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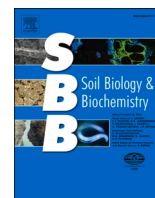
Toward a common set of functional traits for soil protists

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A B S T R A C T

Protists are major actors of soil communities and play key roles in shaping food webs, community assembly, and ecosystem processes, yet their functional diversity is understudied. High-throughput sequencing data have revealed their ubiquity and diversity, but lack of standardized traits has hampered the integration of functional information, limiting our understanding of soil ecosystems. Here, we propose a functional framework for soil protists, identify a set of common traits to characterize their functional diversity, and apply the framework on a broad-scale, real-world dataset. We reviewed studies on soil protists to identify the traits used in the literature, and define a framework based on 10 key traits that satisfy two criteria: availability of information, and applicability to most taxa. The framework was tested on a dataset of environmental DNA metabarcoding data from 1123 soil samples collected in 48 glacier forelands worldwide. Traits were assigned to the 570 Molecular Operational Taxonomic Units (MOTUs) detected in our dataset, leading to the production of a global trait-based dataset from glacier forelands. We estimated the functional space of protist communities and evaluated if the selected traits were effective in describing protist diversity. The functional space of protist communities showed that the MOTUs are clustered in three regions, mainly reflecting different nutritional and habitat preferences. The proposed framework is appropriate for multiple applications, including estimation of functional diversity and food web analyses, and provides a basis for ecological studies on soil protists, enabling the functional characterization of this essential but often neglected component of soil biodiversity.

1. Introduction

Soil ecosystems harbor more than half of the species of our planet (Anthony et al., 2023). Such impressive biodiversity provides essential services for life on Earth (Bardgett and Van Der Putten, 2014; Delgado-Baquerizo et al., 2020). Each biotic component of the belowground world interacts with the surrounding environment and other soil organisms, deeply influencing key ecosystem functions such as organic matter decomposition, soil structure, as well as aboveground productivity (Decaëns, 2010; Orgiazzi et al., 2016; Crowther et al., 2019). Nonetheless, due to its hidden nature and complexity, the soil biota has historically been understudied and only in the last decades we have

begun to uncover its essential role (Bardgett and Van Der Putten, 2014; Geisen et al., 2019; Guerra et al., 2020). Advances in molecular techniques have enabled ecologists to investigate the communities of micro-organisms that inhabit soil ecosystems, significantly increasing our knowledge of soil communities and the dynamics and processes regulating them (Geisen et al., 2019; Burki et al., 2021). Significant progress has been made for soil micro-organisms but, whereas for bacteria, fungi, and even archaea the number of studies has greatly increased in recent years, studies on protists have lagged behind (Fig. 1; Geisen et al., 2017; Guerra et al., 2020).

Protists have been defined as "a grab bag including anything eukaryote that is not an animal, land plant, or dikaryon fungus" (Burki
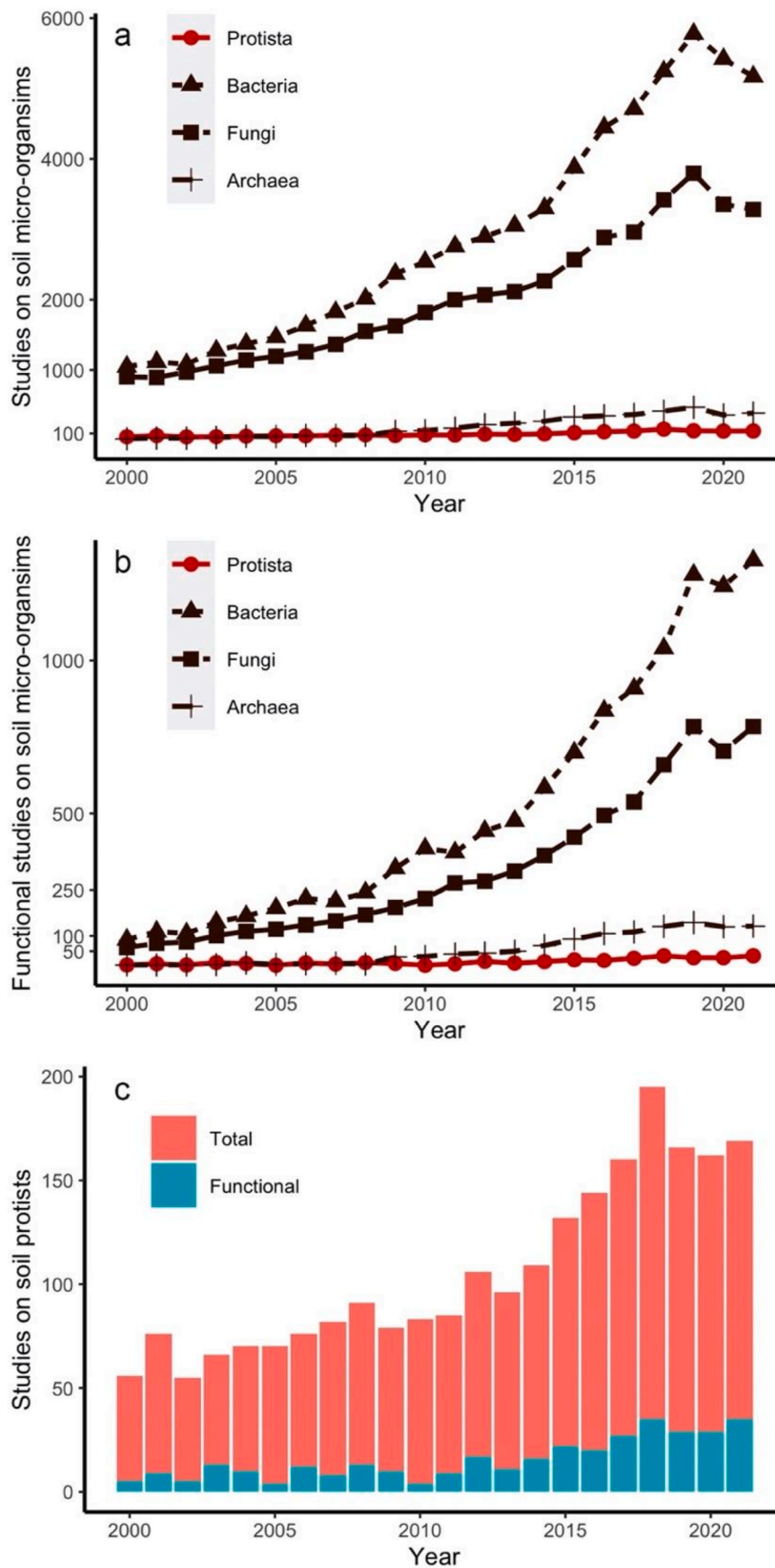

Fig. 1. a) Number of studies on soil microorganisms (bacteria, fungi, archaea and protists); b) number of functional studies for each taxon; c) proportion of functional studies over the total number of studies for protists. See Supplementary Methods 1 for details on the literature search.

et al., 2021), and include an unparalleled diversity of eukaryotes estimated in millions of species belonging to highly diverse lineages (Orgiazzi et al., 2016; Adl et al., 2019; Burki et al., 2021). Soil protists embrace organisms spanning five orders of magnitude in size, ranging from solitary cells measuring a few micrometers, to species of Amoebozoa forming colonies of several centimeters (Geisen et al., 2017). These organisms are an essential component of soil communities, providing an irreplaceable contribution to all soil functions (Bonkowski et al., 2019; Geisen et al., 2020; Oliverio et al., 2020). Despite their small size, protists are so abundant that their global biomass is estimated as 4 Gt, i.e., twice the biomass of all animals on Earth (Bar-On et al., 2018). Protists exhibit a large diversity of feeding modalities influencing different levels of the ecological food web. Phagotrophic protists regulate microbial populations through active predation, releasing nutrients back into the environment and thus strongly influencing plant growth (Clarholm, 1981; Coûteaux and Darbyshire, 1998; Bonkowski, 2004). Phototrophic protists contribute directly to primary production through carbon fixation (Schmidt et al., 2016). Parasitic protists can control populations of much larger organisms such as plants and animals (Mahé et al., 2017), while saprotrophic protists are fundamental for organic matter degradation (Savory et al., 2015). Nevertheless, our understanding of the role played by protists within soil ecosystems is far from exhaustive (Geisen et al., 2017, 2020).

The traits of organisms are known to be closely linked to ecosystem functioning (Loreau et al., 2001; Cardinale et al., 2012; Naeem et al., 2012). Thus, refined information on their functional diversity is required to deepen our understanding of the relationships between soil protists and ecosystem functions, but also their role in biogeochemical cycles, and how abiotic and biotic drivers shape communities in space and time (Cardinale et al., 2012; Naeem et al., 2012; Briones, 2014; Geisen et al., 2023). Functional diversity represents the variety of morphological, ecological, behavioral, and physiological traits exhibited by different species (Hooper et al., 2005; Cadotte et al., 2011). Despite preliminary efforts to define a functional classification for soil protists and identify functional groups (Coûteaux et al., 1998), functional studies on soil protists remain scarce (Fig. 1). This can be related to the complexity of the study of protists ecology (Geisen et al., 2023), but also to the lack of a conceptual framework and comprehensive databases covering key functional traits (Dumack et al., 2020). So far, publicly available datasets are mostly restricted to a few major taxa such as Cercozoa and Endomyxa (Fiore-Donno et al., 2019; Dumack et al., 2020), phytoplankton (Laplace-Treyture et al., 2021), or a subset of Ciliophora and amoeboid protists (Gulin et al., 2022). Furthermore, the few datasets covering phylogenetically diverse taxa are generally limited to a single functional trait, usually the trophic level (Seppey et al., 2020; Mazel et al., 2022; Singer et al., 2021). Nonetheless, a wealth of functional information on protists is available but spread across various sources of the existing literature, including taxonomic articles, functional datasets, and monographs/atlases spanning different taxonomic levels, from phylum (Bahls et al., 2018; Lynn et al., 2018), to family level (Berger, 1999, 2007, 2011; Foissner and Xu, 2007). Still, the absence of standard guidelines or established key functional traits hampers the extraction of information from existing literature.

The aim of this study is to propose a framework for developing a comprehensive database on the functional traits of soil protists that is designed to i) facilitate the retrieval of functional information, ii) accommodate most of soil protist diversity, iii) be exploitable in ecological studies and iv) use consistent terminology. To achieve this, we followed a standardized approach developed for soil fauna (Hedde et al., 2022) and reviewed the literature to define a common set of readily accessible traits to characterize the functional diversity of soil protists, akin to the framework proposed for terrestrial invertebrates (Moretti et al., 2017). To illustrate the efficacy of the proposed framework, we applied it to a dataset containing 570 molecular operational taxonomic units (MOTUs) of protists obtained from the environmental DNA metabarcoding of 1123 soil samples covering a broad range of

environmental conditions and geographic regions.

2. Methods

2.1. Functional traits review

To review and identify the functional traits generally used in soil protists studies, we performed a literature search in Web of Science (October 21st, 2022) with the following string: TS=(soil* AND protist* AND function* AND trait*) OR TS=(soil* AND protozo* AND function* AND trait*) OR TS=(terrestrial AND protist* AND function* AND trait*) OR TS=(terrestrial AND protozo* AND function* AND trait*). We focused on research articles without imposing temporal constraints, i.e. we covered the period 1944–2022. The abstract of each resulting article was read, and non-relevant studies were discarded (e.g., studies analyzing functional traits of other groups, or studies just mentioning protists). To avoid missing relevant literature not available from the Web of Science, we also inspected the references cited by each of the retained studies to identify additional pertinent works. From the retained literature, we extracted information on article type, year of publication, research topic, location and geographical scale, sampling method, sampling environment, taxa considered, the functional traits and functional categories used, and the source of the functional data (Table S1).

The functional traits found through the review were classified into six broad types (Moretti et al., 2017; Dumack et al., 2020): nutrition, morphology, life-history, physiology, behavior, and habitat. From those traits, we selected for our functional framework the ones that satisfied two main criteria: a) information availability, b) broad applicability. Information availability was evaluated considering the possibility to retrieve functional information in the literature (e.g., availability of trait datasets), while the applicability to a broad range of soil protists was assessed by checking if the traits can be used to characterize multiple protist lineages (Table S2). These two criteria of selection are meant to identify a limited number of traits that are useable to characterize soil protists diversity. In some cases, protists exhibit different characters/behaviors depending on features such as the life stage or the environmental conditions (Archibald et al., 2017; Adl et al., 2019), complicating trait assignment. Such organisms (e.g., a parasite with a free-living flagellate stage for dispersal) can be characterized by all the functional categories it can exhibit (e.g., free-living and endoparasite) using fuzzy-coded dummy variables (de Bello et al., 2021), even though researchers can decide to use the predominant one (e.g., endoparasite), depending on the specific aim of the study.

2.2. Application: functional traits of protists from glacier forelands

To illustrate the application of the selected functional traits, we used a dataset containing 570 molecular operational taxonomic units (MOTUs) of protists detected using environmental DNA metabarcoding from 1123 soil samples collected along 48 glacier forelands around the world (Fig. S1). The 48 glacier forelands range from 48°S to 79°N and encompass five continents. Each glacier foreland represents a sequence of dated sites starting from 1 to a maximum of 483 years after glacier retreat (Marta et al., 2021) with a general habitat shift from bare ground to fully vegetated ecosystems (Cantera et al., 2023). MOTUs were derived from the amplification of the eukaryotic marker Euka02 (Guardiola et al., 2015; Taberlet et al., 2018) which amplifies a ~123 bp fragment of the V7 region of the 18S rDNA gene. For details on sample collection, DNA extraction, amplification, sequencing and bioinformatic analyses, see Supplementary Methods 2. After clustering similar sequences (97% threshold; Bonin et al., 2023), a total of 2008 MOTUs belonging to different kingdoms in the Eukaryota domain were detected (Fig. 2). Taxonomy was defined through the NCBI Taxonomy database (Schoch et al., 2020).

We then applied our framework to functionally characterize protist

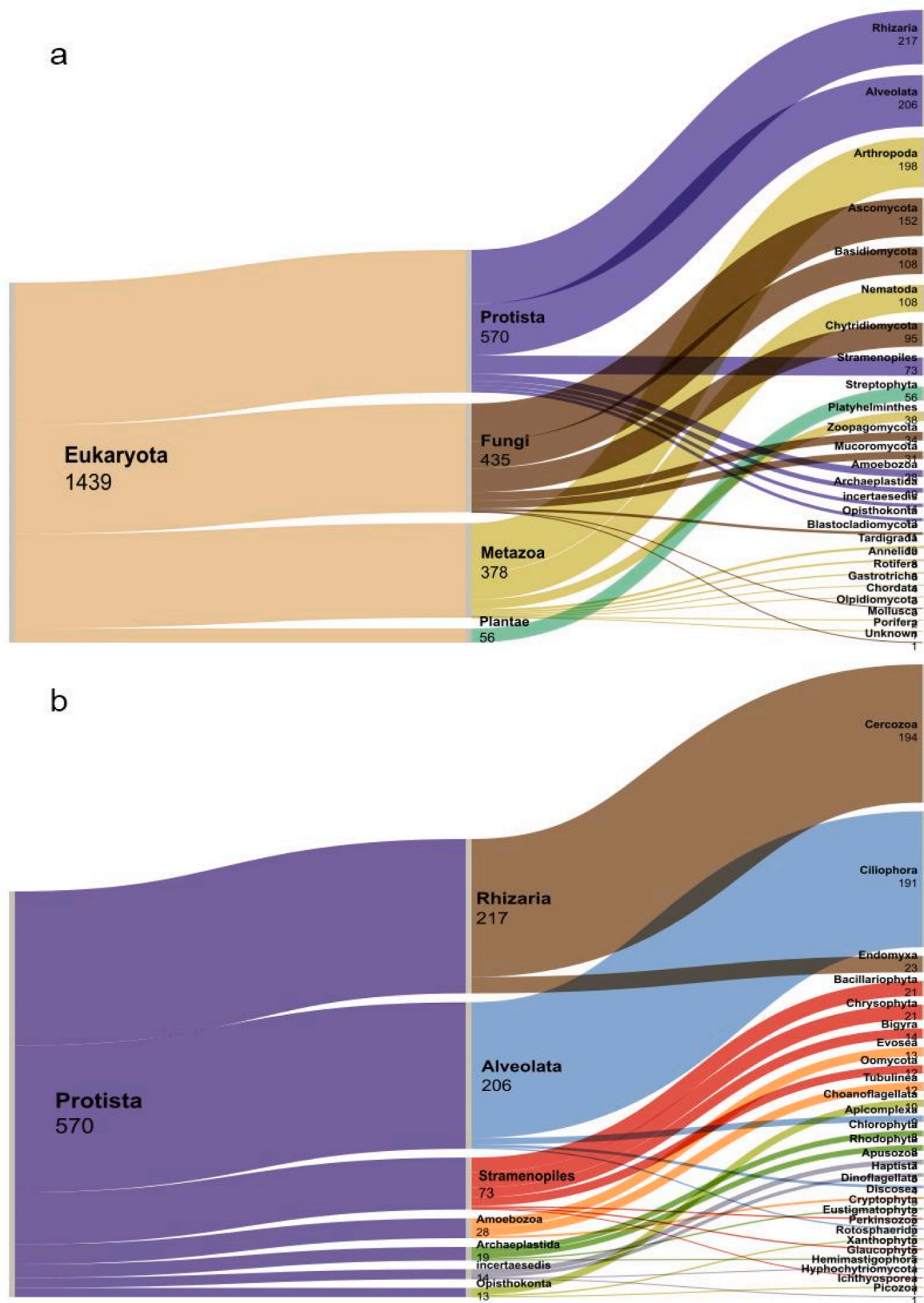**Fig. 2.** Taxonomic composition of MOTUs detected with the general Eukaryota marker Euka02 (Guardiola et al., 2015) of all soil samples collected for the dataset; numbers indicate the number of MOTUs within each taxonomic group. a) All MOTUs identified at least at the kingdom level divided in the four kingdoms Protista, Fungi, Metazoa and Plantae. b) Taxonomic breakdown of MOTUs belonging to the kingdom Protista.

communities in proglacial soils. To assign functional traits, we searched for information on nutrition, morphology, behavior, life-history, and habitat preferences for MOTUs belonging to 27 phyla/clades and to six supergroups (i.e., Alveolata, Amoebozoa, Archaeplastida, Opisthokonta, Rhizaria, Stramenopiles; Fig. 2). In so doing, we conducted an extensive literature search for each taxon included in the dataset using references obtained through our review, plus additional specific searches in Google, Google Scholar and Web of Science. We gathered information from a total of 197 references (176 scientific articles including 17 datasets, 15 books/guides/atlas, and six websites; Table S1). The taxonomic level of identification of MOTUs varied, with some MOTUs identified at fine taxonomic resolution (e.g., *Vorticella sphaeroidalis* or *Euglypha rotunda*), and others identified at coarser resolution (e.g., Cercozoa or Bacillariophyta). Therefore, the assignment of functional traits depended on the taxonomic level of identification of each MOTU. MOTUs that could not be characterized by a unique functional category due to the lack of taxonomic resolution (e.g., MOTU identified as Cercomonadidae), were described using two or more functional categories separated by "or" (e.g., for Cercomonadidae, feeding mode: bacterivore_or_omnivore).

To visualize how the traits proposed in our framework describe the community of protists from the proglacial soil dataset, we estimated the functional space through a principal coordinates analysis (PCoA) using the R package "ape" (Paradis and Schliep, 2019). To build the functional space, we merged MOTUs with the same taxonomic identification, and excluded MOTUs with a taxonomic assignment coarser than family. The remaining MOTUs were characterized by every trait defined in our framework. Categorical traits were coded as dummy variables (de Bello

et al., 2021). Length was log-transformed and scaled between 0 and 1. Dissimilarities among MOTUs were estimated using the Gower distance weighted through the "gawdis" R function to uniform the contribution of different traits (de Bello et al., 2021). Finally, we tested the correlation between the first two PCoA axes and functional traits through the function envfit() from the R package "vegan" (Oksanen et al., 2022). Data manipulation and statistical analyses were done using the R platform v.4.2.2.

3. Results

3.1. Review of functional traits

The Web of Science search resulted in 51 scientific articles, but only 22 actually focused on protists. Inspection of articles and of references therein yielded 10 additional references focusing on functional traits of soil protists. Of the 32 studies, one presents a functional database (Dumack et al., 2020), while the remaining ones are primary research. All retained studies were published between 2015 and 2022, with an increase in publication rate during the last three years (77% of studies published between 2020 and 2022). Studies encompassed different approaches, such as tests of relationships between functional traits and environmental factors (21 studies), network analyses (eight), identification of community assembly processes (four), microcosm experiments (four), and one comparative analysis. Most studies were carried out in Europe (15 studies), followed by Asia (five), South America (two), and Oceania (two); four studies sampled multiple continents. Six studies

Table 1

Overview of the set of the 10 key traits included in the framework with a short description of each trait, the categories composing them, and taxa for which they have been used.

Type	Trait	Description	Categories	Taxa	References
Nutrition	Trophic level	Position of an organism in a food web	consumer; parasite; autotroph	community	Voss et al., 2019; Nguyen et al., 2020, 2021; Oliverio et al., 2020; Seppey et al., 2020; Chen et al., 2021; Mazel et al., 2022; Singer et al., 2021
	Feeding mode	Group of organisms exploiting analogous food resources	bacterivore; omnivore; eukaryvore; animal parasite; plant parasite; other parasite; saprotroph; histophagous	Cercozoa; Endomyxa	Fiore-Donno et al., 2019, 2020, 2022; Dumack et al., 2020; Roshan et al., 2021
				community	Xiong et al., 2018; Schulz et al., 2019; Santos et al., 2020; Ritter et al., 2021; Zhang et al., 2021
Morphology	Body size (length/width/volume)	Multiple measures giving an indication of the dimension of an organism	Continuous traits	testate amoebae	Fournier et al., 2015, 2016; Song et al., 2018
				Chlorophyta	Fang et al. (2021)
				Ciliophora	Krztoń and Kosiba, 2020
				community	Luan et al., 2020; Aslani et al., 2022
	Shell	Protective structure usually composed by proteins and self-secreted minerals	naked; silica; organic	Cercozoa; Endomyxa;	Fiore-Donno et al., 2019, 2020, 2022; Dumack et al., 2020
				amoeboid	Gulin et al. (2022)
	Locomotion structures	Appendages involved in locomotion	flagella; pseudopodia; pseudopodia and flagella; cilia; none	Cercozoa; Endomyxa	Fiore-Donno et al., 2019, 2020, 2022; Dumack et al., 2020
				amoeboid	Gulin et al. (2022)
Behavior	Lifestyle	Living modality of an organism	free-living; sessile; endoparasite	Cercozoa; Endomyxa	Fiore-Donno et al., 2019, 2020, 2022; Dumack et al., 2020
				Ciliophora; amoeboid	Gulin et al. (2022)
				community	Voss et al. (2019)
	Life form	Level of organization of an organism	solitary; colonial; multicellular	Ciliophora; amoeboid	Gulin et al. (2022)
Life history	Resting cyst	Dormant life stage with thick shell	presence/absence	NA	No studies
	Sporulation	Spores for reproduction and dispersal	presence/absence	NA	No studies
Habitat	Habitat preferences	Environments where the species has been sampled	terrestrial; freshwater; ubiquitous; marine	Cercozoa; Endomyxa	Dumack et al. (2020)
				Chlorophyta	Fang et al. (2021)
				Ciliophora; amoeboid	Gulin et al. (2022)

identified protists based on their morphological features, 21 used molecular approaches, and one combined both approaches. Regarding the targeted taxa, 17 articles considered the protist community as a whole, six focused on Cercozoa, five on testate amoebae, three on Ciliophora, two on Oomycota and one on Chlorophyta. Most studies considered nutrition traits (24 studies) and morphology (15 studies), whereas behavioral and physiological traits were included in only seven and two studies, respectively (Table 1). Our review identified 28 distinct traits for the functional characterization of soil protists (Table S2), distributed into five broad types: nutrition (six traits), morphology (11 traits), physiology (two traits), behavior (five traits), and habitat (four traits).

Through the application of the selection criteria (data availability and applicability), we identified a set of functional traits enabling us to describe most of protist diversity with the data available in the literature (Table S2). Below, we describe the eight functional traits obtained from the selection, including two additional life-history traits (i.e., sporulation and resting cyst) that were not considered in the selected articles, but could be relevant to describe the variation in life history and persistence under specific environmental conditions (Geisen et al., 2018). For each trait, we report the definition, ecological relevance, functional categories (for qualitative traits), availability in the literature, and critical aspects. Given the presence of inconsistent terminology for the same traits, we selected the most commonly used terms in the available literature (summarized in Table 1).

3.1.1. Nutrition

Nutrition describes dietary habits, synthesizing the consumer-resource interaction. Information on nutrition is essential to analyze predator-prey dynamics, build food webs, and test relationships with environmental drivers (Briones, 2014; Bonkowski et al., 2019; Potapov et al., 2022). Considering the high diversity of nutrition modalities within protists, we considered two functional traits with different levels of precision. First, the "trophic level" (Seppey et al., 2020; Mazel et al., 2022; Singer et al., 2021) represents general information that allows for the discrimination of three broad trophic categories (consumer, parasite, and autotroph). Subsequently, the "feeding mode" allows for a more detailed identification of dietary habits (Fiore-Donno et al., 2019; Dumack et al., 2020; Zhang et al., 2021).

3.1.1.1. Trophic level

3.1.1.1.1. Definition. The trophic level indicates the position of an organism in a trophic web, based on feeding relationships and energy transfer. Trophic modalities can be characterized by a first broad discrimination between heterotrophs and autotrophs (Voss et al., 2019), but heterotrophic protists can be further distinguished in phagotrophs (i. e., consumers) and parasites (Bonkowski et al., 2019).

3.1.1.1.2. Ecological relevance. The trophic level has been often used to characterize community assembly of soil protists (Singer et al., 2021), verify relationships with environmental factors (Seppey et al., 2020) or detect community changes along elevational gradients (Mazel et al., 2022).

3.1.1.1.3. Functional categories. Three main trophic categories are generally considered (Oliverio et al., 2020; Seppey et al., 2020; Mazel et al., 2022; Singer et al., 2021):

a) Consumer: heterotrophic protists feeding through phagocytosis.
b) Parasite: heterotrophic protists living at the expense of another organism.
c) Autotroph: a non-heterotrophic protist, typically a phototrophic organism that produces energy through photosynthesis.

3.1.1.1.4. Availability in the literature. A comprehensive dataset reporting trophic levels of all protist taxa can be found in Singer et al. (2021), whereas Laplace-Treyture et al. (2021) published a detailed functional dataset for phytoplankton.

3.1.1.1.5. Critical aspects. Due to its coarse characterization, the

trophic level is not appropriate for detailed analyses of the trophic composition of communities, or to infer specific biotic interactions. Furthermore, several taxa can have two trophic levels (e.g., both consumer and autotroph) or are able to switch between different trophic levels (Singer et al., 2021).

3.1.1.2. Feeding mode

3.1.1.2.1. Definition. The feeding mode identifies a group of organisms exploiting analogous food resources (Bonkowski et al., 2019).

3.1.1.2.2. Ecological relevance. Grouping protists in a few well-defined feeding modes is pivotal to understand community assembly (Roshan et al., 2021), test relations among feeding groups and environmental factors (Fiore-Donno et al., 2019, 2020, 2022), infer biotic interactions (e.g., predator-prey), and reconstruct food webs (Briones, 2014; Geisen and Bonkowski, 2018; Potapov, 2022). The assignment of a specific feeding mode is also essential to study key soil nutrient dynamics such as the microbial loop (Bonkowski and Clarholm, 2012) or alternative pathways for nutrient cycling (Seppey et al., 2017; Geisen et al., 2020).

3.1.1.2.3. Functional categories. We use the functional categories reported by Dumack et al. (2020) to discriminate among seven feeding modes, adding the categories "histophagous" and "saprotroph" (Lynn, 2008; Adl et al., 2019).

a) Bacterivore: phagotroph feeding exclusively on prokaryotes.
b) Eukaryvore: phagotroph feeding exclusively on eukaryotes (algae, fungi, other protists, and small metazoans).
c) Omnivore: phagotroph feeding on both prokaryotes and eukaryotes.
d) Animal parasite: protist living at the expense of a metazoan host.
e) Plant parasite: protist living at the expense of a plant host.
f) Other parasites: protists living at the expense of neither a metazoan nor a plant (typically another protist).
g) Histophagous: protist feeding on tissues of unhealthy bodies of metazoans.
h) Saprotroph: protist feeding on decaying organic matter.
i) Autotroph.

3.1.1.2.4. Availability in literature. The feeding mode is one of the most widely used functional traits for soil protists. It can be found in available functional datasets (Adl et al., 2019; Dumack et al., 2020; Calderón-Sanou et al., 2022; Gulin et al., 2022), and can be deducted from monographies or original description articles reporting the food sources exploited by a species or higher taxonomic groups. A synopsis of feeding habits of protists is also found in a recent review on feeding habits of soil consumers (Potapov et al., 2022).

3.1.1.2.5. Critical aspects. Although a good amount of data is available concerning feeding preference of soil protists, for many taxa uncertainty remains regarding their actual food sources and further analyses are needed. For instance, recent studies have discovered that several taxa traditionally defined as strictly bacterivorous actually are omnivores (Dumack et al., 2020). Finally, some species can drastically switch their food sources during their life cycle. For instance, *Chlorella* green algae are usually phototrophs, but under specific environmental conditions they can parasitize animals (Jagielski et al., 2019).

3.1.2. Morphology

Morphology includes all traits describing the size and shape of an organism and its structural features. Morphological traits can be quantitative such as body size, or qualitative such as the presence of a shell, its composition, and the type of structures used for locomotion. Size information is useful to understand functional roles in the community, the potentially exploited microhabitat, and to infer prey-predator interactions (Potapov, 2022). Regarding qualitative traits, major groups such as Ciliophora, Cercozoa, or Amoebozoa have been traditionally described using the terms "ciliates", "flagellate", "amoeba", or "amoeboflagellate", often coupled with information regarding the presence

("testate") or absence ("naked") of a shell (Fiore-Donno et al., 2019; Dumack et al., 2020; Gulin et al., 2022). This terminology derived from the type of structures used for locomotion: cilia for ciliates, flagella for flagellates, pseudopodia for amoebae, and a combination of both flagella and pseudopodia for amoeboflagellates. Here, in order to encompass and better characterize the range of protist lifeforms, the presence of a shell and type of locomotion structures were considered as distinct traits.

3.1.2.1. Body size

3.1.2.1.1. Definition. Size refers to measures of the overall dimensions of an organism. Multiple traits have been used to define body size; the most employed are length, width, volume, and biomass.

3.1.2.1.2. Ecological relevance. Since many soil organisms have generalist feeding (e.g., they eat everything smaller than their size), body size information is useful to infer size-dependent prey selection and can be used as an integrative trait to model food webs (Coûteaux et al., 1998; Potapov, 2022). Size differences can also reflect a vertical stratification of the composition of soil communities, with larger species often limited to the surface soil horizons (Geisen and Bonkowski, 2018; Fiore-Donno et al., 2022; Potapov, 2022).

3.1.2.1.3. Availability in literature. Although measures of body length and width are usually reported in articles describing taxa, databases gathering this information remain scarce (but see Luan et al., 2020; Laplace-Treyture et al., 2021).

3.1.2.1.4. Critical aspects. Length and width measurements are often available but highly dispersed in literature, making it time-consuming to obtain this information, especially if the aim is the functional characterization of many taxa. Furthermore, information on length is almost always available, whereas measures of width can be lacking. Finally, the articles reporting original descriptions often only consider one or a few populations, which may not be representative of the whole species.

3.1.2.2. Shell

3.1.2.2.1. Definition. The shell is a protective structure usually composed of a mixture of proteins and self-secreted minerals such as silica or calcium carbonate (silica or calcium carbonate; Meisterfeld, 2002; Geisen et al., 2018).

3.1.2.2.2. Ecological relevance. The presence of a shell can provide resistance to desiccation and/or represent a defense against predators (Geisen et al., 2018). Protist shells can remain preserved over millennia and are thus widely used to reconstruct past environmental conditions (Adl et al., 2011; Marcisz et al., 2020).

3.1.2.2.3. Functional categories. a) Naked: organism lacking shell structures.
b) Silica: organism protected by a shell structure made of self-fixed silica.
c) Organic or calcareous: organism protected by a shell that is organic, calcareous, or organic with embedded or attached foreign materials.

3.1.2.2.4. Availability in literature. The occurrence of a protective shell and its composition are reported in original description articles. Datasets reporting information on shell structure include Fiore-Donno et al. (2019) and Dumack et al. (2020) for Cercozoa and Endomyxa; some data on amoeboid protists are available in Gulin et al. (2022), while for phytoplankton see Laplace-Treyture et al. (2021).

3.1.2.2.5. Critical aspects. Some taxa exhibit protective structures like spicules or spines that morphologically cannot be considered as a true shell (e.g., members of the family Heterophryidae and Raphidiophryidae; Cavalier-Smith and von der Heyden, 2007).

3.1.2.3. Locomotion structures

3.1.2.3.1. Definition. Locomotion structures are appendages involved in the movement across the environment.

3.1.2.3.2. Ecological relevance. Locomotion structures are directly related to the movement modality (e.g., free swimming, or creeping/gliding on a substrate) and to the microhabitat exploited. For instance, protists with flagella are abundant in humid soils, while their frequency decreases with increasing soil bulk density (Fiore-Donno et al., 2019, 2022; Dumack et al., 2020).

3.1.2.3.3. Functional categories. a) Flagella: presence of one or more flagella (flagellates).
b) Pseudopodia: presence of pseudopodia (amoebae).
c) Pseudopodia and flagella: presence of both pseudopodia and one or more flagella (amoeboflagellates).
d) Cilia: presence of cilia (ciliates).
e) None: absence of locomotion structure.

3.1.2.3.4. Availability in literature. The presence of locomotion structures is reported in original description articles. Datasets summarizing information on locomotion structures use the terminology referred to the morphotypes "flagellate", "amoeba", "amoeboflagellate" (Fiore-Donno et al., 2019; Dumack et al., 2020 for Cercozoa and Endomyxa; Laplace-Treyture et al., 2021 for phytoplankton; Gulin et al., 2022 for amoeboid protists).

3.1.2.3.5. Critical aspects. While for major soil protist groups data are easily available (i.e., Cercozoa, Ameobozoa, Ciliophora), for other taxa extensive literature research is needed. In some taxa, multiple life stages can display different locomotion structures (e.g. Plasmidiophorida; Dumack et al., 2020).

3.1.2.4. Behavior.
Behavioral traits can refer to how protists move throughout their microhabitat (e.g., "free swimming" or "gliding on the substrate"), or the capability to form colonies of multiple individuals (Fiore-Donno et al., 2019; Dumack et al., 2020; Gulin et al., 2022). We considered two general functional traits, "lifestyle" and "life form"; we used coarse categories as taxonomic articles usually only report if taxa are free-living or sessile (without a distinction among modalities of movement), and if they are solitary or capable of organizing colonies.

3.1.2.4.1. Lifestyle

3.1.2.4.1.1. Definition

Lifestyle defines the modality of living of an organism, distinguishing among protists that live freely in the environment, protists that are attached to a substrate and therefore characterized by reduced motility, and protists that live as endoparasites.

3.1.2.4.1.2. Functional categories

a) Free-living: organism capable of moving throughout the environment, including both protists creeping/gliding on substrates and protists swimming in the interstices between soil grains or in water bodies.
b) Sessile: organism living attached to a substrate.
c) Endoparasite: parasites spending most of their life cycle in the cells of their host, although they may have ephemeral flagellate stages for dispersal.

3.1.2.4.1.3. Availability in literature

Information about lifestyle is often present in original descriptions articles. Datasets containing information on lifestyle include Fiore-Donno et al. (2019) and Dumack et al. (2020) for Cercozoa and Endomyxa, and Laplace-Treyture et al. (2021) for phytoplankton.

3.1.2.4.1.4. Critical aspects

Several protists have multiple life stages exhibiting different lifestyles. Many endoparasites, such as dinoflagellates belonging to the Amoebophryaceae family, present a free-living life stage in which they move throughout the environment to find a host (Archibald et al., 2017).

3.1.2.5. Life form

3.1.2.5.1. Definition. Life form identifies the level of organization of an organism in life. Most protists are solitary cells, but some can form colonies composed of multiple unicellular individuals, and some algae develop into truly multicellular organisms.

3.1.2.5.2. Functional categories. a) Solitary: most of the life cycle is spent as solitary unicellular individuals.
b) Colonial: organism capable of organizing in colonies of multiple unicellular individuals.
c) Multicellular: organism composed of multiple cells.

3.1.2.5.3. Availability in literature. Information about life forms is often indicated in original description articles but datasets reporting this information are scarce (but see Laplace-Treyture et al., 2021 for phytoplankton; Gulin et al., 2022 for Ciliophora).

3.1.2.5.4. Critical aspects. Even though the definition of a solitary unicellular protist is generally unambiguous, some taxa can create large multinucleate structures (e.g., plasmodia of Myxomycetes; Archibald et al., 2017). Here we considered these taxa as "solitary" since they are neither colonial nor multicellular.

3.1.3. Life-history

Life-history type represents traits defining the ontogeny, life stages, and reproductive modalities of an organism. Protists show many types of life cycles, each one characterized by peculiar life stages. To limit the number of categories and ease interpretations, we defined two binary traits describing the presence/absence of ecologically relevant life stages, namely spores and resting cysts. Spores are related to reproduction and dispersal, while resistant stages enable organisms to survive in unsuitable conditions (Geisen et al., 2018).

3.1.3.1. Resting cyst

3.1.3.1.1. Definition. The resting or resistant cyst is a life stage of many protists, in which the organism produces a thick shell around its body and enters a dormant phase.

3.1.3.1.2. Ecological relevance. Encystment can enable protists to survive unfavorable environmental conditions, such as drought or extreme temperatures, helping life in harsh environments (Geisen et al., 2017).

3.1.3.1.3. Functional categories. Present/Absent: ability to produce a resting cyst or not.

3.1.3.1.4. Availability in literature. For many soil protists, the resting cyst is a significant life stage, thus information on its presence is usually available in taxonomic articles when resting cysts were observed. Laplace-Treyture et al. (2021) gathered information on the presence of a resting cyst is for algae (but see Geisen et al., 2018 for other few examples).

3.1.3.1.5. Critical aspects. The presence of resting cysts can only be assessed through visual observation, thus defining with certainty whether a species is unable to produce resting cysts is challenging, as their absence could be caused by lack of observation. So far, the ability to produce resting cysts as a functional trait is seldom considered in ecological studies.

3.1.3.2. Sporulation

3.1.3.2.1. Definition. Sporulation involves the production of spores by organisms and is typically related to reproduction and dispersal (Geisen et al., 2018). Soil protists often produce "sporocysts", i.e., spores covered by a protection layer, enabling survival under hostile conditions.

3.1.3.2.2. Ecological relevance. Sporulation can improve survival and dispersal potential (e.g. pioneer species; Geisen et al., 2018).

3.1.3.2.3. Functional categories. Present/Absent: ability to produce spores or not.

3.1.3.2.4. Availability in literature. The ability to produce spores is usually shared at the phylum (or higher) level, therefore handbooks (Archibald et al., 2017) and generic taxonomic articles (Adl et al., 2019) are helpful to find information on this trait.

3.1.3.2.5. Critical aspects. So far, sporulation is seldom considered in ecological studies and datasets (but see Geisen et al., 2018 for some

examples).

3.1.4. Habitat

Although habitat preferences are not always considered in common sets of functional traits (Moretti et al., 2017; Brousseau et al., 2018), discriminating protists inhabiting marine, freshwater, or terrestrial environments provides key information on their adaptations. The definition of habitat for protists is different from that of plants and metazoans. Due to their small size, most freshwater protists can inhabit terrestrial environments with high moisture, where they live within thin water layers, thus many protists inhabit both terrestrial and freshwater environments (Burki et al., 2021). Nonetheless, some species show stronger specialization to specific habitats. Furthermore, metabarcoding data from freshwater ecosystems revealed the presence of taxa traditionally assumed to be specialists of marine habitats (Simon et al., 2015), hence the presence of these protists in soil communities cannot be excluded.

3.1.4.1. Habitat preferences

3.1.4.1.1. Definition. Type of environments in which the species have been sampled (Burki et al., 2021).

3.1.4.1.2. Functional categories. a) Terrestrial: found exclusively in terrestrial environments.
b) Freshwater: found exclusively in freshwater environments.
c) Marine: found exclusively in marine environments.
d) Ubiquitous: found in both terrestrial and aquatic environments.

3.1.4.1.3. Availability in literature. Information on the typical habitat is usually present in taxonomic articles and has been summarized in some datasets (Dumack et al., 2020; Gulin et al., 2022).

3.1.4.1.4. Critical aspects. Data on the type of exploited habitats typically refers to where a specific taxon has been historically found, thus information available in the literature is probably incomplete (Simon et al., 2015).

3.2. Application of the framework to soil protists from glacier forelands

The eDNA metabarcoding of 1123 soil samples yielded 570 MOTUs assigned to protists, encompassing six major supergroups (Alveolata, Amoebozoa, Archaeplastida, Opisthokonta, Stramenopiles, Rhizaria; Fig. 2). The most detected phyla were represented by Cercozoa (194 MOTUs, ~34%) and Ciliophora (191 MOTUs, ~34%) followed by Endomyxa, Bacillariophyta and Chrysophyta (each representing 4% of MOTUs). The taxonomic resolution of detected MOTUs was highly variable, ranging from species to phylum levels. Specifically, 297 MOTUs were identified at the genus level or better, 119 at the family level, 53 at the order level, 33 at the class level, and 68 at the phylum level. After consulting 197 scientific references, we were able to functionally characterize 99.7% of MOTUs identified at the genus level or better, 98.3% of MOTUs identified at the family level, and all MOTUs identified at coarser levels. Due to coarse taxonomic assignment, some MOTUs received an uncertain assignment at one or more categorical traits (trophic level: uncertainty for 1.6% of MOTUs; feeding mode: 27.4%; shell: 9.1%; locomotion structures: 19.1%; lifestyle: 8.2%; life form: 9.3%; resting cysts: 21.6%).

The functional composition of protist communities from proglacial soils was dominated by consumers, mostly omnivores, and was further characterized by the abundance of solitary, free living, ciliates and flagellates, without a shell (Fig. S2a; Fig. S2b). Protists able to produce a resting cyst represented 40% of the total, whereas 15% of MOTUs were known to produce spores. The body length range covered four orders of magnitude, spanning from 2.5 μm (*Siluania monomastiga*) to the >6 cm of the multicellular golden alga *Hydrurus foetidus* (Fig. S2b).

The ordination of MOTUs in the functional space showed that communities of proglacial soils are not evenly distributed across the whole functional space (Fig. 3) but clustered in three regions, mostly reflecting the three broad trophic levels: consumers, parasites, and freshwater
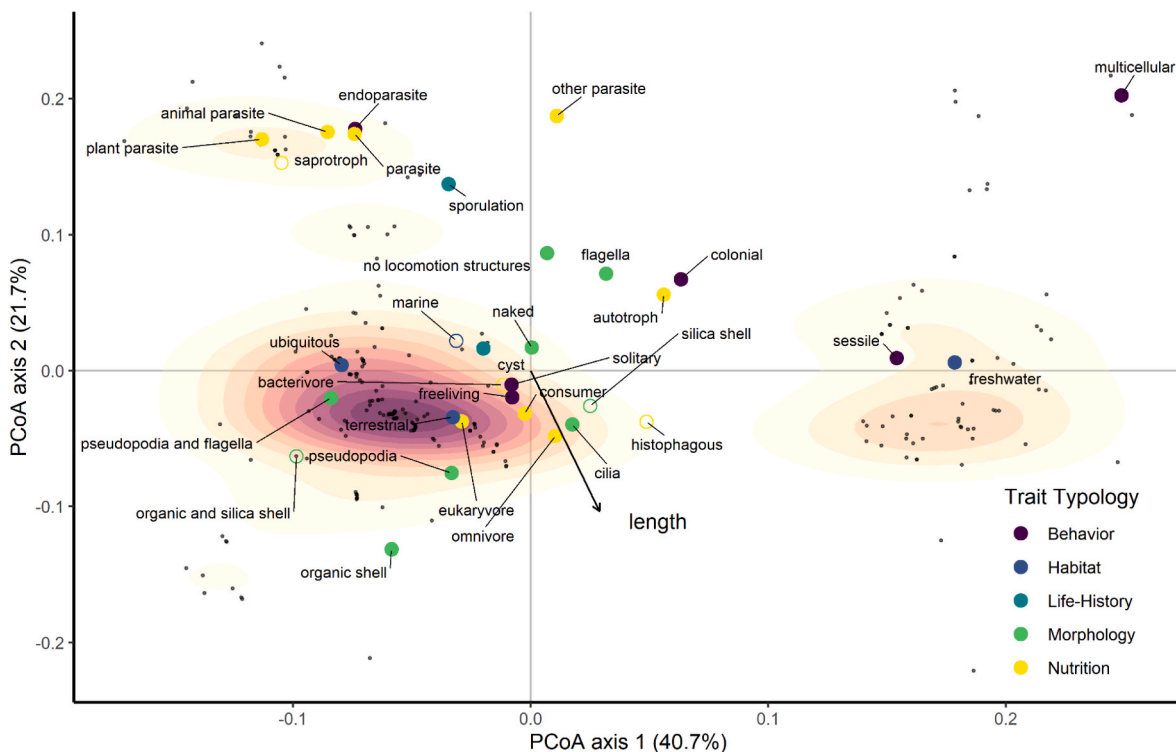
Fig. 3. Functional space of protist communities in proglacial soils. Black dots represent MOTUs; centroids of each category of the categorical traits are represented by larger dots colored by trait type; full dots are the categories significantly related to the first two PCoA axes (Table S3). The arrow indicates the direction and the strength of the correlation between body length and the first and second PCoA axes. Shaded areas represent the density of MOTUs computed with a two-dimensional Kernel density estimation.

autotrophs. A further distinction was represented by habitat preferences, with the typically terrestrial or ubiquitous MOTUs localized around the space characterized by negative values of the first PCoA axis, whereas freshwater MOTUs were positively related to the first PCoA axis. For all traits considered, most categories were significantly related to the first two PCoA axes (Table S3). The first axis of the functional space explained 40.7% of variation and mainly represented traits belonging to types habitat and behavior while the second axis (21.7% of variation) mostly represented morphological, nutritional and life history traits (Fig. 3). The majority of MOTUs were localized in the region of consumers (low values of the first PCoA axis), within which they were mainly discerned by morphological traits. Freshwater autotrophs were positively related to the first PCoA axis and associated with a sessile lifestyle. Parasites were negatively related to both the first and second PCoA axes. This region of the functional space showed a significant association with the endoparasitic lifestyle and the presence of sporulation.

4. Discussion

4.1. Knowledge gaps on functional traits of soil protists

The advancement of sequencing techniques is opening a door to the secretive belowground world and is boosting the study of its amazing biodiversity. Soil protists remain understudied compared to other key microorganisms, despite increasing interest in recent years (Fig. 1). This rise in interest was likely driven by the publication of seminal works that provided large amounts of functional information on protists (Adl et al., 2019; Dumack et al., 2020). Indeed, half of the studies that used functional data from the literature were based on these two aforementioned publications. As observed for most biodiversity research (Martin et al., 2012), there is a severe geographical bias with most studies carried out in Europe, while hyperdiverse tropical areas remain understudied.

Consequently, the availability of functional information is uneven with most data referring to European taxa (Fiore-Donno et al., 2019; Laplace-Treyture et al., 2021; Gulin et al., 2022), even though information remains incomplete also for taxa found in Europe (Geisen et al., 2018). So far, protists lack of a comprehensive trait repository similar to those available for other taxa (e.g., TRY for plants, FAPROTAX for bacteria, or FUNGuild/NEMAGuild for fungi and nematods; Kattge et al., 2011; Louca et al., 2016; Nguyen et al., 2016), limiting the potential of functional analyses and ecological interpretation (Geisen et al., 2018). In fact, most functional studies on soil protists have focused on one or a few traits, often limited to nutrition and morphology – the trait types for which most data are available. While a single-trait approach can reveal how specific features or behaviors are related to environmental factors (Oliverio et al., 2020; Fiore-Donno and Bonkowski, 2021; Singer et al., 2021) or ecological gradients (Mazel et al., 2022), the use of multiple traits is required to investigate the complexity of biotic communities and understand the role their inhabitants play in providing ecosystem functions (Lavorel et al., 2013; Krause et al., 2014; Potapov, 2022).

4.2. A common framework to describe the functional diversity of soil protists

To address the need for more accessible and standardized functional information, we proposed a framework consisting of a set of 10 key traits that summarize current knowledge on soil protist functional diversity (Table 1). The aim of this framework is to promote reproducibility and comparability across existing datasets and repositories, representing a first step toward the development of a common trait database for soil protists. Given that protists are an extremely heterogeneous group, it is debatable whether one comprehensive framework can effectively describe such diverse organisms or, alternatively, a specific functional characterization would be required for each clade/group (e.g., Ciliophora, Cercozoa, testate amoebae). We argue that each approach has

its own purpose. On one hand, clade-specific traits are often used in studies at fine taxonomic resolution, because they allow for a more refined characterization that permits to functionally discriminate similar species or even individuals within the same species. This approach can be used to identify ecological processes (e.g., environmental selection) structuring the composition of specific taxonomic groups (Fiore-Donno et al., 2019), and can enable the detection of local trait adaptation following habitat restoration (Gulin et al., 2022). Clade-specific approaches are also used in palaeoecological studies to reconstruct past environmental conditions (Fournier et al., 2015; Marcisz et al., 2020). However, the clade-specific approach lacks the potential to perform multi-taxa analyses and is not appropriate for datasets with broad taxonomic scope (i.e., high-throughput sequencing data based on generalist markers). In this case, a common set of key traits can facilitate a multi-taxa approach which is essential to explain community assembly (Calderón-Sanou et al., 2022), to reveal the functions and services provided by soil ecosystems (Donald et al., 2021) and to construct ecological networks such as belowground food webs (Potapov, 2022). A multi-taxa approach is frequent in metabarcoding studies (69% of reviewed studies; Table S1), which often use generalist markers providing an overall characterization of protist communities but often show limited taxonomic resolution. A common framework is thus pivotal to exploit the full potential of these datasets and will facilitate relating detected organisms with ecosystem processes and functions (de Bello et al., 2010; Brousseau et al., 2018). The generality and flexibility of this approach can also encourage studies in less investigated areas of the world, reducing the observed geographical bias.

Along with the multi-taxa approach, the common set of traits proposed in our framework enables us to investigate the multifaceted variation of traits in soil protist communities. This is particularly helpful to identify patterns of co-variation in functional traits across taxa (i.e., trait syndromes; Raffard et al., 2017), favouring the discrimination between traits constrained by phylogeny and those related to local environmental conditions and ecological trade-offs (Ellers et al., 2018). A set of multiple traits can also be used to estimate the functional space of the community, which can lead to the identification of broad evolutionary or ecological trajectories represented by the main dimensions of variation in the trait space, such as what was done for size and leaf economics spectrum for plants (Díaz et al., 2016), reproductive strategies for mammals (Bielby et al., 2007) or the maturity index for nematodes (Bongers, 1990). The combination of a multi-taxa and multi-trait approach can also help estimating the relative position and links of an organism in a food web, accounting for traits such as diet, dimensions, or physical protection (Potapov, 2022). A multi-trait approach is particularly important for soil protists, as this group encompasses highly distinct evolutionary lineages. For instance, considering a single trait such as "life-form", which discriminates between solitary and colonial protists, leads to grouping phylogenetically distant protists with differing ecological functions. The integration of multiple traits like feeding mode, morphology, lifestyle, and habitat preferences enables to construct a functional space in which protist communities are well discriminated into distinct clusters. These clusters may suggest that a few traits can effectively summarize the functional diversity of protist communities but could also reflect a strong phylogenetic signal of traits (Goberna and Verdú, 2016).

The application of the framework to a metabarcoding dataset provided a practical example of how these traits can be suitable for high-throughput sequencing data. Nonetheless, characterizing a MOTU identified only to the genus or the family level requires specific adjustments to apply functional information defined at the species level. Such a process is different between trait types. Continuous traits, such as body size, can be defined by averaging the values (e.g., mean length) from the multiple species belonging to high-level MOTUs (e.g., genus or family), whereas categorical traits can be transformed in fuzzy dummy variables (de Bello et al., 2021) to account for the functional variability across species composing the MOTU (i.e., species from the same taxonomic unit

exhibiting diverse ecological features). Due to the lack of a common trait repository, trait assignment required an extensive literature search. The search was particularly challenging for body size data, which are usually only found in taxonomic articles where the species or genus is described or revised. To facilitate data retrieval in future studies, we report the main datasets available for different traits and taxonomic groups, highlighting the ecological relevance and potential issues for each trait (see Results section 3.1). Despite these challenges, the framework allowed us to develop a functional dataset on proglacial soils, representing a starting point toward the creation of a common trait repository. However, the use of a single generic eukaryotic marker limited the ability to target specific taxonomic groups (e.g., Amoebozoa or Heterolobosea; Geisen et al., 2023) and, therefore, the dataset presented is not complete, as widespread soil protist taxa are missing.

5. Conclusion

Exhaustive understanding of soil biodiversity requires the development of multi-taxa approaches to the analysis of protist traits. The widespread adoption of high-throughput sequencing techniques has boosted the study of soil communities and will greatly increase the availability of information in the coming years. To complete the puzzle, we now need to effectively couple the taxonomic data with functional information, in order to obtain a more comprehensive view on the processes structuring belowground ecosystems. The common set of key traits proposed here can be applied to high-throughput sequencing data and is based on traits for which information is available in the existing literature, thus can help achieving these tasks. Nonetheless, further efforts are needed to make functional information on a large number of taxa promptly available, to measure functional data on taxa from the whole globe, and to adapt raw functional information to the specific features of high-throughput sequencing data. We encourage researchers to build upon the proposed framework and functional dataset while tailoring it to their specific research needs.

Author contributions

SG, IC, AC and GFF conceived the ideas and designed methodology; SG and CC performed the literature review; SM, AG, AB and GFF collected and analyzed the environmental DNA metabarcoding data; SG, IC and AC analyzed the data; SG led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2023.109207.

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