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Composition of soil microbial communities by molecular techniques: limits and drawbacks

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Determining microbial biodiversity in soil using cultivation-based methods is said to cover only around 1% of the total diversity. The estimation of the actual number of unique genomes in a sample appears to vary according to what technique is used to perform the estimate. It is, however, obvious that a great number of microbes do not form colonies, even if different media and culture conditions are used. DNA-based techniques do generate a more complete picture, but are susceptible to other biases. The most commonly used methods rely on PCR amplification of isolated total DNA. Limited isolation efficiency generates an underestimate, whereas a factor that pushes in the opposit direction is DNA from intact but dead cells, and possibly even DNA preserved on soil particles. In a rapidly evolving, highly dynamic environment, such as an active compost, the dividing and metabolizing cells clearly represent the current diversity, but the situation is more obscure in e.g. a forest soil determining which DNA sources are viable and which are ghosts is not easy. If the primers used are designed to target certain sequences such as ribosomal (rDNA) genes, one source of bias is the generality (or success in targeting) of these primers. Since the design of the primers is based on available sequence data, anything that is truly novel and unexpected may go undetected even as the databases grow. Direct chemical analysis of isolated cell components such as fatty acids (PLFA) is in a sense even more objective than DNA analysis, but the information obtained is very limited. A serious drawback when using any molecular technique is that there is no isolate to characterize. Knowing only limited DNA stretches says very little about the organism. Sequence data may, however, help in designing specific isolation conditions. With time our picture of the total soil biology is complemented, and to this end different technological approaches,

including also metagenomics and microarrays complement each other.