

Highlight

Thorough high-throughput sequencing analyses unravels huge diversities of soil parasitic protists

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Microbes are by far the most abundant and diverse organisms in soils. Among them, prokaryotic bacteria and archaea as well as eukaryotic fungi constitute the base of the soil food web (Hunt *et al.*, 1987; de Ruiter *et al.*, 1995). Thanks to continuously improving molecular tools, especially high-throughput sequencing (HTS) approaches, deep insights into the previously unknown diversity and community structure of these microbes has now been obtained. For instance, a huge diversity of previously undescribed groups among these microbes were detected in soils; many of those were later shown to provide important ecological functions and ecosystem services. For instance, archaea were shown to represent surprisingly abundant soil prokaryotes (Roesch *et al.*, 2007) and to be key ammonium oxidizers in soils (Leininger *et al.*, 2006). HTS-based methods also helped at disentangling environmental drivers that structure prokaryotic and fungal community compositions; pH was shown to be the major driver of prokaryotic communities (Lauber *et al.*, 2009; Rousk *et al.*, 2010; Griffiths *et al.*, 2011), while fungi seem equally affected by a wider range of variables including pH, plant species and climatic factors (Tedersoo *et al.*, 2014; 2016; Treseder *et al.*, 2014).

Comparably little is known about soil protists, which represent the often forgotten remaining microbial group (Caron *et al.*, 2008). The majority of the few studies investigating the community structure of soil protists applied time-consuming microscopic determinations that require profound taxonomic expertise [e.g. (Finlay and Fenchel, 2001; Scherber *et al.*, 2010; Domonell *et al.*, 2013; Geisen *et al.*, 2014)]. Molecular approaches, nowadays more and more based on HTS, start replacing those classical approaches (Baldwin *et al.*, 2013; Bates

et al., 2013; Lentendu *et al.*, 2014; Geisen *et al.*, 2015b; Fiore-Donno *et al.*, 2016). Still, HTS targeting protists are extremely underrepresented in comparison to other microbes (Mitchell, 2015). The major reason for the profound gap stems from the immense, paraphyletic diversity of protists that span over the entire eukaryotic tree of life with plants, fungi and metazoans representing monophyletic branches at different places therein (Adl *et al.*, 2012; Burki *et al.*, 2016). Therefore, designing universal protist-specific primers is impossible. This is of little relevance when studying microbes in aquatic systems, where protists can be easily extracted and focused upon through subsequent size-filtering (de Vargas *et al.*, 2015). Close attachment of many protists to soil particles eliminates straight-forward extraction, while directly applying universal eukaryotic primers to extracted DNA/RNA will ultimately result in sequencing mostly fungi due to their dominance in soils. To avoid this issue, more focused group specific primers targeting e.g. Cercozoa, Kinetoplastida, Chrysophyceae, acanthamoebae and Myxomycetes have been applied (Lentendu *et al.*, 2014; Bass *et al.*, 2016; Fiore-Donno *et al.*, 2016).

Continuous improvements in sequencing technologies with decreased costs at higher sequencing depth enabled the use of general eukaryotic primers to target soil protists. Bates *et al.* (2013) were the first using this approach and found biogeographic patterns of protist communities that were mainly explained by soil moisture. The key novelty of this initial survey was the finding of an unimagined diversity of soil protists similar to that of bacteria, with many sequences most closely matching potential parasites such as apicomplexans (Bates *et al.*, 2013). This finding was supported by follow-up DNA-based HTS studies (Ramirez *et al.*, 2014; Grossmann *et al.*, 2016) as well as metatranscriptomic approaches (Urich *et al.*, 2008; Geisen *et al.*, 2015b).

In this issue of Environmental Microbiology, Dupont *et al.* (2016) provide further evidence for the widespread diversity of parasitic protists in soils. One of the key novelties in this study is the use of phylogenetic analyses of manually mined HTS data; the authors elegantly reveal an immense diversity of largely unknown parasitic

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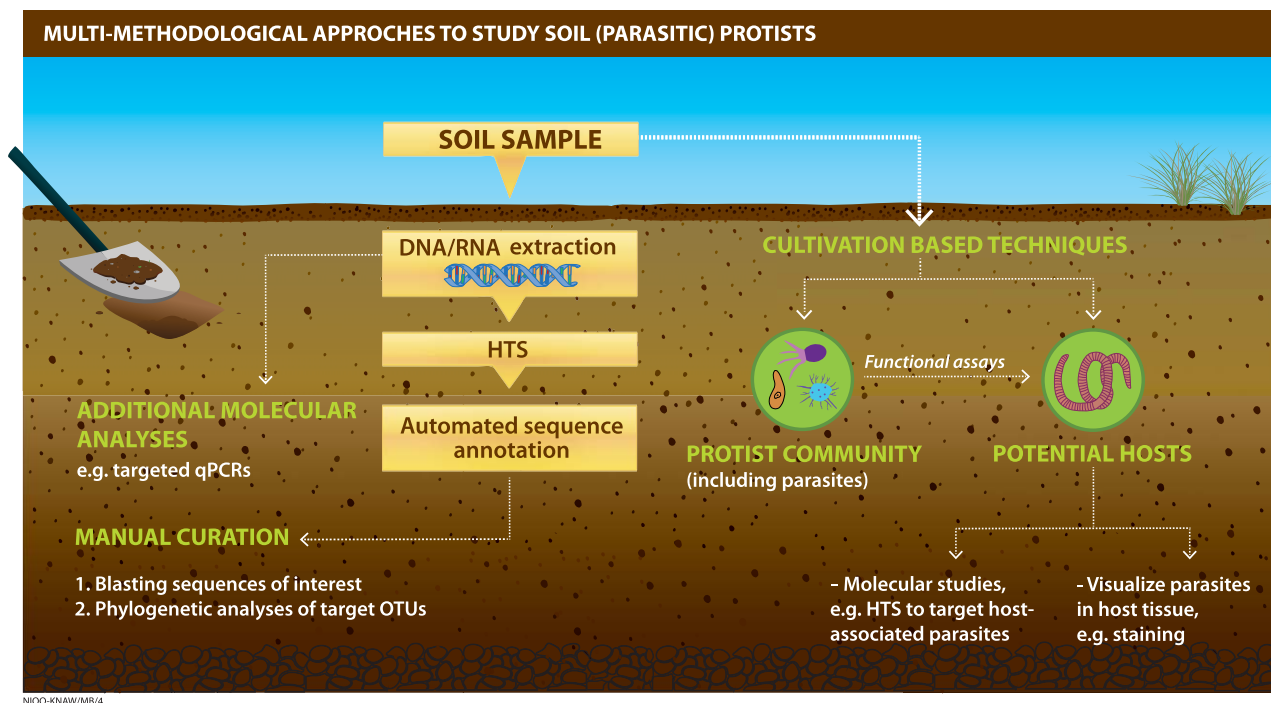


Fig. 1. Conceptual scheme of current methodology to study soil (parasitic) protists; gold, the suggested 'gold standard' methodology to study soil protists; green, additional approaches including manual sequence analyses, molecular and extraction-/cultivation-based techniques to investigate abundances, community structures and functions of protist (parasites) in more detail; HTS: High-throughput sequencing; OTUs: operational taxonomic units.

Apicomplexa and saprotrophic/parasitic Labyrinthulea. To date, few HTS studies made use of phylogenetic analyses to confirm sequence identities of often automated Blast assignments. Even manually blasting HTS-derived operational taxonomic units (OTUs) against online databases often reveals similarities <97% with known species, indicating that these OTUs likely represent other, unknown species. This scenario can be expanded to higher taxonomic ranks, i.e. genus, family, etc., as OTUs with much lower identities are commonly obtained in HTS studies, but nevertheless automatically assigned even to species level. Dupont *et al.* (2016) perfectly illustrate this issue by comparing assignments of identical sequences against two often used and quality controlled databases: SILVA (Pruesse *et al.*, 2007) and PR2 (Guillou *et al.*, 2013); profound differences were shown even at the highest taxonomic ranks, i.e. at phylum/supergroup, which should theoretically give very consistent taxonomic affinities. Automation is without any question unavoidable to deal with the majority of sequences obtained in HTS studies that now easily result in millions of sequences within thousands of OTUs. However, when more reliable, higher resolving information is desired to investigate OTUs of interest, phylogenetic analyses offer unprecedented possibilities.

Another novel finding in the study by Dupont *et al.* (2016) is that entire protist communities are structured

not only by moisture as found before (Bates *et al.*, 2013). In contrast to other studies suggesting that pH is involved in driving soil protist communities (Mitchell *et al.*, 2008; Lentendu *et al.*, 2014; Lara *et al.*, 2016), Dupont *et al.* (2016) revealed that classical free-living bacterivorous taxa are little affected, whereas especially parasites are strongly driven by pH-differences. The authors propose that pH-responsive hosts such as animals and plants might, therefore, be among the key drivers of protist communities, in line with recent findings of high diversities of parasites in soil organisms (Troemel *et al.*, 2008; Geisen *et al.*, 2015a).

In addition to targeted amplicon-based HTS approaches, the application of other HTS methodologies, such as PCR-free metagenomics and metatranscriptomics, will become applicable for a wide range of scientists. These 'omics' approaches are suggested to most reliably unravel soil protist communities (Urich *et al.*, 2008; Turner *et al.*, 2013; Geisen *et al.*, 2015b) and simultaneously allow taxonomic assignments of the entity of microbes and provide information on potential functions (Fierer *et al.*, 2007; Urich *et al.*, 2008; Damon *et al.*, 2012; Fierer *et al.*, 2012a,b; Turner *et al.*, 2013). However, not only in the field of (soil) protistology it is often forgotten that purely HTS-based approaches, the suggested 'gold-standard' to study microbes (Stoeck *et al.*, 2014), cannot stand alone; other approaches

such as classical cultivation based efforts need to go hand in hand with HTS (Fig. 1). This can perfectly be exemplified by the notorious case of parasites; HTS now allows to directly show parasite populations in soils either in approaches targeting the entirety of soil eukaryotes or using group specific primers (Ardila-Garcia *et al.*, 2013; Bass *et al.*, 2015; Dupont *et al.*, 2016) (Fig. 1). To more exactly study parasites, cultivation based approaches can be applied to isolate facultative parasitic taxa; obligate parasites need to be studied *in-vivo* inside potential hosts using parasite-specific visualization approaches or molecular sequencing tools (Fig. 1). Consequently, multi-methodological approaches are essential to link morphological to molecular to functional information of distinct parasites, to decipher abundances, community structures and potential biogeographic patterns of parasites, determine host(s) identity, evaluate whether and how they affect host performance and if these effects differ between hosts (Woolhouse *et al.*, 2001). This holds not only for protist parasites, but for microbes in general; now, soil scientists are in a luxurious situation as easy available, formerly unprecedented methods are ready to be implemented to fill the vast remaining knowledge gaps using multi-methodological approaches.

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