



Royal Netherlands Academy of Arts and Sciences (KNAW) KONINKLIJKE NEDERLANDSE AKADEMIE VAN WETENSCHAPPEN

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

Geisen, Stefan

published in

Environmental Microbiology
2016

DOI (link to publisher)

[10.1111/1462-2920.13309](https://doi.org/10.1111/1462-2920.13309)

document version

Publisher's PDF, also known as Version of record

document license

CC BY

[Link to publication in KNAW Research Portal](#)

citation for published version (APA)

Geisen, S. (2016). Thorough high-throughput sequencing analyses unravels huge diversities of soil parasitic protists. *Environmental Microbiology*, 18(6), 1669-1672. <https://doi.org/10.1111/1462-2920.13309>

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the KNAW public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain.
- You may freely distribute the URL identifying the publication in the KNAW public portal.

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

E-mail address:

pure@knaw.nl

Highlight

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

Stefan Geisen**Department of Terrestrial Ecology, Netherlands Institute for Ecology, Wageningen, The Netherlands*

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

*For correspondence. E-mail s.geisen@nioo.knaw.n; Tel. +31 (0)317 40 634; Fax +31 317473675.

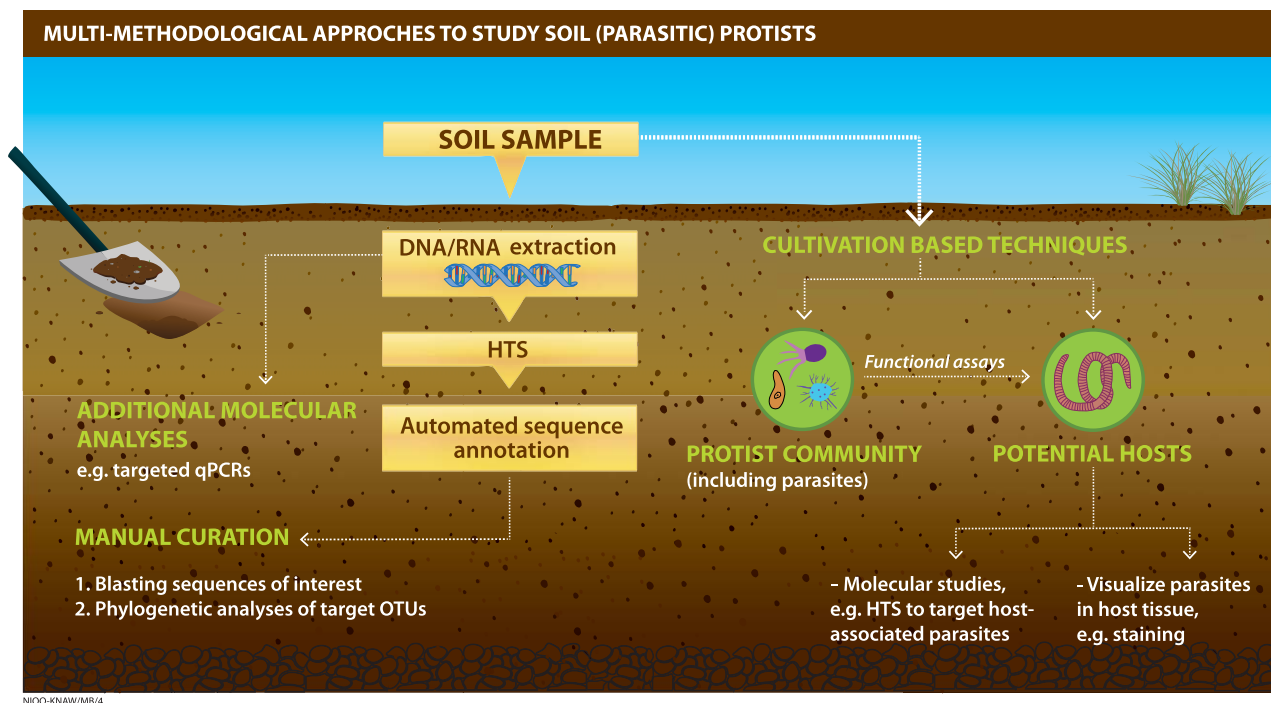


Fig. 1. Conceptual scheme of current methodology to study soil (parasitic) protists; gold, the suggested 'gold standard' methodology to study soil protists; green, additional approaches including manual sequence analyses, molecular and extraction-/cultivation-based techniques to investigate abundances, 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 entirety 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.

Acknowledgements

I want to thank Monique Beijaert for designing the conceptual scheme and for support by the ERC-Adv grant SPECIALS (260-55290) awarded to Wim van der Putten.

References

- Adl, S.M., Simpson, A.G.B., Lane, C.E., Lukeš, J., Bass, D., Bowser, S.S., *et al.* (2012) The revised classification of eukaryotes. *J Eukaryot Microbiol* **59**: 429–514.
- Ardila-Garcia, A.M., Raghuram, N., Sihota, P., and Fast, N.M. (2013) Microsporidian diversity in soil, sand, and compost of the Pacific Northwest. *J Eukaryot Microbiol* **60**: 601–608.
- Baldwin, D.S., Colloff, M.J., Rees, G.N., Chariton, A.A., Watson, G.O., Court, L.N., *et al.* (2013) Impacts of inundation and drought on eukaryote biodiversity in semi-arid floodplain soils. *Mol Ecol* **22**: 1746–1758.
- Bass, D., Stentiford, G.D., Littlewood, D.T.J., and Hartikainen, H. (2015) Diverse applications of environmental DNA methods in parasitology. *Trends Parasitol* **31**: 499–513.
- Bass, D., Silberman, J.D., Brown, M.W., Tice, A.K., Jousset, A., Geisen, S., and Hartikainen, H. (2016) Coprophilic amoebae and flagellates, including *Guttulinopsis*, *Rosculus* and *Helkesimastix*, characterise a divergent and diverse rhizarian radiation and contribute to a large diversity of faecal-associated protists. *Environ Microbiol* **18**: 1604–1619.
- Bates, S.T., Clemente, J.C., Flores, G.E., Walters, W.A., Parfrey, L.W., Knight, R., and Fierer, N. (2013) Global biogeography of highly diverse protistan communities in soil. *ISME J* **7**: 652–659.
- Burki, F., Kaplan, M., Tikhonenkov, D.V., Zlatogursky, V., Minh, B.Q., Radaykina, L.V., *et al.* (2016) Untangling the early diversification of eukaryotes: a phylogenomic study of the evolutionary origins of Centrohelida, Haptophyta and Cryptista. *Proc R Soc Lond B Biol Sci* **283**.
- Caron, D.A., Worden, A.Z., Countway, P.D., Demir, E., and Heidelberg, K.B. (2008) Protists are microbes too: a perspective. *ISME J* **3**: 4–12.
- Damon, C., Lehembre, F., Oger-Desfeux, C., Luis, P., Ranger, J., Fraissinet-Tachet, L., and Marmeisse, R. (2012) Meta-transcriptomics reveals the diversity of genes expressed by eukaryotes in forest soils. *PLoS One* **7**: e28967.
- de Ruiter, P.C., Neutel, A.M., and Moore, J.C. (1995) Energetics, patterns of interaction strengths, and stability in real ecosystems. *Science* **269**: 1257–1260.
- de Vargas, C., Audic, S., Henry, N., Decelle, J., Mahé, F., Logares, R., *et al.* (2015) Eukaryotic plankton diversity in the sunlit ocean. *Science* **348**.
- Domonell, A., Brabender, M., Nitsche, F., Bonkowski, M., and Arndt, H. (2013) Community structure of cultivable protists in different grassland and forest soils of Thuringia. *Pedobiologia* **56**: 1–7.
- Dupont, A., Griffiths, R.I., Bell, T., and Bass, D. (2016) Differences in soil micro-eukaryotic communities over soil pH gradients are strongly driven by parasites and saprotrophs. *Environ Microbiol* **18**: 2010–2024.
- Fierer, N., Breitbart, M., Nulton, J., Salamon, P., Lozupone, C., Jones, R., *et al.* (2007) Metagenomic and small-subunit rRNA analyses reveal the genetic diversity of bacteria, archaea, fungi, and viruses in soil. *Appl Environ Microbiol* **73**: 7059–7066.
- Fierer, N., Lauber, C.L., Ramirez, K.S., Zaneveld, J., Bradford, M.A., and Knight, R. (2012a) Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. *ISME J* **6**: 1007–1017.
- Fierer, N., Leff, J.W., Adams, B.J., Nielsen, U.N., Bates, S.T., Lauber, C.L., *et al.* (2012b) Cross-biome metagenomic analyses of soil microbial communities and their functional attributes. *Proc Natl Acad Sci USA* **109**: 21390–21395.
- Finlay, B.J., and Fenchel, T. (2001) Protozoan community structure in a fractal soil environment. *Protist* **152**: 203–218.
- Fiore-Donno, A.M., Weinert, J., Wubet, T., and Bonkowski, M. (2016) Metacommunity analysis of amoeboid protists in grassland soils. *Sci Rep* **6**: 19068.
- Geisen, S., Bandow, C., Römbke, J., and Bonkowski, M. (2014) Soil water availability strongly alters the community composition of soil protists. *Pedobiologia* **57**: 205–213.
- Geisen, S., Laros, I., Vizcaino, A., Bonkowski, M., and de Groot, G.A. (2015a) Not all are free-living: high-throughput DNA metabarcoding reveals a diverse community of protists parasitizing soil metazoa. *Mol Ecol* **24**: 4556–4569.
- Geisen, S., Tveit, A.T., Clark, I.M., Richter, A., Svenning, M.M., Bonkowski, M., and Urich, T. (2015b) Metatranscriptomic census of active protists in soils. *ISME J* **9**: 2178–2190.

- Griffiths, R.I., Thomson, B.C., James, P., Bell, T., Bailey, M., and Whiteley, A.S. (2011) The bacterial biogeography of British soils. *Environ Microbiol* **13**: 1642–1654.
- Grossmann, L., Jensen, M., Heider, D., Jost, S., Glucksman, E., Hartikainen, H., *et al.* (2016) Protistan community analysis: key findings of a large-scale molecular sampling. *ISME J* (in press). doi: 10.1038/ismej.2016.10.
- Guillou, L., Bachar, D., Audic, S., Bass, D., Berney, C., Bittner, L., *et al.* (2013) The Protist Ribosomal Reference database (PR2): a catalog of unicellular eukaryote small sub-unit rRNA sequences with curated taxonomy. *Nucleic Acids Res* **41**: D597–D604.
- Hunt, H.W., Coleman, D.C., Ingham, E.R., Ingham, R.E., Elliott, E.T., Moore, J.C., *et al.* (1987) The detrital food web in a shortgrass prairie. *Biol Fert Soils* **3**: 57–68.
- Lara, E., Roussel-Delif, L., Fournier, B., Wilkinson, D.M., and Mitchell, E.A.D. (2016) Soil microorganisms behave like macroscopic organisms: patterns in the global distribution of soil euglyphid testate amoebae. *J Biogeogr* **43**: 520–532.
- Lauber, C.L., Hamady, M., Knight, R., and Fierer, N. (2009) Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. *Appl Environ Microbiol* **75**: 5111–5120.
- Leininger, S., Urich, T., Schloter, M., Schwark, L., Qi, J., Nicol, G.W., *et al.* (2006) Archaea predominate among ammonia-oxidizing prokaryotes in soils. *Nature* **442**: 806–809.
- Lentendu, G., Wubet, T., Chatzinotas, A., Wilhelm, C., Buscot, F., and Schlegel, M. (2014) Effects of long-term differential fertilization on eukaryotic microbial communities in an arable soil: a multiple barcoding approach. *Mol Ecol* **23**: 3341–3355.
- Mitchell, E.A.D. (2015) Pack hunting by minute soil testate amoebae: nematode hell is a naturalist's paradise. *Environ Microbiol* **17**: 4145–4147.
- Mitchell, E.A.D., Charman, D.J., and Warner, B.G. (2008) Testate amoebae analysis in ecological and paleoecological studies of wetlands: past, present and future. *Biodivers Conserv* **17**: 2115–2137.
- Pruesse, E., Quast, C., Knittel, K., Fuchs, B.M., Ludwig, W., Peplies, J., and Glöckner, F.O. (2007) SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Res* **35**: 7188–7196.
- Ramirez, K.S., Leff, J.W., Barberán, A., Bates, S.T., Betley, J., Crowther, T.W., *et al.* (2014) Biogeographic patterns in below-ground diversity in New York City's Central Park are similar to those observed globally. *Proc Biol Sci* **281**.
- Roesch, L.F., Fulthorpe, R.R., Riva, A., Casella, G., Hadwin, A.K., Kent, A.D., *et al.* (2007) Pyrosequencing enumerates and contrasts soil microbial diversity. *ISME J* **1**: 283–290.
- Rousk, J., Bååth, E., Brookes, P.C., Lauber, C.L., Lozupone, C., Caporaso, J.G., *et al.* (2010) Soil bacterial and fungal communities across a pH gradient in an arable soil. *ISME J* **4**: 1340–1351.
- Scherber, C., Eisenhauer, N., Weisser, W.W., Schmid, B., Voigt, W., Fischer, M., *et al.* (2010) Bottom-up effects of plant diversity on multitrophic interactions in a biodiversity experiment. *Nature* **468**: 553–556.
- Stoeck, T., Breiner, H.-W., Filker, S., Ostermaier, V., Kammerlander, B., and Sonntag, B. (2014) A morphogenetic survey on ciliate plankton from a mountain lake pinpoints the necessity of lineage-specific barcode markers in microbial ecology. *Environ Microbiol* **16**: 430–444.
- Tedersoo, L., Bahram, M., Põlme, S., Kõljalg, U., Yorou, N.S., Wijesundera, R., *et al.* (2014) Fungal biogeography. Global diversity and geography of soil fungi. *Science* **346**: 1256688.
- Tedersoo, L., Bahram, M., Cajthaml, T., Polme, S., Hiiesalu, I., Anslan, S., *et al.* (2016) Tree diversity and species identity effects on soil fungi, protists and animals are context dependent. *ISME J* **10**: 346–362.
- Treseder, K.K., Maltz, M.R., Hawkins, B.A., Fierer, N., Stajich, J.E., and McGuire, K.L. (2014) Evolutionary histories of soil fungi are reflected in their large-scale biogeography. *Ecol Lett* **17**: 1086–1093.
- Troemel, E.R., Félix, M.-A., Whiteman, N.K., Barrière, A., and Ausubel, F.M. (2008) Microsporidia are natural intracellular parasites of the nematode *Caenorhabditis elegans*. *PLoS Biol* **6**: e309.
- Turner, T.R., Ramakrishnan, K., Walshaw, J., Heavens, D., Alston, M., Swarbrick, D., *et al.* (2013) Comparative metatranscriptomics reveals kingdom level changes in the rhizosphere microbiome of plants. *ISME J* **7**: 2248–2258.
- Urich, T., Lanzén, A., Qi, J., Huson, D.H., Schleper, C., and Schuster, S.C. (2008) Simultaneous assessment of soil microbial community structure and function through analysis of the meta-transcriptome. *PLoS One* **3**: e2527.
- Woolhouse, M.E.J., Taylor, L.H., and Haydon, D.T. (2001) Population biology of multihost pathogens. *Science* **292**: 1109–1112.