Geophysical Research Abstracts, Vol. 9, 09325, 2007
SRef-ID: 1607-7962/gra/EGU2007-A-09325

When fingerprinting joins sequencing comprehensive microbial ecology studies become possible: The Río Tinto model revisited through the new SARST-V6 ribotyping method

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Microbiologists still face technical difficulties studying ecological diversity patterns and their link to natural environments. Recent tag sequencing methods allow for higher throughput when determining microbial community composition. The use of the phylogenetic information in genetically hypervariable regions of the 16S ribosomal RNA gene (rDNA) to fingerprint natural microbial communities directly from sequencing avoids many of the difficulties and assumptions associated with conventional microbial ecological fingerprinting methods. Furthermore, the smaller size of the amplicons used as tags virtually eliminates the PCR bias typically associated with sequencing rDNA clone libraries. The well studied biogeochemistry of the Río Tinto in Spain provides an ideal model for testing the potential of new techniques to provide insights into evolutionary and ecological microbial processes. We used Serial Analysis of Ribosomal Sequence Tags of the V6 hypervariable region (SARST-V6) to explore the bacterial diversity in parts of the northern domain of the Tinto, a river characterized by high heavy metal concentrations and low pH. Using a careful experimental design accompanied by physico-chemical parameter measurements, high-throughput sequencing and appropriate analytical tools, we have carried out an in-depth analysis

of bacterial diversity to contrast theories on microbial community dynamics and biogeography. The patterns of bacterial diversity composition encountered in this study suggest a scenario in which high immigration rates allow for *global* dispersion of ecotypes defined by neutrally divergent microclusters at the 99% similarity cut-off level. These microclusters are likely better adapted to certain environmental conditions, which allows them to prevail over less adapted OTUs that might have emerged *locally*. Our results are in agreement with the hypothesis of "everything is everywhere, but the environment selects", advocating for deterministic forces shaping microbial community composition and challenging neutral models of community assemblage.