High-throughput analysis of soil eukaryotic unicellular microorganisms: from cytometry to pyrosequencing

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Abstract

Soil eukaryotic unicellular microorganisms play an important role in plant growth promotion as well as in the regulation of the carbon and nitrogen budget in the soil, pleading for the necessity to study i.e. their diversity and function. Considering that soil is a complex and heterogeneous environment, community and diversity studies of such organisms are only applicable through direct counting, restricted to cultivable organisms, or through analysis of the overall soil DNA. This includes DNA of low active or dead organisms and avoids separating uni- and pluri-cellular organisms of same groups (e.g. yeast among fungi or unicellular green algae among plants). Therefore, whole unicellular microorganisms were extracted from soil using a cell extraction method which was initially developed for bacteria (Lindahl & Bakken, 1995). This technique rendered a quantitatively measurable part of soil unicellular eukayotes (i.e. algae & yeast) using flow cytometry. First, the quantitative and qualitative effects of the extraction method were assessed by an inoculation-extraction experiment in sterile native soil. Preliminary results show a cell recovering efficiency of 40 % for algal species and 65 % for yeast species. These data encourage further analysis of natural soil communities, when this bias on the observed community composition and abundance is considered. In future experiments, cell sorted subpopulations of unicellular autotroph and heterotroph organisms obtained from a long term fertilization experiment field soil (i.e. Bad

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Lauchst´adt, Germany) will be characterized. High-throughput pyrosequencing will be used to determine community composition of sorted cells which will be compared to the whole cell community DNA and bulk soil DNA, in order to asses effects of the method at the community level. This method combination will also aim to facilitate characterization of poorly described, non-cultivable, soil unicellular organisms (yeast & algae), and to understand the effect of fertilization (organic and mineral) on unicellular eukaryotic community composition.

Keywords: eukaryotic microorganisms, soil, cell extraction, pyrosequencing, community composition