Article:
Geisen, Stefan, Mitchell, Edward A. D., Wilkinson, David M. et al. (44 more authors) (2017) Soil protistology rebooted:30 fundamental questions to start with. Soil Biology and Biochemistry. 94–103. ISSN 0038-0717

https://doi.org/10.1016/j.soilbio.2017.04.001

Soil protistology rebooted: 30 fundamental questions to start with

Stefan Geisen[1, 33*], Edward A. D. Mitchell[2, 3], David M. Wilkinson[4,43], Sina Adl[5], Michael Bonkowski[6], Matthew W. Brown[7], Anna Maria Fiore-Donno[6], Thierry J. Heger[2], Vincent E.J. Jassey[8, 9], Valentyna Krashevska[10], Daniel J. G. Lahr[11], Katarzyna Marcisz[12,44], Matthieu Mulot[2], Richard Payne[13], David Singer[2], O. Roger Anderson[14], Dan J. Charman[15], Flemming Ekelund[16], Bryan S. Griffiths[17], Regin Rønn[16], Alexey Smirnov[18], David Bass[19, 20], Lassaâd Belbahri[2], Cédric Berney[21,22], Quentin Blandenier[2], Antonis Chatzinotas[23,24], Marianne Clarholm[25], Micah Dunthorn[26], Alan Feest[27,] Leonardo D. Fernández[28], Wilhelm Foissner[29], Bertrand Fournier[30], Eleni Gentekaki[31], Michal Hajek[32], Johannes Helder[33], Alexandre Jousset[34], Robert Koller[35], Santosh Kumar[36-37], Antonietta La Terza[37], Mariusz Lamentowicz[12], Yuri Mazei[38,39], Susana S. Santos[40], Christophe V.W. Seppey[2], Frederick W. Spiegel[41], Julia Walochnik[42], Anne Winding[40], Enrique Lara[2]

[1] Department of Terrestrial Ecology, Netherlands Institute of Ecology, 6708 PB Wageningen, The Netherlands

[2] Laboratory of Soil Biodiversity, University of Neuchatel, Rue Emile-Argand 11, Neuchatel 2000, Switzerland

[3]Jardin Botanique de Neuchâtel, Chemin du Perthuis-du-Sault 58, Neuchâtel 2000, Switzerland

[4] Natural Science and Psychology, Liverpool John Moores University, Byrom Street, Liverpool L3 3AF, UK

[5] Department of Soil Sciences, College of Agriculture and Bioresources, University of Saskatchewan, 51 Campus Drive, Saskatoon, Canada

[6] Department of Terrestrial Ecology, University of Cologne, Zuelpicher Str. 47b, 50674 Cologne, Germany

[7] Department of Biological Sciences, Mississippi State University, Mississippi State, MS, 39762

[8] School of Architecture, Civil and Environmental Engineering (ENAC), Ecole Polytechnique Fédérale de Lausanne EPFL, Ecological Systems Laboratory (ECOS), Station 2, 1015 Lausanne, Switzerland

[9] Swiss Federal Institute for Forest, Snow and Landscape Research (WSL), Site Lausanne, Station 2, 1015 Lausanne, Switzerland

[10] Georg August University Göttingen, J.F. Blumenbach Institute of Zoology and Anthropology, Berliner Str. 28, 37073 Göttingen, Germany

[11] Department of Zoology, Institute of Biosciences, University of São Paulo, Brazil, 05508-090

[12] Laboratory of Wetland Ecology and Monitoring & Department of Biogeography and Paleoecology, Adam Mickiewicz University, Dzięgielowa 27, 61-680 Poznań, Poland

[13] Environment, University of York, York YO105DD, UK

[14] Biology and Paleoenvironment, Lamont-Doherty Earth Observatory of Columbia University, Palisades, NY, 10964

[15] Department of Geography, College of Life and Environmental Sciences, University of Exeter, Exeter EX4 4RJ, United Kingdom

[16] Department of Biology, University of Copenhagen, Universitetsparken 15, 2100 Copenhagen, Denmark

[17] SRUC, Crop and Soil Systems Research Group, West Mains Road, Edinburgh EH9 3JG, United Kingdom

[18] Department of Invertebrate Zoology, Faculty of Biology, Saint Petersburg State University, Universitetskaya nab. 7/9, St. Petersburg, 199034, Russia

[19] Department of Life Sciences, The Natural History Museum, London SW7 5BD, United Kingdom

[20] Cefas, Barrack Road, Weymouth, Dorset DT4 8UB, United Kingdom

[21] EPEP—Evolution des Protistes et des Ecosystèmes Pélagiques—team, Sorbonne Universités, UPMC Univ Paris 06, UMR 7144, Station Biologique de Roscoff, Roscoff, France

[22] CNRS, UMR 7144, Station Biologique de Roscoff, Roscoff, France

[23] Helmholtz Centre for Environmental Research – UFZ, Department of Environmental Microbiology, Permoserstr. 15, 04318 Leipzig, Germany

[24] German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Deutscher Platz 5e, 04103 Leipzig, Germany

[25] Department of Forest Mycology and Plant Pathology, SLU, Uppsala, Sweden

[26] Department of Ecology, University of Kaiserslautern, 67663 Kaiserslautern, Germany

[27] Faculty of Engineering, University of Bristol, Bristol BS8 1TR, United Kingdom

[28] Centro de Investigación en Recursos Naturales y Sustentabilidad (CIRENYS), Universidad Bernardo O'Higgins, Fábrica 1990, 2° piso, Santiago, Chile

[29] University of Salzburg, Department of Ecology and Evolution, Hellbrunnerstrasse 34, A-5020 Salzburg, Austria

[30] Université Bourgogne Franche-Comté, Laboratoire Chrono-Environnement UMR 6249 CNRS, 16 route de Gray, 25030 Besançon Cedex, France

[31] School of Science, Mae Fah Luang University, Chiang Rai, 57100, Thailand

[32] Department of Botany and Zoology, Masaryk University, Kotlářská 2, 61137 Brno, Czech Republic

[33] Laboratory of Nematology, Wageningen University, Droevendaalsesteeg 1, 6708 PB, Wageningen, The Netherlands

[34] Department of Ecology and Biodiversity, Utrecht University, 3584 CH Utrecht, The Netherlands

[35] Forschungszentrum Jülich, IBG-2: Plant Sciences, 52425 Jülich, Germany

[36] Department of Biological Sciences, College of Natural Sciences, University of Ulsan, Ulsan 44610, South Korea

[37] School of Bioscience and Veterinary Medicine, University of Camerino, Via Gentile III da Varano, 62032 Camerino (MC), Italy

[38] Department of Hydrobiology, Lomonosov Moscow State University, Leninskiye gory, 1, Moscow 119899, Russia

[38] Department of Zoology and Ecology, Penza State University, Krasnaya str. 40, 440026 Penza, Russia

[40] Department of Environmental Science, Aarhus University, Frederiksborgvej 399, 4000 Roskilde, Denmark

[41] Department of Biological Sciences, University of Arkansas, Fayetteville, AR, 72701

[42] Molecular Parasitology, Institute of Tropical Medicine, Medical University, 1090 Vienna, Austria

[43] School of Life Science, University of Lincoln, UK

[44] Institute of Plant Sciences and Oeschger Centre for Climate Change Research, University of Bern, Altenbergrain 21, CH-3013 Bern, Switzerland

[*] Corresponding author: Stefan Geisen, Department of Terrestrial Ecology, Netherlands Institute of Ecology, 6708 PB Wageningen, the Netherlands.

Tel: +31 (0)317 47 34 00; e-mail: s.geisen@nioo.knaw.nl

Running title: Soil protistology rebooted

Keywords: Soil protists; Protozoa; Microbial interactions; Food web; Biodiversity; Functional diversity

Abstract

Protists are the most diverse eukaryotes. These microbes are keystone organisms of soil ecosystems and regulate essential processes of soil fertility such as nutrient cycling and plant growth. Despite this, protists have received little scientific attention, especially compared to bacteria, fungi and nematodes in soil studies. Recent methodological advances, particularly in molecular biology techniques, have made the study of soil protists more accessible, and have created a resurgence of interest in soil protistology. This ongoing revolution now enables comprehensive investigations of the structure and functioning of soil protist communities, paving the way to a new era in soil biology. Instead of providing an exhaustive review, we provide a synthesis of research gaps that should be prioritized in future studies of soil protistology to guide this rapidly developing research area. Based on a synthesis of expert opinion we propose 30 key questions covering a broad range of topics including evolution, phylogenetics, functional ecology, macroecology, paleoecology, and methodologies. These questions highlight a diversity of topics that will establish soil protistology as a hub discipline connecting different fundamental and applied fields such as ecology, biogeography, evolution, plant-microbe interactions, agronomy, and conservation biology. We are convinced that soil protistology has the potential to be one of the most exciting frontiers in biology.

1. Introduction

Protists are everywhere, in aquatic and terrestrial ecosystems, free-living, and as symbionts (including parasites) of many organisms including humans. These usually single-celled or colonial microorganisms are by far the most diverse eukaryotes (Adl et al., 2012) and their species-numbers might easily exceed 10 million (Global Soil Biodiversity Atlas; www.globalsoilbiodiversity.org). Since the term ‘protista’ was introduced (Haeckel, 1866), profound taxonomic re-orderings have taken place. The vast majority of eukaryotic lineages has been shown to be protists, with the exception of the derived monophyletic multicellular lineages: animals, plants, and some fungi (Burki, 2014).

Electron microscopy and molecular phylogenies have revealed that both algal and protozoan lineages are intermingled throughout the eukaryote phylogenies (Delwiche, 1999; Burki, 2014), and hence it is less confusing to use Haeckel's broader category of 'protist'. Similarly, the classical protozoan morphological categories: flagellates, testate and naked amoebae – but not ciliates - are not monophyletic but distributed across the eukaryotic tree of life (Adl et al., 2012). A snapshot of the immense morphological and phylogenetic diversity of soil protists is visualized in Fig 1. We therefore recommend to use 'protist' as a term for all single celled phototrophic, mixotrophic and heterotrophic eukaryotes, with the exception of fungi.

The huge diversity of protist species has only recently become evident as many morphospecies recognizable under the microscope were shown to hide many cryptic species (Boenigk et al., 2012a). This 'dark matter of biodiversity' suggests that protist taxon richness has been considerably underestimated. A recent study of environmental eukaryotic diversity based on state-of-the-art high-throughput sequencing (HTS) showed that protists are considerably more diverse than plants and animals in the sunlit zone of oceans (de Vargas et al., 2015). HTS studies of soil protists have shown a wide diversity of non-phagotrophic protists and the diversity of protists in soils is at least as diverse as that in aquatic systems (e.g. (Bates et al., 2013; Geisen et al., 2015c). Nevertheless, soil protists are much less well studied than their aquatic counterparts and this gap is increasing (Fig. 2a).

Soil protists have received relatively little attention mainly due to methodological challenges, especially their isolation from the opaque soil matrix. These, however, do not entirely explain why soil protists are relatively less studied than other soil organisms, especially bacteria, fungi and nematodes (Fig. 2b). The volume of work on microbial bacteria and fungi far outweighs protist studies, possibly because of their direct role as primary decomposers, and they represent monophyletic groups that can more easily be studied with various targeted methodological approaches (Foissner, 1987; Mitchell, 2015). Even soil viruses have been subject to more studies than soil protists, despite being extremely challenging to study (Fierer et al., 2007) and their uncertain functional importance in soils. The under-studied nature of soil protists is exemplified by a comparison between research on protists and on soil archaea, a domain erected in 1990 and reported to be functionally important in soil only decade ago (Leininger et al., 2006; Bates et al., 2011). Historically studies mentioning soil protists in the title were eight times more abundant than those including archaea (Fig. 2b, Supplementary Table 2). However, in the last 15 years, this pattern entirely changed; studies on soil protists decreased by 15% while those on other common soil organisms increased by at least 30%, especially soil archaea which increased by 88% (Fig. 2b, Supplementary Table 2).

The relative decline of papers on soil protists strongly contrasts with what we now know about their ubiquity, diversity, and perhaps more importantly, their functional significance. Soil protists can both make an important contribution to primary production (Jassey et al., 2015; Schmidt et al., 2016) and play a key role in the decomposition pathways as consumers of bacteria (Clarholm, 1981; de Ruiter et al., 1995), fungi, other protists, and small invertebrates; they can also act as parasites of plants and animals (Adl and Gupta, 2006; Jassey et al., 2012; Geisen, 2016b). As predators, protists transfer nutrients to higher trophic levels in the soil foodweb (de Ruiter et al., 1995; Crotty et al., 2012). Protist predation also stimulates microbial activity and nutrient cycling via the microbial loop, thus stimulating plant growth (Bonkowski and Clarholm, 2012) and representing an important link between aboveground and belowground components.

The functional significance, abundance, environmental sensitivity, rapid response times and increasing ease of analysis of soil protists also makes them invaluable bioindicators of a variety of aspects of environmental change (Foissner, 1987; Gupta and Yeates, 1997; Payne, 2013). A particular example of this is in paleoecology, where the hard shells of testate amoebae, diatoms and foraminifera are widely used in the reconstruction of past environments and past climate change over a range of timescales (Mitchell et al., 2008; Adl et al., 2011; Charman, 2015).

Most of these applications are, however, based on a few often small-scale studies. Thus significant taxonomic and functional aspects remain largely untouched. Our aim in this report is to pool expert knowledge and opinion across the diverse field of soil protistology and soil microbial ecology to identify major knowledge gaps that need to be addressed and their significance for soil processes and ecosystem services.

2. Materials and Methods

2.1 Approach to identify the 30 most relevant questions

Our aim was to review research gaps both in the field of soil protistology and in general soil biology with a special focus on protists. In line with recent studies (Sutherland et al., 2013; Seddon et al., 2014), we aimed to pool community expertise to identify the most important questions in different broad categories. We modified previously-used methods (Sutherland et al., 2013; Seddon et al., 2014) to obtain a list of most interesting questions through a democratic, transparent, multi-step curation process.

The participants in this process are involved in a wide range of research areas, with self-determined primary research area expressed as being ecology (62%), palaeoecology (12%), evolution (9%), biogeography (6%), phylogeny (6%), taxonomy (3%), parasitology (3%). Each participant formulated up to 10 questions that they believed were most relevant for their future research. The resulting 368 questions were then compiled via an integrative group effort into consensus questions and placed into six major categories following a discussion by 16 of the participants at the German Society for Protozoology meeting in February 2016. We included very broad, general questions as well as highly specialised topics into similar scaled consensus questions.

The resulting consensus questions were then re-evaluated and groupings adjusted in a vote. These questions (Supplementary Table 1) were sent out to all 47 participants, who individually indicated up to 12 priority questions with at least one being allocated in each of the six following categories: (i) Morphology, Phylogeny, Taxonomy, Evolution and Physiology, (ii) Diversity, Community Composition and Biogeography, (iii) Interactions among Protists and other Organisms, (iv) Functions of Protists, (v) Global Change, Bioindicators and Applications, and (vi) Methodology.

All 47 participants were asked to provide their key scientific expertise and literature references for studies that (partly) addressed individual questions. Finally, minor comments raised by individual participants during the vote were integrated to clarify the questions and give consistent formatting without changing the meaning of the questions that had been voted upon.

All individual votes were combined and five questions per category chosen to result in the final list of 30 key questions. When more than one question received the same number of votes (as present in categories 1, 2, and 4), these questions were sent out to all 47 participants for another vote on the selected questions only.

2.2 Potential limitations

Biases in broad-scale studies are impossible to avoid (Sutherland et al., 2013). On the other hand, the more interdisciplinary the panel of authors is in terms of cultural and societal background and specific scientific expertise, the more biases are reduced. Researchers working on soil protists are often ecologists, whereas taxonomists, phylogeneticists, and physiologists more often focus on aquatic taxa that are easier to isolate and cultivate. Indeed, participants who indicated ecology as their first expertise dominated our list of participants (62%). Ecology, however, is a broad field and our division into finer categories such as biogeography, palaeoecology, community structure, and interactions resulted in a broad diversification into different subcategories. Additionally, 21% of the participants indicated topics such as taxonomy, phylogeny, evolution, and physiology as their main expertise corroborating the wide diversity of research fields among the co-authors.

The majority of participants are PhDs (Professor: 38%; Graduated scientists: 29%; Post-doc: 24%) with an average number of publications on protists of 43 (minimum = 1; maximum = 230). A high proportion of the participants work on multiple ecosystems (41%). Many focus on testate amoebae (41%) although 26% of them work on multiple morphogroups (ciliates, heterotrophic flagellates, amoebae, etc.). A majority (74%) of participants have a European background, but Asia, North and South America are also well represented, thus reducing potential impacts of geographic origin. Furthermore, most participants have international collaborations that partly compensate for gaps in the geographic distribution of individuals.

Despite these potential limitations, we found few biases in the way participants replied to questions (Supplementary Results 1). Most participants (70%) selected questions evenly distributed across the six categories, except a small group of people mostly constituted of researchers from the same institute and/or with the same kind of expertise (phylogeny, taxonomy and evolutionary; see Supplementary Results 1). This small group allocated 45% of their votes to the category (ii). Except this small bias, most participants selected questions regardless of their experience, age, geographic background, and most importantly, their expertise and group of interest.

Questions were differently formulated, hence we had to make decisions and remove some nuances as we merged similar questions. This resulted in some discussions about how questions should be best stated and consequently combined and grouped into non-predetermined categories. However, we preferred to receive non-restricted questions to stimulate lateral thinking as previously suggested (Sutherland et al., 2013); due to intensive exchange and to a democratic group effort at all steps of the procedure, we are convinced that we have reached a consensus format.

3. Results and Discussion

3.1 The 30 most relevant consensus questions

From the 107 questions in the final vote, 94% received at least one, 79% two, 67% three and 50% five votes showing that the pre-selected questions had a wide general appeal to the scientific experts involved (Supplementary Table 1). Therefore, all questions seem to be relevant for future studies that focus on soil protists. However, as we aimed at providing a highly specific list of the major research gaps and open challenges in soil protistology, we only provide the top-ranked 30 questions classified in six major categories that most researches voted upon.

3.2 Categories

I Morphology, Phylogeny, Taxonomy, Evolution and Physiology

1. How long can protists survive in an encysted form? What are the tolerances of (encysted) protists to stress and what is the importance of cysts for ecosystem resilience?
2. How much morphological and genetic variability exists within soil protists?
3. How do species that occur in both aquatic and soil systems adapt to differing demands?
4. What are the phylogenetic relations of true soil to aquatic protist taxa and how often have soils been colonized by aquatic protists and vice versa?
5. How widespread is sex in soil protists?

Linking the individual topics of this category is one of the major tasks confronting soil protistology. The coupling of morphology and phylogeny is crucial to obtain a stable taxonomic framework for protists. This is, for instance, crucial to answer evolutionary questions on the origin of eukaryotes (López-García and Moreira, 2015). Soil protists may have an important role to play in such research as most taxa likely remain unknown and novel higher-level taxonomic groups are continuously being discovered (Berney et al., 2015; Bass et al., 2016; Singer et al., 2016; Tice et al., 2016). Soil protists might fill remaining phylogenetic gaps from better-studied aquatic taxa to improve phylogenetic resolution within and between protist clades, as strict soil protist clades seem to be common (Bass et al., 2016). Sequencing whole genomes will reveal ancient traits of eukaryotes and potential changes in their function during the evolution of eukaryotes. In this respect, soil protists must certainly play a key role for understanding the evolution of the eukaryotic cell and, therefore, of life as a whole.

While the morphological and phylogenetic framework for ciliates is reasonably well established (Lynn, 2008; Foissner, 2016), it remains rudimentary for other morphogroups as well as phylogenetic clades of protists (Kosakyan et al., 2016). The taxonomy of the groups has profoundly benefited from (mainly) 18S rRNA gene-based characterisations that have often led to drastic changes in phylogenetic placements of individual species, genera, families or even orders (Boenigk et al., 2012a; Berney et al., 2015; Bass et al., 2016). This is exemplified by the morphologically and functionally diverse Cercozoa, which was the first protist clade inferred solely based on molecular phylogenetic information, and has become home to ever more morphologically different organisms (Bass et al., 2016). Therefore, the true extent of morphological and genetic variability in different groups of soil protists remains largely unknown and is a key missing gap for future studies (Q2).

A key feature of soil protist species is their capacity for cyst formation as this allows them to resist constantly changing conditions, especially with respect to moisture and temperature. Furthermore, given that protists can excyst after decades, even millennia (Shmakova et al., 2016), cyst formation may protect species from becoming extinct at local or even at the global scales, influence population dynamics and maintain biodiversity (Corliss and Esser, 1974; Jones and Lennon, 2010) The importance of the cyst bank in ecosystem functioning and resilience remains largely unknown and have consequently been identified as a key element for future studies (Q1). More generally, this applies to all specific (physiological) adaptations of soil protists in comparison to their aquatic relatives (Q3, Q4) and to reproduction (Q5)..

II Diversity, Community Composition and Biogeography

6. What is the real diversity and community structure of soil protists in different systems (e.g. soils, rhizosphere, (plant) endosphere)?
7. How similar are the diversity patterns of soil protists and other soil biota along ecological gradients, and to what extent do different environmental factors shape their respective diversity?
8. What abiotic environmental factors influence the distribution and community composition of protists, and how?
9. How cosmopolitan are protists and how many endemic soil protist species are there?
10. What are dominant groups of soil protists in terms of turnover, abundance and biomass?

We are progressively shedding light into the soil ‘black box’; however, knowledge on protists lags behind that of other groups (Fig. 2) (Wilkinson, 2008). Traditional studies have focused exclusively on a few of the ‘classic’ morphogroups, especially ciliates and testate amoebae, at least partly due to their ease of isolation and feature-rich morphologies (Foissner, 1999). Despite dominating many soil protist communities in terms of numbers and diversity, flagellates and naked amoebae have remained understudied, due to their often smaller sizes, lack of diagnostic features when studied by light microscopy, and the need to establish specialised enrichment cultivation for their isolation from soils (Berthold and Palzenberger, 1995; Foissner, 1999; Smirnov and Brown, 2004; Tikhonenkov et al., 2010). The development of molecular tools such as DNA barcoding and metabarcoding has considerably improved the situation in the last decade (Pawlowski et al., 2012) and allowed a phylogenetically based (morphogroup-independent) and consequently much more detailed analysis of the entirety of soil protist communities. These studies have revealed an enormous diversity of protists inhabiting soils, a phylogenetic diversity that might be similar to that of bacteria (e.g., (Bates et al., 2013; Geisen et al., 2015c; Mahé et al., 2017). Also, groups of protists previously almost unknown from soils have been shown to be common e.g. choanoflagellates, foraminifera, dinoflagellates, parasitic apicomplexans and pathogenic oomycetes (Bates et al., 2013; Geisen et al., 2015c; Grossmann et al., 2016; Mahé et al., 2017). Therefore, we are only beginning to understand the diversity of soil protists (Q10), which part is active, how this diversity differs in different soil environments (Q6), how protist communities are structured by, e.g., abiotic factors (Q7, Q8) (Geisen et al., 2014a; Lentendu et al., 2014; Geisen et al., 2015c; Dupont et al., 2016).

In addition, the biogeography of (soil) protists has been addressed in some studies, but it is still unclear which and how many groups display a restricted biogeography and what the factors are that shape these distributions (Q8, Q9). Although distribution of bacteria may support the hypothesis that "everything is everywhere, but, the environment selects" (Baas-Becking, 1934), its extrapolation to protists has been countered, particularly, by work on ciliates and testate amoebae (Foissner, 2006, 2008; Fernandez et al., 2016). The diversity and biogeographical distribution of protists, is, therefore, one of degree (rather than all cosmopolitan or all limited) and the possibility exists that the investigation of cryptic diversity within morphospecies will allow a finer-scale resolution of these questions.

III Interactions among Protists and other Organisms

11. How do protist taxa affect the composition of the soil microbiome and what other important interactions take place?
12. What are the biotic interactions of soil protists with other taxonomic groups, and how are protists linked within the soil food web?
13. What is the relative contribution of nutrient cycling (i.e. the microbial loop) versus modification of the rhizosphere microbiome in protist-induced stimulation of plant growth?
14. What are the mechanisms by which individual soil protist species affect plant performance, and do those mechanisms differ between plant species?
15. What is the impact of protists on the community functioning of other soil microbes?

Soil protists are still predominantly considered as being mainly bacterivorous (Bradford, 2016; Geisen, 2016a). Differential feeding by protists stimulated by bacterial volatiles modifies the community composition of bacteria (Bonkowski, 2004; Glücksman et al., 2010; Schulz-Bohm et al., 2017), which results in functional changes in the bacterial community structure (see next section). Many free-living bacteria can, in turn, defend themselves against certain protist predators and even kill them (Greub and Raoult, 2004; Jousset et al., 2006). Several bacteria, viruses, and even other protists can also parasitize protist hosts (Barker and Brown, 1994; Raoult and Boyer, 2010).

The prey spectrum of protists has, however, repeatedly been shown to be much more diverse than bacteria. Indeed, archaea (Ballen-Segura et al., 2017), fungi (Gupta and Germida, 1988; Ekelund, 1998; Adl and Gupta, 2006; Geisen et al., 2016), other protists (Page, 1977; Jassey et al., 2012), and nematodes (Bjørnlund and Rønn, 2008; Geisen et al., 2015b) constitute prey for diverse protist species. Recently, HTS approaches have revealed the ubiquitous presence and dominant roles of protist parasites and pathogens in soils, and they likely represent a key component controlling other soil organisms including larger soil metazoans (animals) and plants (Geisen et al., 2015a; Dupont et al., 2016; Geisen, 2016b). This draws attention to the enormous complexity and importance of protist interactions with other organisms (Bonkowski, 2004).

Due to our limited knowledge of protist diversity and because most studies have used only one or few protists as models, we lack understanding about most aspects of how soil protist communities interact with other organisms. Disentangling the diverse interactions of protists with other soil organisms (Q11, Q12, Q15), the exact mechanisms (Q14) and the resulting importance for functioning (Q13, Q14), therefore, are key knowledge gaps necessitating future research.

IV Functions of Protists

16 What is the importance of soil protists in biogeochemical cycling?

17 How much functional redundancy is there in the soil protist community?

18 Does increased protist diversity affect ecosystem functioning?

19 What is the comparative importance of eukaryotic microbes vs. prokaryotes in driving key soil processes?

20 Which individual functions are performed by distinct groups, and what is the entire functional diversity of soil protists?

Many acknowledged functions of soil protists are attributed to interactions with other organisms as outlined above. Especially important is the role of protists in driving the microbial loop, i.e. releasing nutrients (particularly nitrogen) bound in bacterial prey. The microbial loop has been demonstrated both in aquatic (Azam et al., 1983) and soil systems (Clarholm, 1985). This ground-breaking research identified protists as important drivers of the global ecosystem. Subsequent work on the microbial loop demonstrated that differential feeding by protists on bacterial prey is beneficial for plant growth (Bonkowski, 2004; Rosenberg et al., 2009). The main focus in earlier studies was, however, mainly on nitrogen cycling, and the importance of protists for cycling of other elements such as carbon and phosphorus has been relatively neglected, with few exceptions (Cole et al., 1977; Gupta and Germida, 1988; Treonis and Lussenhop, 1997; Frey et al., 2001; Murase et al., 2011; Eisenhauer et al., 2012; Jassey et al., 2015). Protists might even play a role in silica cycling as some use Si as reinforcing elements or in an exoskeleton (Aoki et al., 2007; Creevy et al., 2016). More thorough investigations about the functional roles of additional protist species and communities as a whole will likely reveal insights into the importance of protists in biogeochemical nutrient cycling. This was identified by most participants of this study as *the* most important question for future research (Q16).

In contrast to free-living protists, plant pathogenic protists, such as oomycetes or plasmodiophorids, have, not surprisingly, attracted considerable attention due to their agro-economic impact (Anderson et al., 2004; Bell et al., 2006; Neuhauser et al., 2014). These were, however, until very recently often considered as ‘fungi’ (Schardl and Craven, 2003; Gams et al., 2011). Similarly, soil protists with immediate relevance for human diseases such as those directly harmful to humans (Schuster, 2002; Siddiqui and Ahmed Khan, 2012; Geisen et al., 2014b) and those that act as “Trojan horses” harbouring human-pathogenic bacteria (Brown and Barker, 1999; Molmeret et al., 2005) have received considerable attention. In turn, the role of protists in plant disease control due to, e.g., increasing bacterial biocontrol agents (Jousset, 2012) or by directly feeding on plant pathogens (Old and Chakraborty, 1986; Geisen et al., 2016) has received comparatively little attention. In line with their importance in nutrient cycling and as biocontrol agents, the role of individual protist species and that of protist diversity for the general functioning of soils and ecosystems (Q17, Q18, Q20), also in comparison to other groups of microbes (Q19), were identified as important questions to be addressed in future studies.

V Global Change, Bioindicators and Applications

21. How do changing climatic patterns affect the diversity of, community structure of and ecosystem services provided by soil protists?
22. Which protist clades can be used as bioindicators to assess soil properties, ecosystem state, and anthropogenic impacts? How could this be implemented?
23. Why are some species more sensitive to environmental change than others, why do some respond faster to environmental factors?
24. How can protists be used for nutrient mobilization and biocontrol in cropping systems?
25. What is the importance of soil protists for biodiversity conservation and ecosystem management and restoration? Should we protect particular species or habitats?

Protist communities are often studied as bioindicators of past and present climatic conditions, land use changes and pollution (Gupta and Yeates, 1997; Mitchell et al., 2008). Abiotic changes affect protists in species-specific ways, thus forming the basis for their use as bioindicators (Fournier et al., 2012). They may, for instance, provide information on soil state in agro-ecosystems (Foissner, 1997, 1999; Bharti et al., 2015). Testate amoebae and their subfossil remains have been used to evaluate wetland hydrological conditions, applied, for instance, in studies of peatland restoration (Marcisz et al., 2014) and reconstruction of Holocene environmental change (Turner et al., 2014; Lamentowicz et al., 2015; Payne et al., 2015). However, more generally, there has been little progress on evaluating protists as bioindicators even though reliable indicators to assess soil quality continue to be of high relevance (Griffiths et al., 2016) as also revealed here (Q22). Application of protists for stimulating plant performance in terms of nutrition, growth, productivity and disease suppression holds great promise but has received little attention (Q24)

Effects of ongoing global climate change and human impact on the environment are the focus of increasing scientific attention. Global warming has been shown to alter the abundance and community structure of protists (Tsyganov et al., 2011; Jassey et al., 2013) in the limited number of studies that have been done. Predicted changes in precipitation regime will likely affect water availability, which will impact protist communities directly (Clarholm, 1981; Bates et al., 2013; Geisen et al., 2014a). Elevated atmospheric CO_2 has also been shown to increase abundance and changes community structure of rhizosphere protists, possibly due to increased plant productivity and enhanced release of root organic exudates (e.g., (Treonis and Lussenhop, 1997; Anderson and Griffin, 2001; Rønn et al., 2002)). Increased air pollution by nitrogen, sulphur, tropospheric ozone and metals are also likely to alter protist abundance and diversity (Meyer et al., 2012; Payne et al., 2012; Payne et al., 2013). Most of these studies focused on testate amoebae, but it is important to study how global environmental changes affect entire protist communities (Q21, Q23, Q25) as these changes are likely to have significant impacts on ecosystem functioning/services and, consequently, on human welfare, and may provide more informative markers of environmental change.

VI Methodology

26 What is the most practical taxonomic unit to measure protist diversity?

27. How can we standardize and calibrate cultivation based and molecular methods to reliably quantify soil protist abundance, diversity and activity?
28. How should sampling be performed to adequately evaluate soil protist diversity?
29. At what scales (temporal, spatial/physical, morphological, phylogenetic) should we study protists to fully understand their diversity and function in soil; which one should be prioritized?
30. How can we infer functional traits of soil protists based on morphology or phylogenetic affiliation, and what taxonomic resolution is needed?

Diverse methods are used to study community structures of soil protists. Even with respect to more classical culturing and morphological observational techniques, the application of methods of non-protistological disciplines, such as mycology, have the potential of broadening our perspectives on the soil protist community (Spiegel et al., 2004). However, especially recent developments in molecular sequencing technologies, have changed and will continue to change our knowledge about protist diversity and community structure in soils (Bates et al., 2013; Geisen et al., 2015c). However, some issues relating to HTS-based efforts remain as they provide relative abundances of taxa without providing information on absolute abundances. For example PCR-based HTS efforts have been shown to artificially alter the observed community structure of soil protists, a problem which needs to be solved to decipher their real community structure (Geisen et al., 2015a). PCR-free 'omics-approaches', i.e. metagenomics and metatranscriptomics, might resolve some of these issues (Geisen et al., 2015c; Jacquiod et al., 2016). Indeed, these sequence-based omics approaches and sequence-independent metaproteomics provide valuable information not only on taxonomic diversity but also on their potential functions (Prosser, 2015). Calibrating, standardizing and adopting community-defined methodologies to study soil protists will, consequently, be key for cross-study comparisons (Q27) and correct sampling and analyses through different scales need to be defined *a priory* (Q28, Q29). Furthermore, it is essential to identify the most meaningful taxonomic levels to use in the study of diversity and functioning of soil protists (Q26), but even the definition of a species remains a challenge (Boenigk et al., 2012b) and integrating morphology to phylogeny to function remains missing (Q30).

In addition, medical and novel imaging techniques applied to soil are revolutionising *in situ* work allowing us to study protist species in undisturbed soil and on plant roots. These include applications of NanoSIMS technology to precisely locate isotopic markers and isotopic composition of material in fixed preparations and to study dynamics of nutrient fluxes (Stockdale et al., 2009), which allows tracing nutrient flow from microbial prey to protist predator and further in the food web in high resolution. This will allow detailed investigations how protists selective interact in microsites with their prey, how nutrients become released and where they are translocated. Applications of a variety of X-ray based synchrotron spectroscopy and tomography with undisturbed soil is becoming technically feasible and permits the study of dynamics and fluxes at a very fine resolution without interfering with the matrix (Keyes et al., 2013). The ability to use soils with intact fine roots, and examining undisturbed natural soil communities finally provides access to rhizosphere processes. Techniques to measure and analyse chemically soil community molecular interactions and communications are now only a few steps away.

3.3 (Partial) knowledge gaps and future directions

In this paper we provide a guide to 30 highly relevant questions for future studies in soil protistology. Research has already been conducted on many of these questions. Literature searches and personal knowledge of the literature allowed us to identify studies that addressed 91 % of the initial and 97 % of the final questions. However, many of these studies focus on organisms other than soil protists (e.g. aquatic protists or non-protist microbes), and may not be directly applicable to the situation with soil protists. The fact that these 30 questions have been identified by our pool of experts strongly implies that previous research has been insufficient to provide conclusive answers. In Supplementary Table 1 we provide an extensive bibliography of previous research relevant to addressing these questions. This bibliography will be a valuable literature guide to the current state of the art on soil protistology.

We are beginning to understand many aspects of soil protist biology, as we are identifying the hyperdiverse nature of protist communities, determining their (a)biotic drivers, deciphering interactions with other organisms, and shedding light on their importance in ecosystem dynamics. So far, however, we are only seeing the tip of the iceberg. Addressing many of the 30 questions highlighted here will undoubtedly reveal novel insights, not only into soil protists, but also into other organisms, soils, and fundamental ecological processes. We hope that these questions will be used to catalyse soil protistology and to build research agendas for the future. More specifically, we encourage both protistologists and researchers in closely related fields to consider these questions carefully and to use them to develop new and innovative individual and collaborative projects. With newly available techniques, an increase in knowledge and a growing awareness of the importance of soil protists, we are at the start of a bright future for soil protist research!

Acknowledgements

We thank Alex K. Tice for providing pictures of *Acrasis* and *Rosculus* included in Fig.1. SG thanks Wim van der Putten and his ERC-Adv grant 260-55290 (SPECIALS). We further acknowledge funding by the Swiss Contribution to the enlarged European Union (Project CLIMPEAT: PSPB-013/2010) to ML, KM, MM, VEJJ and EADM, the Russian Scientific Fund (no 14-14-00891) to RJP and YuM, the São Paulo Research Foundation (FAPESP) (Young Investigator Award no 2013/04585-3) to DJGL, the Swiss National Science Foundation to VEJJ (no 315260_149807), EL, QB and DS (grants no 143960 and 163254), EADM & TH (no 141188 and 163431), the Chilean Comisión Nacional de Investigación Científica y Tecnológica (CONICYT) (doctoral grants no 21110037 and 78130011), the Universidad de Concepcion (Chile) and the Wildlife Conservation Society (Karukinka-grant no. 2012) to LDFP, and the University of Neuchâtel to LDFP & CVWS. This is publication XXXX of the NIOO-KNAW.

All authors declare no conflict of interest.

Author contributions

SG, EADM and EL designed the study; all authors sent initial questions. SG, TH, VEJJ, DJGL, DS and DW compiled the information and analysed data for Fig. 2. JW, MB, AF, EL, VK, ALT, SK, FS, AS, FE, SG provided photographs that DS, SG, EL and EADM compiled in Fig. 1. SG, EL, EADM, MB, VK, KM, DJGL, MWB, MM, DMW, BSG, SA, RR, AMFD integrated, modified and established the final questions being sent out to all participants. All authors voted on the final list of questions and provided references for separate questions. SG and EL wrote the first draft of the manuscript and all authors contributed substantially to revisions.

References

Adl, M.S., Gupta, V.V.S.R., 2006. Protists in soil ecology and forest nutrient cycling. Can. J. For. Res. 36, 1805–1817.

Adl, S., Girard, V., Breton, G., Lak, M., Maharning, A., Mills, A., Perrichot, V., Trionnaire, M., Vullo, R., Néraudeau, D., 2011. Reconstructing the soil food web of a 100 million-year-old forest: The case of the mid-Cretaceous fossils in the amber of Charentes (SW France). Soil Biology and Biochemistry 43, 726-735.

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. Journal of Eukaryotic Microbiology 59, 429-514.

Anderson, P.K., Cunningham, A.A., Patel, N.G., Morales, F.J., Epstein, P.R., Daszak, P., 2004. Emerging infectious diseases of plants: pathogen pollution, climate change and agrotechnology drivers. Trends in Ecology and Evolution 19, 535-544.

Anderson, R.O., Griffin, K.L., 2001. Abundances of protozoa in soil of laboratory grown wheat plants cultivated under low and high atmospheric CO2 concentrations. Protistology 2.

Aoki, Y., Hoshino, M., Matsubara, T., 2007. Silica and testate amoebae in a soil under pine–oak forest. Geoderma 142, 29-35.

Azam, F., Fenchel, T., Field, J., Gray, J., Meyer-Reil, L., Thingstad, F., 1983. The ecological role of water-column microbes in the sea. Marine Ecology Progress Series 10, 257-263.

Baas-Becking, L.G.M., 1934. Geobiologie, of inleiding tot de milieukunde. Van Stockum.

Ballen-Segura, M., Felip, M., Catalan, J., 2017. Some Mixotrophic Flagellate Species Selectively Graze on Archaea. Applied and environmental microbiology 83.

Barker, J., Brown, M.R.W., 1994. Trojan Horses of the microbial world: protozoa and the survival of bacterial pathogens in the environment. Microbiology 140, 1253-1259.

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. Environmental Microbiology 18, 1604–1619.

Bates, S.T., Berg-Lyons, D., Caporaso, J.G., Walters, W.A., Knight, R., Fierer, N., 2011. Examining the global distribution of dominant archaeal populations in soil. The ISME journal 5, 908-917.

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 Journal 7, 652-659.

Bell, T., Freckleton, R.P., Lewis, O.T., 2006. Plant pathogens drive density-dependent seedling mortality in a tropical tree. Ecology Letters 9, 569-574.

Berney, C., Geisen, S., Van Wichelen, J., Nitsche, F., Vanormelingen, P., Bonkowski, M., Bass, D., 2015. Expansion of the 'Reticulosphere': diversity of novel branching and network-forming amoebae helps to define Variosea (Amoebozoa). Protist 166, 271-295.

Berthold, A., Palzenberger, M., 1995. Comparison between direct counts of active soil ciliates (Protozoa) and most probable number estimates obtained by Singh's dilution culture method. Biology and Fertility of Soils 19, 348-356.

Bharti, D., Kumar, S., La Terza, A., 2015. Two gonostomatid ciliates from the soil of Lombardia, Italy; including note on the soil mapping project. Journal of Eukaryotic Microbiology 62, 762-772.

Bjørnlund, L., Rønn, R., 2008. 'David and Goliath' of the soil food web – Flagellates that kill nematodes. Soil Biology and Biochemistry 40, 2032-2039.

Boenigk, J., Ereshefsky, M., Hoef-Emden, K., Mallet, J., Bass, D., 2012a. Concepts in protistology: species definitions and boundaries. Eur. J. Protistol. 48, 96-102.

Boenigk, J., Ereshefsky, M., Hoef-Emden, K., Mallet, J., Bass, D., 2012b. Concepts in protistology: species definitions and boundaries. European Journal of Protistology 48, 96-102.

Bonkowski, M., 2004. Protozoa and plant growth: the microbial loop in soil revisited. New Phytologist 162, 617-631.
Bonkowski, M., Clarholm, M., 2012. Stimulation of plant growth through interactions of bacteria and protozoa: testing the auxiliary microbial loop hypothesis. Acta Protozool. 51, 237-247.
Bradford, M.A., 2016. Re-visioning soil food webs. Soil Biology and Biochemistry 102, 1-3.
Brown, M.R.W., Barker, J., 1999. Unexplored reservoirs of pathogenic bacteria: protozoa and biofilms. Trends in Microbiology 7, 46-50.
Burki, F., 2014. The eukaryotic tree of life from a global phylogenomic perspective. Cold Spring Harbor Perspectives in Biology 6, a016147.
Charman, D.J., 2015. Testate amoebae, In: Shennan, I., Long, A.J. and Horton, B.P. (Eds). (Ed.), Handbook of Sea-Level Research. Wiley, Chichester, pp. 281-294.
Clarholm, M., 1981. Protozoan grazing of bacteria in soil—impact and importance. Microb. Ecol. 7, 343-350.
Clarholm, M., 1985. Interactions of bacteria, protozoa and plants leading to mineralization of soil nitrogen. Soil Biol. Biochem. 17, 181-187.
Cole, C.V., Elliott, E.T., Hunt, H.W., Coleman, D.C., 1977. Trophic interactions in soils as they affect energy and nutrient dynamics. V. Phosphorus transformations. Microbial ecology 4, 381-387.
Corliss, J.O., Esser, S.C., 1974. Comments on the role of the cyst in the life cycle and survival of free-living protozoa. Transactions of the American Microscopical Society 93, 578-593.
Creevy, A.L., Fisher, J., Puppe, D., Wilkinson, D.M., 2016. Protist diversity on a nature reserve in NW England—With particular reference to their role in soil biogenic silicon pools. Pedobiologia 59, 51-59.
Crotty, F., Adl, S., Blackshaw, R., Murray, P., 2012. Protozoan pulses unveil their pivotal position within the soil food web. Microbial ecology 63, 905-918.
de Ruiter, P.C., Neutel, A.M., 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., Lara, E., Berney, C., Le Bescot, N., Probert, I., Carmichael, M., Poulain, J., Romac, S., Colin, S., Aury, J.-M., Bittner, L., Chaffron, S., Dunthorn, M., Engelen, S., Flegontova, O., Guidi, L., Horák, A., Jaillon, O., Lima-Mendez, G., Lukeš, J., Malviya, S., Morard, R., Mulot, M., Scalco, E., Siano, R., Vincent, F., Zingone, A., Dimier, C., Picheral, M., Searson, S., Kandels-Lewis, S., Coordinators, T.O., Acinas, S.G., Bork, P., Bowler, C., Gorsky, G., Grimsley, N., Hingamp, P., Iudicone, D., Not, F., Ogata, H., Pesant, S., Raes, J., Sieracki, M.E., Speich, S., Stemmann, L., Sunagawa, S., Weissenbach, J., Wincker, P., Karsenti, E., 2015. Eukaryotic plankton diversity in the sunlit ocean. Science 348.
Delwiche, C.F., 1999. Tracing the thread of plastid diversity through the tapestry of life. The American Naturalist 154, S164-S177.
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.
Eisenhauer, N., Cesarz, S., Koller, R., Worm, K., Reich, P.B., 2012. Global change belowground: impacts of elevated CO2, nitrogen, and summer drought on soil food webs and biodiversity. Global Change Biol. 18, 435–447.
Ekelund, F., 1998. Enumeration and abundance of mycophagous protozoa in soil, with special emphasis on heterotrophic flagellates. Soil Biology and Biochemistry 30, 1343-1347.
Fernandez, L.D., Fournier, B., Rivera, R., Lara, E., Mitchell, E.A.D., Hernandez, C.E., 2016. Water-energy balance, past ecological perturbations and evolutionary constraints shape the latitudinal diversity gradient of soil testate amoebae in south-western South America. Global Ecology and Biogeography 25, 1216-1227.
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. Applied and Environmental Microbiology 73, 7059-7066.

Foissner, W., 1987. Soil protozoa: fundamental problems, ecological significance, adaptations in ciliates and testaceans, bioindicators, and guide to the literature. Prog. Protistol. 2, 69-212.
Foissner, W., 1997. Protozoa as bioindicators in agroecosystems, with emphasis on farming practices, biocides, and biodiversity. Agr. Ecosyst. Environ. 62, 93-103.
Foissner, W., 1999. Soil protozoa as bioindicators: pros and cons, methods, diversity, representative examples. Agriculture, Ecosystems & Environment 74, 95-112.
Foissner, W., 2006. Biogeography and dispersal of micro-organisms: a review emphasizing protists. Acta Protozool. 45, 111-136.
Foissner, W., 2008. Protist diversity and distribution: some basic considerations. Biodivers. Conserv. 17, 235-242.
Foissner, W., 2016. Terrestrial and semiterrestrial ciliates (Protozoa, Ciliophora) from Venezuela and Galápagos. Denisia 35, 1-912.
Fournier, B., Malysheva, E., Mazei, Y., Moretti, M., Mitchell, E.A.D., 2012. Toward the use of testate amoeba functional traits as indicator of floodplain restoration success. European Journal of Soil Biology 49, 85-91.
Frey, S.D., Gupta, V.V.S.R., Elliott, E.T., Paustian, K., 2001. Protozoan grazing affects estimates of carbon utilization efficiency of the soil microbial community. Soil Biology and Biochemistry 33, 1759-1768.
Gams, W., Diederich, P., Poldmaa, K., 2011. Biodiversity of fungi: inventory and monitoring methods, In: Mueller, G.M., Bills, G.F., Foster, M.S. (Ed.), Biodiversity of Fungi: Inventory and Monitoring Methods. Elsevier.
Geisen, S., 2016a. The bacterial-fungal energy channel concept challenged by enormous functional versatility of soil protists. Soil Biology and Biochemistry 102, 22-25.
Geisen, S., 2016b. Thorough high-throughput sequencing analyses unravels huge diversities of soil parasitic protists. Environmental Microbiology 18, 1669-1672.
Geisen, S., Bandow, C., Römbke, J., Bonkowski, M., 2014a. Soil water availability strongly alters the community composition of soil protists. Pedobiologia 57, 205–213.
Geisen, S., Fiore-Donno, A.M., Walochnik, J., Bonkowski, M., 2014b. *Acanthamoeba* everywhere: high diversity of *Acanthamoeba* in soils. Parasitology Research 113, 3151-3158.
Geisen, S., Koller, R., Hünninghaus, M., Dumack, K., Urich, T., Bonkowski, M., 2016. The soil food web revisited: Diverse and widespread mycophagous soil protists. Soil Biology and Biochemistry 94, 10-18.
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., Urich, T., Bonkowski, M., 2015b. Pack hunting by a common soil amoeba on nematodes. Environmental Microbiology 17, 4538-4546.
Geisen, S., Tveit, A.T., Clark, I.M., Richter, A., Svenning, M.M., Bonkowski, M., Urich, T., 2015c. Metatranscriptomic census of active protists in soils. The ISME Journal 9, 2178-2190.
Glücksman, E., Bell, T., Griffiths, R.I., Bass, D., 2010. Closely related protist strains have different grazing impacts on natural bacterial communities. Environ. Microbiol. 12, 3105-3113.
Greub, G., Raoult, D., 2004. Microorganisms resistant to free-living amoebae. Clinical Microbiology Reviews 17, 413-433.
Griffiths, B.S., Römbke, J., Schmelz, R.M., Scheffczyk, A., Faber, J.H., Bloem, J., Pérès, G., Cluzeau, D., Chabbi, A., Suhadolc, M., Sousa, J.P., Martins da Silva, P., Carvalho, F., Mendes, S., Morais, P., Francisco, R., Pereira, C., Bonkowski, M., Geisen, S., Bardgett, R.D., de Vries, F.T., Bolger, T., Dirilgen, T., Schmidt, O., Winding, A., Hendriksen, N.B., Johansen, A., Philippot, L., Plassart, P., Bru, D., Thomson, B., Griffiths, R.I., Bailey, M.J., Keith, A., Rutgers, M., Mulder, C., Hannula, S.E., Creamer, R., Stone, D., 2016. Selecting cost effective and policy-relevant biological indicators for European monitoring of soil biodiversity and ecosystem function. Ecological Indicators 69, 213-223.

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.

Gupta, V.V., Yeates, G., 1997. Soil microfauna as bioindicators of soil health. CAB International.

Gupta, V.V.S.R., Germida, J.J., 1988. Distribution of microbial biomass and its activity in different soil aggregate size classes as affected by cultivation. Soil Biology and Biochemistry 20, 777-786.

Haeckel, E., 1866. Generelle Morphologie der Organismen. G. Reimer, Berlin.

Jacquiod, 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., Chiapusio, G., Binet, P., Buttler, A., Laggoun-Défarge, F., Delarue, F., Bernard, N., Mitchell, E.A.D., Toussaint, M.-L., Francez, A.-J., Gilbert, D., 2013. Above- and belowground linkages in Sphagnum peatland: climate warming affects plant-microbial interactions. Global Change Biol. 19, 811-823.

Jassey, V.E.J., Shimano, S., Dupuy, C., Toussaint, M.L., Gilbert, D., 2012. Characterizing the feeding habits of the testate amoebae *Hyalosphenia papilio* and *Nebela tincta* along a narrow "fen-bog" gradient using digestive vacuole content and 13C and 15N isotopic analyses. Protist 163, 451-464.

Jassey, V.E.J., Signarbieux, C., Hättenschwiler, S., Bragazza, L., Buttler, A., Delarue, F., Fournier, B., Gilbert, D., Laggoun-Défarge, 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. Scientific Reports 5, 16931.

Jones, S.E., Lennon, J.T., 2010. Dormancy contributes to the maintenance of microbial diversity. Proceedings of the National Academy of Sciences 107, 5881-5886.

Jousset, A., 2012. Ecological and evolutive implications of bacterial defences against predators. Environmental Microbiology 14, 1830–1843.

Jousset, A., Lara, E., Wall, L.G., Valverde, C., 2006. Secondary metabolites help biocontrol strain *Pseudomonas fluorescens* CHA0 to escape protozoan grazing. Appl. Environ. Microbiol. 72, 7083-7090.

Keyes, S.D., Daly, K.R., Gostling, N.J., Jones, D.L., Talboys, P., Pinzer, B.R., Boardman, R., Sinclair, I., Marchant, A., Roose, T., 2013. High resolution synchrotron imaging of wheat root hairs growing in soil and image based modelling of phosphate uptake. New Phytologist 198, 1023-1029.

Kosakyan, A., Gomaa, F., Lara, E., Lahr, D.J.G., 2016. Current and future perspectives on the systematics, taxonomy and nomenclature of testate amoebae. European Journal of Protistology in press.

Lamentowicz, M., Gałka, M., Lamentowicz, Ł., Obremska, M., Kühl, N., Lücke, A., Jassey, V., 2015. Climate change over the last 4000 years in a Baltic bog in northern Poland revealed by a trait-based approach, biotic proxies, and stable isotopes. Palaeogeography Palaeoclimatology Palaeoecology 418, 261-277.

Leininger, S., Urich, 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.

López-García, P., Moreira, D., 2015. Open questions on the origin of eukaryotes. Trends in Ecology and Evolution 30, 697-708.

Lynn, D., 2008. The ciliated protozoa: characterization, classification, and guide to the literature, 3rd edition ed. Springer Science & Business Media, Dordrecht.

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.M., Mitchell, E.A.D., Seppey, C.V.W., Egge, E., Wirth, R., Trueba, G., Dunthorn, M., 2017. Parasites dominate hyperdiverse soil protist communities in Neotropical rainforests. Nature Ecology and Evolution 1:0091.

Marcisz, K., Lamentowicz, Ł., Słowińska, S., Słowiński, M., Muszak, W., Lamentowicz, M., 2014. Seasonal changes in Sphagnum peatland testate amoeba communities along a hydrological gradient. European Journal of Protistology 50, 445-455.
Meyer, C., D.Gilbert, Gillet, F., Moskura, M., Franchi, M., Bernard, N., 2012. Using "bryophytes and their associated testate amoeba" microsystems as indicators of atmospheric pollution. Ecological Indicators 13, 144-151.
Mitchell, E.A.D., 2015. Pack hunting by minute soil testate amoebae: nematode hell is a naturalist's paradise. Environmental Microbiology 17, 4145-4147.
Mitchell, E.A.D., Charman, D.J., Warner, B.G., 2008. Testate amoebae analysis in ecological and paleoecological studies of wetlands: past, present and future. Biodiversity & Conservation 17, 2115-2137.
Molmeret, M., Horn, M., Wagner, M., Santic, M., Kwaik, Y.A., 2005. Amoebae as training grounds for intracellular bacterial pathogens. Applied and Environmental Microbiology 71, 20-28.
Murase, J., Hordijk, K., Tayasu, I., Bodelier, P.L.E., 2011. Strain-specific incorporation of methanotrophic biomass into eukaryotic grazers in a rice field soil revealed by PLFA-SIP. FEMS Microbiol. Ecol. 75, 284-290.
Neuhauser, S., Kirchmair, M., Bulman, S., Bass, D., 2014. Cross-kingdom host shifts of phytomyxid parasites. BMC Evol. Biol. 14, 33.
Old, K.M., Chakraborty, S., 1986. Mycophagous soil amoebae: their biology and significance in the ecology of soil-borne plant pathogens. Progress in protistology 1, 163-194.
Page, F.C., 1977. The genus *Thecamoeba* (Protozoa, Gymnamoebia) species distinctions, locomotive morphology, and protozoan prey. J. Nat. Hist. 11, 25-63.
Pawlowski, J., Audic, S., Adl, S., Bass, D., Belbahri, L., Berney, C., Bowser, S.S., Cepicka, I., Decelle, J., Dunthorn, M., Fiore-Donno, A.M., Gile, G.H., Holzmann, M., Jahn, R., Jirků, M., Keeling, P.J., Kostka, M., Kudryavtsev, A.A., Lara, E., Lukeš, J., Mann, D.G., Mitchell, E.A.D., Nitsche, F., Romeralo, M., Saunders, G.W., Simpson, A.G.B., Smirnov, A.V., Spouge, J.L., Stern, R.F., Stoeck, T., Zimmermann, J., Schindel, D., de Vargas, C., 2012. CBOL protist working group: barcoding eukaryotic richness beyond the animal, plant, and fungal kingdoms. Plos Biology 10, e1001419.
Payne, R.J., 2013. Seven reasons why protists make useful bioindicators. Acta Protozoologica 52, 105.
Payne, R.J., Jassey, V.E., Leith, I.D., Sheppard, L.J., Dise, N.B., Gilbert, D., 2013. Ammonia exposure promotes algal biomass in an ombrotrophic peatland. Soil Biology and Biochemistry 57, 936-938.
Payne, R.J., Malysheva, E., Tsyganov, A., Pampura, T., Novenko, E., Volkova, E., Babeshko, K., Mazei, Y., 2015. A multi-proxy record of Holocene environmental change, peatland development and carbon accumulation from Staroselsky Moch peatland, Russia. The Holocene 26, 314-326.
Payne, R.J., Mitchell, E.A., Nguyen-Viet, H., Gilbert, D., 2012. Can pollution bias peatland paleoclimate reconstruction? Quaternary Research 78, 170-173.
Prosser, J.I., 2015. Dispersing misconceptions and identifying opportunities for the use of 'omics' in soil microbial ecology. Nature Reviews Microbiology 13, 439-446.
Raoult, D., Boyer, M., 2010. Amoebae as genitors and reservoirs of giant viruses. Intervirology 53, 321-329.
Rønn, R., Gavito, M., Larsen, J., Jakobsen, I., Frederiksen, H., Christensen, S., 2002. Response of free-living soil protozoa and microorganisms to elevated atmospheric CO2 and presence of mycorrhiza. Soil Biology and Biochemistry 34, 923-932.
Rosenberg, K., Bertaux, J., Krome, K., Hartmann, A., Scheu, S., Bonkowski, M., 2009. Soil amoebae rapidly change bacterial community composition in the rhizosphere of *Arabidopsis thaliana*. ISME Journal 3, 675-684.
Schardl, C.L., Craven, K.D., 2003. Interspecific hybridization in plant-associated fungi and oomycetes: a review. Molecular Ecology 12, 2861-2873.
Schmidt, O., Dyckmans, J., Schrader, S., 2016. Photoautotrophic microorganisms as a carbon source for temperate soil invertebrates. Biology Letters 12.

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

Schuster, F.L., 2002. Cultivation of pathogenic and opportunistic free-living amebas. Clinical Microbiology Reviews 15, 342-354.

Seddon, A.W.R., Mackay, A.W., Baker, A.G., Birks, H.J.B., Breman, E., Buck, C.E., Ellis, E.C., Froyd, C.A., Gill, J.L., Gillson, L., Johnson, E.A., Jones, V.J., Juggins, S., Macias-Fauria, M., Mills, K., Morris, J.L., Nogués-Bravo, D., Punyasena, S.W., Roland, T.P., Tanentzap, A.J., Willis, K.J., Aberhan, M., van Asperen, E.N., Austin, W.E.N., Battarbee, R.W., Bhagwat, S., Belanger, C.L., Bennett, K.D., Birks, H.H., Bronk Ramsey, C., Brooks, S.J., de Bruyn, M., Butler, P.G., Chambers, F.M., Clarke, S.J., Davies, A.L., Dearing, J.A., Ezard, T.H.G., Feurdean, A., Flower, R.J., Gell, P., Hausmann, S., Hogan, E.J., Hopkins, M.J., Jeffers, E.S., Korhola, A.A., Marchant, R., Kiefer, T., Lamentowicz, M., Larocque-Tobler, I., López-Merino, L., Liow, L.H., McGowan, S., Miller, J.H., Montoya, E., Morton, O., Nogué, S., Onoufriou, C., Boush, L.P., Rodriguez-Sanchez, F., Rose, N.L., Sayer, C.D., Shaw, H.E., Payne, R., Simpson, G., Sohar, K., Whitehouse, N.J., Williams, J.W., Witkowski, A., 2014. Looking forward through the past: identification of 50 priority research questions in palaeoecology. Journal of Ecology 102, 256-267.

Shmakova, L., Bondarenko, N., Smirnov, A., 2016. Viable species of *Flamella* (Amoebozoa: Variosea) isolated from ancient arctic permafrost sediments. Protist 167, 13-30.

Siddiqui, R., Ahmed Khan, N., 2012. Biology and pathogenesis of *Acanthamoeba*. Parasites and Vectors 5, 1-13.

Singer, D., Lara, E., Steciow, M.M., Seppey, C.V.W., Paredes, N., Pillonel, A., Oszako, T., Belbahri, L., 2016. High-throughput sequencing reveals diverse oomycete communities in oligotrophic peat bog micro-habitat. Fungal Ecology 23, 42-47.

Smirnov, A.V., Brown, S., 2004. Guide to the methods of study and identification of soil gymnamoebae. Protistology 3, 148-190.

Spiegel, F.W., Stephenson, S.L., Keller, H.W., Moore, D.L., Cavender, J.C., 2004. Biodiversity of fungi: inventory and monitoring methods, In: Mueller, G.M., Foster, M.S., Bills, G.F. (Eds.), Biodiversity of Fungi, Inventory and Monitoring Methods. Elsevier Academic Press, Burlington, MA, pp. 547-576.

Stockdale, A., Davison, W., Zhang, H., 2009. Micro-scale biogeochemical heterogeneity in sediments: A review of available technology and observed evidence. Earth-Science Reviews 92, 81-97.

Sutherland, W.J., Freckleton, R.P., Godfray, H.C.J., Beissinger, S.R., Benton, T., Cameron, D.D., Carmel, Y., Coomes, D.A., Coulson, T., Emmerson, M.C., Hails, R.S., Hays, G.C., Hodgson, D.J., Hutchings, M.J., Johnson, D., Jones, J.P.G., Keeling, M.J., Kokko, H., Kunin, W.E., Lambin, X., Lewis, O.T., Malhi, Y., Mieszkowska, N., Milner-Gulland, E.J., Norris, K., Phillimore, A.B., Purves, D.W., Reid, J.M., Reuman, D.C., Thompson, K., Travis, J.M.J., Turnbull, L.A., Wardle, D.A., Wiegand, T., 2013. Identification of 100 fundamental ecological questions. Journal of Ecology 101, 58-67.

Tice, A.K., Shadwick, L.L., Fiore-Donno, A.M., Geisen, S., Kang, S., Schuler, G.A., Spiegel, F.W., Wilkinson, K.A., Bonkowski, M., Dumack, K., Lahr, D.J., Voelcker, E., Clauss, S., Zhang, J., Brown, M.W., 2016. Expansion of the molecular and morphological diversity of Acanthamoebidae (Centramoebida, Amoebozoa) and identification of a novel life cycle type within the group. Biology Direct 11, 69.

Tikhonenkov, D.V., Mazei, Y.A., Embulaeva, E.A., 2010. Effect of ecosystem type on soil heterotrophic flagellate communities under forest-steppe conditions. Protistology 6.

Treonis, A.M., Lussenhop, J.F., 1997. Rapid response of soil protozoa to elevated CO2. Biology and fertility of soils 25, 60-62.

Tsyganov, A.N., Nijs, I., Beyens, L., 2011. Does climate warming stimulate or inhibit soil protist communities? A test on testate amoebae in high-arctic tundra with free-air temperature increase. Protist 162, 237-248.

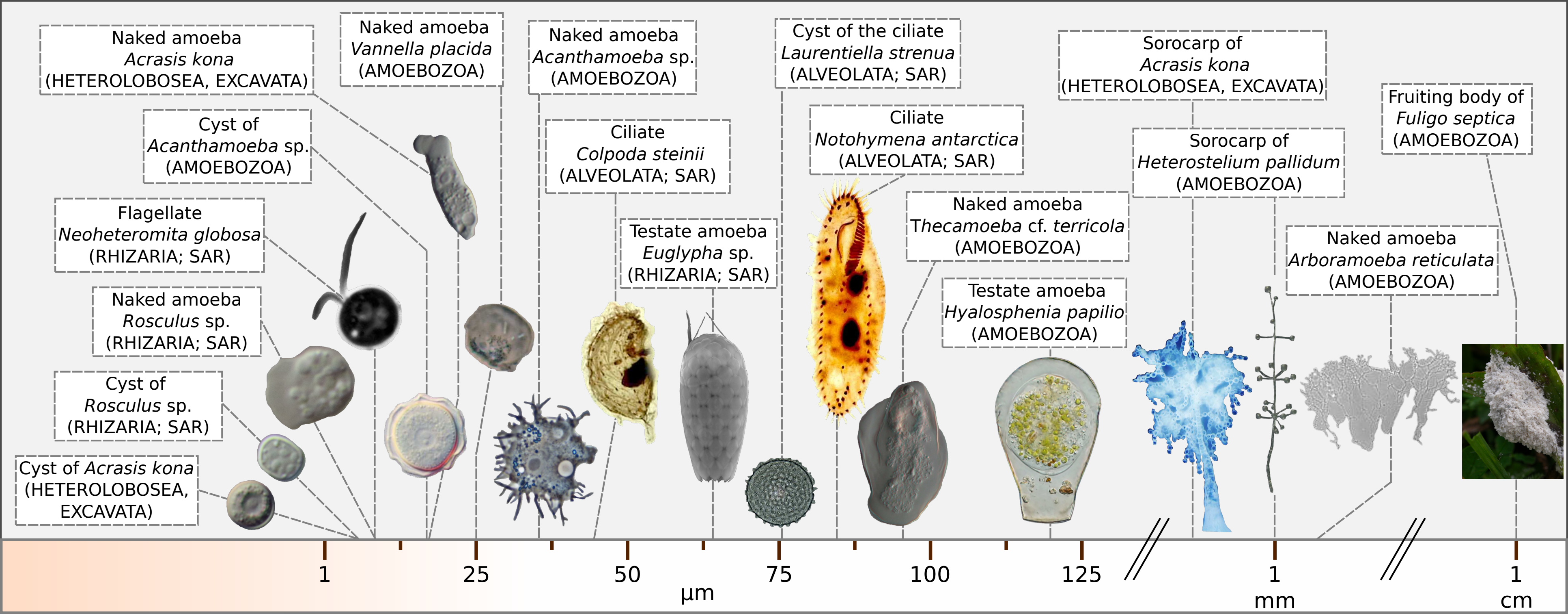
Turner, T.E., Swindles, G.T., Roucoux, K.H., 2014. Late Holocene ecohydrological and carbon dynamics of a UK raised bog: impact of human activity and climate change. Quaternary Science Reviews 84, 65-85.

Wilkinson, D.M., 2008. Testate amoebae and nutrient cycling: peering into the black box of soil ecology. Trends in Ecology & Evolution 23, 596-599.

Figures

Fig. 1: Common free-living soil protists as visualized by size (lengths), morphology and phylogenetic affiliation. Note, soil protists belong to a wide range of supergroups (in brackets), whereas animals are only placed in the supergroup Opisthokonta. Furthermore, soil protists span a much wider size range as commonly assumed. With the exception of ciliates, morphogroups are not phylogenetically conserved and are placed in different eukaryotic supergroups. Most soil protists can occur in different life forms including active form (amoeba, flagellate, ciliate), but most form cysts, while some can form special reproduction structures (sorocarps and fruiting bodies).

Fig. 2: (a) Overview of studies specifically mentioning protists in the title in 5-year intervals since 1980. Soil studies represent only about a fraction of aquatic studies (separated into freshwater, marine and those that more broadly indicate aquatic) showing a strong increase in protist research in aquatic, but not soil environments; (b) Comparison of soil studies specifically mentioning protists in the title with those on other micro-sized organisms including viruses (blue filled circles), archaea (green open circles), bacteria (red diamonds), fungi (orange crosses) and nematodes (green triangles). See Supplementary Methods for details on the search.



Naked amoeba
Acrasis kona
(HETEROLOBOSEA, EXCAVATA)
Naked amoeba
Vannella placida
(AMOEBOZOA)
Naked amoeba
Acanthamoeba sp.
(AMOEBOZOA)
Cyst of the ciliate
Laurentiella strenua
(ALVEOLATA; SAR)
Sorocarp of
Acrasis kona
(HETEROLOBOSEA, EXCAVATA)
Fruiting body of
Fuligo septica
(AMOEBOZOA)
Cyst of
Acanthamoeba sp.
(AMOEBOZOA)
Ciliate
Colpoda steinii
(ALVEOLATA; SAR)
Ciliate
Notohymena antarctica
(ALVEOLATA; SAR)
Sorocarp of
Heterostelium pallidum
(AMOEBOZOA)
Flagellate
Neoheteromita globosa
(RHIZARIA; SAR)
Testate amoeba
Euglypha sp.
(RHIZARIA; SAR)
Naked amoeba
Thecamoeba cf. terricola
(AMOEBOZOA)
Naked amoeba
Arboramoeba reticulata
(AMOEBOZOA)
Naked amoeba
Rosculus sp.
(RHIZARIA; SAR)
Testate amoeba
Hyalosphenia papilio
(AMOEBOZOA)
Cyst of
Rosculus sp.
(RHIZARIA; SAR)
Cyst of Acrasis kona
(HETEROLOBOSEA,
EXCAVATA)
1
25
50
75
100
125
µm
1
mm
1
cm

A
Protist studies
marine
freshwater
aquatic
soil
number of publications
2500
2000
1500
1000
500
0
1980 - 1985
1985 - 1990
1990 - 1995
1995 - 2000
2000 - 2005
2005 - 2010
2010 - 2015
B
Soil studies
bacteria
fungi
nematodes
archaea
viruses
protist
number of publications
4000
3000
2000
1000
0
1980 - 1985
1985 - 1990
1990 - 1995
1995 - 2000
2000 - 2005
2005 - 2010
2010 - 2015

Highlights

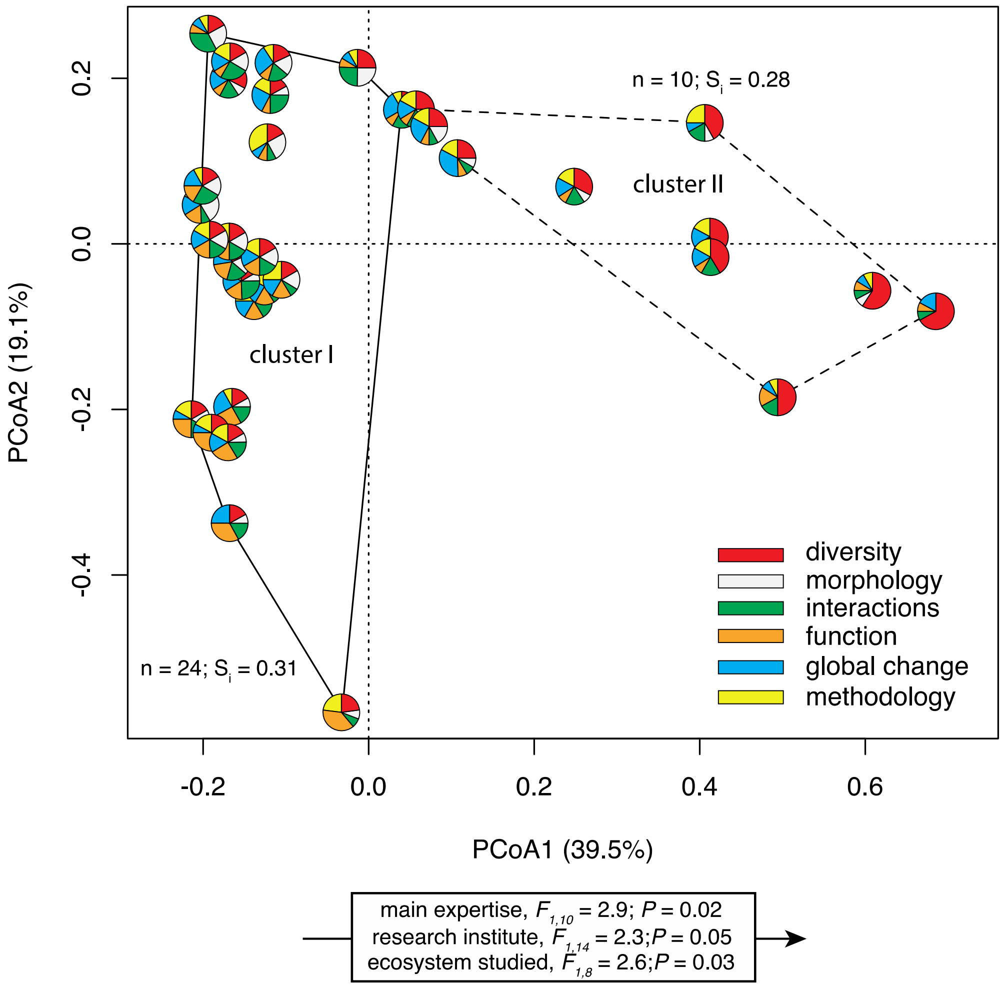
- Protists are the most diverse eukaryotes in soils
- They are key elements in the soil food web and are essential for plant functioning
- Nevertheless, protists are highly understudied compared to other microorganisms
- We here provide an overview of missing research gaps to guide future studies
- This will allow bridging protistology to general microbiology and ecology in soils

We aimed at providing a list of major questions without ranking them by numbers of votes. Despite we obtained these numbers (Supplementary Table 1), we restrained from including them into the main text as the questions were often hard to compare in terms of their scope and due to the bias included through the selection of participants – which, however, turned out to be absent or minor (see bias results below). We therefore include an overview of the analyses based on votes here.

Most votes (117) were placed into the category (ii) Diversity, Community Composition and Biogeography. (iv) Functions of Protists (103), received second most votes (99), followed by (vi) Methodology (99), (i) Morphology, Phylogeny, Taxonomy, Evolution and Physiology (81), (iii) Interactions among Protists and other Organisms (80), and (v) Global Change, Bioindicators and Applications (76). However, the highest number of votes per question was found for the categories with the least numbers of questions, i.e. (v) Global Change, Bioindicators and Applications (6.3 votes per question) and (i) Morphology, Phylogeny, Taxonomy, Evolution and Physiology (6.2 votes per question). We note that both rankings are biased, as most votes were placed in the categories where most questions were sent while the highest vote per question was affected by the necessity that researchers place at least one vote in each of the six categories.

Potential biases

Based on the votes, the fuzzy c-Means ordination showed that participants aggregated in two clusters (Supplementary Figure 1): cluster I composed of 24 participants and cluster II composed of 10 participants. The silhouette width showed that all participants well belonged to their respective clusters with an average silhouette of 0.31 and 0.28 for cluster I and II, respectively. Cluster I was composed of participants which equally voted to the proposed categories of questions, while cluster II was characterised by participants who mostly voted in the category Diversity, Community Composition and Biogeography. Main expertise, research institute and the type ecosystem studied mainly explained such grouping in the ordination space (Supplementary Figure 1). Cluster II was indeed characterised by participants from the same research area (phylogeny, taxonomy, and evolutionary), studying multiple systems and/or working in the same institution. Furthermore, variables such as experience, age, type of position and group of interest were not significantly related to the fuzzy c-Means ordination space.



Supplementary Figure 1: Principal co-ordinate ordination associated with c-Means fuzzy clustering representing the dissimilarity of votes among participants. Solid and dashed lines define the different clusters found along axis PCoA1. The average membership of the participants (sites) to each cluster can be seen with the silhouette width value (S_i). Pie charts show the proportion of votes for each question category and for each participant occurring in clusters I and II. Significant effect of explanatory variables (ANOVAs) between the PCoA axes and the individual explanatory variables are shown in boxes.

Supplementary Table 1: Questions sent for the final vote with category, total number of votes and references that (partially) addressed the respective question before.

Nr	Question	Category	Votes	References
1	How long can protists survive in an encysted form? What are the tolerances of (encysted) protists to stress and what is the importance of cysts for ecosystem resilience?	1	14	[1-6]
2	How much morphological and genetic variability exists within soil protists?	1	11	[7-15]
3	How do species that occur in both aquatic and soil systems adapt to differing demands?	1	9	[16]
4	What are the phylogenetic relations of true soil to aquatic protist taxa and how often have soils been colonized by aquatic protists and vice versa?	1	7	[8, 146-148]
5	How widespread is sex in soil protists?	1	7	[17, 18]
6	What is the real diversity and community structure of soil protists in different systems (e.g. soils, rhizosphere, (plant) endosphere)?	2	11	[26-29]
7	How similar are the diversity patterns of soil protists and other soil biota along ecological gradients, and to what extent do different environmental factors shape their respective diversity?	2	10	[30-37]
8	What abiotic environmental factors influence the distribution and community composition of protists, and how?	2	10	[30, 32, 37-48]
9	How cosmopolitan are protists and how many endemic soil protist species are there?	2	10	[49-60]
10	What are dominant groups of soil protists in terms of turnover, abundance and biomass?	2	7	[44, 62, 164, 165]
11	How do protist taxa affect the composition of the soil microbiome and what other important interactions take place?	3	14	[64-74]
12	What are the biotic interactions of soil protists with other taxonomic groups, and how are protists linked within the soil food web?	3	12	[44, 66, 75-83]
13	What is the relative contribution of nutrient cycling (i.e. the microbial loop) versus modification of the rhizosphere microbiome in protist-induced stimulation of plant growth?	3	9	[44, 82, 84-88]
14	What are the mechanisms by which individual soil protist species affect plant performance, and do those mechanisms differ between plant species?	3	8	[78, 85, 88-93]
15	What is the impact of protists on the community functioning of other soil microbes?	3	7	[74, 94-98]
16	What is the importance of soil protists in biogeochemical cycling?	4	18	[26, 99-106]
17	How much functional redundancy is there in the soil protist community?	4	12	[107-109]
18	Does increased protist diversity affect ecosystem functioning?	4	12	[26]
19	What is the comparative importance of eukaryotic microbes vs. prokaryotes in driving key soil processes?	4	9	[93]
20	Which individual functions are performed by distinct groups, and what is the entire functional diversity of	4	9	[110, 111]

	soil protists?			
21	How do changing climatic patterns affect the diversity of, community structure of and ecosystem services provided by soil protists?	5	12	[36, 112-116]
22	Which protist clades can be used as bioindicators to assess soil properties, ecosystem state, and anthropogenic impacts? How could this be implemented?	5	11	[44, 47, 82, 117-127]
23	Why are some species more sensitive to environmental change than others, why do some respond faster to environmental factors?	5	10	[128]
24	How can protists be used for nutrient mobilization and biocontrol in cropping systems?	5	8	[129]
25	What is the importance of soil protists for biodiversity conservation and ecosystem management and restoration? Should we protect particular species or habitats?	5	8	[130-133]
26	What is the most practical taxonomic unit to measure protist protist diversity?	6	20	[9, 11, 106, 134-136]
27	How can we standardize and calibrate cultivation based and molecular methods to reliably quantify soil protist abundance, diversity and activity?	6	9	[44, 137, 138]
28	How should sampling be performed to adequately evaluate soil protist diversity?	6	9	[138-140]
29	At what scales (temporal, spatial/physical, morphological, phylogenetic) should we study protists to fully understand their diversity and function in soil; which one should be prioritized?	6	8	
30	How can we infer functional traits of soil protists based on morphology or phylogenetic affiliation, and what taxonomic resolution is needed?	6	7	[26, 118, 128, 141, 142]
31	How much change in soil protists has there been over geological time?	1	7	[19-25]
32	Which taxa should be prioritized for genome and transcriptome sequencing analysis? And how should they be selected?	1	7	[143-145]
33	To what extent does horizontal gene transfer drive protist communities and ecosystem function/ecosystem services?	1	6	[149]
34	How do protists defend themselves against abiotic and biotic stressors?	1	5	[47, 150-152]
35	How often has social behaviour evolved in soil protists and do social protists dominate terrestrial ecosystems?	1	3	[153]
36	Can low Reynolds number physics be used to explain the reality of so called adaptations of soil protists?	1	2	
37	How did the association between intracellular (potentially human pathogens) bacteria and their soil protist hosts evolve?	1	2	[154, 155]
38	Could bacteria and bacterial feeding soil protists be used as a model to experimentally study genome evolution - such as horizontal gene transfer events?	1	1	[156-158]
49	Do soil protists have latitudinal, longitudinal and altitudinal diversity patterns? If so, are these patterns the same as those exhibited by macroorganisms?	2	7	[30, 37, 49, 159-163]

40	How much of the soil protist community is active in different ecosystems - what part, when, and why?	2	7	[61-63]
41	How do protist communities vary over time?	2	6	[138, 166-175]
42	How does the micro-heterogeneity of soils affect protist diversity and distribution?	2	6	[46, 108, 176-181]
43	What types of processes (i.e. ecological, historical, evolutionary) produce latitudinal, longitudinal or altitudinal diversity patterns in soil protists?	2	6	[37, 48, 182, 183]
44	How are soil protist communities structured in the soil profile?	2	5	[41, 174, 184-192]
45	How localized is the adaptation of protists to environmental conditions and environmental changes?	2	5	[193-196]
46	How much of the environmental DNA of protists in soils does not not belong to any organism actually living there?	2	4	[197]
47	What is the importance of historical factors in shaping soil protist communities in comparison to local conditions?	2	4	[35, 37, 183, 198]
48	How resilient / resistant is the active/dormant soil protist community?	2	4	[44, 45, 199, 200]
49	What is the diversity of active parasitic protists in soils and what are their hosts?	2	3	[32, 201-204]
50	What is the intraspecific diversity of soil protists?	2	3	[205-210]
51	Which soil habitats host the highest abundance and diversity of soil protists?	2	3	[175, 180, 211-217]
52	What are (unique) adaptations for protists to inhabit soils?	2	2	[195, 218]
53	How many and which soil protist taxa species have an expanding distribution, are invasive or are threatened of extinction?	2	2	[219]
54	Which are keystone taxa of soil protists in certain systems and how can we find them?	2	1	[220]
55	What is the diversity of soil protists that serves as intracellular refuges for human and animal pathogenic microorganisms?	2	1	[221]
56	How (phylogenetically) widespread is the ability of soil protists to live under anaerobic conditions?	2	0	[222]
57	What are the main parasites and pathogens of soil protists, and what impacts do they have?	3	5	[223-228]
58	How important are protist-protist interactions in structuring soil protist communities?	3	4	[99, 229]
59	Is there an especially strong connection between bacteria and soil protists as both groups have interacted for more than a billion years, and how does it compare with other microbes interacting in soils?	3	4	[230]
60	How do plant species (e.g. via exudates, volatiles etc.) affect the composition of soil protist communities?	3	4	[91, 231]
61	Is there a link between morphology and body size of soil protists and does this link to their position and role in food webs?	3	3	[72, 232]
62	Which protistan traits are most useful to predict in situ trophic interactions between bacterivorous soil protist and bacteria?	3	2	
63	Which signals/receptors are involved in food recognition of soil protists?	3	2	[233-238]
64	How does the parasitic relationship between soil protist host and parasite establish itself?	3	1	[228, 239]

65	What is known about mutualistic associations between soil protists and other organisms?	3	1	[240-242]
66	What mechanisms are used by soil protists to crack (physically) or lyse (enzymatically) their prey?	3	1	[243, 244]
67	How widespread is intra-guild predation of protists in soils (= protists feeding on other protists)?	3	1	[245-248]
68	Which chemical signals exchanged by soil protists and their prey increase stimulate protist activity resulting in e.g. increased feeding efficiency?	3	1	
69	How does addition of nutrients affect resources utilization, stoichiometry and interactions between protists and other (micro)organisms in soil?	3	1	[85, 93]
70	Are protists important for bacterial dispersion in soil, and if so which taxa are mainly responsible?	3	0	[129, 249]
71	What is the impact of protists on the process of decomposition?	4	8	[100, 250]
72	What is the abundance, diversity and functional role of the many novel and unknown soil protist clades that have been revealed by molecular environmental sampling?	4	5	[110, 251, 252]
73	What is the potential role of protists as disease suppression and biocontrol agents in soils?	4	5	[44, 82]
74	What is the relative functional importance of non-bacterivorous soil protists and how does this differ between soils/biomes/ecosystems?	4	4	[32, 253]
75	How can we extrapolate soil protist activity, diversity, and community changes to those of general microbial and higher trophic level communities, and consequently ecosystem functioning?	4	4	[93]
76	How much C is channelled through protists to higher trophic levels in soil?	4	4	[254]
77	What is the importance/function of (plant- and animal-)parasitic protists in soil?	4	4	[32, 255]
78	What are the main metabolic pathways that affect soil protist communities and ecosystem function?	4	3	[256]
79	What is the primary production input from soil protists?	4	2	[26, 241]
80	Which soil protist groups belong to the rare biosphere and are they functionally important?	4	2	[7, 201, 252, 257]
81	What is the proportion of omnivorous soil protists and are they functionally important?	4	1	
82	How does environmental filtering impact soil protist functional diversity between species (trait selection) or within species (phenotypic plasticity)?	4	1	[47]
83	What drives productivity in soil protists?	4	0	[258, 259]
84	Could soil protists be a novel mine of genetic resources with potential value for human well-being?	5	8	
85	How can we maximise the accuracy and precision of protist bioindicators?	5	6	[119, 183, 260-262]
86	How do anthropogenic activities affect soil protists?	5	6	[219, 263-268]
87	How can we model community changes including soil protists in response to global change?	5	5	[232, 269, 270]
88	Were there rapid local adaptations within soil protists that may have lead to niche changes during the Holocene, thereby obscuring palaeoreconstructions.	5	1	[128]
89	How can soil protists be used for resource production such as hydrogen?	5	1	[271]
90	How does a reduction of habitat space (e.g. due forest conversion) alter trophic level positions of indiviual	5	0	

	or the entire community of soil protists?			
91	How can we develop a universal, molecular high-throughput sequencing approach to target the entirety of soil protist diversity?	6	6	[201, 272, 273]
92	How should protist (functional) diversity be treated so as to be incorporated in food web/microbial loop models?	6	6	[138, 274]
93	How can we define standard protocols for measuring the most important functional traits for the ecology of soil protists?	6	5	[117]
94	How can we use ecological network analysis (or other such association techniques) to link protists with other soil organisms or functions?	6	5	[275-280]
95	How can eDNA and genomic experiments targeting soil protists reveal the most meaningful, functional results?	6	4	[281, 282]
96	How can we use soil protists as model organisms to study community assembly, biodiversity, trophic interactions, and eco-evolutionary processes?	6	4	[283]
97	Do transcriptome molecular genetic analyses reliably reveal the genetic basis for the ecophysiological roles of soil protists under different conditions?	6	3	[7, 64, 284]
98	How many, and which, soil protist clades are missed with current molecular methods as well as with cultivation based methods and how can we identify them?	6	2	[7, 145, 284]
99	How can we select ecologically relevant soil protist "model species" to investigate plant-fungal-bacteria interactions in specific types of soil?	6	2	[44]
100	How can we define standard protocols for using soil protists in micro-mesocosm experiments (e.g. in eco-evo studies)?	6	2	[283]
101	How can we best visualise the distribution and form of protists in soils (e.g. Micro-CT)?	6	2	
102	How reliable are identifications of soil protists based solely on morphology (no molecular barcoding) to answer (palaeo-) ecological questions and can they be optimized?	6	2	[285]
103	How consistent are results obtained in different ecological and taxonomic studies of soil protists considering identification biases, different resolutions and evolving taxonomy?	6	1	[202]
104	Which methods allow food preferences of protists to be studied in vivo?	6	1	[221, 245]
105	What is, and how can we access, the 'long-branch biosphere' (those with highly diverging sequences in barcoding regions) of soil protists?	6	1	[145]
106	Which soil protists are really unculturable and which can be cultivated using appropriate media/conditions?	6	0	
107	How can we design representative "synthetic soil protist communities" that successfully establish in soils?	6	0	

References

1. Shatilovich A, Stoupin D, Rivkina E. Ciliates from ancient permafrost: Assessment of cold resistance of the resting cysts. Eur J Protistol. 2015;51(3):230-40.
2. Mazur T, Hadaś E, Iwanicka I. The duration of the cyst stage and the viability and virulence of *Acanthamoeba* isolates. Tropical Medical Parasitology. 1995;46(2):106-8.
3. Feest A. Numbers of myxogastrids and other protozoa recovered from Bohemian soils. EKOLOGIA CSFR. 1986;5(2):125-33.
4. Corliss JO, Esser SC. Comments on the role of the cyst in the life cycle and survival of free-living protozoa. Transactions of the American Microscopical Society. 1974;93(4):578-93. doi: 10.2307/3225158.
5. Goodey T. Note on the remarkable retention of vitality by protozoa from old stored soils. Annals of Applied Biology. 1915;1(3-4):395-9. doi: 10.1111/j.1744-7348.1915.tb08008.x.
6. Shmakova L, Bondarenko N, Smirnov A. Viable species of *Flamella* (Amoebozoa: Variosea) isolated from ancient arctic permafrost sediments. Protist. 2016;167(1):13-30. doi: http://dx.doi.org/10.1016/j.protis.2015.11.001.
7. Bass D, Silberman JD, Brown MW, Tice AK, Jousset A, Geisen S, et al. 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. Environmental Microbiology. 2016;18(5):1604–19. doi: 10.1111/1462-2920.13235. PubMed PMID: 26914587.
8. Berney C, Geisen S, Van Wichelen J, Nitsche F, Vanormelingen P, Bonkowski M, et al. Expansion of the 'Reticulosphere': diversity of novel branching and network-forming amoebae helps to define Variosea (Amoebozoa). Protist. 2015;166(2):271-95. doi: http://dx.doi.org/10.1016/j.protis.2015.04.001.
9. Howe AT, Bass D, Vickerman K, Chao EE, Cavalier-Smith T. Phylogeny, taxonomy, and astounding genetic diversity of Glissomonadida ord. nov., the dominant gliding zooflagellates in soil (Protozoa: Cercozoa). Protist. 2009;160(2):159-89.
10. Howe AT, Bass D, Chao EE, Cavalier-Smith T. New genera, species, and improved phylogeny of Glissomonadida (Cercozoa). Protist. 2011;162(5):710-22.
11. Bass D, Howe AT, Mylnikov AP, Vickerman K, Chao EE, Smallbone JE, et al. Phylogeny and classification of Cercomonadida (Protozoa, Cercozoa): *Cercomonas*, *Eocercomonas*, *Paracercomonas*, and *Cavernomonas* gen. nov. Protist. 2009;160(4):483-521.
12. Foissner W. Diversity and ecology of soil flagellates. In: Patterson DJ, Larsen, J., editor. The Biology of Free-Living Heterotrophic Flagellates. 45. Oxford: Clarendon Press; 1991. p. 93-112.
13. Foissner W, Agatha S, Berger H. Soil ciliates (Protozoa, Ciliophora) from Namibia (Southwest Africa), with emphasis on two contrasting environments, the Etosha region and the Namib Desert: Denisia; 2002.
14. Foissner W, Berger H, Xu K, Zechmeister-Boltenstern S. A huge, undescribed soil ciliate (Protozoa: Ciliophora) diversity in natural forest stands of Central Europe. Biodiversity and Conservation. 2005;14(3):617-701.
15. Foissner W. Terrestrial and semiterrestrial ciliates (Protozoa, Ciliophora) from Venezuela and Galápagos: Denisia; 2016.
16. Brown T, Cursons R, Keys E. Amoebae from antarctic soil and water. Applied and Environmental Microbiology. 1982;44(2):491-3.
17. Lahr DJG, Parfrey LW, Mitchell EAD, Katz LA, Lara E. The chastity of amoebae: re-evaluating evidence for sex in amoeboid organisms. Proc R Soc Ser B-Bio. 2011;278(1715):2081-90.

18. Tekle YI, Anderson OR, Lecky AF. Evidence of parasexual activity in “Asexual Amoebae” *Cochliopodium* spp. (Amoebozoa): extensive cellular and nuclear fusion. Protist. 2014;165(5):676-87.
19. Singh V, Pandita SK, Tewari R, van Hengstum PJ, Pillai SS, Agnihotri D, et al. Thecamoebians (testate amoebae) straddling the Permian-Triassic boundary in the Guryul Ravine Section, India: evolutionary and palaeoecological implications. Plos One. 2015;10(8):e0135593.
20. Foissner W, Schiller W. Stable for 15 million years: scanning electron microscope investigation of Miocene euglyphid thecamoebians from Germany, with description of the new genus *Scutiglypha*. Eur J Protistol. 2001;37(2):167-80.
21. Schmidt AR, Schönborn W, Schäfer U. Diverse fossil amoebae in German Mesozoic amber. Palaeontology. 2004;47(2):185-97.
22. Fiz-Palacios O, Romeralo M, Ahmadzadeh A, Weststrand S, Ahlberg PE, Baldauf S. Did terrestrial diversification of amoebas (Amoebozoa) occur in synchrony with land plants? PloS one. 2013;8(9):e74374. doi: 10.1371/journal.pone.0074374.
23. Martín-González A, Wierzchos J, Gutiérrez JC, Alonso J, Ascaso C. Morphological stasis of protists in Lower Cretaceous amber. Protist. 2008;159(2):251-7.
24. Girard V, Adl SM. Amber microfossils: on the validity of species concept. Comptes Rendus Palevol. 2011;10(2):189-200.
25. Lahr DJ, Bosak T, Lara E, Mitchell EA. The Phanerozoic diversification of silica-cycling testate amoebae and its possible links to changes in terrestrial ecosystems. PeerJ. 2015;3:e1234.
26. Jassey VEJ, Signarbieux C, Hättenschwiler S, Bragazza L, Buttler A, Delarue F, et al. An unexpected role for mixotrophs in the response of peatland carbon cycling to climate warming. Scientific Reports. 2015;5:16931. doi: 10.1038/srep16931

http://www.nature.com/articles/srep16931#supplementary-information.
27. Berg G, Rybakova D, Grube M, Koberl M. The plant microbiome explored: implications for experimental botany. Journal of Experimental Botany. 2016;67(4):995-1002. doi: 10.1093/jxb/erv466. PubMed PMID: 26547794.
28. Harder CB, Ronn R, Brejnrod A, Bass D, Al-Soud WA, Ekelund F. Local diversity of heathland Cercozoa explored by in-depth sequencing. The ISME Journal. 2016;10(10):2488-97. doi: 10.1038/ismej.2016.31. PubMed PMID: 26953604; PubMed Central PMCID: PMCPMC5030685.
29. Ploch S, Rose LE, Bass D, Bonkowski M. High Diversity Revealed in Leaf-Associated Protists (Rhizaria: Cercozoa) of Brassicaceae. Journal of Eukaryotic Microbiology. 2016;63(5):635-41. doi: 10.1111/jeu.12314. PubMed PMID: 27005328; PubMed Central PMCID: PMCPMC5031217.
30. Bates ST, Clemente JC, Flores GE, Walters WA, Parfrey LW, Knight R, et al. Global biogeography of highly diverse protistan communities in soil. The ISME Journal. 2013;7(3):652-9. doi: 10.1038/ismej.2012.147.
31. Ramirez KS, Leff JW, Barberán A, Bates ST, Betley J, Crowther TW, et al. Biogeographic patterns in below-ground diversity in New York City's Central Park are similar to those observed globally. Proc Biol Sci. 2014;281(1795). Epub 2014/10/03. doi: 10.1098/rspb.2014.1988. PubMed PMID: 25274366; PubMed Central PMCID: PMCPmc4213626.
32. Dupont AO, Griffiths RI, Bell T, Bass D. Differences in soil micro-eukaryotic communities over soil pH gradients are strongly driven by parasites and saprotrophs. Environmental Microbiology. 2016;18(6):2010-24. doi: 10.1111/1462-2920.13220. PubMed PMID: 26768496.
33. Glaser K, Kuppardt A, Boenigk J, Harms H, Fetzer I, Chatzinotas A. The influence of environmental factors on protistan microorganisms in grassland soils along a land-use gradient. Science of the Total Environment. 2015;537:33-42.

34. Marcisz K, Lamentowicz Ł, Słowińska S, Słowiński M, Muszak W, Lamentowicz M. Seasonal changes in Sphagnum peatland testate amoeba communities along a hydrological gradient. European Journal of Protistology. 2014;50(5):445-55. doi: http://dx.doi.org/10.1016/j.ejop.2014.07.001.
35. Mazei YA. Biodiversity patterns in protozoan communities: linking processes and scales. Protistology. 2008;5(4).
36. Tsyganov AN, Aerts R, Nijs I, Cornelissen JH, Beyens L. Sphagnum-dwelling testate amoebae in subarctic bogs are more sensitive to soil warming in the growing season than in winter: the results of eight-year field climate manipulations. Protist. 2012;163(3):400-14.
37. Fernández LD, Fournier B, Rivera R, Lara E, Mitchell EAD, Hernández CE. Water–energy balance, past ecological perturbations and evolutionary constraints shape the latitudinal diversity gradient of soil testate amoebae in south-western South America. Global Ecology and Biogeography. 2016;25(10):1216-27.
38. Lentendu G, Wubet T, Chatzinotas A, Wilhelm C, Buscot F, Schlegel M. Effects of long-term differential fertilization on eukaryotic microbial communities in an arable soil: a multiple barcoding approach. Mol Ecol. 2014;23(13):3341-55. doi: 10.1111/mec.12819.
39. Liu Q-S, Yan S-Z, Chen S-L. Species diversity of myxomycetes associated with different terrestrial ecosystems, substrata (microhabitats) and environmental factors. Mycological Progress. 2015;14(5):1-13.
40. Heger TJ, Mitchell, E. A. D., Leander, B. S. Holarctic phylogeography of the testate amoeba *Hyalosphenia papilio* (Amoebozoa: Arcellinida) reveals extensive genetic diversity explained more by environment than dispersal limitation. Molecular Ecology. 2013;in press, DOI: 10.1111/mec.12449.
41. Scharroba A, Dibbern D, Hünninghaus M, Kramer S, Moll J, Butenschoen O, et al. Effects of resource availability and quality on the structure of the micro-food web of an arable soil across depth. Soil Biol Biochem. 2012;50(0):1-11. doi: 10.1016/j.soilbio.2012.03.002.
42. Romeralo M, Moya-Laraño J, Lado C. Social amoebae: environmental factors influencing their distribution and diversity across south-western Europe. Microbial Ecology. 2011;61(1):154-65.
43. Rønn R, Thomsen IK, Jensen B. Naked amoebae, flagellates, and nematodes in soils of different texture. European Journal of Soil Biology. 1995;31(3):135-41.
44. Feest A. The quantitative ecology of soil mycetozoa. Progress in Protistology. 1987;2:331-61.
45. Feest A, Stephenson S. The response of myxogastrids to soil amendments. Mycosphere. 2014;5(6):821-9.
46. Fernández LD. Source-sink dynamics shapes the spatial distribution of soil protists in an arid shrubland of northern Chile. . Journal of Arid Environments 2015;113:121-5.
47. Fournier B, Malysheva E, Mazei Y, Moretti M, Mitchell EA. Toward the use of testate amoeba functional traits as indicator of floodplain restoration success. European Journal of Soil Biology. 2012;49:85-91.
48. Fournier B, Coffey EED, van der Knaap WO, Fernández LD, Bobrov A, Mitchell EAD. A legacy of human-induced ecosystem changes: spatial processes drive the taxonomic and functional diversities of testate amoebae in *Sphagnum* peatlands of the Galápagos. Journal of Biogeography. 2016;43(3):533-43. doi: 10.1111/jbi.12655.
49. Lara E, Roussel-Delif L, Fournier B, Wilkinson DM, Mitchell EAD. Soil microorganisms behave like macroscopic organisms: patterns in the global distribution of soil euglyphid testate amoebae. Journal of Biogeography. 2016;43:520–32 doi: 10.1111/jbi.12660.
50. Heger TJ, Booth RK, Sullivan ME, Wilkinson DM, Warner BG, Asada T, et al. Rediscovery of *Nebela ansata* (Amoebozoa: Arcellinida) in eastern North America: biogeographical implications. Journal of Biogeography. 2011;38(10):1897-906.

51. Yang J, Smith HG, Sherratt TN, Wilkinson DM. Is there a size limit for cosmopolitan distribution in free-living microorganisms? A biogeographical analysis of testate amoebae from polar areas. Microbial Ecology. 2010;59(4):635-45.
52. Bass D, Richards TA, Matthai L, Marsh V, Cavalier-Smith T. DNA evidence for global dispersal and probable endemicity of protozoa. BMC Evolutionary Biology. 2007;7. doi: 162

10.1186/1471-2148-7-162. PubMed PMID: WOS:000252444400001.
53. Azovsky A, Mazei Y. Do microbes have macroecology? Large-scale patterns in the diversity and distribution of marine benthic ciliates. Global Ecology and Biogeography. 2013;22(2):163-72.
54. Fernández LD, Lara, E., Mitchell, E. A. D. . Checklist diversity and distirbution of testate amoebae in Chile. Eur J Protistol. 2015;51:409-24.
55. Foissner W, Blatterer H, Foissner I. The hemimastigophora (*Hemimastix amphikineta* nov. gen., nov. spec.), a new protistan phylum from gondwanian soils. Eur J Protistol. 1988;23(4):361-83. doi: http://dx.doi.org/10.1016/S0932-4739(88)80027-0.
56. Chao A, C. Li P, Agatha S, Foissner W. A statistical approach to estimate soil ciliate diversity and distribution based on data from five continents. Oikos. 2006;114(3):479-93. doi: 10.1111/j.2006.0030-1299.14814.x.
57. Foissner W. Biogeography and dispersal of micro-organisms: a review emphasizing protists. Acta Protozool. 2006;45(2):111-36. PubMed PMID: WOS:000237675700001.
58. Foissner W. An updated compilation of world soil ciliates (Protozoa, Ciliophora), with ecological notes, new records, and descriptions of new species. European Journal of Protistology. 1998;34(2):195-235. doi: 10.1016/s0932-4739(98)80028-x. PubMed PMID: WOS:000074484700011.
59. Foissner W. Protist diversity: estimates of the near-imponderable. Protist. 1999;150(4):363-8. doi: 10.1016/S1434-4610(99)70037-4. PubMed PMID: 10714770.
60. Foissner W. Protist diversity and distribution: some basic considerations. Biodivers Conserv. 2008;17(2):235-42. doi: DOI 10.1007/s10531-007-9248-5. PubMed PMID: WOS:000252689400001.
61. Anderson OR. Experimental evidence for non-encysted, freeze-resistant stages of terrestrial naked amoebae capable of resumed growth after freeze-thaw events. Acta Protozoologica. 2016;55(1):1925.
62. Geisen S, Tveit AT, Clark IM, Richter A, Svenning MM, Bonkowski M, et al. Metatranscriptomic census of active protists in soils. ISME Journal. 2015;9(10):2178-90. doi: 10.1038/ismej.2015.30. PubMed PMID: 25822483; PubMed Central PMCID: PMCPMC4579471.
63. Ekelund F, Frederiksen HB, Rønn R. Population dynamics of active and total ciliate populations in arable soil amended with wheat. Applied and Environmental Microbiology. 2002;68(3):1096-101.
64. Song C, Mazzola M, Cheng X, Oetjen J, Alexandrov T, Dorrestein P, et al. Molecular and chemical dialogues in bacteria-protozoa interactions. Scientific Reports. 2015;5.
65. Fieseler L, Doyscher D, Loessner MJ, Schuppler M. Acanthamoeba release compounds which promote growth of Listeria monocytogenes and other bacteria. Applied and Environmental Microbiology. 2014;98(7):3091-7.
66. Rønn R, Vestergård M, Ekelund F. Interactions between bacteria, protozoa and nematodes in soil. Acta Protozool. 2012;51:223-35.
67. Eisenmann H, Harms H, Meckenstock R, Meyer EI, Zehnder AJ. Grazing of a *Tetrahymena* sp. on adhered bacteria in percolated columns monitored by in situ hybridization with fluorescent oligonucleotide probes. Applied and Environmental Microbiology. 1998;64(4):1264-9.

68. Pedersen AL, Ekelund F, Johansen A, Winding A. Interaction of bacteria-feeding soil flagellates and *Pseudomonas* spp. Biology and Fertility of Soils. 2010;46(2):151-8.
69. Pedersen AL, Winding A, Altenburger A, Ekelund F. Protozoan growth rates on secondary-metabolite-producing *Pseudomonas* spp. correlate with high-level protozoan taxonomy. FEMS Microbiology Letters. 2011;316(1):16-22.
70. Rønn R, McCaig AE, Griffiths BS, Prosser JI. Impact of protozoan grazing on bacterial community structure in soil microcosms. Applied and Environmental Microbiology. 2002;68(12):6094-105.
71. Saleem M, Fetzer I, Harms H, Chatzinotas A. Diversity of protists and bacteria determines predation performance and stability. The ISME Journal. 2013;7(10):1912-21. Epub 2013/06/15. doi: 10.1038/ismej.2013.95. PubMed PMID: 23765100; PubMed Central PMCID: PMCPMC3965320.
72. Glücksman E, Bell T, Griffiths RI, Bass D. Closely related protist strains have different grazing impacts on natural bacterial communities. Environmental Microbiology. 2010;12(12):3105-13. doi: 10.1111/j.1462-2920.2010.02283.x. PubMed PMID: 20602629.
73. Krivtsov V, Liddell K, Bezginova T, Salmond R, Garside A, Thompson J, et al. Ecological interactions of heterotrophic flagellates, ciliates and naked amoebae in forest litter of the Dawyck Cryptogamic Sanctuary (Scotland, UK). Eur J Protistol. 2003;39(2):183-98.
74. Wilkinson DM, Creevy AL, Kalu CL, Schwartzman DW. Are heterotrophic and silica-rich eukaryotic microbes an important part of the lichen symbiosis? Mycology. 2015;6(1):4-7.
75. Parry JD, Allen I. Laskin JWB, Geoffrey MG. Protozoan grazing of freshwater biofilms. Adv Appl Microbiol. 2004;54:167-96. doi: 10.1016/S0065-2164(04)54007-8. PubMed PMID: 15251281.
76. Boenigk J, Arndt H. Bacterivory by heterotrophic flagellates: community structure and feeding strategies. Anton Leeuw. 2002;81(1-4):465-80. PubMed PMID: 12448743.
77. Rosenberg K, Bertaux J, Krome K, Hartmann A, Scheu S, Bonkowski M. Soil amoebae rapidly change bacterial community composition in the rhizosphere of *Arabidopsis thaliana*. ISME Journal. 2009;3(6):675-84.
78. Kreuzer K, Adamczyk J, Iijima M, Wagner M, Scheu S, Bonkowski M. Grazing of a common species of soil protozoa (*Acanthamoeba castellanii*) affects rhizosphere bacterial community composition and root architecture of rice (*Oryza sativa* L.). Soil Biology and Biochemistry. 2006;38(7):1665-72. doi: 10.1016/j.soilbio.2005.11.027.
79. Pedersen AL, Nybroe O, Winding A, Ekelund F, Bjørnlund L. Competitive success of biocontrol strains, *Pseudomonas fluorescens* CHA0 and *P*. sp. DSS73, depends on feeding mode of bacterial feeders (the nematode *Caenorhabditis elegans* or the flagellate *Cercomonas longicaudata*) in flow cytometric assessed microcosms. Microbial Ecology. 2009;57:501-9.
80. Bjørnlund L, Rønn R. 'David and Goliath' of the soil food web - Flagellates that kill nematodes. Soil Biology and Biochemistry. 2008;40(8):2032-9.
81. Rønn R, Grunert J, Ekelund F. Protozoan response to addition of the bacteria *Mycobacterium chlorophenolicum* and *Pseudomonas chlororaphis* to soil microcosms. Biology and Fertility of Soils. 2001;33(2):126-31.
82. Stephenson S.L., Feest A. Ecology of soil Eumycetozoans. Acta Protozoologica. 2013;51:201-8.
83. Petz W, Foissner W, Adam H. Culture, food selection and growth rate in the mycophagous ciliate *Grossglockneria acuta* Foissner, 1980: first evidence of autochthonous soil ciliates. Soil Biology and Biochemistry. 1985;17(6):871-5. doi: 10.1016/0038-0717(85)90149-x.

84. Bonkowski M, Clarholm M. Stimulation of plant growth through interactions of bacteria and protozoa: testing the auxiliary microbial loop hypothesis. Acta Protozool. 2012;51(3):237-47. doi: Doi 10.4467/16890027ap.12.019.0765. PubMed PMID: WOS:000314646400005.
85. Koller R, Rodriguez A, Robin C, Scheu S, Bonkowski M. Protozoa enhance foraging efficiency of arbuscular mycorrhizal fungi for mineral nitrogen from organic matter in soil to the benefit of host plants. New Phytologist. 2013;199(1):203-11. doi: 10.1111/nph.12249.
86. Herdler S, Kreuzer K, Scheu S, Bonkowski M. Interactions between arbuscular mycorrhizal fungi (*Glomus intraradices*, Glomeromycota) and amoebae (*Acanthamoeba castellanii*, Protozoa) in the rhizosphere of rice (*Oryza sativa*). Soil Biology and Biochemistry. 2008;40(3):660-8. doi: 10.1016/j.soilbio.2007.09.026.
87. Gilbert D, Amblard C, Bourdier G, Francez A-J. The microbial loop at the surface of a peatland: structure, function, and impact of nutrient input. Microbial Ecology. 1998;35(1):83-93.
88. Bonkowski M. Protozoa and plant growth: the microbial loop in soil revisited. New Phytologist. 2004;162(3):617-31. doi: 10.1111/j.1469-8137.2004.01066.x. PubMed PMID: WOS:000221913500005.
89. Koller R, Scheu S, Bonkowski M, Robin C. Protozoa stimulate N uptake and growth of arbuscular mycorrhizal plants. Soil Biol Biochem. 2013;65(0):204-10. doi: http://dx.doi.org/10.1016/j.soilbio.2013.05.020.
90. Clarholm M. Interactions of bacteria, protozoa and plants leading to mineralization of soil nitrogen. Soil Biol Biochem. 1985;17(2):181-7. doi: 10.1016/0038-0717(85)90113-0. PubMed PMID: WOS:A1985AHA5000011.
91. Porazinska DL, Bardgett RD, Blaauw MB, Hunt HW, Parsons AN, Seastedt TR, et al. Relationships at the aboveground-belowground interface: plants, soil biota, and soil processes. Ecol Monogr. 2003;73(3):377-95. doi: Doi 10.1890/0012-9615(2003)073[0377:Rataip]2.0.Co;2. PubMed PMID: WOS:000184866400003.
92. Pankhurst C, Ophel-Keller K, Doube B, Gupta V. Biodiversity of soil microbial communities in agricultural systems. Biodiversity and Conservation. 1996;5(2):197-209.
93. Trap J, Bonkowski M, Plassard C, Villenave C, Blanchart E. Ecological importance of soil bacterivores for ecosystem functions. Plant Soil. 2016;398(1):1-24. doi: 10.1007/s11104-015-2671-6.
94. Friman VP, Buckling A. Effects of predation on real-time host–parasite coevolutionary dynamics. Ecology Letters. 2013;16(1):39-46.
95. Murase J, Noll M, Frenzel P. Impact of protists on the activity and structure of the bacterial community in a rice field soil. Applied and Environmental Microbiology. 2006;72(8):5436-44. doi: 10.1128/aem.00207-06.
96. Liess A, Diehl S. Effects of enrichment on protist abundances and bacterial composition in simple microbial communities. Oikos. 2006;114(1):15-26.
97. Lawler SP, Morin PJ. Food web architecture and population dynamics in laboratory microcosms of protists. American Naturalist. 1993:675-86.
98. Winding A, Oberender J. Effects of the biological control agent *Pseudomonas fluorescens* CHA0 on soil protozoa. Acta Protozoologica. 2011;51:249-58.
99. Krashevska V, Sandmann D, Maraun M, Scheu S. Moderate changes in nutrient input alter tropical microbial and protist communities and belowground linkages. The ISME journal. 2014;8(5):1126-34. doi: 10.1038/ismej.2013.209.
100. Bonkowski M, Griffiths B, Scrimgeour C. Substrate heterogeneity and microfauna in soil organic ‘hotspots’ as determinants of nitrogen capture and growth of ryegrass. Appl Soil Ecol. 2000;14(1):37-53. doi: http://dx.doi.org/10.1016/S0929-1393(99)00047-5.

101. Alphei J, Bonkowski M, Scheu S. Protozoa, Nematoda and Lumbricidae in the rhizosphere of *Hordelymus europeaus* (Poaceae): faunal interactions, response of microorganisms and effects on plant growth Oecologia. 1996;106(1):111-26. doi: 10.1007/bf00334413.
102. Anderson OR. The role of amoeboid protists and the microbial community in moss-rich terrestrial ecosystems: biogeochemical implications for the carbon budget and carbon cycle, especially at higher latitudes. J Eukaryot Microbiol. 2008;55(3):145-50. doi: 10.1111/j.1550-7408.2008.00319.x.
103. Gadd GM, Raven JA. Geomicrobiology of eukaryotic microorganisms. Geomicrobiology Journal. 2010;27(6-7):491-519.
104. Osler GH, Sommerkorn M. Toward a complete soil C and N cycle: incorporating the soil fauna. Ecology. 2007;88(7):1611-21.
105. Wilkinson DM, Mitchell EAD. Testate amoebae and nutrient cycling with particular reference to soils. Geomicrobiology Journal. 2010;27(6-7):520-33.
106. Adl SM, Gupta VS. Protists in soil ecology and forest nutrient cycling. Canadian Journal of Forest Research. 2006;36(7):1805-17.
107. Mitra A, Flynn KJ, Tillmann U, Raven JA, Caron D, Stoecker DK, et al. Defining planktonic protist functional groups on mechanisms for energy and nutrient acquisition: incorporation of diverse mixotrophic strategies. Protist. 2016;167(2):106-20. doi: http://dx.doi.org/10.1016/j.protis.2016.01.003.
108. Anderson OR. Laboratory and field-based studies of abundances, small-scale patchiness, and diversity of gymnamoebae in soils of varying porosity and organic content: evidence of microbiocoenoses. J Eukaryot Microbiol. 2002;49(1):17-23. doi: 10.1111/j.1550-7408.2002.tb00334.x.
109. Kuikman P, Van Elsas J, Jansen A, Burgers S, Van Veen J. Population dynamics and activity of bacteria and protozoa in relation to their spatial distribution in soil. Soil Biology and Biochemistry. 1990;22(8):1063-73.
110. Coûteaux M-M, Darbyshire JF. Functional diversity amongst soil protozoa. Applied Soil Ecology. 1998;10(3):229-37.
111. Fitter A, Gilligan C, Hollingworth K, Kleczkowski A, Twyman R, Pitchford J. Biodiversity and ecosystem function in soil. Functional Ecology. 2005;19(3):369-77.
112. Krashevska V, Sandmann D, Maraun M, Scheu S. Consequences of exclusion of precipitation on microorganisms and microbial consumers in montane tropical rainforests. Oecologia. 2012;170(4):1067-76. doi: 10.1007/s00442-012-2360-6.
113. Eisenhauer N, Cesarz S, Koller R, Worm K, Reich PB. Global change belowground: impacts of elevated CO2, nitrogen, and summer drought on soil food webs and biodiversity. Global Change Biology. 2012;18(2):435-47.
114. Tsyganov AN, Nijs I, Beyens L. Does climate warming stimulate or inhibit soil protist communities? A test on testate amoebae in high-arctic tundra with free-air temperature increase. Protist. 2011;162(2):237-48.
115. Smith H, Crook M. Temperature relations of soil protists in the Antarctic zone as an indicator of climatic change. Acta Zoologica Fennica. 1995;(196):183-5.
116. Royles J, Amesbury MJ, Convey P, Griffiths H, Hodgson DA, Leng MJ, et al. Plants and soil microbes respond to recent warming on the Antarctic Peninsula. Current Biology. 2013;23(17):1702-6.
117. Fournier B, Gillet F, Le Bayon RC, Mitchell EA, Moretti M. Functional responses of multitaxa communities to disturbance and stress gradients in a restored floodplain. Journal of Applied Ecology. 2015;52(5):1364-73.
118. Lamentowicz M, Gałka M, Obremska M, Kühl N, Lücke A, Jassey V. Reconstructing climate change and ombrotrophic bog development during the last 4000years in northern Poland using biotic proxies, stable isotopes and trait-based approach. Palaeogeography, Palaeoclimatology, Palaeoecology. 2015;418:261-77.

119. Lara E, Acosta-Mercado D. A molecular perspective on ciliates as soil bioindicators. European Journal of Soil Biology. 2012;49:107-11. doi: 10.1016/j.ejsobi.2011.11.001. PubMed PMID: WOS:000302736500015.
120. Meyer C, Gilbert D, Gillet F, Moskura M, Franchi M, Bernard N. Using "bryophytes and their associated testate amoeba" microsystems as indicators of atmospheric pollution. Ecological Indicators. 2012;13(1):144-51. doi: 10.1016/j.ecolind.2011.05.020. PubMed PMID: WOS:000296042500015.
121. Payne RJ. Seven reasons why protists make useful bioindicators. Acta Protozoologica. 2013;52(3):105.
122. Foissner W. Protozoa as bioindicators in agroecosystems, with emphasis on farming practices, biocides, and biodiversity. Agriculture, Ecosystems & Environment. 1997;62(2):93-103.
123. Valentine J, Davis SR, Kirby JR, Wilkinson DM. The use of testate amoebae in monitoring peatland restoration management: case studies from North West England and Ireland. Acta Protozoologica. 2013;52(3):129.
124. Foissner W. Soil protozoa as bioindicators: pros and cons, methods, diversity, representative examples. Agriculture, Ecosystems & Environment. 1999;74(1-3):95-112. doi: 10.1016/s0167-8809(99)00032-8. PubMed PMID: WOS:000082010000007.
125. Heger TJ, Straub F, Mitchell EA. Impact of farming practices on soil diatoms and testate amoebae: A pilot study in the DOK-trial at Therwil, Switzerland. European Journal of Soil Biology. 2012;49:31-6.
126. Foissner W. Soil protozoa: fundamental problems, ecological significance, adaptations in ciliates and testaceans, bioindicators, and guide to the literature. Progress in Protistology. 1987;2:69-212.
127. Foissner W. Soil protozoa as bioindicators in ecosystems under human influence. In: J.F. D, editor. Soil Protozoa. Wallingford, Oxon: CAB International; 1994. p. 147-93.
128. Fournier B, Lara E, Jassey VEJ, Mitchell EAD. Functional traits as a new approach for interpreting testate amoeba palaeo-records in peatlands and assessing the causes and consequences of past changes in species composition. The Holocene. 2015;25(9):1375-83. doi: 10.1177/0959683615585842. PubMed PMID: WOS:000360004500001.
129. Rubinstein RL, Kadilak AL, Cousens VC, Gage DJ, Shor LM. Protist-facilitated particle transport using emulated soil micromodels. Environmental science & technology. 2015;49(3):1384-91. doi: 10.1021/es503424z.
130. Cotterill F, Al-Rasheid K, Foissner W. Conservation of protists: is it needed at all? Biodiversity and Conservation. 2008;17(2):427-43.
131. Qin Y, Payne R, Yang X, Yao M, Xue J, Gu Y, et al. Testate amoebae as indicators of water quality and contamination in shallow lakes of the Middle and Lower Yangtze Plain. Environmental Earth Sciences. 2016;75(7):1-11.
132. Cairns Jr J. Can microbial species with a cosmopolitan distribution become extinct? Speculations in Science and Technology. 1993;16(1):69-73.
133. Wilkinson DM. Do we need to worry about the conservation of microorganisms. Earthy realism: the meaning of Gaia. 2007:52-9.
134. Boenigk J, Ereshefsky M, Hoef-Emden K, Mallet J, Bass D. Concepts in protistology: species definitions and boundaries. Eur J Protistol. 2012;48(2):96-102.
135. Pawlowski J, Audic S, Adl S, Bass D, Belbahri L, Berney C, et al. CBOL protist working group: barcoding eukaryotic richness beyond the animal, plant, and fungal kingdoms. Plos Biol. 2012;10(11):e1001419.
136. Tekle YI. DNA barcoding in Amoebozoa and challenges: the example of *Cochliopodium*. Protist. 2014;165(4):473-84. doi: http://dx.doi.org/10.1016/j.protis.2014.05.002.

137. Santos SS, Nielsen TK, Hansen LH, Winding A. Comparison of three DNA extraction methods for recovery of soil protist DNA. Journal of Microbiological Methods. 2015;115:13-9. doi: http://dx.doi.org/10.1016/j.mimet.2015.05.011.
138. Feest A, Madelin M. A method for the enumeration of myxomycetes in soils and its application to a wide range of soils. FEMS Microbiology Ecology. 1985;1(2):103-9.
139. Bahram M, Kohout P, Anslan S, Harend H, Abarenkov K, Tedersoo L. Stochastic distribution of small soil eukaryotes resulting from high dispersal and drift in a local environment. The ISME journal. 2016;10(4):885-96. doi: 10.1038/ismej.2015.164. PubMed PMID: 26394006; PubMed Central PMCID: PMCPMC4796928.
140. Engel M, Behnke A, Bauerfeld S, Bauer C, Buschbaum C, Volkenborn N, et al. Sample pooling obscures diversity patterns in intertidal ciliate community composition and structure. FEMS Microbiology Ecology. 2012;79(3):741-50. Epub 2011/11/19. doi: 10.1111/j.1574-6941.2011.01255.x. PubMed PMID: 22093023.
141. Jassey VEJ, Lamentowicz Ł, Robroek BJM, Gąbka M, Rusińska A, Lamentowicz M. Plant functional diversity drives niche-size-structure of dominant microbial consumers along a poor to extremely rich fen gradient. J Ecol. 2014;102(5):1150-62. doi: 10.1111/1365-2745.12288.
142. Mitchell EA, Lamentowicz M, Payne RJ, Mazei Y. Effect of taxonomic resolution on ecological and palaeoecological inference–a test using testate amoeba water table depth transfer functions. Quaternary Science Reviews. 2014;91:62-9.
143. del Campo J, Sieracki ME, Molestina R, Keeling P, Massana R, Ruiz-Trillo I. The others: our biased perspective of eukaryotic genomes. Trends in Ecology and Evolution. 2014;29(5):252-9.
144. Keeling PJ, Burki F, Wilcox HM, Allam B, Allen EE, Amaral-Zettler LA, et al. The Marine Microbial Eukaryote Transcriptome Sequencing Project (MMETSP): illuminating the functional diversity of eukaryotic life in the oceans through transcriptome sequencing. Plos Biol. 2014;12(6):e1001889.
145. Kolisko M, Boscaro V, Burki F, Lynn DH, Keeling PJ. Single-cell transcriptomics for microbial eukaryotes. Current Biology. 2014;24(22):R1081-R2.
146. Koch TA, Ekelund F. Strains of the heterotrophic flagellate *Bodo designis* from different environments vary considerably with respect to salinity preference and SSU rRNA gene composition. Protist. 2005;156(1):97-112.
147. Parfrey LW, Walters WA, Lauber CL, Clemente JC, Berg-Lyons D, Teiling C, et al. Communities of microbial eukaryotes in the mammalian gut within the context of environmental eukaryotic diversity. Frontiers in Microbiology. 2014;5:298. doi: 10.3389/fmicb.2014.00298. PubMed PMID: PMC4063188.
148. Heger TJ, Mitchell EAD, Todorov M, Golemansky V, Lara E, Leander BS, et al. Molecular phylogeny of euglyphid testate amoebae (Cercozoa: Euglyphida) suggests transitions between marine supralittoral and freshwater/terrestrial environments are infrequent. Molecular Phylogenetics and Evolution. 2010;55(1):113-22. doi: http://dx.doi.org/10.1016/j.ympev.2009.11.023.
149. Belbahri L, Calmin G, Mauch F, Andersson JO. Evolution of the cutinase gene family: evidence for lateral gene transfer of a candidate *Phytophthora* virulence factor. Gene. 2008;408(1):1-8.
150. Rygiewicz PT, Monleon VJ, Ingham ER, Martin KJ, Johnson MG. Soil life in reconstructed ecosystems: initial soil food web responses after rebuilding a forest soil profile for a climate change experiment. Applied Soil Ecology. 2010;45(1):26-38.
151. Darby BJ, Housman DC, Zaki AM, Shamout Y, Adl SM, Belnap J, et al. Effects of altered temperature and precipitation on desert protozoa associated with biological soil crusts. Journal of Eukaryotic Microbiology. 2006;53(6):507-14.

152. Anderson OR. The effects of release from cold stress on the community composition of terrestrial gymnamoebae: A laboratory-based ecological study simulating transition from winter to spring. Acta Protozoologica. 2004;43(1):21-8.
153. Romeralo M, Escalante R, Baldauf SL. Evolution and diversity of dictyostelid social amoebae. Protist. 2012;163(3):327-43.
154. Gomez-Valero L, Rusniok C, Buchrieser C. *Legionella pneumophila*: population genetics, phylogeny and genomics. Infection, Genetics and Evolution. 2009;9(5):727-39.
155. Bui XT, Winding A, Qvortrup K, Wolff A, Bang DD, Creuzenet C. Survival of *Campylobacter jejuni* in co-culture with *Acanthamoeba castellanii*: role of amoeba-mediated depletion of dissolved oxygen. Environmental Microbiology. 2012;14(8):2034-47.
156. Clarke M, Lohan AJ, Liu B, Lagkouvardos I, Roy S, Zafar N, et al. Genome of *Acanthamoeba castellanii* highlights extensive lateral gene transfer and early evolution of tyrosine kinase signaling. Genome Biol. 2013;14(2):R11. Epub 2013/02/05. doi: 10.1186/gb-2013-14-2-r11. PubMed PMID: 23375108; PubMed Central PMCID: PMCPmc4053784.
157. He D, Fu CJ, Baldauf SL. Multiple origins of eukaryotic cox15 suggest horizontal gene transfer from bacteria to jakobid mitochondrial DNA. Molecular Biology and Evolution. 2016;33(1):122-33. doi: 10.1093/molbev/msv201. PubMed PMID: 26412445.
158. Gilbert C, Cordaux R. Horizontal transfer and evolution of prokaryote transposable elements in eukaryotes. Genome Biology and Evolution. 2013;5(5):822-32.
159. Krashevska V, Bonkowski M, Maraun M, Scheu S. Testate amoebae (protista) of an elevational gradient in the tropical mountain rain forest of Ecuador. Pedobiologia. 2007;51(4):319-31. doi: 10.1016/j.pedobi.2007.05.005.
160. Lamentowicz M, Bragazza L, Buttler A, Jassey VEJ, Mitchell EAD. Seasonal patterns of testate amoeba diversity, community structure and species–environment relationships in four Sphagnum-dominated peatlands along a 1300 m altitudinal gradient in Switzerland. Soil Biol Biochem. 2013;67(0):1-11. doi: http://dx.doi.org/10.1016/j.soilbio.2013.08.002.
161. Sowerby A, Emmett B, Beier C, Tietema A, Penuelas J, Estiarte M, et al. Microbial community changes in heathland soil communities along a geographical gradient: interaction with climate change manipulations. Soil Biology and Biochemistry. 2005;37(10):1805-13.
162. Heger TJ, Derungs N, Theurillat J, Mitchell E. Testate amoebae like it hot: species richness decreases along a subalpine-alpine altitudinal gradient in both natural *Calluna vulgaris* litter and transplanted *Minuartia sedoides* cushions. Microbial Ecology. 2016;71:725-34.
163. Mazei YA, Marfina O, Chernyshov V. Distribution of soil-inhabiting testate amoebae along a mountain slope (Baikal Lake region, Khamar-Daban ridge, Cherskii peak). Biology Bulletin. 2012;39(10):800-4.
164. Cowling AJ. Protozoan distribution and adaptation. In: (ed.) DJ, editor. Soil Protozoa. Wallingford: CAB International; 1994. p. 5-42.
165. Ekelund F, Rønn R, Griffiths BS. Quantitative estimation of flagellate community structure and diversity in soil samples. Protist. 2001;152(4):301-14. doi: 10.1078/1434-4610-00069. PubMed PMID: 11822659.
166. Anderson OR. Abundance of terrestrial gymnamoebae at a northeastern U. S. site: a four-year study, including the El Nino winter of 1997–1998. J Eukaryot Microbiol. 2000;47(2):148-55. doi: 10.1111/j.1550-7408.2000.tb00024.x.
167. Feest A, Madelin M. Seasonal population changes of myxomycetes and associated organisms in four woodland soils. FEMS Microbiology Ecology. 1988;4(3-4):133-40.

168. Feest A, Madelin M. Seasonal population changes of myxomycetes and associated organisms in five non-woodland soils, and correlations between their numbers and soil characteristics. FEMS Microbiology Ecology. 1988;4(3-4):141-52.
169. Coûteaux MM. Quelques aspects des relations entre les Thécamoebiens et les sols. Revue d'Écologie et de Biologie du Sol. 1975;12:45-57.
170. Coûteaux MM. Dynamisme de l'equilibre des thecamobiens dans qelques sols climaciques. Memoires du Museum National D'Histoire Naturelle Serie A Zoologie. 1976;96:1-183.
171. Heal O. Observations on the seasonal and spatial distribution of testacea (Protozoa: Rhizopoda) in *Sphagnum*. The Journal of Animal Ecology. 1964:395-412.
172. Wanner M, Xylander WE. Biodiversity development of terrestrial testate amoebae: is there any succession at all? Biology and Fertility of Soils. 2005;41(6):428-38.
173. Smith H. The Signy Island terrestrial reference sites: III. Population ecology of *Corythion dubium*. British Antarctic Survey Bulletin. 1973;33&34:123-35.
174. Mazei YA, Tsyganov AN. Species composition, spatial distribution and seasonal dynamics of testate amoebae community in a sphagnum bog (Middle Volga region, Russia). Protistology. 2007;5(2/3):156-206.
175. Mazei YA, Bubnova O. Species composition and structure of testate amoebae community in a *Sphagnum* bog at the initial stage of its formation. Biology Bulletin. 2007;34(6):619-28.
176. Jassey VEJ, Chiapusio G, Mitchell EAD, Binet P, Toussaint M-L, Gilbert D. Fine-scale horizontal and vertical micro-distribution patterns of testate amoebae along a narrow Fen/Bog gradient. Microbial Ecology. 2011;61(2):374-85. doi: 10.1007/s00248-010-9756-9.
177. Smirnov AV, Thar R. Spatial distribution of gymnamoebae (Rhizopoda, Lobosea) in brackish-water sediments at the scale of centimeters and millimeters. Protist. 2003;154(3–4):359-69. doi: http://dx.doi.org/10.1078/143446103322454121.
178. Adl SM. Motility and migration rate of protozoa in soil columns. Soil Biology and Biochemistry. 2007;39(2):700-3.
179. Winding A, Rønn R, Hendriksen NB. Bacteria and protozoa in soil microhabitats as affected by earthworms. Biol Fertil Soils. 1997;24(2):133-40. doi: 10.1007/s003740050221.
180. Mazei YA, Tsyganov A, Bubnova O. The species composition, distribution, and structure of a testate amoeba community from a moss bog in the middle Volga river basin. Zoologicheskiĭ Zhurnal. 2007;86:1155-67.
181. Mazei YA, Chernyshov V. Testate amoebae communities in the southern tundra and forest-tundra of Western Siberia. Biology Bulletin. 2011;38(8):789-96.
182. Andersen R, Chapman S, Artz R. Microbial communities in natural and disturbed peatlands: a review. Soil Biology and Biochemistry. 2013;57:979-94.
183. Tsyganov AN, Mityaeva OA, Mazei YA, Payne RJ. Testate amoeba transfer function performance along localised hydrological gradients. Eur J Protistol. in press.
184. Smirnov AV. Vertical distribution and abundance of gymnamoebae (Rhizopoda) in bottom sediments of the brackish water Nivå Bay (Baltic Sea, The Sound). Protist. 2002;153(3):239-50. doi: 10.1078/1434-4610-00101. PubMed PMID: 12389813.
185. Smirnov AV, Thar R. Vertical distribution of gymnamoebae (Rhizopoda, Lobosea) in the top layer of brackish-water sediments. Protist. 2004;155(4):437-46. doi: 10.1078/1434461042650352. PubMed PMID: 15648723.

186. Harvey RW, Kinner NE, Bunn A, Macdonald D, Metge D. Transport behavior of groundwater protozoa and protozoan-sized microspheres in sandy aquifer sediments. Applied and Environmental Microbiology. 1995;61(1):209-17.
187. Foissner W. Ciliates in rapid gravity filters of waterworks exploiting deep groundwaters. Microscopy research and technique. 1996;33(1):12-22.
188. Kinner N, Harvey R, Blakeslee K, Novarino G, Meeker L. Size-selective predation on groundwater bacteria by nanoflagellates in an organic-contaminated aquifer. Applied and Environmental Microbiology. 1998;64(2):618-25.
189. Euringer K, Lueders T. An optimised PCR/T-RFLP fingerprinting approach for the investigation of protistan communities in groundwater environments. Journal of Microbiological Methods. 2008;75(2):262-8. doi: http://dx.doi.org/10.1016/j.mimet.2008.06.012.
190. Griebler C, Lueders T. Microbial biodiversity in groundwater ecosystems. Freshwater Biology. 2009;54(4):649-77.
191. Bischoff PJ. An analysis of the abundance, diversity and patchiness of terrestrial gymnamoebae in relation to soil depth and precipitation events following a drought in southeastern U.S.A. Acta Protozool. 2002;41(2):183-9.
192. Ehrmann O, Puppe D, Wanner M, Kaczorek D, Sommer M. Testate amoebae in 31 mature forest ecosystems–densities and micro-distribution in soils. Eur J Protistol. 2012;48(3):161-8.
193. Fiore-Donno AM, Weinert J, Wubet T, Bonkowski M. Metacommunity analysis of amoeboid protists in grassland soils. Scientific Reports. 2016;6:19068.
194. Boenigk J, Pfandl K, Garstecki T, Harms H, Novarino G, Chatzinotas A. Evidence for geographic isolation and signs of endemism within a protistan morphospecies. Appl Environ Microbiol. 2006;72(8):5159-64. doi: 10.1128/aem.00601-06.
195. Boenigk J, Jost S, Stoeck T, Garstecki T. Differential thermal adaptation of clonal strains of a protist morphospecies originating from different climatic zones. Environ Microbiol. 2007;9(3):593-602. doi: 10.1111/j.1462-2920.2006.01175.x.
196. Bailly J, Fraissinet-Tachet L, Verner MC, Debaud JC, Lemaire M, Wesolowski-Louvel M, et al. Soil eukaryotic functional diversity, a metatranscriptomic approach. ISME J. 2007;1:632-42. doi: 10.1038/ismej.2007.68. PubMed PMID: WOS:000250894300006.
197. Carini P, Marsden PJ, Leff JW, Morgan EE, Strickland MS, Fierer N. Relic DNA is abundant in soil and obscures estimates of soil microbial diversity. bioRxiv. 2016. doi: 10.1101/043372.
198. Esteban GF, Clarke KJ, Olmo JL, Finlay BJ. Soil protozoa — an intensive study of population dynamics and community structure in an upland grassland. Appl Soil Ecol. 2006;33(2):137-51. doi: http://dx.doi.org/10.1016/j.apsoil.2005.07.011.
199. Murase J, Shinohara Y, Yokoe K, Matsuda R, Asakawa S, Hashimoto T. Impact of soil solarization on the ciliate community structure of a greenhouse soil. Soil Science and Plant Nutrition. 2015;61(6):927-33.
200. Griffiths BS, Bonkowski M, Roy J, Ritz K. Functional stability, substrate utilisation and biological indicators of soils following environmental impacts. Appl Soil Ecol. 2001;16(1):49-61. doi: 10.1016/s0929-1393(00)00081-0.
201. Grossmann L, Jensen M, Heider D, Jost S, Glücksman E, Hartikainen H, et al. Protistan community analysis: key findings of a large-scale molecular sampling. ISME Journal. 2016;10:2269–79. doi: 10.1038/ismej.2016.10.
202. Geisen S, Laros I, Vizcaíno A, Bonkowski M, de Groot GA. Not all are free-living: high-throughput DNA metabarcoding reveals a diverse community of protists parasitizing soil metazoa. Mol Ecol. 2015;24(17):4556–69. doi: DOI: 10.1111/mec.13238. PubMed PMID: 25966360.

203. da Silva JB, Piva C, Falavigna-Guilherme AL, Rossoni DF, de Ornelas Toledo MJ. Spatial distribution and enteroparasite contamination in peridomiciliar soil and water in the Apucaraninha Indigenous Land, southern Brazil. Environmental Monitoring and Assessment. 2016;188(4):217.
204. Wegener Parfrey L. Mock communities highlight the diversity of host-associated eukaryotes. Molecular Ecology. 2015;24(17):4337-9.
205. Lara E, Heger TJ, Scheihing R, Mitchell EaD. COI gene and ecological data suggest size-dependent high dispersal and low intra-specific diversity in free-living terrestrial protists (Euglyphida: Assulina). Journal of Biogeography. 2011;38(4):640-50. doi: 10.1111/j.1365-2699.2010.02426.x.
206. Kosakyan A, Heger TJ, Leander BS, Todorov M, Mitchell EAD, Lara E. COI barcoding of nebelid testate amoebae (Amoebozoa: Arcellinida): extensive cryptic diversity and redefinition of the Hyalospheniidae Schultze. Protist. 2012;163(3):415-34. doi: 10.1016/j.protis.2011.10.003.
207. Bobrov A, Mazei Y. Morphological variability of testate amoebae (Rhizopoda: Testacealobosea and Testaceafilosea) in natural populations. Acta Protozoologica. 2004;43(2):133-46.
208. Tsyganov AN, Mazei YA. Morphology, biometry and ecology of *Arcella gibbosa* Penard 1890 (rhizopoda, testacealobosea). Protistology. 2006;4(3).
209. Tsyganov AN, Mazei YA. Morphology and biometry of *Arcella intermedia* (Deflandre, 1928) comb. nov. from Russia and a review of hemispheric species of the genus *Arcella* (Testcealobosea, Arcellinida). Protistology. 2006;4(4).
210. Fucikova K, Lahr DJ. Uncovering cryptic diversity in two amoebozoan species using complete mitochondrial genome sequences. Journal of Eukaryotic Microbiology. 2016;63(1):112-22. doi: 10.1111/jeu.12253. PubMed PMID: 26211788.
211. Domonell A, Brabender M, Nitsche F, Bonkowski M, Arndt H. Community structure of cultivable protists in different grassland and forest soils of Thuringia. Pedobiologia. 2013;56(1):1-7. doi: 10.1016/j.pedobi.2012.07.001.
212. Malysheva E, Mazei YA, Yermokhin M. Testate amoebae community pattern in different types of boundary structures at the water-land contact zone. Biology Bulletin. 2013;40(10):823-31.
213. Mazei YA, Tsyganov A, Bubnova O. Structure of a community of testate amoebae in a *Sphagnum* dominated bog in upper sura flow (Middle Volga Territory). Biology Bulletin. 2007;34(4):382-94.
214. Mazei YA, Tsyganov A, Bubnova O. The species composition and community structure of testate amoebae in *Sphagnum* bogs of northern Karelia (The White Sea Lowland). Zoologicheskiĭ Zhurnal. 2009;88:1-12.
215. Mazei YA, Malysheva E, Lapteva E, Komarov A, Taskaeva A. The role of the floodplain gradient in structuring of testate amoebae communities in the Ilych River. Biology Bulletin. 2012;39(4):368-74.
216. Bobrov A, Mazei Y, Chernyshov V, Gong Y, Feng W. Testate amoebae communities from some freshwater and soil habitats in China (Hubei and Shandong Provinces). Frontiers of Earth Science. 2012;6(1):1-9.
217. Mazei YA, Bubnova O. Testate amoebae from *Sphagnum* biotopes of forested mires. Zoologicheskiĭ Zhurnal. 2009;88(4):387-97.
218. Korganova G. Adaptive morphological structures in the evolution of soil testate amoebae (Protista, Testacea). Zoologicheskiĭ Zhurnal. 2003;83:S67.
219. Wilkinson DM. Have we underestimated the importance of humans in the biogeography of free-living terrestrial microorganisms? Journal of Biogeography. 2010;37(3):393-7. doi: 10.1111/j.1365-2699.2009.02236.x. PubMed PMID: WOS:000273949700001.
220. Berry D, Widder S. Deciphering microbial interactions and detecting keystone species with co-occurrence networks. Front Microbiol. 2014;5:219. Epub 2014/06/07. doi: 10.3389/fmicb.2014.00219. PubMed PMID: 24904535; PubMed Central PMCID: PMCPmc4033041.

221. Amaro F, Wang W, Gilbert JA, Anderson OR, Shuman HA. Diverse protist grazers select for virulence-related traits in *Legionella*. The ISME Journal. 2015;9(7):1607-18.
222. Ginger ML, Fritz-Laylin LK, Fulton C, Cande WZ, Dawson SC. Intermediary metabolism in protists: a sequence-based view of facultative anaerobic metabolism in evolutionarily diverse eukaryotes. Protist. 2010;161(5):642-71.
223. de Vargas C, Audic S, Henry N, Decelle J, Mahé F, Logares R, et al. Eukaryotic plankton diversity in the sunlit ocean. Science. 2015;348(6237). doi: 10.1126/science.1261605.
224. Gleason FH, Lilje O, Marano AV, Sime-Ngando T, Sullivan BK, Kirchmair M, et al. Ecological functions of zoosporic hyperparasites. Frontiers in microbiology. 2014;5(244). doi: 10.3389/fmicb.2014.00244.
225. Lara E, Belbahri L. SSU rRNA reveals major trends in oomycete evolution. Fungal Diversity. 2011;49(1):93-100.
226. Barker J, Brown MRW. Trojan Horses of the microbial world: protozoa and the survival of bacterial pathogens in the environment. Microbiology. 1994;140(6):1253-9. doi: 10.1099/00221287-140-6-1253.
227. Raoult D, Boyer M. Amoebae as genitors and reservoirs of giant viruses. Intervirology. 2010;53(5):321-9. doi: Doi 10.1159/000312917. PubMed PMID: WOS:000278955300009.
228. Foissner I, Foissner W. *Ciliomyces spectabilis*, nov. gen., nov. spec., a zoosporic fungus which parasitizes cysts of the ciliate *Kahliella simplex*. Zeitschrift für Parasitenkunde. 1986;72(1):29-41. doi: 10.1007/bf00927732.
229. Krashevska V, Maraun M, Ruess L, Scheu S. Carbon and nutrient limitation of soil microorganisms and microbial grazers in a tropical montane rain forest. Oikos. 2010;119(6):1020-8. doi: 10.1111/j.1600-0706.2009.18169.x.
230. Matsuo J, Oguri S, Nakamura S, Hanawa T, Fukumoto T, Hayashi Y, et al. Ciliates rapidly enhance the frequency of conjugation between *Escherichia coli* strains through bacterial accumulation in vesicles. Research in Microbiology. 2010;161(8):711-9.
231. Ledeganck P, Nijs I, Beyens L. Plant functional group diversity promotes soil protist diversity. Protist. 2003;154(2):239-49. doi: http://dx.doi.org/10.1078/143446103322166536. PubMed PMID: 13677451.
232. Jassey VEJ, Chiapusio G, Binet P, Buttler A, Laggoun-Défarge F, Delarue F, et al. Above- and belowground linkages in Sphagnum peatland: climate warming affects plant-microbial interactions. Global Change Biol. 2013;19(3):811-23. doi: 10.1111/gcb.12075.
233. Maeda Y, Mayanagi T, Amagai A. Folic acid is a potent chemoattractant of free-living amoebae in a new and amazing species of protist, *Vahlkampfia* sp. Zoological Science. 2009;26(3):179-86.
234. Chattwood A, Bolourani P, Weeks G. RasG signaling is important for optimal folate chemotaxis in *Dictyostelium*. BMC Cell Biology. 2014;15(1):13.
235. Láng J, Rákász V, Magyar A, Pállinger É, Kőhidai L. Chemotactic effect of odorants and tastants on the ciliate *Tetrahymena pyriformis*. Journal of Receptors and Signal Transduction. 2011;31(6):423-33.
236. Wilks SA, Sleigh MA. Lectin binding sites on *Euplotes mutabilis* (Tuffrau, 1960) and the implications for food particle selection. Eur J Protistol. 2004;40(2):153-62.
237. Fenchel T. Orientation in two dimensions: chemosensory motile behaviour of *Euplotes vannus*. Eur J Protistol. 2004;40(1):49-55.
238. Bovee EC. Studies of Feeding Behavior of Amebas. I. Ingestion of Thecate Rhizopods and Flagellates by Verrucosid Amebas, particularly *Thecamoeba sphacronucleolüs*. The Journal of Protozoology. 1960;7(1):55-60. doi: 10.1111/j.1550-7408.1960.tb00708.x.

239. Matz C, Kjelleberg S. Off the hook-how bacteria survive protozoan grazing. Trends in microbiology. 2005;13(7):302-7.
240. Schmitz-Esser S, Toenshoff ER, Haider S, Heinz E, Hoenninger VM, Wagner M, et al. Diversity of bacterial endosymbionts of environmental *Acanthamoeba* isolates. Applied and Environmental Microbiology. 2008;74(18):5822-31.
241. Schmidt O, Dyckmans J, Schrader S. Photoautotrophic microorganisms as a carbon source for temperate soil invertebrates. Biology Letters. 2016;12(1):20150646.
242. Neuhauser S, Kirchmair M, Bulman S, Bass D. Cross-kingdom host shifts of phytomyxid parasites. BMC Evolutionary Biology. 2014;14(1):33.
243. Old KM. Giant soil amoebae cause perforation of conidia of *Cochliobolus sativus*. Transactions of the British Mycological Society. 1977;68(2):277-81.
244. Ogden CG. The ultrastructure of *Heleopera petricola* an agglutinate soil amoeba; with comments on feeding and silica deposition. Eur J Protistol. 1991;27(3):238-48.
245. Jassey VEJ, Shimano S, Dupuy C, Toussaint M-L, Gilbert D. Characterizing the feeding habits of the testate amoebae *Hyalosphenia papilio* and *Nebela tincta* along a narrow "fen-bog" gradient using digestive vacuole content and 13C and 15N isotopic analyses. Protist. 2012;163(3):451-64. doi: 10.1016/j.protis.2011.07.006.
246. Gilbert D, Amblard C, Bourdier G, Francez A-J, Mitchell EAD. Le regime alimentaire des thecamoebiens (Protista, Sarcodina). L'Année Biologique. 2000;39(2000):57-68.
247. Page FC. The genus *Thecamoeba* (Protozoa, Gymnamoebia) species distinctions, locomotive morphology, and protozoan prey. J Nat Hist. 1977;11(1):25-63.
248. Page FC. The genus *Thecamoeba* (Protozoa, Gymnamoebia) species distinctions, locomotive morphology, and protozoan prey. Journal of Natural History. 1977;11(1):25-63.
249. Doyscher D, Fieseler L, Dons L, Loessner MJ, Schuppler M. *Acanthamoeba* feature a unique backpacking strategy to trap and feed on *Listeria* monocytogenes and other motile bacteria. Environ Microbiol. 2013;15(2):433-46. doi: 10.1111/j.1462-2920.2012.02858.x.
250. Koller R, Robin C, Bonkowski M, Ruess L, Scheu S. Litter quality as driving factor for plant nutrition via grazing of protozoa on soil microorganisms. FEMS Microbiology Ecology. 2013;85(2):241-50. doi: 10.1111/1574-6941.12113. PubMed PMID: 23521364.
251. Tirjaková E. Structure and dynamics of communities of ciliated Protozoa (Ciliophora) in field communities. Succession, genus-species relationships, nutrition. Ekologia (Slovak Republic). 1997.
252. Dumack K, Schuster J, Bass D, Bonkowski M. A novel lineage of 'naked filose amoebae'; *Kraken carinae* gen. nov. sp. nov. (Cercozoa) with a remarkable locomotion by disassembly of its cell body. Protist. 2016;167(2):93-105.
253. Ekelund F. Enumeration and abundance of mycophagous protozoa in soil, with special emphasis on heterotrophic flagellates. Soil Biology and Biochemistry. 1998;30(10-11):1343-7. doi: Doi 10.1016/S0038-0717(97)00266-6. PubMed PMID: WOS:000074866400015.
254. Anderson OR, McGuire K. C-biomass of bacteria, fungi, and protozoan communities in Arctic tundra soil, including some trophic relationships. Acta Protozoologica. 2013;52(4):217.
255. Hudson PJ, Dobson AP, Lafferty KD. Is a healthy ecosystem one that is rich in parasites? Trends in Ecology and Evolution. 2006;21(7):381-5.
256. Uberto R, Moomaw EW. Protein similarity networks reveal relationships among sequence, structure, and function within the Cupin superfamily. Plos One. 2013;8(9):e74477. doi: 10.1371/journal.pone.0074477.

257. Bass D, Chao EE-Y, Nikolaev S, Yabuki A, Ishida K-i, Berney C, et al. Phylogeny of novel naked filose and reticulose Cercozoa: Granofilosea cl. n. and Proteomyxidea revised. Protist. 2009;160(1):75-109.
258. Zwart K, Burgers S, Bloem J, Bouwman L, Brussaard L, Lebbink G, et al. Population dynamics in the belowground food webs in two different agricultural systems. Agriculture, Ecosystems & Environment. 1994;51(1):187-98.
259. Lousier JD. Population dynamics and production studies of species of Nebelidae (Testacea, Rhizopoda) in an aspen woodland soil. Acta Protozoologica. 1984;23(3):145-59.
260. Zhao F, Xu K, He Y. Application of the Ludox-QPS method for estimating ciliate diversity in soil and comparison with direct count and DNA fingerprinting. European Journal of Soil Biology. 2012;49:112-8.
261. Metcalf JL, Xu ZZ, Weiss S, Lax S, Van Treuren W, Hyde ER, et al. Microbial community assembly and metabolic function during mammalian corpse decomposition. Science. 2016;351(6269):158-62.
262. Metcalf JL, Parfrey LW, Gonzalez A, Lauber CL, Knights D, Ackermann G, et al. A microbial clock provides an accurate estimate of the postmortem interval in a mouse model system. Elife. 2013;2:e01104.
263. Balik V. The effect of the road traffic pollution on the communities of testate amoebae(Rhizopoda, Testacea) in Warsaw(Poland). Acta Protozoologica. 1991;30(1):5-11.
264. Ekelund F, Olsson S, Johansen A. Changes in the succession and diversity of protozoan and microbial populations in soil spiked with a range of copper concentrations. Soil Biology and Biochemistry. 2003;35(11):1507-16. doi: http://dx.doi.org/10.1016/S0038-0717(03)00249-9.
265. Postma-Blaauw MB, de Goede RGM, Bloem J, Faber JH, Brussaard L. Soil biota community structure and abundance under agricultural intensification and extensification. Ecology. 2010;91(2):460-73.
266. Johansen A, Pedersen AL, Jensen KA, Karlson U, Hansen BM, Scott-Fordsmand JJ, et al. Effects of C60 fullerene nanoparticles on soil bacteria and protozoans. Environmental Toxicology and Chemistry. 2008;27(9):1895-903. Epub 2008/12/17. PubMed PMID: 19086316.
267. Gilbert D, Jakobsen HH, Winding A, Mayer P. Co-transport of polycyclic aromatic hydrocarbons by motile microorganisms leads to enhanced mass transfer under diffusive conditions. Environmental science & technology. 2014;48(8):4368-75. Epub 2014/03/15. doi: 10.1021/es404793u. PubMed PMID: 24625194.
268. Imparato V, Santos SS, Johansen A, Geisen S, Winding A. Stimulation of bacteria and protists in rhizosphere of glyphosate-treated barley. Applied Soil Ecology. 2016;98:47-55. doi: http://dx.doi.org/10.1016/j.apsoil.2015.09.007.
269. Newsham K, Garstecki T. Interactive effects of warming and species loss on model Antarctic microbial food webs. Functional ecology. 2007;21(3):577-84.
270. Payne RJ, Creevy A, Malysheva E, Ratcliffe J, Andersen R, Tsyganov AN, et al. Tree encroachment may lead to functionally-significant changes in peatland testate amoeba communities. Soil Biology and Biochemistry. 2016;98:18-21.
271. Tsaousis AD, Nyvltova E, Sutak R, Hrdy I, Tachezy J. A nonmitochondrial hydrogen production in *Naegleria gruberi*. Genome Biology and Evolution. 2014;6(4):792-9. doi: 10.1093/gbe/evu065. PubMed PMID: 24682152; PubMed Central PMCID: PMCPMC4007538.
272. Czechowski P, Clarke LJ, Breen J, Cooper A, Stevens MI. Antarctic eukaryotic soil diversity of the Prince Charles Mountains revealed by high-throughput sequencing. Soil Biology and Biochemistry. 2016;95:112-21.

273. de Groot GA, Laros I, Geisen S. Molecular Identification of Soil Eukaryotes and Focused Approaches Targeting Protist and Faunal Groups Using High-Throughput Metabarcoding. In: Martin F, Uroz S, editors. Microbial Environmental Genomics (MEG). Methods in Molecular Biology. 1399. New York, NY: Springer New York; 2016. p. 125-40.
274. Wilkinson DM. Fundamental processes in ecology: an earth systems approach. Oxford and New York: Oxford University Press; 2006.
275. Valverde A, Makhalanyane TP, Seely M, Cowan DA. Cyanobacteria drive community composition and functionality in rock–soil interface communities. Molecular Ecology. 2015;24(4):812-21.
276. Lentendu G, Wubet T, Chatzinotas A, Wilhelm C, Buscot F, Schlegel M. Effects of long-term differential fertilization on eukaryotic microbial communities in an arable soil: a multiple barcoding approach. Molecular Ecology. 2014;23(13):3341-55.
277. Tan S, Zhou J, Zhu X, Yu S, Zhan W, Wang B, et al. An association network analysis among microeukaryotes and bacterioplankton reveals algal bloom dynamics. Journal of Phycology. 2015;51(1):120-32. doi: 10.1111/jpy.12259.
278. Jacquiod S, Stenbaek J, Santos SS, Winding A, Sorensen SJ, Prieme A. Metagenomes provide valuable comparative information on soil microeukaryotes. Research in Microbiology. 2016;167(5):436-50. doi: 10.1016/j.resmic.2016.03.003. PubMed PMID: 27020245.
279. Consortium Q. Networking our way to better ecosystem service provision. Trends in Ecology and Evolution. 2016;31(2):105-15. Epub 2016/01/19. doi: 10.1016/j.tree.2015.12.003. PubMed PMID: 26777789.
280. Spitz A, Gimmler A, Stoeck T, Zweig KA, Horvat EA. Assessing Low-Intensity Relationships in Complex Networks. Plos One. 2016;11(4):e0152536. Epub 2016/04/21. doi: 10.1371/journal.pone.0152536. PubMed PMID: 27096435; PubMed Central PMCID: PMCPMC4838277.
281. Guidi L, Chaffron S, Bittner L, Eveillard D, Larhlimi A, Roux S, et al. Plankton networks driving carbon export in the oligotrophic ocean. Nature. 2016;532(7600):465-70. doi: 10.1038/nature16942. PubMed PMID: 26863193; PubMed Central PMCID: PMCPMC4851848.
282. Aylward FO, Eppley JM, Smith JM, Chavez FP, Scholin CA, DeLong EF. Microbial community transcriptional networks are conserved in three domains at ocean basin scales. Proceedings of the National Academy of Sciences of the United States of America. 2015;112(17):5443-8. doi: 10.1073/pnas.1502883112. PubMed PMID: PMC4418921.
283. Altermatt F, Fronhofer EA, Garnier A, Giometto A, Hammes F, Klecka J, et al. Big answers from small worlds: a user's guide for protist microcosms as a model system in ecology and evolution. Meth Ecol Evol. 2015;6(2):218–31. doi: 10.1111/2041-210X.12312.
284. Bass D, Stentiford GD, Littlewood D, Hartikainen H. Diverse applications of environmental DNA methods in parasitology. Trends in Parasitology. 2015;31(10):499-513.
285. Payne RJ, Lamentowicz M, Mitchell EAD. The perils of taxonomic inconsistency in quantitative palaeoecology: experiments with testate amoeba data. Boreas. 2011;40(1):15-27. doi: 10.1111/j.1502-3885.2010.00174.x.

Supplementary Table 2: Terms used to identify differences of protist research between different aquatic versus soil habitats (see Figure 1A).

	Habitat terms	*Protist terms*
Soil	Soil Soils Terrestrial	Alga Algae Algal Protist Protistan Protists Protozoa Protozoan Protozoans
Freshwater	Fresh water Freshwater Lake Pond River	
Marine	Marine Ocean Salt water Sea	
Aquatic	Aquatic Water Waters	

Supplementary Table 3: Terms used to compare research efforts for different soil organisms (see Figure 1B).

	Habitat terms
Soil	Soil
	Soils
	Terrestrial

	Organism terms
Protists	Alga
	Algae
	Algal
	Protist
	Protistan
	Protists
	Protozoa
	Protozoan
	Protozoans
Fungi	Fungal
	Fungi
	Fungus
Nematodes	Nematoda
	Nematode
	Nematodes
Bacteria	Bacteria
	Bacterial
Archaea	Archaea
	Archaeal
	Archea
Viruses	Bacteriophage
	Bacteriophages
	Phage
	Phages
	Viral
	Virus
	Viruses

Supplementary Table 4: Numbers of papers with the respective search terms in different time intervals (See Figure 1).

		1800-1980	1981-1985	1986-1990	1991-1995	1996-2000	2001-2005	2006-2010	2011-2015	2016
Protist terms	**Soil**	989	257	196	255	268	273	234	232	41
	Freshwater	2603	740	732	783	966	1108	1415	1482	150
	Marine	4334	1095	1023	1174	1445	1685	1950	2209	200
	Aquatic	1795	564	473	622	758	1072	1301	1344	142
	Freshwater, marine and aquatic	8732	2399	2228	2579	3169	3865	4666	5035	492
Common soil organisms	**Protists**	989	257	196	255	268	273	234	232	41
	Fungi	2686	796	749	887	1048	1358	2038	2612	321
	Nematodes	1689	434	477	530	611	684	970	979	84
	Bacteria	2202	541	554	793	1180	1559	2799	3881	491
	Archaea	0	0	0	0	12	33	123	283	27
	Viruses	514	141	184	154	118	162	239	87	14

Supplementary Table 5: Summary of the explanatory variables used to describe the participants

Variable	What does it describe?
Main and secondary expertise	This describes the research area of the participants
Research institute	This shows where participants are working
Age	Age of the participants
Type of position	Whether the participants are Professors, Scientists (PhD but not Professor), Post docs or PhD students
Number of publications on protists	This shows the experience of participants on the subject
Main topic	Whether protists are the main interest of the participants
Ecosystem studied	The type of ecosystem studied (e.g. forests, grasslands, wetlands, peatlands, freshwater, etc)
Morphogroup of interest	Whether the participants focused on diverse morphogroups or specifically on amoebae, ciliates, heterotrophic flagellates, naked amoebae, microeukaryotes, testate amoebae, etc