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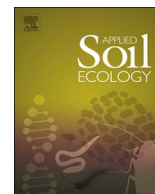
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Short communication

Methodological advances to study the diversity of soil protists and their functioning in soil food webs

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ABSTRACT

Soils host the most complex communities of organisms, which are still largely considered as an unknown ‘black box’. A key role in soil food webs is held by the highly abundant and diverse group of protists. Traditionally, soil protists are considered as the main consumers of bacteria in soils. However, recent insights obtained using new methodologies, provide clear evidence for the trophic diversity of microbial eukaryotes, showing that non-bacterivorous soil protists (fungivores, omnivores, predators of other protists and nematodes), photosynthetic taxa and plant-as well as animal parasites might be equally important.

Here we provide an overview of methodologies to study these important soil organisms. Major gaps of knowledge are highlighted, which can be addressed using a combination of now available methods. These studies will undeniably reveal an even higher functional diversity of protists and likely raise awareness of their ecological importance in soils.

1. Introduction

Disentangling the drivers of decomposition and the flow of energy through the soil food web remains one of the greatest challenges in soil biology (Scheu, 2002). Traditionally, soil organisms were extracted and visually identified leading to the widest knowledge on large organisms such as earthworms (Brown, 1995; Edwards and Bohlen, 1995; Lee, 1985). In line, prokaryotic bacteria and eukaryotic fungi at the base of the soil food web are comparably well studied, due to (1) their key functional roles for decomposition and the mineralisation of nutrients (de Ruiter et al., 1995; Hunt et al., 1987), (2) the availability of an immense repertoire of techniques to study them (Alef and Nannipieri, 1995; Frostegård et al., 1993; Martin-Laurent et al., 2001; Paul, 2014). Knowledge on the smallest primary consumers of bacteria and fungi is least, despite their suggested major roles in nutrient cycling and energy transfer to higher trophic levels in the soil food web (de Ruiter et al., 1995; Hunt et al., 1987). These groups are nematodes and, predominantly, protists (Hunt et al., 1987; Ingham et al., 1985).

2. Taxonomic and functional diversity of soil protists

While nematodes by virtue of their morphologically distinct feeding structures can immediately be grouped into different trophic guilds

(Ettema, 1998; Sohlenius, 1980; Yeates, 1979), soil protists have been mainly considered as bacterial feeders (Bezemer et al., 2010; de Ruiter et al., 1995; Hunt et al., 1987) and grouped according to their locomotion into the morphotypes of flagellates, ciliates and (naked and testate) amoebae (Darbyshire, 1994; Ekelund and Rønn, 1994). The full taxonomic diversity of protists (Adl et al., 2005, 2012), which are suggested to reach numbers between 10,000 and 100,000 individuals per gram of soil, is only gradually being acknowledged in soil biology. So far, concepts integrating this taxonomic diversity at the base of soil food webs is lacking (Geisen, 2016a; Geisen et al., 2016) (Fig. 1, grey boxes).

Developing techniques revolutionized not only our understanding on the species richness of prokaryotes and fungi (Fierer et al., 2007; Rousk et al., 2010; Urich et al., 2008), but also on protists. Potential parasites and pathogens of plants and animals were shown to be very common and abundant members of soil protist communities (Bass et al., 2016; Bates et al., 2013; Geisen et al., 2015c; Grossmann et al., 2016). Many protist taxa found in soil surveys seem to symbiotically inhabit other eukaryotes of the soil meso- and macrofauna, such as mites, collembolans, and especially earthworms (Field and Michiels, 2005, 2006; Geisen et al., 2015a; Pizl, 1986; Purrini, 1984; Velavan et al., 2009) (Fig. 1, red italicized). Purely phototrophic (algae) or mixotrophic protists are mainly associated with soil crusts and might

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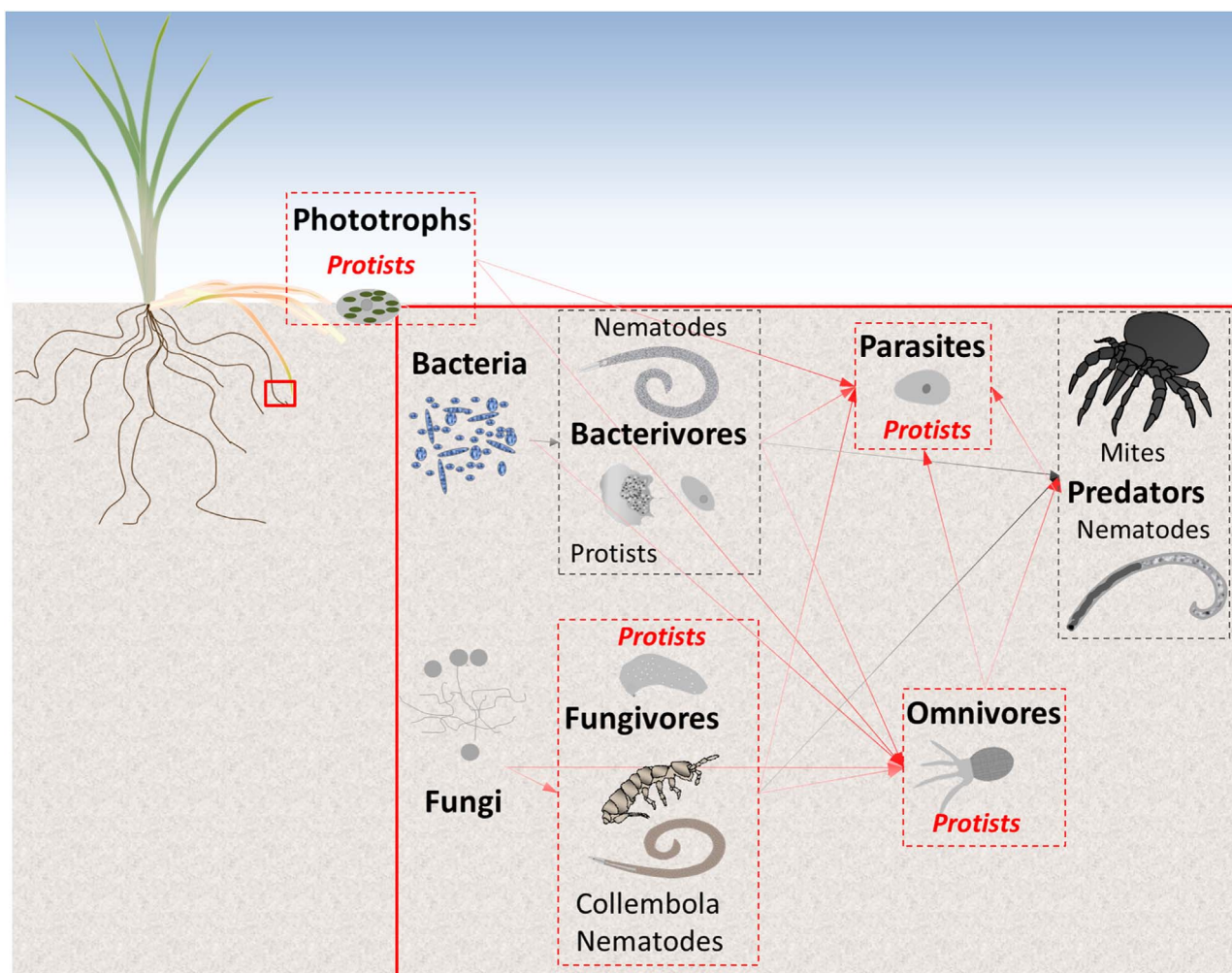


Fig. 1. The base of soil food webs with focus on the diversity of soil protists; red arrows connecting red boxes with italicized term *Protists*: previously non-considered nutrient flow from or to functional units of protists; grey: commonly used standard nutrient flow through the soil food web. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Note: Additional functional units of protists present in soils including mixotrophs and predators are not shown here; other groups of soil organisms (e.g. mites and collembolan) are also often more diverse than depicted in here; higher trophic levels omitted for simplification.

represent an important carbon input into soil systems that is only beginning to be appreciated (Jassey et al., 2015; Seppely et al., 2017). Non-surprisingly, classical cultivation based approaches have profoundly furthered our knowledge on soil protist functioning, showing that a taxonomically wide range of soil protist taxa are fungal feeders (Chakraborty and Old, 1982; Ekelund, 1998; Geisen et al., 2016; Heal, 1963; Petz et al., 1986), but their distribution in soil and functional importance are largely unknown (Fig. 1, red italicized). More recently, the discovery of omnivorous protists with a significant role as nematode predators (Bjørnlund and Rønn, 2008; Geisen et al., 2015b; Rønn et al., 2012), has dramatically changed our perception of microbial food webs and highlighted for the first time the importance of feedback loops in nutrient flows (Geisen, 2016a; Geisen et al., 2015b) (Fig. 1, red italicized).

3. Methodology to study soil protists

These examples focusing on protists illustrate the discrepancy in soil food web models that virtually have not changed since their introduction almost 30 years ago (Hunt et al., 1987); this is even more surprising taken into account the cumulative review article by Scheu (2002) proposing that methodological improvements should be implemented to study soil food webs 15 years ago. Biochemical methods, such as the analyses of phospholipid fatty acids (PLFA), have provided a

thorough understanding of abundances and a rough idea of the community composition of bacteria and fungi (Frostegård and Bååth, 1996; Frostegård et al., 1993). Despite commonly being applied, the usefulness of PLFAs to specifically study protists has been disproven as the 'protist markers', such as 20:2 ω 6c, 20:3 ω 6c, 20:4 ω 6c, are present in nematodes and other soil fauna (Ruess and Chamberlain, 2010).

Meanwhile, many methods have already been replaced by new approaches. Especially molecular tools now allow comparably non-expensive, user-friendly high-throughput analyses of soil organisms and have revolutionized our understanding of the abundances (qPCR approaches) and community structure (targeted amplicon high-throughput sequencing (AS)) of prokaryotic bacteria and archaea (Fierer et al., 2009; Leininger et al., 2006; Roesch et al., 2007) and fungi (Buée et al., 2009; Davison et al., 2015; Tedersoo et al., 2014). However protists, as the remaining third microbial group have received little attention (Bates et al., 2013; Lentendu et al., 2014), most prominently exemplified by the lack of molecular tools to reliably study abundances of the entity or even specific protist taxa. This is illustrated by the fact that even closely related protist species fundamentally differ in copy numbers of targeted barcode genes which makes copy-number to abundance information such as used in qPCR inapplicable at least to target a wider range of protists. More information is needed on the applicability of qPCR methods, but at present qPCR seems rather inapplicable to quantify most protist taxa if not rigorously tested with

defined mock communities. Copy number differences also impair AS, and primer biases might furthermore artificially alter the ‘true’ community composition of soil protists (Geisen et al., 2015a). Nevertheless, in focused studies where the same methodology is being used AS provides an invaluable tool that allows an untargeted analysis of nearly the entire diversity of so far often unknown protists in soils (Bates et al., 2013; Mahé et al., 2017)

In addition to AS, new molecular methods such as metagenomics and metatranscriptomics are starting to be applied to unravel soil communities (Carvalho et al., 2012; Fierer et al., 2012; Urich et al., 2008). These tools still suffer from differences due to copy numbers of marker genes analysed, but avoid biases introduced by the PCR step in AS. These ‘omics’ datasets have been rarely mined for protists and soil animals at higher trophic levels, despite the high potential of these tools to unravel the identities of organisms in complex soil communities (Bailey et al., 2007; Geisen et al., 2015; Jacquiod et al., 2016; Urich et al., 2008). Coupled with isotope labelling, we get detailed information on the functional roles of specific taxa in natural soils (Kramer et al., 2016). These and other omics approaches such as metaproteomics (Stopnisek et al., 2015) and meta-metabolomics (Turnbaugh and Gordon, 2008) when coupled with network analyses (Consortium, 2016; Creamer et al., 2016; Morriën et al., 2017), are likely to give novel insights into the real complexity of belowground food webs, and of their functions as (transcribed) genes, proteins and metabolites can be studied at once (Damon et al., 2012; Franzosa et al., 2015; Prosser, 2015; Turnbaugh and Gordon, 2008; Tveit et al., 2013) (Fig. 2).

Taken together, a wide range of microscopic, molecular and visualization methods is already available to get new insights into the community composition and functioning of soil protists in relation to

microorganisms and soil fauna (Fig. 2 and Table 1).

4. Summary and outlook

We here provide a brief overview of available methodologies to study the widely unknown diversity and community structure of soil protists that resulted in a recent revival of the field of soil protistology (Geisen et al., 2017). We have to realize that protist functioning in soils is not restricted to “bacterivory”, and that gradually the exact players and their contribution to food web functioning are being revealed. This rules out that the entire functional diversity of soil protists can be integrated into existing food web models; instead, food web models have to be revised so that protists find their places in different functional nodes in existing food webs, such as present for nematodes. This will undoubtedly result in novel insights how the entity of soil organisms is interlinked in soil food webs (Geisen, 2016a).

Last, we want to list some knowledge gaps on soil protists which, by addressing them, will likely further our knowledge on soil food webs in general.

Establishing and standardizing methodologies to study soil protists

This will allow inter-study comparisons independent of studied system, investigator, and applied method. Many classical approaches have studied protist taxa that were only roughly described morphologically without providing molecular barcodes and can now not reliably be placed taxonomically. The lack of comparability between classical cultivation-based and molecular studies greatly hampers advance in the identification of the dominant taxa and the abundance of protists, and

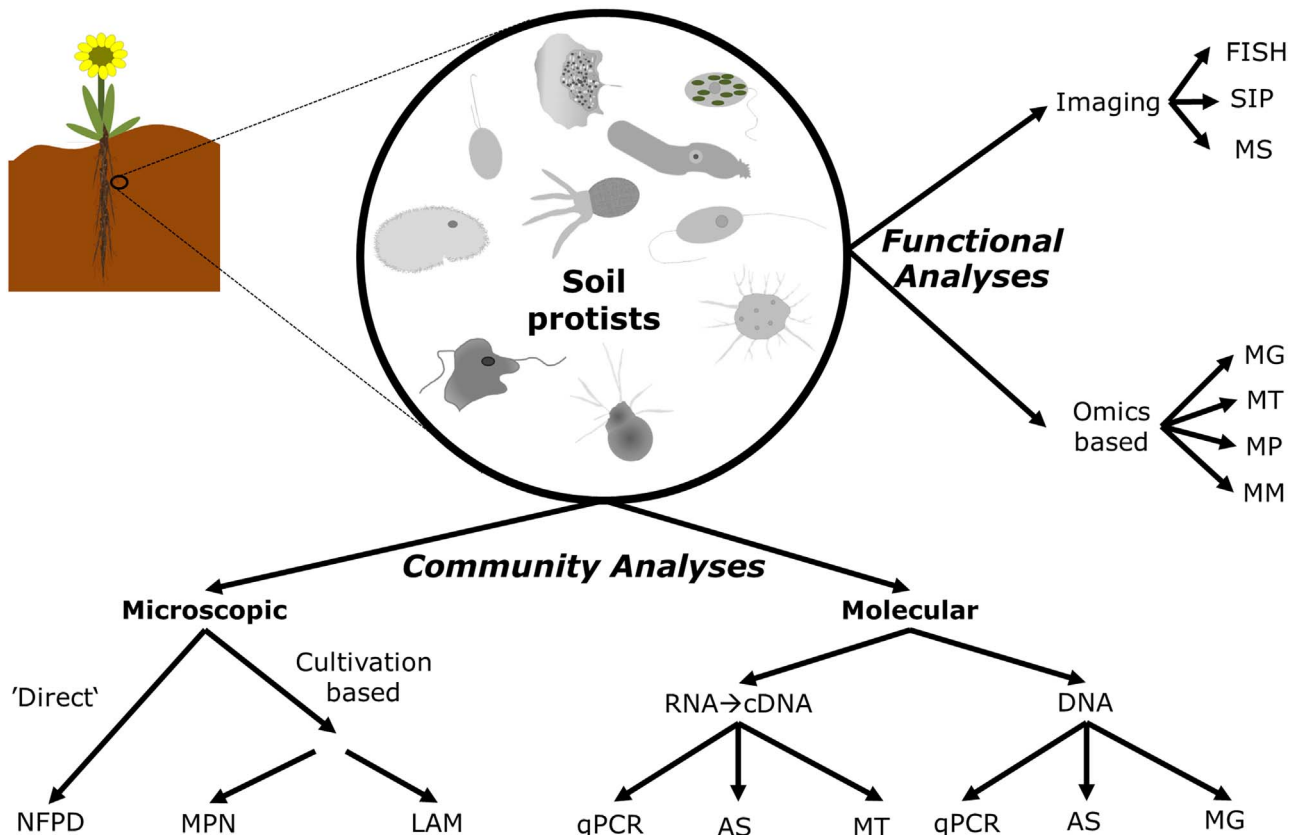


Fig. 2. Overview of common methods to study soil protists, which are separated into two major approaches, one that uses microscopic analyses to identify protists to differential resolutions, one using molecular methods on extracted nucleic acids (DNA or cDNA after reverse transcription of RNA) (see Table 1 for further information on the different methods). Functional analyses can follow at different points of this workflow such as after cultivation based efforts, MG and MT data can be harvested for functional genes and additional techniques such as stable isotope probing can be integrated.

NFPD: non-flooded petri dish method; MPN: Most probable number technique; LAM: Liquid aliquot method; qPCR: quantitative PCR; MT: Metatranscriptomic high-throughput sequencing (HTS); AS: Amplicon HTS; MG: Metagenomic HTS.

Table 1

Overview of techniques to study soil protists; NDPD: Non flooded petri dish method; MPN: Most probably number technique; LAM: Liquid aliquot method; qPCR: quantitative PCR; AS: Amplicon high-throughput sequencing (HTS); MG: Metagenomic HTS; MT: Metatranscriptomic HTS.

Method	Description	Pros	Cons	Protist specific references
Direct counting	A soil suspension is stained with Anilin blue to distinguish empty and full tests of amoebae, sieved the filtrate between 250 µm and 25 µm mesh size is filled up to 10 ml and analysed drop by drop under a microscope.	Cost-efficient Direct quantification of active and dead (empty) testate amoebae	Very time consuming, expert knowledge to identify taxa needed	(Krashevskaya et al., 2007; Schönborn, 1978)
NFPD	Dried soil or litter is saturated, but not flooded, in a Petri dish and organisms are being identified and enumerated in the water that drains off when the Petri dish is tilted.	Cost-efficient Fast Direct examination of active, non-encysted taxa	Expert knowledge in identifying taxa needed Time consuming Only actively swimming taxa (ciliates, some flagellates) identifiable	(Foissner, 1987)
MPN	Serial dilution of a soil suspension followed by a cultivation period before identification of grown taxa	Estimation of production of protists in experiments Cost-efficient Fast Medium throughput	No differentiation between active and encysted protists Culture medium is selective Inaccurate quantification Only differentiation into rough morphogroups	(Darbyshire et al., 1974)
LAM	High dilution of a soil suspension followed by a cultivation period before identification of grown taxa	Cost-efficient Medium taxonomic resolution	Time consuming Expert knowledge in identifying taxa needed	(Butler and Rogerson, 1995; Geisen et al., 2014)
qPCR	Using group specific primers on extracted DNA or cDNA after reverse transcription of RNA	Cost-efficient Fast Abundance information High throughput Active taxa targeted (RNA)	No community differentiation unless many differential primer pairs are used	To be evaluated
AS	Using general eukaryotic primers to target all eukaryotes or protist group specific primers on extracted DNA or cDNA	High taxonomic resolution High throughput Active taxa targeted (RNA)	Sophisticated bioinformatic facilities and knowledge required Primer and PCR biases	(Bates et al., 2013; de Groot et al., 2016)
MG	Shotgun sequencing of all extracted DNA and followed by bioinformatic analyses	High taxonomic resolution No primer and PCR biases Entire genomes targeted	Very costly Sophisticated bioinformatic facilities and knowledge required Low sample throughput Mostly non-protist information recovered	(Jacquiod et al., 2016)
MT	Random reverse transcription of RNA followed by sequencing and bioinformatic analyses	High taxonomic resolution No primer and PCR biases Entire genomes targeted Active taxa targeted	Very costly Sophisticated bioinformatic facilities and knowledge required Low sample throughput Mostly non-protist information recovered	(Geisen et al., 2015a,b,c; Urich et al., 2008)

Note: Classical molecular techniques such as DGGE, T-RFLP or ELISA that have been replaced by HTS are not shown.

thus the general understanding microbial food webs.

Disentangling the taxonomic diversity of soil protists

HTS studies revealed an astounding diversity of protist taxa, but whole large clades are still only known from sequences. Much more effort is therefore needed in cultivating and describing the immense unknown diversity of soil protist taxa. This is needed for reliable assignments of protist traits and functions in sequence-based studies, and provides the base for deciphering the real structure of soil protist communities. Furthermore, more thorough sequence annotations and analyses are needed to decipher the true diversity of protist species beyond the OTU level (Dupont et al., 2016; Geisen, 2016b)

Deciphering the functional diversity of soil protists

Using cultivated species, studies that address how different protist taxa interact with other soil organisms are pivotal (Dumack et al., 2016; Geisen, 2016b; Geisen et al., 2015b). Sequence based analyses will further help at identifying potential functional genes in the genome of protist taxa that can be investigated in more detail for their relevance in nature. Tracing of stable isotopes after differential labelling of protist prey will reveal insights into species-specific feeding differences of protists and how nutrients are being transferred to higher trophic levels in the soil food web (Crotty et al., 2012; Kramer et al., 2016). Sequencing and imaging technologies will help in unravelling hosts of

protist parasites and pathogens and where they are located.

Future integrated work in these three major areas will further our understanding of the diversity, community structure and functioning of soil protists and soil biodiversity *per se*. Only that will allow us to reliable study soil organisms for more general (ecological) questions such as their response to environmental changes or to estimate their importance in ecosystem functioning.

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References

- Adl, S.M., Simpson, A.G., Farmer, M.A., Andersen, R.A., Anderson, O.R., Barta, J.R., Bowser, S.S., Brugerolle, G., Fensome, R.A., Fredericq, S., James, T.Y., Karpov, S., Kugrens, P., Krug, J., Lane, C.E., Lewis, L.A., Lodge, J., Lynn, D.H., Mann, D.G., McCourt, R.M., Mendoza, L., Moestrup, O., Mozley-Standridge, S.E., Nerad, T.A., Shearer, C.A., Smirnov, A.V., Spiegel, F.W., Taylor, M.F., 2005. The new higher level

- classification of Eukaryotes with emphasis on the taxonomy of Protists. *J. Eukaryot. Microbiol.* 52, 399–451.
- Adl, S.M., Simpson, A.G.B., Lane, C.E., Lukeš, J., Bass, D., Bowser, S.S., Brown, M.W., Burki, F., Dunthorn, M., Hampl, V., Heiss, A., Hoppenrath, M., Lara, E., le Gall, L., Lynn, D.H., McManus, H., Mitchell, E.A.D., Mozley-Stanridge, S.E., Parfrey, L.W., Pawlowski, J., Rueckert, S., Shadwick, L., Schoch, C.L., Smirnov, A., Spiegel, F.W., 2012. The revised classification of eukaryotes. *J. Eukaryot. Microbiol.* 59, 429–514.
- Alef, K., Nannipieri, P., 1995. Methods in applied soil microbiology and biochemistry. Academic Press.
- Bailey, J., Fraissinet-Tachet, L., Verner, M.C., Debaud, J.C., Lemaire, M., Wesolowski-Louvel, M., Marmeisse, R., 2007. Soil eukaryotic functional diversity, a metatranscriptomic approach. *ISME J.* 1, 632–642.
- Bass, D., Silberman, J.D., Brown, M.W., Tice, A.K., Jousset, A., Geisen, S., 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., Fierer, N., 2013. Global biogeography of highly diverse protistan communities in soil. *ISME J.* 7, 652–659.
- Bezemer, T.M., Fountain, M.T., Barea, J.M., Christensen, S., Dekker, S.C., Duyts, H., van Hal, R., Harvey, J.A., Hedlund, K., Maraun, M., Mikola, J., Mladenov, A.G., Robin, C., de Ruiter, P.C., Scheu, S., Setälä, H., Šmilauer, P., van der Putten, W.H., 2010. Divergent composition but similar function of soil food webs of individual plants: plant species and community effects. *Ecology* 91, 3027–3036.
- Bjørnlund, L., Rønn, R., 2008. 'David and Goliath' of the soil food web – Flagellates that kill nematodes. *Soil Biol. Biochem.* 40, 2032–2039.
- Brown, G.G., 1995. How do earthworms affect microfloral and faunal community diversity? In: Collins, H.P., Robertson, G.P., Klug, M.J. (Eds.), *The Significance and Regulation of Soil Biodiversity: Proceedings of the International Symposium on Soil Biodiversity*, Held at Michigan State University, East Lansing, May 3–6, 1993. Springer, Netherlands, Dordrecht, pp. 247–269.
- Buée, M., Reich, M., Murat, C., Morin, E., Nilsson, R.H., Uroz, S., Martin, F., 2009. 454 Pyrosequencing analyses of forest soils reveal an unexpectedly high fungal diversity. *New Phytol.* 184, 449–456.
- Butler, H., Rogerson, A., 1995. Temporal and spatial abundance of naked amoebae (Gymnamoebae) in marine benthic sediments of the Clyde Sea Area, Scotland. *J. Eukaryot. Microbiol.* 42, 724–730.
- Carvalho, L.C., Dennis, P.G., Tyson, G.W., Schenk, P.M., 2012. Application of metatranscriptomics to soil environments. *J. Microbiol. Methods* 91, 246–251.
- Chakraborty, S., Old, K., 1982. Mycophagous soil amoeba: interactions with three plant pathogenic fungi. *Soil Biol. Biochem.* 14, 247–255.
- Consortium, T.Q., 2016. Networking our way to better ecosystem service provision. *Trends Ecol. Evol.* 31, 105–115.
- Creamer, R.E., Hannula, S.E., Leeuwen, J.P.V., Stone, D., Rutgers, M., Schmelz, R.M., Ruiter, P.C. d. Hendriksen, N.B., Bolger, T., Bouffaud, M.L., Buee, M., Carvalho, F., Costa, D., Dirilgen, T., Francisco, R., Griffiths, B.S., Griffiths, R., Martin, F., Silva P.M. d. Mendes, S., Morais, P.V., Pereira, C., Philippot, L., Plassart, P., Redecker, D., Römcke, J., Sousa, J.P., Wouterse, M., Lemanceau, P., 2016. Ecological network analysis reveals the inter-connection between soil biodiversity and ecosystem function as affected by land use across Europe. *Appl. Soil Ecol.* 97, 112–124.
- Crotty, F., Adl, S., Blackshaw, R., Murray, P., 2012. Protozoan pulses unveil their pivotal position within the soil food web. *Microb. Ecol.* 63, 905–918.
- Damon, C., Lehenbre, F., Oger-Desfeux, C., Luis, P., Ranger, J., Fraissinet-Tachet, L., Marmeisse, R., 2012. Metatranscriptomics reveals the diversity of genes expressed by eukaryotes in forest soils. *PLoS One* 7, e28967.
- Darbyshire, J.F., Whitley, R.E., Graebes, M.P., Inkson, R.H.E., 1974. A rapid micromethod for estimating bacterial and protozoan populations in soil. *Rev. Ecol. Biol. Sol.* 11, 465–475.
- Darbyshire, J., 1994. *Soil Protozoa*. CAB International, Wallingford.
- Davison, J., Moora, M., Öpik, M., Adholean, A., Ainsaar, L., Bå, A., Burla, S., Diedhiou, A.G., Hiiesalu, I., Jairus, T., Johnson, N.C., Kane, A., Koorem, K., Kõrjar, M., Ndiaye, C., Pärtel, M., Reier, Ü., Saks, Ü., Singh, R., Vasar, M., Zobel, M., 2015. Global assessment of arbuscular mycorrhizal fungus diversity reveals very low endemism. *Science* 349, 970–973.
- Dumack, K., Müller, M.E.H., Bonkowski, M., 2016. Description of *Lecythium terrestris* sp. nov. (Chlamydomonadophyceae: Cercozoa), a soil dwelling protist feeding on fungi and algae. *Protist* 167, 93–105.
- Dupont, A.O., Griffiths, R.I., Bell, T., 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.
- Edwards, C.A., Bohlen, P.J., 1995. *Biology and Ecology of Earthworms*. Springer, Netherlands.
- Ekelund, F., Rønn, R., 1994. Notes on protozoa in agricultural soil with emphasis on heterotrophic flagellates and naked amoebae and their ecology. *FEMS Microbiol. Rev.* 15, 321–353.
- Ekelund, F., 1998. Enumeration and abundance of mycophagous protozoa in soil: with special emphasis on heterotrophic flagellates. *Soil Biol. Biochem.* 30, 1343–1347.
- Ettema, C.H., 1998. Soil nematode diversity: species coexistence and ecosystem function. *J. Nematol.* 30, 159–169.
- Field, S.G., Michiels, N.K., 2005. Parasitism and growth in the earthworm *Lumbricus terrestris*: fitness costs of the gregarine parasite *Monocystis* sp. *Parasitology* 130, 397–403.
- Field, S.G., Michiels, N.K., 2006. Does the acephaline gregarine *Monocystis* sp. modify the surface behaviour of its earthworm host *Lumbricus terrestris*? *Soil Biol. Biochem.* 38, 1334–1339.
- Fierer, N., Breitbart, M., Nulton, J., Salamon, P., Lozupone, C., Jones, R., Robeson, M., Edwards, R.A., Felts, B., Rayhawk, S., Knight, R., Rohwer, F., Jackson, R.B., 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., Strickland, M.S., Liptzin, D., Bradford, M.A., Cleveland, C.C., 2009. Global patterns in belowground communities. *Ecol. Lett.* 12, 1238–1249.
- Fierer, N., Leff, J.W., Adams, B.J., Nielsen, U.N., Bates, S.T., Lauber, C.L., Owens, S., Gilbert, J.A., Wall, D.H., Caporaso, J.G., 2012. Cross-biome metagenomic analyses of soil microbial communities and their functional attributes. *Proc. Natl. Acad. Sci. U. S. A.* 109, 21390–21395.
- Foissner, W., 1987. Soil protozoa: Fundamental problems, ecological significance, adaptations in ciliates and testaceans, bioindicators, and guide to the literature. *Progr. Protistol.* 2, 69–121.
- Franzosa, E.A., Hsu, T., Sirota-Madi, A., Shafiqat, A., Abu-Ali, G., Morgan, X.C., Huttenhower, C., 2015. Sequencing and beyond: integrating molecular 'omics' for microbial community profiling. *Annu. Rev. Microbiol.* 13, 360–372.
- Frostgård, A., Bååth, E., 1996. The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. *Biol. Fertil. Soils* 22, 59–65.
- Frostgård, Å., Bååth, E., Tunlio, A., 1993. Shifts in the structure of soil microbial communities in limed forests as revealed by phospholipid fatty acid analysis. *Soil Biol. Biochem.* 25, 723–730.
- Geisen, S., Bandow, C., Römcke, J., Bonkowski, M., 2014. Soil water availability strongly alters the community composition of soil protists. *Pedobiologia* 57, 205–213.
- Geisen, S., Laros, I., Vizcaíno, A., Bonkowski, M., 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., Rosengarten, J., Koller, R., Mulder, C., Ulrich, T., Bonkowski, M., 2015b. Pack hunting by a common soil amoeba on nematodes. *Environ. Microbiol.* 17, 4538–4546.
- Geisen, S., Tveit, A.T., Clark, I.M., Richter, A., Svenning, M.M., Bonkowski, M., Ulrich, T., 2015c. Metatranscriptomic census of active protists in soils. *ISME J.* 9, 2178–2190.
- Geisen, S., Koller, R., Hünninghaus, M., Dumack, K., Ulrich, T., Bonkowski, M., 2016. The soil food web revisited: Diverse and widespread mycophagous soil protists. *Soil Biol. Biochem.* 94, 10–18.
- Geisen, S., Mitchell, E.A.D., Wilkinson, D.M., Adl, S., Bonkowski, M., Brown, M.W., Fiore-Donno, A.M., Heger, T.J., Jassey, V.E.J., Krashevskaya, V., Lahr, D.J.G., Marcisz, K., Mulot, M., Payne, R., Singer, D., Anderson, O.R., Charman, D.J., Ekelund, F., Griffiths, B.S., Rønn, R., Smirnov, A., Bass, D., Belbahri, L., Berney, C., Blandenier, Q., Chatzinotas, A., Clarholm, M., Dunthorn, M., Feest, A., Fernández, L.D., Foissner, W., Fournier, B., Gentekaki, E., Hájek, M., Helder, J., Jousset, A., Koller, R., Kumar, S., La Terza, A., Lamentowicz, M., Mazei, Y., Santos, S.S., Seppye, C.V.W., Spiegel, F.W., Walochnik, J., Winding, A., Lara, E., 2017. Soil protistology rebooted: 30 fundamental questions to start with. *Soil Biol. Biochem.* 111, 94–103.
- Geisen, S., 2016a. The bacterial-fungal energy channel concept challenged by enormous functional versatility of soil protists. *Soil Biol. Biochem.* 102, 22–25.
- Geisen, S., 2016b. Thorough high-throughput sequencing analyses unravels huge diversities of soil parasitic protists. *Environ. Microbiol.* 18, 1659–1672.
- Grossmann, L., Jensen, M., Heider, D., Jost, S., Glucksman, E., Hartikainen, H., Mahamdallie, S.S., Gardner, M., Hoffmann, D., Bass, D., Boenigk, J., 2016. Protistan community analysis: key findings of a large-scale molecular sampling. *ISME J.* 10, 2269–2279.
- Heal, O.W., 1963. Soil fungi as food for amoebae. In: Doeksen, J., Van der Drift, J. (Eds.), *Soil Organisms*. North-Holland Publishing Company, Amsterdam, pp. 289–297.
- Hunt, H.W., Coleman, D.C., Ingham, E.R., Ingham, R.E., Elliott, E.T., Moore, J.C., Rose, S.L., Reid, C.P.P., Morley, C.R., 1987. The detrital food web in a shortgrass prairie. *Biol. Fertil. Soils* 3, 57–68.
- Ingham, R.E., Trofymow, J.A., Ingham, E.R., Coleman, D.C., 1985. Interactions of bacteria, fungi, and their nematode grazers: effects on nutrient cycling and plant growth. *Ecol. Monogr.* 55, 119–140.
- Jacquioud, S., Stenbaek, J., Santos, S.S., Winding, A., Sorensen, S.J., Prieme, A., 2016. Metagenomes provide valuable comparative information on soil microeukaryotes. *Res. Microbiol.* 167, 436–450.
- Jassey, V.E.J., Signarbieux, C., Hättenschwiler, S., Bragazza, L., Buttler, A., Delarue, F., Fournier, B., Gilbert, D., Lagoun-Déferge, F., Lara, E.T.E., Mills, R., Mitchell, E.A.D., Payne, R.J., Robroek, B.J.M., 2015. An unexpected role for mixotrophs in the response of peatland carbon cycling to climate warming. *Sci. Rep.* 5, 16931.
- Kramer, S., Döberner, D., Moll, J., Huenninghaus, M., Koller, R., Krueger, D., Marhan, S., Ulrich, T., Wubet, T., Bonkowski, M., Buscot, F., Lueders, T., Kandeler, E., 2016. Resource partitioning between bacteria, fungi, and protists in the detritusphere of an agricultural soil. *Front. Microbiol.* 7.
- Krashevskaya, V., Bonkowski, M., Maraun, M., Scheu, S., 2007. Testate amoebae (Protista) of an elevational gradient in the tropical mountain rain forest of Ecuador. *Pedobiologia* 51, 319–331.
- Lee, K.E., 1985. *Earthworms: Their Ecology and Relationships with Soils and Land Use*. Academic Press Inc.
- Leininger, S., Ulrich, T., Schloter, M., Schwark, L., Qi, J., Nicol, G.W., Prosser, J.I., Schuster, S.C., Schleper, C., 2006. Archaea predominate among ammonia-oxidizing prokaryotes in soils. *Nature* 442, 806–809.
- Lentendu, G., Wubet, T., Chatzinotas, A., Wilhelm, C., Buscot, F., 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.
- Mahé, F., de Vargas, C., Bass, D., Czech, L., Stamatakis, A., Lara, E., Singer, D., Mayor, J., Bunge, J., Sernaker, S., Siemensmeyer, T., Trautmann, I., Romac, S., Berney, C., Kozlov, A., Mitchell, E.A.D., Seppye, C.V.W., Egge, E., Lentendu, G., Wirth, R., Trueba, G., Dunthorn, M., 2017. Parasites dominate hyperdiverse soil protist communities in Neotropical rainforests. *Nat. Ecol. Evol.* 1, 0091.
- Martin-Laurent, F., Philippot, L., Hallet, S., Chaussod, R., Germon, J.C., Soulas, G.,

- Catroux, G., 2001. DNA extraction from soils: old bias for new microbial diversity analysis methods. *Appl. Environ. Microbiol.* 67, 2354–2359.
- Morriën, E., Hannula, S.E., Snoek, L.B., Helmsing, N.R., Zweers, H., de Hollander, M., Soto, R.L., Bouffaud, M.-L., Buée, M., Dimmers, W., Duyts, H., Geisen, S., Girlanda, M., Griffiths, R.I., Jørgensen, H.-B., Jensen, J., Plassart, P., Redecker, D., Schmelz, R.M., Schmidt, O., Thomson, B.C., Tisserant, E., Uroz, S., Winding, A., Bailey, M.J., Bonkowski, M., Faber, J.H., Martin, F., Lemanceau, P., de Boer, W., van Veen, J.A., van der Putten, W.H., 2017. Soil networks become more connected and take up more carbon as nature restoration progresses. *Nat. Commun.* 8, 14349.
- Paul, E.A., 2014. *Soil microbiology, ecology and biochemistry*. Academic Press.
- Petz, W., Foissner, W., Wirmsberger, E., Krautgartner, W.D., Adam, H., 1986. Mycophagy, a new feeding strategy in autochthonous soil ciliates. *Naturwissenschaften* 73, 560–562.
- Pizl, V., 1986. The effect of the herbicide Zeazin 50 on the earthworm infection by monocyetid gregarines. *Pedobiologia* 28, 399–402.
- Prosser, J.I., 2015. Dispersing misconceptions and identifying opportunities for the use of ‘omics’ in soil microbial ecology. *Nat. Rev. Micro.* 13, 439–446.
- Purrini, K., 1984. Light and electron microscope studies on *Helicospiridium* sp. parasitizing oribatid mites (OribateiAcarina) and collembola (Apterygota, Insecta) in forest soils. *J. Invertebr. Pathol.* 44, 18–27.
- Rønn, R., Vestergård, M., Ekelund, F., 2012. Interactions between bacteria, protozoa and nematodes in soil. *Acta Protozool.* 51, 223–235.
- Roesch, L.F., Fulthorpe, R.R., Riva, A., Casella, G., Hadwin, A.K., Kent, A.D., Daroub, S.H., Camargo, F.A., Farmerie, W.G., Triplett, E.W., 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., Knight, R., Fierer, N., 2010. Soil bacterial and fungal communities across a pH gradient in an arable soil. *ISME J.* 4, 1340–1351.
- Ruess, L., Chamberlain, P.M., 2010. The fat that matters: soil food web analysis using fatty acids and their carbon stable isotope signature. *Soil Biol. Biochem.* 42, 1898–1910.
- Schönborn, W., 1978. Untersuchungen zur Produktion der Boden-Testaceen. *Pedobiologia* 18, 373–377.
- Scheu, S., 2002. The soil food web: structure and perspectives. *Eur. J. Soil Biol.* 38, 11–20.
- Seppely, C.V.W., Singer, D., Dumack, K., Fournier, B., Belbahri, L., Mitchell, E.A.D., Lara, E., 2017. Distribution patterns of soil microbial eukaryotes suggests widespread algivory by phagotrophic protists as an alternative pathway for nutrient cycling. *Soil Biol. Biochem.* 112, 68–76.
- Sohlenius, B., 1980. Abundance, biomass and contribution to energy flow by soil nematodes in terrestrial ecosystems. *Oikos* 34, 186–194.
- Stopnisek, N., Zuhlke, D., Carlier, A., Barberan, A., Fierer, N., Becher, D., Riedel, K., Eberl, L., Weisskopf, L., 2015. Molecular mechanisms underlying the close association between soil *Burkholderia* and fungi. *ISME J.* 10, 253–264.
- Tedersoo, L., Bahram, M., Pöhlme, S., Kõljalg, U., Yorou, N.S., Wijesundera, R., Ruiz, L.V., Vasco-Palacios, A.M., Thu, P.Q., Suija, A., Smith, M.E., Sharp, C., Saluveer, E., Saitta, A., Rosas, M., Riit, T., Ratkowsky, D., Pritsch, K., Põldmaa, K., Piepenbring, M., Phosri, C., Peterson, M., Parts, K., Pärtel, K., Otsing, E., Nouhra, E., Njouonkou, A.L., Nilsson, R.H., Morgado, L.N., Mayor, J., May, T.W., Majuakim, L., Lodge, D.J., Lee, S.S., Larsson, K.-H., Kohout, P., Hosaka, K., Hiiesalu, I., Henkel, T.W., Harend, H., Guo, L.-d., Greslebin, A., Grelet, G., Geml, J., Gates, G., Dunstan, W., Dunk, C., Drenkhan, R., Dearnaley, J., De Kesel, A., Dang, T., Chen, X., Buegger, F., Brearley, F.Q., Bonito, G., Anslan, S., Abell, S., Abarenkov, K., 2014. Fungal biogeography. Global diversity and geography of soil fungi. *Science* 346, 1256688.
- Turnbaugh, P.J., Gordon, J.I., 2008. An invitation to the marriage of metagenomics and metabolomics. *Cell* 134, 708–713.
- Tveit, A., Schwacke, R., Svenning, M.M., Urich, T., 2013. Organic carbon transformations in high-Arctic peat soils: key functions and microorganisms. *ISME J.* 7, 299–311.
- Urich, T., Lanzén, A., Qi, J., Huson, D.H., Schleper, C., Schuster, S.C., 2008. Simultaneous assessment of soil microbial community structure and function through analysis of the meta-transcriptome. *PLoS One* 3, e2527.
- Velavan, T.P., Weller, S., Schulenburg, H., Michiels, N.K., 2009. High genetic diversity and heterogeneous parasite load in the earthworm *Lumbricus terrestris* on a German meadow. *Soil Biol. Biochem.* 41, 1591–1595.
- Yeates, G.W., 1979. Soil nematodes in terrestrial ecosystems. *J. Nematol.* 11, 213–229.
- de Groot, G.A., Laros, I., Geisen, S., 2016. Molecular identification of soil eukaryotes and focused approaches targeting protist and faunal groups using high-throughput metabarcoding. In: Martin, F., Uroz, S. (Eds.), *Microbial Environmental Genomics (MEG)*. Springer New York, New York, NY, pp. 125–140.
- de Ruyter, P.C., Neutel, A.-M., Moore, J.C., 1995. Energetics, pattern of interaction strength and stability in real ecosystems. *Science* 269, 1257–1260.