen mineralization were also described.

The results suggests that, in general, there is concordance between the structure of microbial communities estimated by PCR-TGGE of 16S rDNA genes and the functions these microorganisms have in the environment, as estimated from their biochemical properties. The methodology used may provide new lines of research that could be developed and simplified in the future (use of primers specific to different taxonomic units) and therefore, with potential for use as indicators of anthropogenic activity. This work is a preliminary step in such research and more studies are required to investigate and estimate the impact of anthropogenic actions by use of molecular indicators.