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Short communication

# Extracellular DNA extraction is a fast, cheap and reliable alternative for multi-taxa surveys based on soil DNA

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ABSTRACT

DNA metabarcoding on soil samples is increasingly used for large-scale and multi-taxa biodiversity studies. However, DNA extraction may be a major bottleneck for such wide uses. It should be cost/time effective and allow dealing with large sample volumes so as to maximise the representativeness of both micro- and macro-organisms diversity. Here, we compared the performances of a fast and cheap extracellular DNA extraction protocol with a total DNA extraction method in retrieving bacterial, eukaryotic and plant diversity from tropical soil samples of ca. 10 g. The total DNA extraction protocol yielded more high-quality DNA. Yet, the extracellular DNA protocol provided similar diversity assessments although it presented some differences in clades relative abundance and undersampling biases. We argue that extracellular DNA is a good compromise between cost, labor, and accuracy for high-throughput DNA metabarcoding studies of soil biodiversity.



Implementing efficiently soil diversity surveys across taxa and ecosystem types is a formidable challenge. DNA metabarcoding is a most promising monitoring technique to meet this challenge (Bik et al., 2012; Taberlet et al., 2012b; Orgiazzi et al., 2015; Thomsen and Willerslev, 2015), as it provides a high-throughput, standardized and cost-effective assessment of soil diversity (Bik et al., 2012; Taberlet et al., 2012b). The approach is useful for uncovering the diversity of a large array of microorganisms, such as bacteria, protists or fungi (Lauber et al., 2009; Bates et al., 2013; Tedersoo et al., 2014) as well as macroorganisms such as arthropods, plants, or mammals (Andersen et al., 2012; Hiiesalu et al., 2012; Yoccoz et al., 2012; Yang et al., 2014). It also has limitations that are the focus of active research, such as a limited taxonomic resolution in some taxonomic groups (Tang et al., 2012; Grattepanche et al., 2014) or PCR or sequencing biases (Wintzingerode et al., 1997; Huse et al., 2007; Schloss et al., 2011; Thomsen and Willerslev, 2015).

One major problem in implementing DNA metabarcoding for large-scale applications remains the extraction of DNA from soil samples. Soil DNA is encapsulated within complex cell walls or adsorbed onto soil particles, and can be coextracted with variable amounts of humic substances that may inhibit PCR amplification (Wintzingerode et al., 1997). Many laboratory protocols or commercial kits have been developed to maximize extracted DNA purity/yield and downstream PCR success, and some of them now comply with the ISO standard (Martin-Laurent et al., 2001; Philippot et al., 2012). However, these protocols were optimized for assessing microbial diversity. This might hamper PCR amplification of other components of the soil biota due to the overwhelming biomass of bacteria (Taberlet et al., 2012a,b). Also, commercial kits rely on sample sizes typically ranging from 0.25 to 1 g of wet soil, which provide less consistent and representative pictures of local microbial communities than larger sample volumes (Ranjard et al., 2003). This sampling bias would be even worse for targeting larger organisms, unless many replicates are taken (Andersen et al., 2012). Finally, these kits are expensive, and the time and facilities needed for the DNA extraction process is

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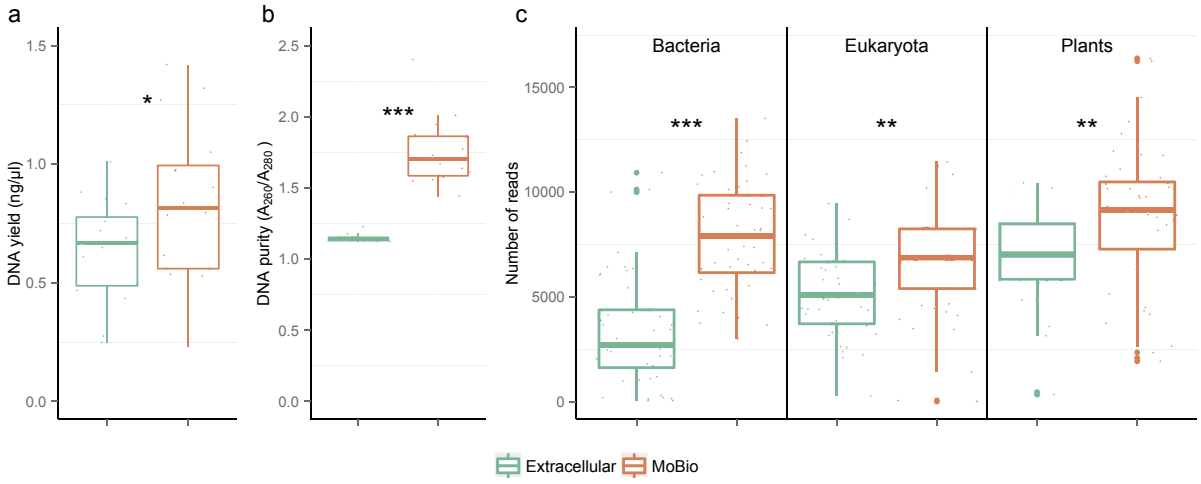


**Fig. 1.** Quantitative comparison of products from the two extraction methods in terms of (a) DNA yield, (b) extraction purity and (c) number of reads after PCR amplification and sequencing, which we used here as a proxy for amplicons DNA concentration and PCR success. Significance was assessed with a Wilcoxon rank-sum test and is indicated with the following code: ***p < 0.001, **p < 0.01, *p < 0.05.

incompatible with the requirements of routine-monitoring surveys.

Taberlet et al. (2012a) proposed a protocol for extracting extracellular DNA that can deal with large soil sample volume (Table S1, Supplementary Methods). It is cheap, rapid and can be implemented with minimal equipment facilities. This protocol can therefore be applied in rugged field conditions within a few hours after sampling. Hence it preserves samples from microbial growth and community change that may occur during sample storage and/or transportation (Rubin et al., 2013). The method has also been shown to provide picture of plant diversity that is consistent with botanical inventories in alpine tundra (Taberlet et al., 2012a). However, its efficiency as compared to classical DNA extraction methods that co-extract extra- and intracellular DNA remains to be assessed. Here we compared this protocol against a total DNA extraction kit adapted to large size samples. More precisely, we aimed at assessing the performance of both methods with regard to (i) DNA extraction efficiency, (ii) PCR efficiency and (iii) consistency and reliability of the resulting diversity patterns at the plot scale for (iv) both micro- and macro-organisms.

We collected 16 soil samples in a 1 ha permanent plot in a tropical forest of French Guiana, South America. DNA samples were extracted from 15 g of material with the extracellular DNA protocol proposed by Taberlet et al. (2012a) and also with the PowerMax® Soil DNA Isolation Kit (MoBio Laboratories, Cambridge, UK; hereafter referred to as extracellular and MoBio methods, respectively). The resulting DNA extracts were PCR-amplified using three universal markers targeting each Eukaryota, Bacteria, and vascular plants. The amplicons were sequenced on Illumina platforms, and the resulting sequences were subjected to a data-curation pipeline (Supplementary Methods) using the OBITools package (Boyer et al., 2015) and scripts in R (R Development Core Team, 2013).

DNA yield and purity were significantly lower with the extracellular protocol, but this represented only a 1.3–1.5 fold decrease respectively (Fig. 1a–b). PCR-amplification success was also lower when using the extracellular protocol (Fig. 1c, ~1.2 fold decrease of the number of reads with the eukaryotic and plant primer pairs and ~2 fold decrease with the bacterial marker). This suggests that the multiple PCR inhibitors removal steps included in the MoBio protocol (Table S1) have noticeable but minor impacts on data acquisition for these soil samples. As would be expected, skipping the cell lysis step primarily diminished the amount of bacterial DNA in the extracts (Taberlet et al., 2012a).

Yet, the overall composition was congruent between the two extraction methods (Fig. S1). We found a strong correlation in the average relative abundances across bacterial phyla, eukaryotic phyla, and plant families (Spearman's $\rho = 0.97$, 0.92 and 0.76 respectively, $p < 0.001$). This observation also held true at the OTU level (Spearman's $\rho = 0.57$, 0.52 and 0.44 respectively, $p < 0.001$). However, some bacterial and eukaryotic clades were found to be enriched in one method compared with the other (Fig. 2) as measured by the average fold change (AFC, ratio of relative

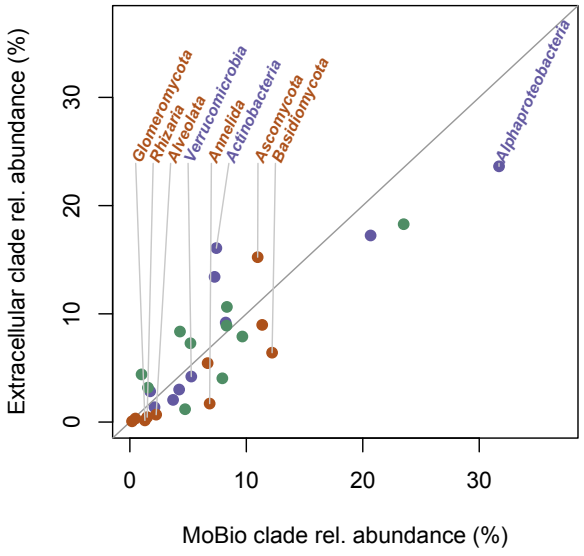


**Fig. 2.** Comparison of clades averaged relative abundances with the extracellular and MoBio method for Bacteria (violet), Eukaryota (orange) and Plants (green). The solid line indicates a 1:1 relationship. Only clades names for which we detected a significant enrichment (after Bonferroni p-values adjustment) that is besides of at least 1.25 are shown (see Table S2 for a more detailed analysis). The top 10 most abundant clades were used for drawing the plot. The SD values for average relative abundances are provided in Table S2. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

abundances in one versus the other method, averaged across sampled, Table S2).

Next, we explored whether the two methods provided similar patterns of diversity at the plot-scale. First, OTU accumulation curves yielded lesser OTUs with the extracellular protocol (Fig. S2a–c), where both rare and abundant OTUs also tended to display lower occupancy (Fig. S2c–d). These features indicate undersampling conditions for all three tested markers. These are known to inflate beta diversity estimates (Beck et al., 2013), as observed here with the extracellular protocol (Fig. S3). Nevertheless, beta diversity patterns were congruent between DNA extraction methods for plants (Spearman's $\rho = 0.43$, $p < 0.001$) and in a lesser extent for eukaryotes (Spearman's $\rho = 0.27$, $p < 0.04$). These might be due to the strong heterogeneity of the plant cover at the study site while the soil environment is relatively homogeneous. Hence difference between samples would be more visible for plants than for the other clades, irrespective of the extraction method. Comparing this feature on soils of more contrasted edaphic conditions would be necessary to validate the extracellular method on these aspects.

To summarize, our results show that extracellular and MoBio extraction methods yield some differences, supporting previous observations on total and extracellular DNA (Wagner et al., 2015). Soil communities tend to be undersampled with the extracellular method, and some clades tend to be biased one way or the other. This could be due to the lower cell lysis intensity of the extracellular method, which might be incomplete for the whole community and/or exclude particular taxa with recalcitrant cell walls, typically Actinobacteria. However, methods with similar cell lysis intensities also yield compositional biases both for bacteria (Terrat et al., 2012) and fungi (Tedersoo et al., 2010), and Actinobacteria were enriched with the extracellular protocol here. In addition, the clades favoured with the extracellular method were previously found to be dominant in active communities assessed through microscopy (Ushio et al., 2014) or RNA-based approaches (Baldrian et al., 2012). Active communities continuously release DNA in the environment through biomass turnover, and could hence be better reflected by the extracellular method, although further tests are needed to validate this assumption. Above all, the extracellular protocol is easy to implement, allows the processing of large volumes of material, and represents a major gain in cost (5-fold) and completion time (Table S1). We conclude that the extracellular DNA extraction protocol may not be adapted to obtain fine estimates of the local diversity, but it remains a reliable and effective compromise for broad taxonomic and high-throughput diversity assessments.

## Competing financial interests

P.T. is co-inventor of patents related to the gh primers and the use of the P6 loop of the chloroplast trnL (UAA) intron for plant identification using degraded template DNA. These patents only restrict commercial applications and have no impact on the use of this locus by academic researchers.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2016.01.008.

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