Soil filtration-sedimentation improves shelled protist discovery in eukaryotic eDNA surveys

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Abstract

A large part of the soil protist diversity is missed in metabarcoding studies based on 0.25 g of soil environmental DNA (eDNA) and universal primers due to ca. 80 % co-amplification of non-target plants, animals and fungi. To overcome this problem, enrichment of the substrate used for eDNA extraction is an easyly implemented option but its effect has not yet been tested. In this study, we evaluated the effect of a 150 µm mesh size filtration and sedimentation method to improve the recovery of protist eDNA, while reducing the co-extraction of plant, animal and fungal eDNA, using a set of contrasted forest and alpine soils from La Réunion, Japan, Spain and Switzerland. Biodiversity of the whole eukaryotic community was estimated with V4 18S rRNA metabarcoding and classical amplicon sequence variant calling. A 2-3-fold enrichment in shelled protists (Euglyphida, Arcellinida and Chrysophyceae) was observed at the sample level with the proposed method, with, at the same time, a 2-fold depletion of Fungi and a 3-fold depletion of Embryophyceae. Protist alpha diversity was slightly lower in filtered samples due to reduced coverage in Variosea and Sarcomonadea, but significant differences were observed in only one region. Beta diversity was mostly impacted by region and habitat, and explained the same variance in bulk soil and filtered samples. The increase resolution in the soil protist diversity provided by the filtration-sedimentation method is a strong argument to include it in the standard preparation of any future soil for protist eDNA metabarcoding studies.

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