SOIL ORGANISMS 92 (3) · December 2020 pp. 189–196

Soil protist life matters!

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Received 30 August 2020 | Accepted 2 November 2020
Published online at www.soil-organisms.de 1 December 2020 | Printed version 15 December 2020
DOI 10.25674/so92iss3pp189

Abstract

Soils host most biodiversity on Earth, with a major fraction of its taxonomic diversity still to be uncovered and most of its functional knowledge to be determined. Much focus has been - and still is - on bacteria, fungi and animals. Clearly, without any of those components, soils would not function as they do. However, the group that constitutes the bulk of eukaryotic diversity and plays a central role for soil functioning is missing: protists. As the main consumers of the microbiome, protists shape its composition and functioning. Other less known functions performed by protists may be equally important. Protists also include primary producers, decomposers, animal parasites and plant pathogens. We briefly review the many functions protists perform in soils and argue that soil biodiversity studies that ignore protists miss some potential mechanistic insight into the drivers of observed patterns. We highlight that the immense functional repertoire of protist affects virtually every soil process, from carbon cycling to primary production, including crop production. Therefore, we call for truly integrated biodiversity assessments including protists, without which the soil food-web and processes cannot reliably be understood: protists matter!

Keywords Soil biodiversity | Soil functioning | Plant-soil interactions | Soil food-webs | Microbiome

1. Introduction

Soil biodiversity is receiving increased recognition due to its profound role underlying ecosystem processes. As such, soil organisms can help in meeting many of the sustainable development goals defined by the UN including zero hunger, climate action and life on land (Griggs et al. 2013). Indeed, soil organisms are the major component of life on Earth, with respect to abundance, biomass and diversity (Bar-On et al. 2018, Geisen et al. 2019, Decaëns 2010). Soils are teeming with life ranging from nano-scale viruses (if considered alive) to microorganisms (bacteria, archaea, fungi and protists), to differentially sized animals that form complex soil networks and in synchrony drive soil functions (Geisen et al. 2019). Research intensity – and resulting knowledge – on these groups is uneven (Geisen et al. 2017). Here we stress that this biased view on a fraction of soil organisms, particularly bacteria, fungi and to some extent animals is no-longer acceptable as soils would not function as they do without all their biodiversity components, and especially not without protists. Many of the points we raise here are not new and have been reviewed in the last years and decades (Geisen et al. 2017, Geisen et al. 2018, Gao et al. 2019, Wilkinson 1998). However, we still feel the need to bring protists to the attention of a broader

ISSN 1864-6417 (print) · ISSN 2509-9523 (online)

group of soil biodiversity experts by highlighting some novel findings and by showing why soil biodiversity without protists cannot be considered soil biodiversity!

2. A heads up on (soil) protists

Most protists are microorganisms invisible to the naked eye (Caron et al. 2008). Their size range is broad from small in the low micrometer scale (commonly known as nanoplankton in aquatic systems) to the largest single-celled organisms (Geisen et al. 2017). Protists dominate eukaryotic diversity in virtually any system on Earth (Adl et al. 2019, Burki et al. 2020). Whereas fungi, plants and animals represent single monophyletic branches within distinct supergroups, protists are paraphyletic and spread across all supergroups in the entire eukaryotic tree of life (Burki et al. 2020). Considering the major lineages, almost all eukaryotes are protists. Potentially millions of protist species exist (Orgiazzi et al. 2016), but as species delineation and identification classically required tedious or nearly impossible cultivation or fixation processes, more reliable estimates have only been possible with high throughput sequencing (HTS) of phylogenetic marker genes, a method commonly termed metabarcoding or amplicon sequencing. These approaches clearly demonstrated that protists indeed make up the bulk of eukaryotic diversity (Geisen et al. 2018, Sunagawa et al. 2020). With such huge taxonomic diversity, it is logical that protists functional importance is equally wide. In aquatic systems protists compose the major fraction of algae that fix half of Earth´s carbon (Falkowski 2002), while heterotrophic protists are just as abundant and important as consumers that drive the microbial loop (Worden et al. 2015, de Vargas et al. 2015). Protists in soil have received less attention due to methodological challenges, but new tools and especially HTS now allow rapid progress, confirming their numerical and functional importance: similar to aquatic systems, soil protists are indeed the major consumers of the microbiome including bacteria, archaea and micro-eukaryotes (i.e. fungi, other protists and micro fauna such as nematodes and rotifers) (Bonkowski & Clarholm 2012, Geisen et al. 2016, Ekelund 1998). A major fraction of up to 50 % of protist diversity can be parasitic and thereby potentially control and shape animal communities (Mahé et al. 2017). Even phototrophic protists (and mixotrophs [Jassey et al. 2015]) seem to play an important role as carbon fixers in many soils (George et al. 2019, Oliverio et al. 2020), with some protists appearing to be specialized algivores (Seppey et al. 2017). A last thing to note is that protists are morphologically diverse and offer a beautiful palette of forms and structures as illustrated in Fig. 1. The huge phylogenetic diversity of protists results from a plethora of independent billion year-long evolutionary trajectories (Adl et al. 2019, Burki et al. 2020) and has led to an extreme morphological diversification. This includes many protective structures of the cell, either proteinaceous or mineral, which do not readily decompose but remain for various amounts of time depending on their composition and the intensity of decomposition in the environment. Among terrestrial ecosystems, preservation of such shells is highest in the nutrient-poor, acidic and waterlogged organic soils of peatlands, where the subfossil records of mostly testate amoebae provide invaluable information about past environmental conditions (Charman 2001, Marcisz et al. 2020).

3. Discrepancies and commonalities between protists and other members of soil biodiversity

We still only have a crude understanding of protists diversity at the global scale (Oliverioet al. 2020), likely even more limited than our knowledge on the global distribution of bacteria (Delgado-Baquerizo et al. 2018), fungi (Tedersoo et al. 2014), nematodes (van den Hoogen et al. 2019) and earthworms (Phillips et al. 2019). Nevertheless, the existing data (Fournier et al. 2020, Zhao et al 2019) suggest that patterns structuring protist communities are fundamentally different to those of other soil biodiversity groups, most strikingly considering that most soil protists depend on bacteria and fungi as prey. While other factors like pH matter the most for bacteria (Delgado-Baquerizo et al. 2018) or the amount of soil organic matter for fungi (Tedersoo et al. 2014), soil moisture is by far the key driver of protistan communities (Oliverio et al. 2020). Also the composition of bacterial communities as their suggested main prey seems not to be a major determinant of protist communities (Oliverio et al. 2020). This is in line with the idea that protists are functionally much more diverse than previously thought and do not only depend on bacteria as major nutrient sources, in contradiction to what is still presented in most food web models (de Ruiter et al. 1995).

The methodology to study protists diversity is rapidly becoming accessible even for non-experts. Based on commonly used DNA/RNA extraction techniques, the same nucleic acid extract that is used to study bacteria and fungi can also be used to study protists (Geisen & Bonkowski 2018). This makes it possible to add protists to former studies if nucleic acids have been preserved with only different primers to be applied that target the 18S

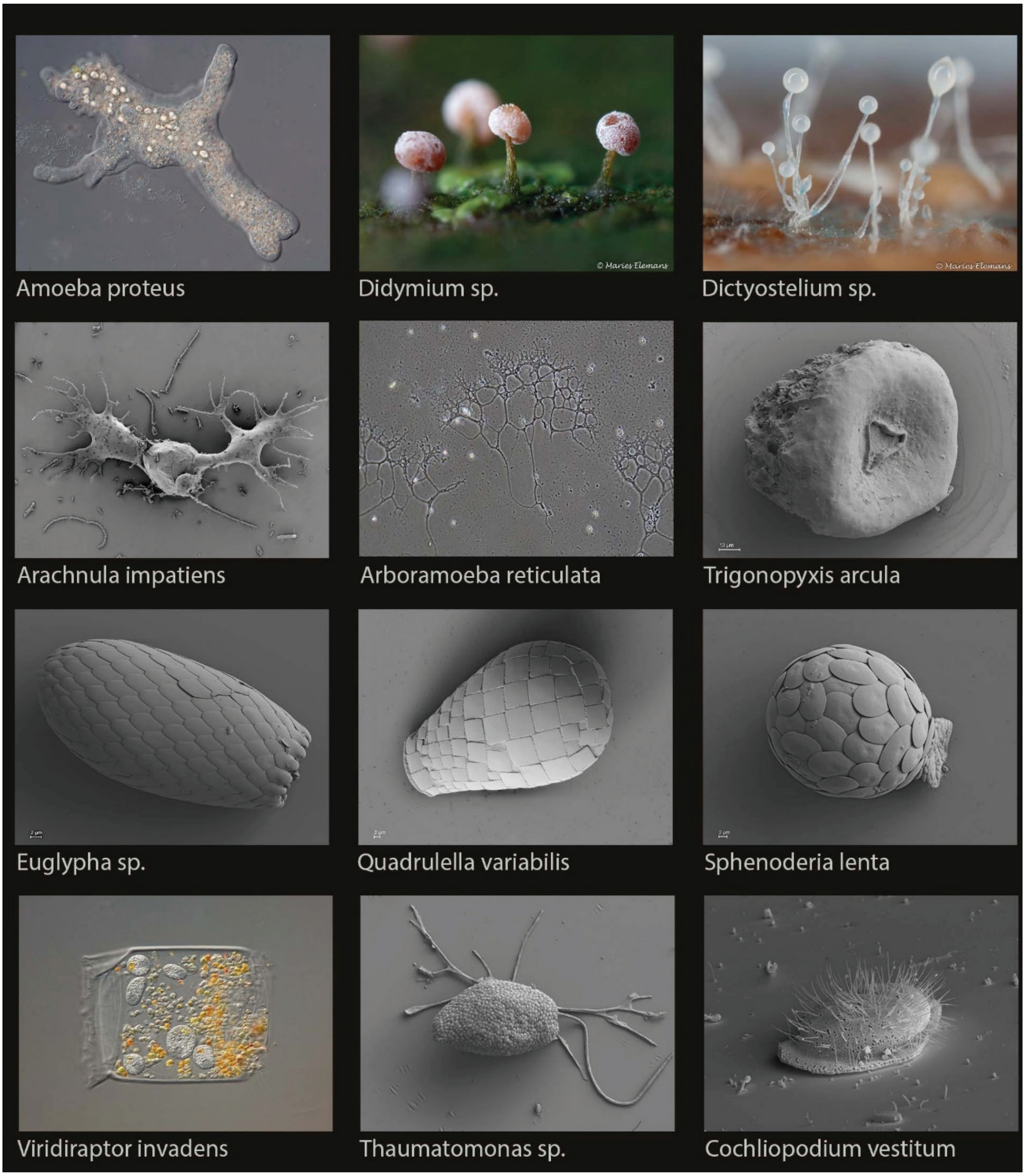

Figure 1. An illustration of the morphological diversity of protists including naked amoebae (the well-known aquatic model species *Amoeba proteus*, the omnivorous *Arachnula impatiens* and the network-forming *Arboramoeba reticulata*), the semi-naked amoeba *Cochliopodium vestitum* (the shell is covering the amoeba only from above), fruiting bodies of slime moulds (*Didymium* and *Dicyostelium* sp), the flagellates *Viridiraptor invadens* (inside an algal cell) and *Thaumatomonas* sp. as well as a range of testate amoebae that build shells from various materials.

rRNA gene for eukaryotes and not the 16S rRNA gene for prokaryotes or the ITS region for fungi. Similar to fungi (Nguyen et al. 2016), we have a rather good knowledge of the basic nutritional uptake modes of major protist groups in soils as some common features such as parasitism, phototrophy or phagotrophy are often conserved across major taxonomic units (Adl et al. 2019). This information is entirely missing for most soil bacteria. Therefore, functional units can be defined for protists based on easily obtainable environmental DNA sequences. In addition to plain taxonomic composition, functional units provide better insights in explaining changes in the systems and their importance for soil functioning (Geisen et al. 2019).

4. Why should protists be considered in soil biodiversity initiatives?

Without protists, biodiversity in soils but even aboveground would be very different and much reduced considering the estimated species richness of many millions (Orgiazzi et al. 2016). In this part we explain why including protists in soil biodiversity studies is essential given their roles in soil nutrient dynamics and as major controllers of animal and plant communities.

4.1 Nutrient uptake and food webs

Protists are key contributors to all soil functions, as summarized in Fig. 2. Phototrophic protists are important carbon fixers, especially in regions with little plant cover, sometimes together with fungal partners in lichen symbioses. This represents an important carbon source for carbon-poor soils, thus providing the basis for soil life and functions (Schmidt et al. 2016), but even might add more labile carbon to carbon-rich soils (Seppey et al. 2017). Consumer protists, those preying mostly on other smaller microbes, represent by far the dominant fraction of soil protists (Geisen et al. 2018, Oliverio et al. 2020). Their feeding actions are of uttermost importance in driving the microbial loop— that summarizes the release of nutrients bound in microorganisms into the environment (Bonkowski & Clarholm 2012). This nutrient ‘recycling’ allows plants to take up previously chemically inaccessible compounds such as nitrogen and phosphorous that lead to an increase in plant growth (Geisen et al. 2018). Also other, more active microbes take up the released nutrients that, together with specialized feeding of protists on microbial prey (Schulz-Bohm et al. 2017, Glücksman et al. 2010, Jousset et al. 2009), results in microbial community changes (Gao et al. 2019). Plants can respond to these community changes when pathogen-suppressive microbes increase or when growth stimulants such as auxins are excreted (Gao et al. 2019, Jousset et al. 2009, Krome et al. 2010). By increasing microbial activity, and hence microbe-derived processes such as decomposition, protists can stimulate litter decomposition (Geisen et al. 2020 [in press]) and thereby contribute to CO_2 emissions (Kuikman et al. 1990). Most of these functional studies were performed with only one or few protist species. Knowing the phylogenetic diversity of protists and that distinct species have specific prey preferences, many new and important findings can be expected in the near future!

4.2 Beyond the classical food-chain: inverted food-webs, parasites and pathogens

Food-chains are often considered to proceed linearly with increasing sizes classes, from small to large. Beside the classical top-down food chains, other interactions affect diversity and functionality of a system. In soils, hardly any example exists similar to those in macroscopic systems such as piranhas, wolves or other smaller organisms that pack-hunt larger prey. However, some protists have this ability. Very common small testate (shelled) amoebae have been shown to increase in reproduction by actively preying, in groups, on much larger nematodes (Geisen et al. 2015). Arguably even more common is the role of protists as animal parasites and plant pathogens. Often forgotten in food-web models, parasites or other minute disease agents are major drivers of the population dynamics and evolution of macroscopic organisms, for example, the most devastating human disease is malaria caused by the protist *Plasmodium falciparum*. Parasites can profoundly affect phototrophic carbon fixation in both marine and terrestrial systems (Paseka et al. 2020 [in press]). Plant pathogenic protists are ubiquitous in soils (Oliverio et al. 2020) and cause significant losses in crops and forests; the most notorious group is the Peronosporomycetes (=oomycetes), which include the potato blight that contributed to the Great Irish Famine in the 19th Century (Kamoun 2001).

4.3 Some unique features of protists vs other soil dwellers

Recent major findings have revealed why protists are special among soil organisms. They might be the most responsive organisms to changes in soil condition, across season, fertilization practices and land use (Zhao et al. 2020, Krashevska et al. 2014). As such, protists can be valuable indicators of soil quality. Protists also seem to determine plant health early and through the entire plant growth phase. Protist communities differed clearly in the presence of a plant pathogen with respect to naïve plants much before plants developed visible symptoms; this effect was much more obvious than in any other microbial group (Xiong et al. 2020). Protist communities were even better predictors for plant health than the abundance of the pathogen (Xiong et al. 2020)! The sensitivity of soil protist communities to environmental disturbances has even lead to applications in forensics to provide precise information on death incidences (Szelecz et al. 2014).

4.4 What would happen in soils without protists?

Short answer: It is hard to imagine and luckily not realistic in any natural soil and even extremely difficult to establish under laboratory conditions as protist spores are common in the air and quickly fill all new habitats (Altenburger et al. 2010). But let's be speculative and assume a situation where protists are not present (Fig. 2), as it is often implicitly suggested even in complex soil biodiversity assessments.

Nutrients would be locked up much more in bacterial and fungal biomass. Part of the role of protist consumers would be taken over by viruses, predatory bacteria and fungi, nematodes and larger soil animals (van den Hoogen et al. 2019, Williamson et al. 2017, Petters et al. 2018). However, phagotrophic protists are likely by far the key predators of especially bacteria (de Ruiter et al. 1995), a role that can hardly be compensated for; viruses are very specific towards single hosts, other organisms are less mobile and can only access bacterial biofilms, while most other microbial predators have a reduced ability to access their prey in small soil aggregates. This would have cascading effects on the entire food web; the missing size class most protists fall into will reduce the prey availability for many larger soil organisms and, likely, would lead to reduced food web lengths. The (extended) microbial loop would largely not exist as it is nearly entirely based on protist predation (Bonkowski & Clarholm 2012). This means that plants without protists would work with a different set of metabolites (Kuppardt et al. 2018) likely leading to a reduction of nutrient-uptake, while also the microbial community would likely be much less plant beneficial (Jousset et al. 2009, Henkes et al. 2018). In sum: plants would not do well and this of course would affect crops! But protists can also harm plants as plant pathogens do, which might be good for (e.g. potato) farmers. Plant communities in nature would lose biodiversity, as parasites are most effective in reducing the dominance of abundant species (Paseka et al. (2020 [in press]). Although we know much less about the impact of protist parasites on animals, the sheer diversity of parasitic protists (Mahé et al. 2017) suggest similar effects on animal communities.

5. Conclusions

We briefly summarized the main justifications for having a more open and integrative view on soil biodiversity as illustrated by the example of protists. This perspective, however, should be expanded to include other groups of soil life including viruses and archaea, as well as little studied animal groups that are also currently underrepresented in research efforts. The functional importance of some of

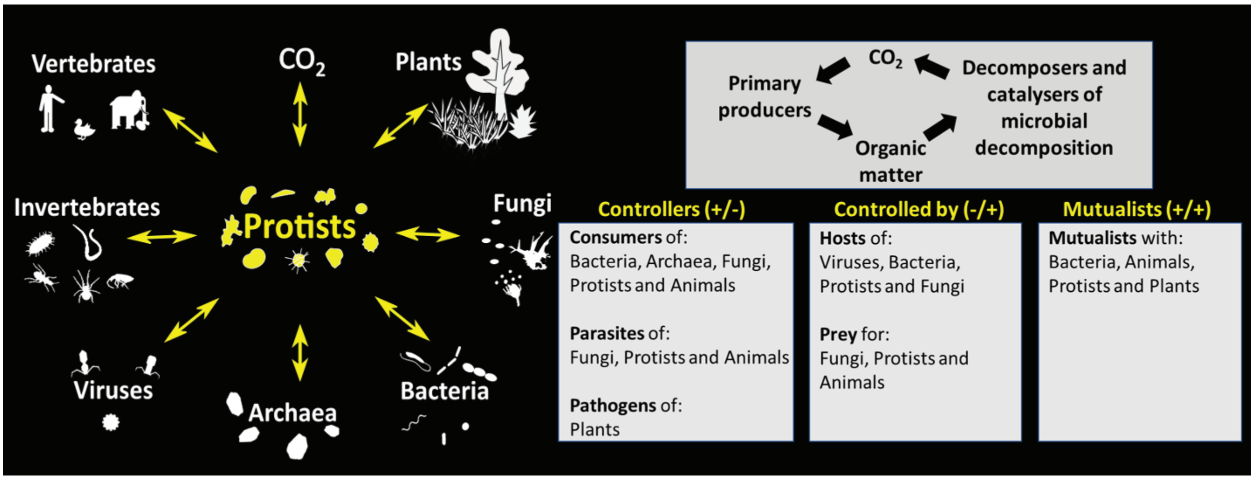

Figure 2. The central role of protists in soils showing their functional diversity and links to all other groups of below - as well as aboveground life. Figure made in Inkscape.

these groups in soils might vary among ecosystems, which then also calls for more comparative studies. We believe that no major group of soil life is redundant in function with any other to the point of not deserving to being studied. This is even less the case when the group is highly diverse and abundant. This is exemplified for termites, a group often ignored by soil ecologists because it is almost absent from temperate zones where most scientists are located or work in, but that is functionally essential at lower latitudes. The same likely holds to various degrees for all groups of soil life. Coming back to protists: the very strong recent increase in research that has found its way into broad-scale scientific journals illustrates the necessity to include protists in biodiversity studies and calls on soil biodiversity. Ongoing developments in molecular tools such as (meta)genomics will bridge the knowledge gap in the currently often-performed diversity inventories to better estimate the real functional diversity of protists. Also real organism numbers and biomass need to be determined in addition to relative sequence data currently obtained to show the quantitative importance of protists (Piwosz et al. 2020). Many (but still a minority of) protists, unlike most bacteria, can also readily be cultivated (Domonell et al. 2013) — a possibility that allows a reliable functional characterization of the distinct species (Keeling 2019). As such, protists need to find their way into broad ecological studies. Otherwise, those researches will miss the chance to obtain a complete picture on major biodiversity groups underlying crucial ecological questions. Soil protist life indeed matters!

6. Acknowledgements

We were inspired by the call to show that protists matter. VK was funded by the Deutsche Forschungsgemeinschaft (DFG), project number 192626868–SFB 990. EL was funded by grants from the Spanish Government PGC2018-094660-B-I00 (MCIU/AEI/FEDER, UE) and the program 'Atracción de Talento Investigador' from the Communidad de Madrid (2017-T1/AMB-5210). EM was funded by Swiss National Science Foundation grants no 31003A_182531 and 31003A_163431.

7. References

Adl, S. M., D. Bass, C. E. Lane, J. Lukeš, C. L. Schoch & A. Smirnov (2019): Revisions to the classification, nomenclature, and diversity of eukaryotes. – Journal of Eukaryotic Microbiology **66**: 4–119.

Altenburger, A., F. Ekelund & C. S. Jacobsen (2010): Protozoa and their bacterial prey colonize sterile soil fast. – Soil Biology and Biochemistry **42**: 1636–1639.

Bar-On, Y. M., R. Phillips & R. Milo (2018): The biomass distribution on Earth. – Proceedings of the National Academy of Sciences **115**: 6506–6511.

Burki, F., A. J. Roger, M. W. Brown & A. G. B. Simpson (2020): The new tree of eukaryotes. – Trends in Ecology & Evolution **35**: 43–55.

Bonkowski, M. & M. Clarholm (2012): Stimulation of plant growth through interactions of bacteria and protozoa: testing the auxiliary microbial loop hypothesis. – Acta Protozoologica **51**: 237–247.

Caron, D. A., A. Z. Worden, P. D. Countway, E. Demir & K. B. Heidelberg (2008): Protists are microbes too: a perspective. – The ISME Journal **3**: 4–12.

Charman, D. J. (2001): Biostratigraphic and palaeoenvironmental applications of testate amoebae. – Quaternary Science Reviews **20**: 1753–1764.

de Vargas, C., S. Audic, N. Henry, J. Decelle, F. Mahe & R. Logares (2015): Ocean plankton. Eukaryotic plankton diversity in the sunlit ocean. – Science **348**: 1261605.

de Ruiter, P. C., A. M. Neutel & J. C. Moore (1995): Energetics, patterns of interaction strengths, and stability in real ecosystems. – Science **269**: 1257–1260.

Decaëns, T. (2010): Macroecological patterns in soil communities. – Global Ecology and Biogeography **19**: 287–302.

Delgado-Baquerizo, M., A. M. Oliverio, T. E. Brewer, A. Benavent-González, D. J. Eldridge & R. D. Bardgett (2018): A global atlas of the dominant bacteria found in soil. – Science **359**: 320–325.

Domonell, A., M. Brabender, F. Nitsche, M. Bonkowski & H. Arndt (2013): Community structure of cultivable protists in different grassland and forest soils of Thuringia. – Pedobiologia **56**: 1–7.

Ekelund, F. (1998): Enumeration and abundance of mycophagous protozoa in soil, with special emphasis on heterotrophic flagellates. – Soil Biology and Biochemistry **30**: 1343–1347.

Falkowski, P. G. (2002): The ocean's invisible forest. – Scientific American **287**: 54–61.

Fournier, B., E. Samaritani, B. Frey, C. V. W Seppey., E. Lara & T. J. Heger (2020): Higher spatial than seasonal variation in floodplain soil eukaryotic microbial communities. – Soil Biology and Biochemistry **147**: 107842.

Gao, Z., I. Karlsson, S. Geisen, G. Kowalchuk & A. Jousset (2019): Protists: Puppet Masters of the Rhizosphere Microbiome. – Trends in Plant Science **24**: 165–176.

Griggs, D., M. Stafford-Smith, O. Gaffney, J. Rockström, M. C. Öhman & P. Shyamsundar (2013): Sustainable development goals for people and planet. – Nature **495**: 305–307.

Geisen, S. & M. Bonkowski (2018): Methodological advances to study the diversity of soil protists and their functioning in soil food webs. – Applied Soil Ecology **123**: 328–333.

Geisen, S., D. H. Wall & W. H. van der Putten (2019): Challenges and opportunities for soil biodiversity in the anthropocene. – Current Biology **29**: R1036–R1044.

Geisen, S., S. Hu, T. E. E. de la Cruz & G. F. Veen (in press 2020): Protists as catalyzers of microbial litter breakdown and carbon cycling at different temperature regimes. – The ISME Journal.

Geisen, S., J. Rosengarten, R. Koller, C. Mulder, T. Urich & M. Bonkowski (2015): Pack hunting by a common soil amoeba on nematodes. – Environmental Microbiology **17**: 4538–4546.

Geisen, S., R. Koller, M. Hünninghaus, K. Dumack, T. Urich & M. Bonkowski (2016): The soil food web revisited: Diverse and widespread mycophagous soil protists. – Soil Biology and Biochemistry **94**: 10–18.

Geisen, S., E. A. D. Mitchell, S. Adl, M. Bonkowski, M. Dunthorn, & F. Ekelund (2018): Soil protists: a fertile frontier in soil biology research. – FEMS Microbiology Reviews **42**: 293–323.

Geisen, S., E. A. D. Mitchell, D. M. Wilkinson, S. Adl, M. Bonkowski & M. W. Brown (2017): Soil protistology rebooted: 30 fundamental questions to start with. – Soil Biology and Biochemistry **111**: 94–103.

Geisen, S., M. J. I. Briones, H. Gan, V. M. Behan-Pelletier, V.-P. Friman & G. A. de Groot (2019): A methodological framework to embrace soil biodiversity. – Soil Biology and Biochemistry **136**: 107536.

George, P. B. L., D. Lallias, S. Creer, F. M. Seaton, J. G. Kenny & R. M. Eccles (2019): Divergent national-scale trends of microbial and animal biodiversity revealed across diverse temperate soil ecosystems. – Nature Communications **10**: 1107.

Glücksman, E., Bell, T., Griffiths, R. I. & Bass, D. (2010): Closely related protist strains have different grazing impacts on natural bacterial communities. – Environmental Microbiology **12**: 3105–3113.

Henkes, G. J., E. Kandeler, S. Marhan, S. Scheu & M. Bonkowski (2018): Interactions of mycorrhiza and protists in the rhizosphere systemically alter microbial community composition, plant shoot-to-root ratio and within-root system nitrogen allocation. – Frontiers in Environmental Science **6**: 117.

Jassey, V. E. J., C. Signarbieux, S. Hättenschwiler, L. Bragazza, A. Buttler & F. Delarue (2015): An unexpected role for mixotrophs in the response of peatland carbon cycling to climate warming. – Scientific Reports **5**: 16931.

Jousset, A., L. Rochat, M. Pechy-Tarr, C. Keel, S. Scheu & M. Bonkowski (2009): Predators promote defence of rhizosphere bacterial populations by selective feeding on non-toxic cheaters. – The ISME Journal **3**: 666–674.

Kamoun, S. (2001): Nonhost resistance to *Phytophthora*: novel prospects for a classical problem. – Current Opinion in Plant Biology **4**: 295–300.

Keeling, P. J. (2019): Combining morphology, behaviour and genomics to understand the evolution and ecology of microbial eukaryotes. – Philosophical Transactions of the Royal Society B: Biological Sciences **374**: 20190085.

Krashevska, V., D. Sandmann, M. Maraun & S. Scheu (2014): Moderate changes in nutrient input alter tropical microbial and protist communities and belowground linkages. – The ISME Journal **8**: 1126–1134.

Krome, K., K. Rosenberg, C. Dickler, K. Kreuzer, J. Ludwig-Müller & C. Ullrich-Eberius (2010): Soil bacteria and protozoa affect root branching via effects on the auxin and cytokinin balance in plants. – Plant Soil **328**: 191–201.

Kuikman, P. J., A. G. Jansen, J. A. van Veen & A. J. B. Zehnder (1990): Protozoan predation and the turnover of soil organic carbon and nitrogen in the presence of plants. – Biology and Fertility of Soils **10**: 22–28.

Kuppardt, A., T. Fester, C. Hartig & A. Chatzinotas (2018): Rhizosphere protists change metabolite profiles in *Zea mays*. – Frontiers in Microbiology **9**: 857.

Mahé, F., C. de Vargas, D. Bass, D., L. Czech, A. Stamatakis, E. Lara (2017): Parasites dominate hyperdiverse soil protist communities in Neotropical rainforests. – Nature Ecology & Evolution **1**: 0091.

Marcisz, K., V. E. J. Jassey, A. Kosakyan, V. Krashevska, D. J. G. Lahr & E. Lara (2020): Testate amoeba functional traits and their use in paleoecology. – Frontiers in Ecology and Evolution **8**: 575966.

Nguyen, N. H., Z. Song, S. T. Bates, S. Branco, L. Tedersoo & J. Menke (2016): FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. – Fungal Ecology **20**: 241–248.

Oliverio, A. M., S. Geisen, M. Delgado-Baquerizo, F. T. Maestre, B. L. Turner & N. Fierer (2020): The global-scale distributions of soil protists and their contributions to belowground systems. – Science Advances **6**: eaax8787.

Orgiazzi, A., R. D. Bardgett & E. Barrios (2016): Global soil biodiversity atlas – European Commission.

Paseka, R. E., L. A. White, D. B. Van de Waal, A. T. Strauss, A. L. González & R. A. Everett (in press 2020): Disease-mediated ecosystem services: Pathogens, plants, and people. – Trends in Ecology & Evolution.

Petters, S., A. Soellinger, M. M. Bengtsson & T. Urich (2018): The soil microbial food web revisited with metatranscriptomics - predatory Myxobacteria as keystone taxon? – bioRxiv.

Phillips, H. R. P., C. A. Guerra, M. L. C. Bartz, M. J. I. Briones, G. Brown & T. W. Crowther (2019): Global distribution of earthworm diversity. – Science **366**: 480.

Piwosz, K., T. Shabarova, J. Pernthaler, T. Posch, K. Šimek & P. Porcal (2020): Bacterial and eukaryotic small-subunit amplicon data do not provide a quantitative picture of microbial communities, but they are reliable in the context of ecological interpretations. – mSphere **5**: e00052–00020.

Seppey, C. V. W., D. Singer, K. Dumack, B. Fournier, L. Belbahri & E. A. D. Mitchell (2017): Distribution patterns of soil microbial eukaryotes suggests widespread algivory by phagotrophic protists as an alternative pathway for nutrient cycling. – Soil Biology and Biochemistry **112**: 68–76.

Schmidt, O., J. Dyckmans & S. Schrader (2016): Photoautotrophic microorganisms as a carbon source for temperate soil invertebrates. – Biology Letters **12**: 20150646.

Schulz-Bohm, K., S. Geisen, E. R. J. Wubs, C. Song, W. de Boer & P. Garbeva (2017): The prey's scent - Volatile organic compound mediated interactions between soil bacteria and their protist predators. – The ISME Journal **11**: 817–820.

Sunagawa, S., S. G. Acinas, P. Bork, C. Bowler, S. G. Acinas & M. Babin (2020): Tara Oceans: towards global ocean ecosystems biology. – Nature Reviews Microbiology **18**: 428–445.

Szelecz, I., B. Fournier, C. Seppey, J. Amendt & E. A. D. Mitchell (2014): Can soil testate amoebae be used for estimating the time since death? A field experiment in a deciduous forest. – Forensic Science International **236**: 90–98.

Tedersoo, L., M. Bahram, S. Põlme, U. Kõljalg, N. S. Yorou & R. Wijesundera (2014): Fungal biogeography. Global diversity and geography of soil fungi. – Science **346**: 1256688.

van den Hoogen, J., S. Geisen, D. Routh, H. Ferris, W. Traunspurger & D. A. Wardle (2019): Soil nematode abundance and functional group composition at a global scale. – Nature **572**: 194–198.

Wilkinson, D. M. (1998): Fragments of an entangled bank: do ecologists study most of ecology? – Oikos **82**: 393–394.

Williamson, K. E., J. J. Fuhrmann, K. E. Wommack & M. Radosevich (2017): Viruses in soil ecosystems: an unknown quantity within an unexplored territory. – Annual Review of Virology **4**: 201–219.

Worden, A. Z., M. J. Follows, S. J. Giovannoni, S. Wilken, A. E. Zimmerman & P. J. Keeling (2015): Environmental science. Rethinking the marine carbon cycle: factoring in the multifarious lifestyles of microbes. – Science **347**: 1257594.

Xiong, W., Y. Song, K. Yang, Y. Gu, Z. Wei & G. A. Kowalchuk (2020): Rhizosphere protists are key determinants of plant health. – Microbiome **8**: 27.

Zhao, Z.-B., J.-Z. He, S. Geisen, L.-L. Han, J.-T. Wang & J.-P. Shen (2019): Protist communities are more sensitive to nitrogen fertilization than other microorganisms in diverse agricultural soils. – Microbiome **7**: 33.

Zhao, Z.-B., J.-Z. He, Z. Quan, C.-F. Wu, R. Sheng & L.-M. Zhang (2020): Fertilization changes soil microbiome functioning, especially phagotrophic protists. – Soil Biology and Biochemistry **148**: 107863.