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Functional responses of biotic communities after glacier retreat

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ABSTRACT

Climate change is causing glaciers to retreat worldwide, exposing large land surfaces that are soon colonized by several organisms, forming new biotic communities. Predicting how these ecosystems will develop is critical for anticipating drastic environmental changes and informing adaptation and mitigation strategies. Predicting future postglacial ecosystems requires a deep knowledge of the global dynamics driving the organization of biotic communities along glacier forelands. Broad-scale, multi-taxa studies investigating multiple dimensions of biodiversity of these rapidly changing environments are urgently needed. Here, I analyzed the functional responses of soil communities after glacier retreat using an innovative approach integrating environmental DNA metabarcoding with functional traits to estimate functional diversity, infer biotic interactions, and reconstruct soil food webs. The first part of the thesis focused on soil protists, an understudied but crucial component of soil communities, playing key roles in shaping biotic interactions, community assembly, and ecosystem functioning. First, I addressed the scarcity of accessible functional information, a major limitation in functional ecology, proposing a common set of 10 functional traits for soil protists. Then, I assigned the 10 traits to an environmental DNA dataset from 1251 soil samples along 46 forelands across four continents to estimate changes in functional diversity of soil protist communities after glacier retreat. Results showed that, although the overall functional diversity of protist communities increased over time since glacier retreat, phototrophic protists (i.e., algae) declined, causing a shift in the trophic composition from early to late communities, with potential cascading effects on the whole food web. In the second part, I worked at the level of the whole community, assigning traits to several other taxa from the same environmental DNA dataset to analyze the changes in the food web structure along the forelands. Results showed that food web complexity increased over time since glacier retreat, driven by higher functional diversity which enhanced food web stability through increased functional redundancy and connectance. Furthermore, although early food webs varied with local temperature, they converged to greater similarity in later communities, suggesting that ecological successions after glacier retreat tended to develop similar food webs worldwide, providing crucial information for predicting future postglacial ecosystems. In conclusion, combining environmental DNA and functional traits proved to be a valuable approach to describing community dynamics at a broad spatial scale. Overcoming the challenges related to this approach while incorporating additional methodologies and tools will help ecologists to rapidly achieve an exhaustive understanding of ecosystem dynamics following glacier retreat.

SUMMARY

CHAPTER 1

Introduction

Introduction

The paragraph 1.4 is extensively based on the following publication:

Cantera, I., Giachello, S., Münkemüller, T., Caccianiga, M., Gobbi, M., Losapio, G., Marta, S., Valle, B., Zawierucha, K., Thuiller, W., & Ficetola, F. G. (in press). Describing functional diversity of communities from environmental DNA. Trends in Ecology & Evolution.

1 INTRODUCTION

1.1 THE GLOBAL RETREAT OF GLACIERS

1.1.1 Glacier retreat in a warming climate

Anthropogenic climate change is impacting our planet at an increasingly higher rate. 2024 is set to become the warmest year ever recorded, with the global mean surface air temperature of the period January-September being 1.54 (±0.13) °C above pre-industrial level (World Meteorological Organization, 2024). However, the global mean temperature does not capture the whole picture, as temperatures are rising unevenly across the globe, with lands warming faster than the oceans and the high latitude regions experiencing the strongest increases (Fig.1.1; Intergovernmental Panel On Climate Change, 2023). Such disproportionate effects of global warming have a particularly strong impact on high mountain and arctic glaciers, which are retreating worldwide as the loss of ice mass is strongly related to increasing local surface temperature (Huss et al., 2017; Rounce et al., 2023). Many high mountain glaciers have experienced a considerable retreat since the end of the nineteenth century, which has further accelerated in the last decades (Hugonnet et al., 2021; Sommer et al., 2020). Temperate and tropical regions are already witnessing the extinction of some glaciers, like the Calderone glacier in Italy (Pecci et al., 2008) or the glaciers in the Venezuelan Andes (Braun & Bezada, 2013).

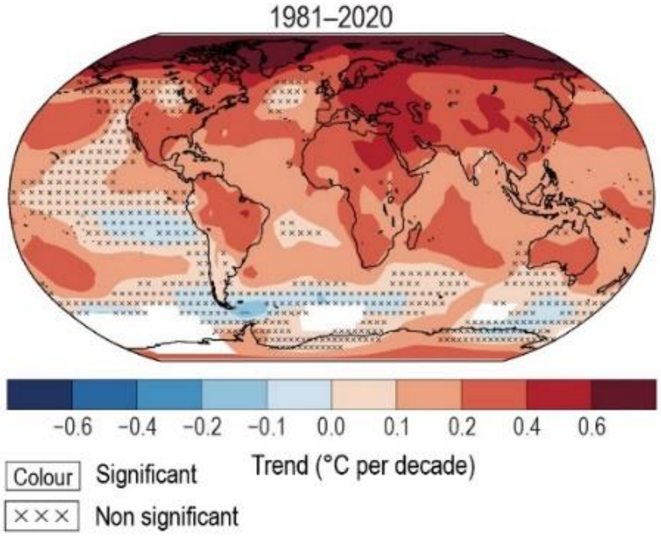

Figure 1.1. *Global observed spatial trends of surface temperature (°C per decade). Colours indicate changes in surface temperature, while 'xxx' represent areas that did not experience significant shifts in temperatures. Figure from Intergovernmental Panel On Climate Change (2023).*

The current climate is already warm enough to commit the continued retreat of glaciers for decades, probably centuries, regardless of the entity of future temperature increases, with projections predicting the disappearance of half of the world's glaciers by the end of the century (Intergovernmental Panel On Climate Change, 2023a; Rounce et al., 2023). The areas that will undergo the highest relative shrinkage of glaciers are the mountain ranges situated at low and mid-latitudes, like the Alps in Europe, the Karakorum and the Himalayas in central Asia, and the Andes in South America. In absolute terms, most of the ice surface will be lost in central Asia, Alaska, and coastal Greenland (Bosson et al., 2023; Rounce et al., 2023).

1.1.2 The unknown future of deglaciated terrains

Retreating glaciers are determining one of the fastest shifts of ecosystems that civilization has ever experienced, causing critical impacts on biodiversity, society, and the economy (Bosson et al., 2023; Cauvy-Fraunié & Dangles, 2019; Huss et al., 2017). If we fail to reduce greenhouse gas emissions, projections show that by 2100, recently deglaciated terrains will cover a surface comparable to a country like Italy (339,000 ± 99,000 km2 of glacier loss under the high-emissions scenario SSP5-8.5; Fig 1.2; Bosson et al., 2023). How postglacial ecosystems will develop in those large newly exposed areas is still unclear. Could deglaciated terrains serve as refugia for cold-adapted species? Will they act as carbon sinks or carbon sources? How much time is required to develop complex communities? Will postglacial ecosystems differ depending on local environmental conditions (e.g., mean temperature)? Predicting the formation of postglacial ecosystems is not only essential for answering these and many other questions but is also critical to favour the development of management strategies for the preservation of these rapidly changing environments, limiting ecological and socio-economic impacts (Cauvy-Fraunié & Dangles, 2019; Lecomte, 2023).

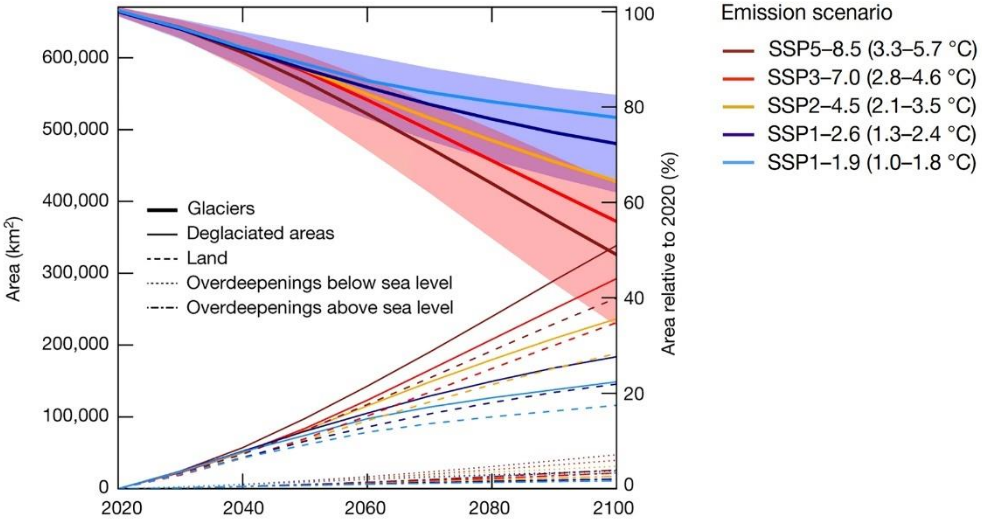

Figure 1.2*. Predicted changes in glacier surface and deglaciated areas. Colours indicate different emission scenarios. Figure from Bosson et al. (2023).*

1.1.3 Glacier forelands to understand ecosystem formation

Predicting the development of postglacial ecosystems requires a deep understanding of the dynamics underlying the organization of biotic communities in recently deglaciated terrains at the global scale. Glacier forelands offer great opportunities to investigate the development of ecosystems after deglaciation. A glacier foreland is a complex geomorphological structure created by a retreating glacier which leaves evident traces of its retreat on the landscape (e.g., moraines). The analyses of those traces can provide estimates of when the glacier retreated from a specific site enabling the reconstruction of a chronological series of dated sites (i.e., a chronosequence), in which the age of the site corresponds to the time since the biotic community started to develop (Ficetola et al., 2021; Marta et al., 2021). The chronosequence of a glacier foreland typically ranges from recently deglaciated sites in proximity to the glacier front characterized by harsh environmental conditions, bare soil, low resource availability, and limited biodiversity, to late successional stages deglaciated since tens or hundreds of years in which environmental conditions are milder, soils are more mature, and the biological community is richer and more complex (Fig. 1.3). Studying glacier forelands can thus provide information on how biotic communities develop after deglaciation accounting for different factors such as time since glacier retreat, local mean temperature, soil properties, biotic interactions or the influence of the glacier itself (Ficetola et al., 2024; Stibal et al., 2020).

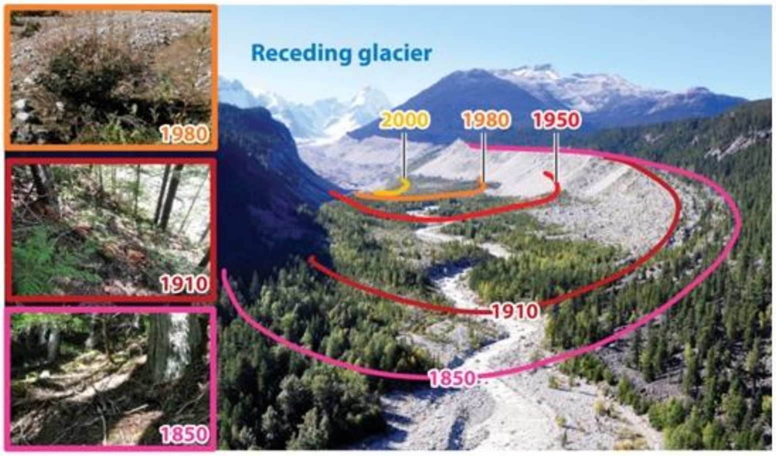

***Figure 1.3**. Picture of the foreland of the Tiedemann Glacier (Canada) showing highly different environments for the recently deglaciated sites (e.g., 1980) and the older sites (e.g., 1850). The dates indicate the year since glacier retreat. Figure from Ficetola et al. (2021).*

Although glacier forelands have been studied for more than a century as models of primary ecological successions (Clements, 1916), the majority of analyses have been performed on one specific taxon (e.g., plants or specific taxa of arthropods), while multi-taxa studies at large spatial scale are scanty, yet urgently needed. A recent meta-analysis showed that most soil taxa increase in richness and abundance over time since glacier retreat following the increase in nutrient availability and plant richness (Pothula & Adams, 2022). The overall increase in biodiversity along the succession was further validated by a multi-taxa study including glacier forelands from four continents which showed that the increase in taxonomic diversity over time is caused by multiple factors such as more favourable environmental conditions, increased biotic interactions, especially with plants, and higher probability of colonization (Ficetola et al., 2024). The common patterns that emerged from these recent studies provided key insights to grasp the development of ecosystems after deglaciation. Still, the analysis of the sole taxonomic diversity limits our understanding of the mechanisms and dynamics occurring in the extremely complex soil communities. Taxonomic diversity needs to be integrated with more information describing biotic interactions and the functions provided by each component of soil communities to better represent the complexity of below-ground ecosystems and their development after deglaciation.

1.2 SOIL COMMUNITIES AND ECOSYSTEM FUNCTIONS

1.2.1 Soil communities host the highest biodiversity on Earth

Soil is a thin layer of organic matter blanketing the land surface of our planet. Without this thin layer, life in terrestrial ecosystems would be severely limited. Soil is now considered to be the most biodiverse ecosystem on Earth, harbouring millions of species spanning from microorganisms like bacteria, fungi, archaea, viruses, and protists to a wide range of metazoans of all sizes, from the minuscule nematodes to much bigger ecosystem engineers like earthworms and moles (Fig. 1.4; (Anthony et al., 2023; Guerra et al., 2020).

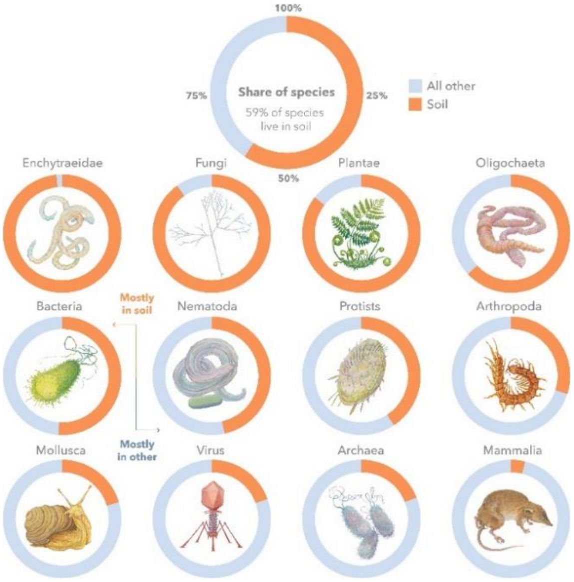

***Figure 1.4**. Figure representing the proportion of biodiversity (i.e., the share of species) living in soil (orange color) for different taxonomic groups. Figure from Anthony et al. (2023).*

1.2.2 Key role of soil organisms on ecosystem functioning and service

The immense complexity and diversity of soil communities have posed challenges to their study, delaying their recognition as critical actors in driving key processes underlying the functioning of our planet. However, the advancements of molecular techniques in the last two decades, together with the still developing functional classification, are enabling ecologists to understand soil tremendous importance in governing biogeochemical fluxes driving several ecosystem functions and services such as the turnover of organic matter, carbon, and nutrient cycling, food production, and even climate regulation (Fig. 1.5; Bardgett & Van Der Putten, 2014; Crowther et al., 2019; Guerra et al., 2020). Such a wide variety of soil organisms composing the soil biota expresses an equal variety of functional roles. Soil organisms can thus be characterized using a functional classification distinguishing organisms in functional groups based on their feeding habits, body size, or life forms, determining their vertical stratification in soil (Potapov, Beaulieu. For example, epigeic earthworms are large decomposers living on the soil surface where they feed on decaying organic matter, favouring its turnover and enhancing microbial activity, while nematode predators are consumers feeding on small organisms contributing to top-down control in soil food webs. Such functional classifications are particularly useful to overcome the limits imposed by taxonomy and link the functional composition of soil communities to environmental factors and processes, increasing our understanding of the roles soil organisms play in ecosystem dynamics (Potapov, Beaulieu, et al., 2022). Considering the central role of soil communities in shaping ecosystem processes, it comes as no surprise that aboveground ecosystems, as well as most human activities, rely on soil biodiversity (Anthony et al., 2023; Bardgett & Van Der Putten, 2014). In particular, the functioning of aboveground ecosystems appeared not to be much influenced by soil taxonomic richness but rather more by its functional diversity and the structure of the trophic interactions occurring among its components.

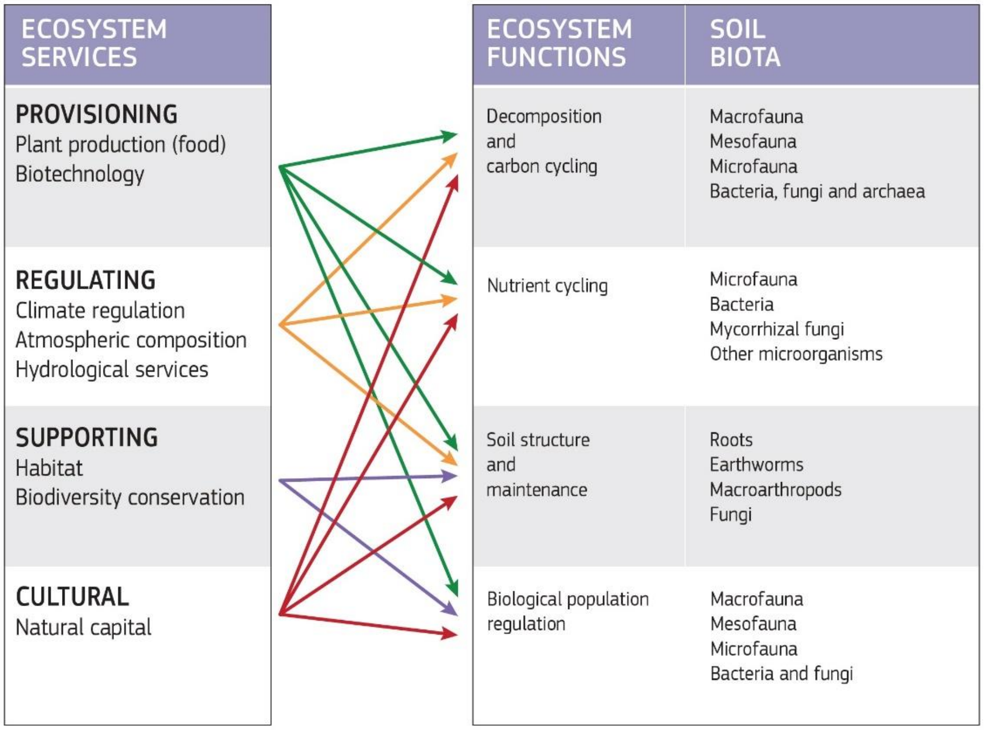

Figure 1.5. *Graphical scheme linking the soil biota to ecosystem services and ecosystem functions. Figure from (Orgiazzi et al., 2016).*

For example, the functional diversity of decomposer organisms has been linked to key ecosystem processes such as soil respiration, litter decomposition, and nutrient cycling across different biomes (Handa et al., 2014; Heemsbergen et al., 2004). Another study reconstructed the soil food webs of several locations in Europe, showing that carbon and nitrogen cycling are deeply affected by changes in the structure of soil food webs (De Vries et al., 2013). Soil food web structure and variation in soil energy fluxes were also linked to changes in below- and aboveground biodiversity of tropical ecosystems subjected to different land use (Potapov et al., 2024). Such findings highlight the importance of considering the functional roles of soil organisms rather than their taxonomy to reveal the functioning and dynamics of ecosystems. Understanding the patterns underlying the development and the organization of soil communities across the globe is, therefore, essential for predicting the responses of ecosystems to radical environmental changes such as the global glacier retreat. Nevertheless, our current knowledge of how the functional composition of soil communities develop along the environmental gradient created by a retreating glacier (i.e., a glacier foreland) is extremely limited, and more research at a large spatial scale is required (Bardgett & Van Der Putten, 2014; Guerra et al., 2020).

1.2.3 Successional dynamics of soil communities along glacier forelands

Previous studies along glacier forelands have shown that recently deglaciated terrains are characterized by low diversity, no or few plants, and simple food webs due to strong abiotic filters mainly related to cold temperatures and a short activity season (Hågvar et al., 2024; Raso et al., 2014; Sint et al., 2019). These simple soil communities are composed mainly of microbial communities of heterotrophic and photosynthetic bacteria, algae, and pioneer metazoans exhibiting high ecological flexibility and great dispersal capacities like tardigrades, springtails, mites, and predator arthropods (Hågvar et al., 2020, 2024; Rosero et al., 2021; Sint et al., 2019). Such traits are typical of colonizers and favours the colonization of the harsh recently deglaciated terrains, as confirmed by a study on nematode communities, which showed that nematodes occurring in the early stages of the succession showed a high dispersal capacity and r-ecological strategies while in the latest stages, persister taxa were more abundant (Guerrieri et al., 2024). Early soil food webs are thus dominated by arthropod predators exercising a top-down control. These generalist predators prey upon micro-arthropods like springtails and mites, which represent the most abundant primary consumers of these harsh environments, or capture the animals that are blown by the wind (Fig. 1.6; Hågvar, 2012; Hågvar et al., 2024). Due to scarce resources, intraguild predation also represents a common feeding strategy in these habitats (Raso et al., 2014). Although simple, early soil food webs are crucial to facilitate the arrival of more organisms (e.g., plants), triggering pedogenesis through accumulating organic matter and nutrients in the early soils. As time since glacier retreat increases, soils start to develop, and plants can establish, soon becoming the most important primary producers (Khedim et al., 2021). The establishment of plants attracts more herbivores and detritivores, which in turn, favour the arrival of more predators due to a higher availability of preys (Hågvar et al., 2020; König et al., 2011). Even in the later stages of the succession, predators are mostly generalist, so intraguild predation remains important (König et al., 2011; Raso et al., 2014). Soil communities in the later stages are thus richer in biodiversity, supporting more species, more individuals, higher functional diversity, and more complex food webs (König et al., 2011; Rosero et al., 2021; Sint et al., 2019).

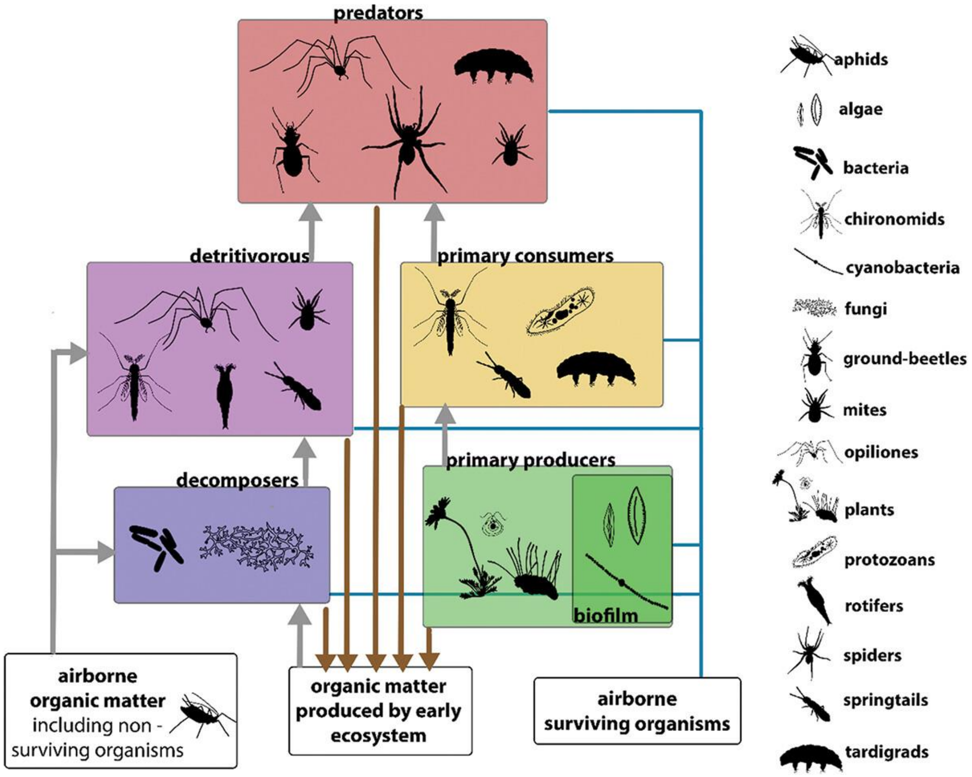

Figure 1.6. *Representation of a hypothesized food webs of soil communities from recently deglaciated terrains (< 10 years after deglaciation). Arrows indicate energy flows between the components of the food web. Figure from Hågvar et al. (2024).*

Although recent studies provided valuable insights into the development of soil ecosystems, most investigated only one or a few glacier forelands or focused on specific taxonomic groups or interactions, limiting the possibility of detecting common patterns or dynamics of ecosystem formation. For example, it is still not clear how and when the transition from simple to complex food webs occurs and what the factors or processes driving this transition are. Furthermore, most of the studies focused on bacteria, plants, or animals, but we know almost nothing about the successional dynamics of protist communities after glacier retreat, even though protists are the most abundant and diverse eukaryotes in soil ecosystems (Geisen et al., 2020; Potapov, Beaulieu, et al., 2022). To gain a more comprehensive view of the processes driving the formation of ecosystems after glacier retreat, we need to investigate soil communities at a large spatial scale, coupling a multi-taxa approach accounting for all the components of soil ecosystems to trait information in order to describe patterns of functional diversity and predict biotic interactions (Ficetola et al., 2021). Understanding the mechanisms driving the formation of soil communities after glacier retreat is crucial not only to understand their role in regulating biogeochemical cycles and supporting below- and aboveground biodiversity but also for gaining insights into how these ecosystems respond to rapid environmental changes, thus improving predictions of ecosystem trajectories under global climate change scenarios (Bardgett & Van Der Putten, 2014; Crowther et al., 2019; Guerra et al., 2020).

1.3 FUNCTIONAL DIVERSITY, TRAITS, AND FOOD WEBS

1.3.1 Going beyond taxonomic diversity

Ecosystems are made of living organisms interacting with each other and with the abiotic components of the environment (e.g., water, minerals, nutrients) providing ecosystem services and functions (Bardgett & Van Der Putten, 2014; Schulze &

Mooney, 1994). The quantity and the type of ecosystem services and functions provided are strictly linked to the diversity of the biotic communities (Cardinale et al., 2006; Loreau et al., 2001). Traditionally, studies described the diversity of a community using indexes of species abundance or richness and demonstrated how ecosystem functions like productivity are related to taxonomic diversity (Naeem et al., 1994; Tilman et al., 1996; Whittaker, 1972). Nonetheless, taxonomic diversity is only one of the many dimensions composing biodiversity; other measures include genetic diversity, phylogenetic diversity, and functional diversity (Naeem et al., 2012; Petchey & Gaston, 2002). Describing the diversity of communities only using an index of taxonomic diversity (e.g., species richness) hinders our understanding of the influence of biodiversity on ecosystems because all species are assumed to be ecologically equivalent and overlooks their functional roles (Lyashevska & Farnsworth, 2012). Thus, in the last 30 years, ecologists started to study not only the diversity of species but also the diversity of functions (functional diversity) provided by each species, i.e., the ecological role of an organism in a community (Díaz & Cabido, 2001; Tilman et al., 1997). This relatively recent approach uses trait information to define 'functional groups' or 'functional entities' grouping species based on shared traits, enabling the estimation of functional indexes that are more directly linked to ecosystem functions (Díaz & Cabido, 2001; Heemsbergen et al., 2004; Isbell et al., 2011; Naeem et al., 2012; Schleuter et al., 2010). Several approaches have been used to evaluate the functional variation of communities based on species traits. For instance, species can be plotted in a bi-dimensional functional space in which the coordinates of the species are estimated using the distance between species based on their trait values (Fig. 1.7; Cantera et al., 2022; Carmona et al., 2021; Díaz et al., 2016; Mouillot et al., 2021).

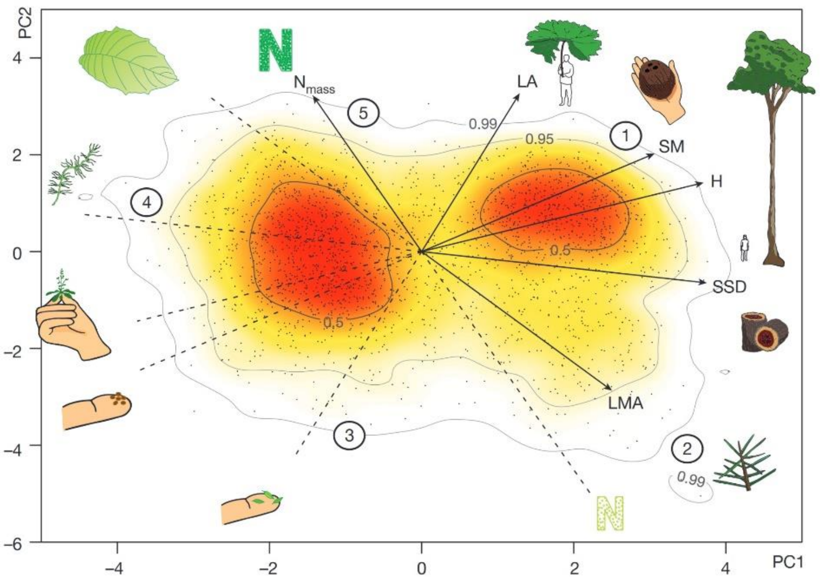

Figure 1.7*. Bidimensional functional space of vascular plant species. The space is defined by the principal component axes 1 and 2. Plant species are plotted in the functional space based on trait distance according to six traits indicated by the arrows and the icons. The colour gradient and contour lines represent the density of species sharing similar traits and thus occupying a similar position in the functional space. Figure from Díaz et al. (2016).*

Plotting species or functional entities in a functional space provides a visual representation of the functional diversity of a community, enabling a better interpretation of the distribution of the functions (i.e., traits) in a community, which can be quantified by several functional indexes (Mouillot et al., 2013, 2021). Some of the most used functional indexes include functional identity, functional richness, functional redundancy, functional complementarity, functional evenness, and functional specialization (Mouchet et al., 2010; Mouillot et al., 2013; Ricotta et al., 2016). Functional identity represents the position of a species in the functional space, averaging the values of the traits expressed by the species. Functional identity has been demonstrated to be a strong predictor of ecosystem functions and food web structure due to its property of summarizing the functional role of a species in a community (Gagic et al., 2015; Laigle et al., 2018; Mouchet et al., 2010). Functional richness quantifies the area of the functional space occupied by all the species in a community, and it is often correlated with species richness (Mammola et al., 2021). Functional redundancy measures how close the species are in the functional space, being the opposite of functional complementarity. High values of functional redundancy indicate that many species exhibit the same trait values, i.e. provide the same functions, vice versa low values of functional redundancy correspond to high values of complementarity indicating that each species in the community provides a different function. Functional redundancy has often been linked to ecosystem stability because it enables maintaining high values of functional richness even in case of extinction events (Carmona et al., 2021). For example, Isbell et al. (2011) showed that functional redundancy in grassland plant communities is needed to maintain multiple ecosystem functions in time and space under changing environmental conditions. Functional evenness describes the regularity of the distribution of species in the functional space. Communities expressing high levels of functional evenness are usually considered more stable, while low values of functional evenness are often associated with communities subjected to harsh conditions or high disturbance, which allow the persistence of only a few species exhibiting specific traits favouring their survival in hostile environments (Ellers et al., 2018; Gerisch et al., 2012; Ochoa-Ochoa et al., 2019). Functional specialization quantifies how far species are from the center of the functional space, indicating the presence of specialist species characterized by extreme trait combinations. Functional specialization is particularly useful in detecting the loss of specialist taxa. For example, Villéger et al. (2010) showed that estuarine fish communities subjected to disturbance experienced a loss of functional diversity due to a decrease in functional specialization even though species richness increased.

To summarize, analyses of functional diversity are needed to predict ecosystem functioning and detect community dynamics that taxonomic diversity cannot fully explain. Functional indexes capture different variations in community composition, though many are often correlated (Mouchet et al., 2010). However, estimating functional indexes or defining functional groups relies on trait information, which is often scarce, hindering the use of functional approaches.

1.3.2 Traits

Traits describe the ecology of species differentiating organisms by their diet, morphology, life history, behaviour, or physiology (Gravel et al., 2016; Moretti et al., 2017). Traits are at the foundation of functional diversity studies, but their use presumes the direct measurement of the traits on the individuals/specimens or their collection in the available databases or literature. Each of these two approaches has its pros and cons. The direct measurement of the traits of the individuals allows to estimate the intraspecific variability in trait expression and, not less importantly, provides information relevant to your study area since individuals of the same species can exhibit different trait values based on differing environmental conditions or diverse populations (Bolnick et al., 2011; Violle et al., 2012). Although the direct measurement of traits on individuals provides excellent trait information, it is very time consuming and can be prohibitive for studies covering a large spatial scale or analysing a wide range of taxonomic groups. In such cases, online available trait databases such as TRY for plants (Kattge et al., 2011), FUNGuild /NEMAGuild for fungi and nematodes (N. H. Nguyen et al., 2016), BETSI for soil fauna (Joimel et al., 2021), or Dumack et al. (2020) for Cercozoa, are valuable source of trait information to functionally characterize species or taxonomic units for which it was not possible to measure the traits directly. For example, the use of trait databases allowed the evaluation of the changes in the functional composition of nematode communities during the colonization of proglacial landscapes (Guerrieri et al., 2024), estimate the erosion of functional diversity for plants and vertebrates at a global scale (Carmona et al., 2021), or describe the global spectrum of plant form and function (Díaz et al., 2016). Even though extremely useful, the existing trait databases are far from complete for most of the taxa and show strong spatial biases, with the most biodiverse areas (i.e., tropical regions) being the least studied (Etard et al., 2020). For instance, FUNGuild (N. H. Nguyen et al., 2016) contains information only for 11% of the 150.000 described fungal taxa (Phukhamsakda et al., 2022); for amphibians and reptiles, traits are available only for 47% and 46% of the species respectively (mean trait coverage from Etard et al., 2020) while plants, mammals and birds are the most studied organisms, with trait data for over 80% of the species (81% for plants, 89% for mammals, 84% for birds; Etard et al., 2020; Kattge et al., 2011). Additionally, for many soil organisms such as protists, springtails, earthworms, or insects, trait databases are limited to specific taxonomic groups or not available at all (Ellers et al., 2018). The scarcity of trait databases is a major constraint for the application of functional diversity measures to studies covering a large spatial scale or considering entire biotic communities. Such studies are further hindered by the resolution of many available trait classifications that are taxon-specific. Traits are intrinsically related to taxonomy, and different taxonomic groups are described using different types of traits due to their highly different ecology. For example, one of the most used traits for insects describes the presence or the absence of wings. Applying this trait to non-winged taxonomic groups like nematoda or springtails makes no sense. The differences in traits between taxonomic groups are even more obvious if we compare plant traits, such as leaf area, with animal traits, like eye position. Thus, the different ecology of different taxonomic groups leads to the development of taxon-specific trait classification or framework (some examples are Dumack et al., 2020 for Cercozoa; Gravel et al., 2016 for animals; Kattge et al., 2011 for plants; Moretti et al., 2017 for terrestrial invertebrates; Nguyen et al., 2016 for fungi) which are difficult to compare and combine when analysing the functional diversity of entire communities. Furthermore, the ‘same’ trait can be estimated differently based on the groups of organisms considered. For example, a trait describing the capacity of dispersal of carabid beetles is different from a trait describing the capacity of dispersal of phagotrophic protists like Cercozoa because the two taxonomic groups are ecologically very different and use different strategies to disperse. The taxon-specificity of many traits makes it particularly challenging to characterize the functional diversity of an entire biological community, which is typically composed of organisms ranging from prokaryotic bacteria to plants, fungi, protists, and animals. Comparing the diversity of such ecologically and phylogenetically distant taxonomic groups can be achieved mainly through two approaches. The first approach uses the taxon-specific traits for each taxonomic group to estimate functional gradients that can be compared across taxa. The use of taxon-specific traits provides a more precise characterization of the functional role of an organism, allowing the detection of functional differences even among closely related taxa. For example, Neyret et al. (2024) estimated a 'slow-fast' trait gradient for more than 2800 above- and belowground taxa belonging to 14 'trophic guild' to study the effect of land use intensification. By estimating a trait gradient for each of the 14 trophic guilds, Neyret et al. (2024) revealed an effect of land use intensification that could have been overlooked if common and broader traits had been used to characterize the whole biotic community. The second approach consists of characterizing all the organisms of the community using very broad traits that can be applied to all the taxa, like the type of trophic group (e.g., autotrophs, parasites, or consumers) or the size. For example, (2022) investigated the importance of size in global patterns of community assembly for a wide range of soil eukaryotes, including fungi, protists, and animals. Characterizing the entire community using the same broad traits is increasingly used to estimate biotic interactions between all the trophic/functional groups of the community, thus allowing the reconstruction of food webs (Potapov, 2022; Potapov et al., 2024).

Despite the scarcity of trait information for some organisms (e.g., soil protists, earthworms, or springtails), the growing availability of trait databases facilitates the estimation of functional diversity at the community level. Trait-based approaches are also proving particularly valuable for reconstructing food webs, offering deeper insights into ecological interactions and ecosystem functioning.

1.3.3 Food webs

Food webs are a representation of the ecological interactions occurring in biotic communities, from microorganisms like bacteria to apex predators, and can be used to provide estimates of energy flows between nodes or trophic levels (Barnes et al., 2018). A food web is a unipartite ecological network in which any two nodes (taxonomic or functional groups) can be connected by trophic interactions (Dunne, 2006). Integrating biodiversity estimates with the structure of ecological interactions makes food webs a powerful tool to assess community dynamics, complexity, and stability (Rooney & McCann, 2012; Saint-Béat et al., 2015) and identify the predictors governing ecosystem functioning (Gravel et al., 2016). Food web structure can be described using multiple food web properties or metrics. Some of the most used food web properties are the number of nodes, the number of links, connectance, modularity, nestedness, or complementarity (Delmas et al., 2019). The number of nodes is an estimate of biodiversity. If nodes are species, the number of nodes is equal to species richness; if nodes are functional groups, it is a measure of functional richness. The number of links quantifies the total number of interactions occurring in the community, representing the size of the food web (Delmas et al., 2019). Connectance represents the proportion of realized interactions relative to all potential interactions. Connectance provides insights into how interactions are distributed in a food web, is one of the most important predictors of food web structure (Braga et al., 2019), and is often related to community dynamics. For example, Dunne et al. (2002) found that in food webs experiencing species loss, connectance increased the robustness of ecosystems, decreasing the number of cascading extinction events (species going extinct due to the previous loss of other species). Modularity or compartmentalization quantifies how nodes are closely connected, grouping them into modules that are characterized by closely connected internal nodes that are less connected with the nodes outside the module (A. E. Krause et al., 2003; Olesen et al., 2007). Modular networks were found to be more resistant to disturbances because modules buffer the effect of the perturbation, limiting its spread throughout the community (Gilarranz et al., 2017), suggesting that food webs with higher levels of modularity could be more resilient to environmental changes. Another property used to explain ecological processes in communities is nestedness. Food webs with a nested structure, i.e., the interactions of specialist species or functional groups are subsets of the interactions of more generalist nodes, are hypothesized to enhance biodiversity by reducing competition among species within a community (Bastolla et al., 2009). Although different, most of these food web properties are often collinear and highly dependent on the diversity (number of nodes) and size (number of links) of the food webs, challenging the ecological interpretation of the results (Poisot & Gravel, 2014).

To overcome the problem of correlation between food web properties, null models are often applied to compare the observed results to random expectation, providing a more mechanistic understanding of the factors driving food web structure and the organization of ecological communities (Pellissier et al., 2018). In food web ecology, null models are usually applied, keeping constant the number of nodes while randomly permuting the abundance of each node, thus changing the probability of interaction between nodes (Caruso et al., 2022). For example, Calderón-Sanou et al. (2024) used null models to understand if trophic groups (i.e., nodes) with more interactions were randomly distributed in the food web or formed pairs of linked groups.

The reconstruction of food webs can also provide information for identifying potential keystone species, i.e., species having a disproportionate importance in the community (Mills & Doak, 1993). For example, Schwarzmüller et al. (2015) found that large-bodied consumers, defined as ‘trophic whales,’ can preserve ecosystem stability in environments subjected to continuous disturbances. Centrality indexes represent quantitative approaches to identifying potential keystone species from food webs (Jordán, 2009). Centrality indexes are measures defining how ‘central’ is a node in a network; some of the most used are degree centrality, closeness centrality, and betweenness centrality. Degree centrality is simply the number of interactions per node, closeness centrality indicates how close a node is to all the other nodes in the food web, and betweenness centrality quantifies how many times a node is found between a pair of connected nodes, for example, a node with high values of betweenness centrality is defined as a ‘connector’ for its role of intermediate between other interacting nodes (Delmas et al., 2019).

Despite the high potential explanatory power of food webs, collecting empirical data to assess real trophic interactions for all the components of a community remains a major challenge hindering the reconstruction of real and comprehensive food webs (Evans et al., 2016; Potapov et al., 2023). Since traits represent the ecological role of a species in a community, biotic interactions are governed by species traits, which are also correlated with the position of a species in a food web (Laigle et al., 2018). Trait-based approaches can thus be applied to define functional groups based on species trait similarity and predict biotic interactions following general assumptions from food web theory stating that species interact with each other based on their traits such as feeding preferences or body size (Fig. 1.8; Coux et al., 2016; Laigle et al., 2018; Potapov, 2022). Despite the growing use of multiple dimensions of biodiversity to describe biotic communities, linking biodiversity to ecosystem functions remains one of the main challenges in ecology (Antunes et al., 2024). High-throughput techniques like environmental DNA metabarcoding can be used to perform fast and exhaustive samplings of soil communities (Ficetola & Taberlet, 2023), providing taxonomical data that can be integrated into a trait-based approach to reconstruct soil food webs at a large spatial scale(Potapov, 2022).

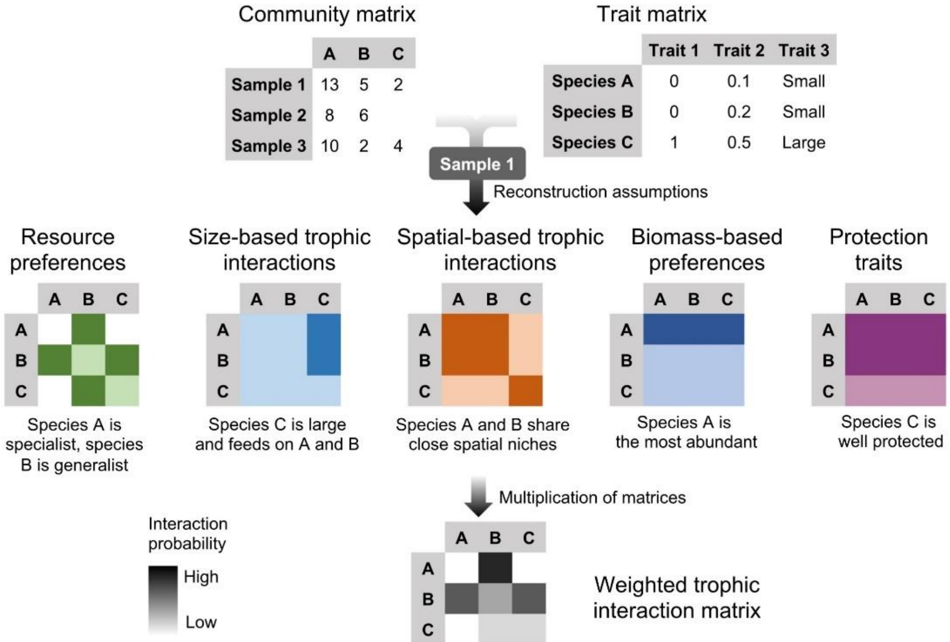

***Figure 1.8**. Approach of Potapov (2022) to estimate trophic interactions using traits and reconstruct food webs. Taxonomic composition of communities (community matrix) is combined with trait data of the taxa present in the communities (trait matrix) to estimate N matrices of interaction probability, one for each of the N traits. The N matrices are then multiplied to obtain a unique weighted trophic interaction matrix, which is used to build the food webs. Figure from Potapov (2022).*

By estimating food web properties and identifying keystone species, food webs can reveal how communities organize after deglaciation, leading to the formation of more complex and stable ecosystems. However, modelling soil food web combining a trait-approach with environmental DNA data poses several challenges that remain to be addressed (Pereira et al., 2023).

1.4 DESCRIBING FUNCTIONAL DIVERSITY OF COMMUNITIES FROM ENVIRONMENTAL DNA

1.4.1 eDNA metabarcoding to assess functional diversity

eDNA metabarcoding has emerged as a powerful tool for rapidly compiling biodiversity inventories across diverse taxonomic groups, habitats, and ecosystems (Beng & Corlett, 2020; Taberlet et al., 2018). This method facilitates extensive biodiversity assessments across numerous sites, achieving goals that would be unattainable with traditional sampling techniques. eDNA-based inventories of communities effectively capture taxonomic diversity (Fediajevaite et al., 2021), thereby revolutionising the acquisition of standardised, large-scale biodiversity data. While conventional biodiversity metrics predominantly focus on taxonomic diversity, there is a growing recognition of functional diversity as a more proximate measure of ecosystem functions (Díaz et al., 2006; Mammola et al., 2021; Reiss et al., 2009). Functional traits, such as body size, feeding habits, photosynthetic capacity, reproductive strategies, and dispersal ability, are intricately linked to community structure and dynamics (Nolte et al., 2019). Analysing these traits reveals how they influence ecosystem functions (effect traits) and how they respond to environmental drivers (response traits) (Lavorel et al., 2013). When thoughtfully selected (see Streit & Bellwood, 2023), traits can enhance our understanding of ecosystem functions (Lavorel et al., 2011), enabling the quantification and prediction of biodiversity's impacts on ecosystem performance (Gobbi & Fontaneto, 2008; Mouillot et al., 2013). Recent years have seen a growing number of studies adopting a functional perspective through eDNA, with over 100 articles published between 2012 and 2024, assessing various taxonomic groups of eukaryotes (e.g., protists, plants, animals) and diverse environments (Box 1). These studies characterise the functional diversity of communities by linking traits to taxonomic units identified through eDNA. Traits are gathered from a variety of origins, including scientific literature, open databases, and direct observations or measurements of organisms collected independently from the eDNA samples (Box 1). This approach, hereafter referred to as 'Fun-eDNA' (Figure 1.10), enables three key types of trait-based applications. First, functional groups can be identified at sampling sites based on the presence of traits indicative of specific functions, such as trophic level or growth form (Jauss et al., 2021). Second, researchers can calculate indices that synthesise the variability of multiple traits, thereby characterising aspects of functional diversity for a given community (e.g., functional richness and redundancy Cantera et al., 2023). Finally, trait values can be attributed to taxonomic units to derive mean community values for specific traits (e.g., body size Aslani et al., 2022). The resulting functional groups, indices, and community trait values can then be related to environmental parameters. By assigning traits to eDNA-based inventories, researchers can consider a broader array of functions within the sampled communities compared with assigning traits to traditional inventories, due to the reduced selectivity towards specific traits, taxa or habitats inherent in eDNA sampling (Aglieri et al., 2021; Marques, Castagné, et al., 2021; Seymour et al., 2021). Furthermore, the Fun-eDNA approach offers novel insights into difficult-to-sample communities, such as microscopic organisms arduous to identify (Labouyrie et al., 2023; Singer et al., 2021), or species-rich ecosystems (Coutant et al., 2023), which often require extensive expertise for taxonomic identification.

Box 1. Literature review of articles using the Fun-eDNA approach

We reviewed studies assigning traits to taxonomic units obtained from eDNA ('Fun-eDNA approach'). The review was conducted through a Web of Science query (on 12 September 2024). See the supplemental information online for the search string and the selection of the relevant articles. We identified 129 published articles employing the Fun-eDNA approach starting from 2012, with a notable increase in the past 5 years (Figure 1.9A). In these studies, a very diverse range of taxa (Figure 1.9B) was assessed by analysing the eDNA obtained from the water, soil, sediment, or organic material (e.g., faeces, plant organs) (Figure 1.9C). Aims were heterogeneous across studies (Figure 1.9D), with the majority of articles testing the methodological robustness of functional assessments and assessing the natural (e.g., climate, soil properties) and/or anthropogenic drivers (e.g., deforestation) of functional diversity. Feeding characteristics were largely the traits most frequently used to assess functional diversity, followed by habitat and morphology (Figure 1.9E). Traits were assigned to eDNA-based inventories by gathering information from different sources (Figure 1.9F). Functional diversity was characterised using three general traitbased applications. Over 70% of studies inferred functional diversity on the basis of the number of functional groups (e.g., herbivores, decomposers, predators) or from the proportion/number of taxa within defined functional groups. Over 25% of studies calculated functional indices by combining information of several traits for each taxa to summarise different aspects of the functional structure of a given community (e.g., functional richness, redundancy, specialisation). Finally, few studies attributed values for a given trait to the taxonomic unit and derived mean trait values for the entire community (e.g., mean community values) by averaging the values of the taxa detected in the sample.

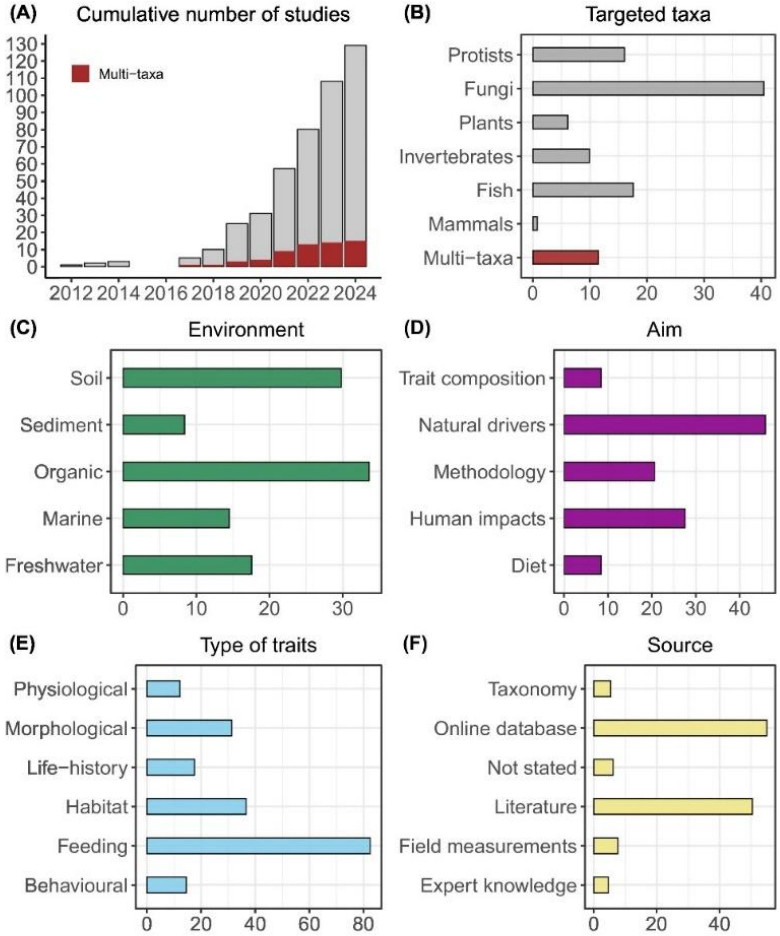

***Figure 1.9**. Results of the literature review of the articles using the Fun-eDNA approach. (A) Cumulative number of studies using the Fun-eDNA approach for each year from 2012 to 2024. Multi-taxa studies in red correspond to the studies assessing the traits of at least three different taxonomic groups from the same eDNA sample. We then report the percentages of Fun-eDNA studies categorised by the sampled taxa (B), sampled environments (C), research aims (D), trait typologies (E), and the source from which the traits were collected (F). See the supplemental information online for details on the definition of the categories and the list of selected articles.*

Despite its potential to advance functional and ecological studies, trait-based assessments from eDNA data face major conceptual and technical challenges. Anticipating an increasing number of studies embracing this functional perspective, we summarise the major achievements of the Fun-eDNA approach, identify recurring challenges, and recommend solutions while acknowledging their limitations. Finally, we propose future research avenues aimed at addressing the challenges posed by this emerging and dynamic approach.

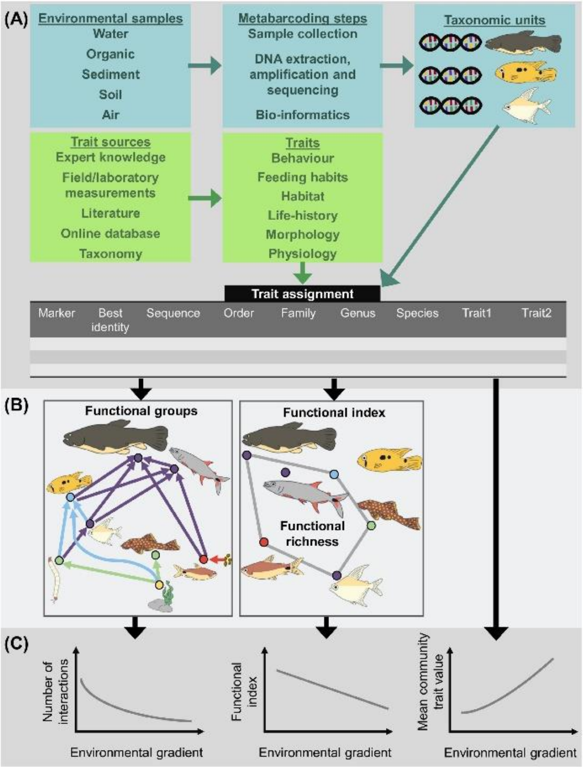

Figure 1.10. *Fun-eDNA procedure. (A) The Fun-eDNA approach links eDNA (blue) and functional ecology (green) methods to assign traits to taxonomic units identified*

with eDNA. Traits from various sources are assigned to taxonomic units enabling characterisation of the functional diversity of communities. (B) Functional diversity can be characterised through the identification of functional groups (categorised based on specific traits), community indexes, or community mean trait values. Functional groups can be used to reconstruct food-webs, from which different variables can be derived (e.g., number of interactions). Community indexes, synthesising the variability of multiple traits in a given community, can be also quantified. Here, we represented functional richness, as the convex hull volume occupied by the community in a functional space. Values for a given trait can be attributed to taxa to calculate mean community values for the trait. (C) The obtained functional variables can be related to natural or anthropogenic drivers. In (B), arrows represent the flow of energy and matter from one organism to another, indicating the direction of consumption (from the resource to the consumer). Coloured dots represent trophic groups: yellow = primary producer, red = detrivore, green = herbivore, blue = omnivore, purple = secondary consumer.

1.4.2 The untapped potential of integrating eDNA with trait-based approaches

eDNA-based inventories provide efficient means for conducting biodiversity analyses across multiple sites within reasonable timeframes. A significant advantage of Fun-eDNA lies in its ability to integrate trait information into large-scale inventories, thereby offering additional insights into ecological questions at broad spatial and temporal resolutions (Seymour et al., 2021). For instance, Fun-eDNA has identified key drivers of functional diversity at both continental (Labouyrie et al., 2023) and global scales (Aslani et al., 2022; Guerrieri et al., 2024), illustrating consistent effects of ecological factors on traits (see Figure 1.11A for an example). As global changes accelerate, large-scale spatio-temporal patterns derived from Fun-eDNA can enhance rapid assessments of biodiversity alterations. Traits assigned to comprehensive eDNA-based inventories can elucidate the impacts of global stressors at regional and continental scales, yielding valuable implications for management. For instance, Fun-eDNA revealed a functional homogenization of fish communities in human-impacted rivers in French Guiana and showed that assemblages poorly monitored by the Water Framework Directive exhibited unique traits from the regional pool of species' traits (Coutant et al., 2023). In Europe, assessing the impacts of land-use changes on the structure and functions of soil communities with Fun-eDNA revealed higher microbial richness in croplands compared with less-disturbed environments, with potential negative impacts on ecosystems due to increased proportion of fungal pathogens (Labouyrie et al., 2023). Another advantage of Fun-eDNA is its applicability to multi-taxa inventories. Characterising functional diversity across many taxonomic groups is inherently challenging, requiring diverse expertise and multiple sampling techniques. Fun-eDNA allows for the simultaneous coverage of functional groups from various trophic levels and domains of life from a single sample, using multiple markers or a generalist one (Ficetola & Taberlet, 2023; Li et al., 2020). Analysing traits across multiple taxa is crucial for understanding the intricate relationships between biodiversity losses and ecosystem functions (Reiss et al., 2009) and for identifying whole-ecosystem responses to environmental drivers (Montagna et al., 2018).

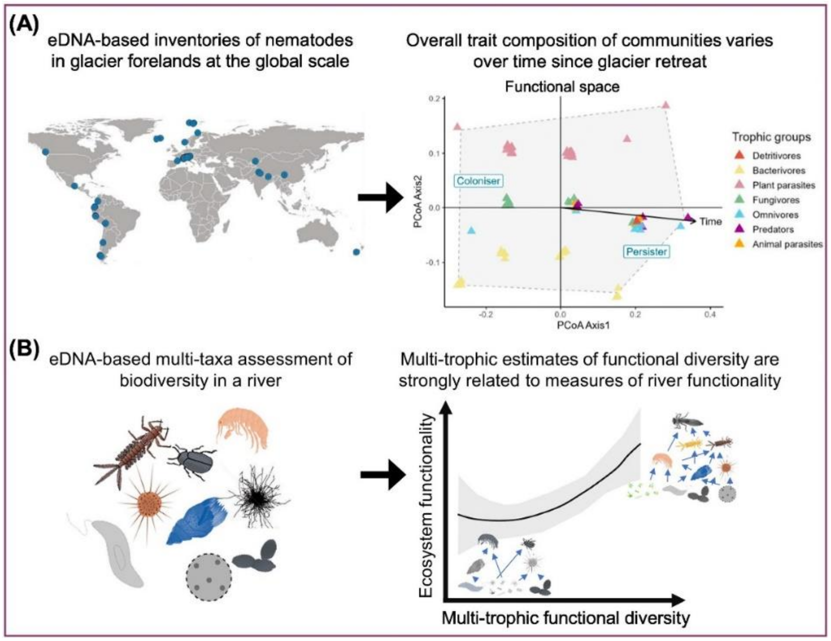

Figure 1.11*. Major advances enabled by the Fun-eDNA approach. Exploiting the full potential of Fun-eDNA allows for the assessment of functional diversity in communities across spatial scales and taxonomic extents that would be challenging with traditional methods. (A)* Guerrieri et al. (2024) *used soil eDNA to describe the functional diversity of nematodes in 46 glacier forelands distributed across the world (blue dots). Building a functional space by ordinating nematode taxonomic units (triangles) based on their traits showed a general shift in trait composition over time since glacier retreat. In recently deglaciated sites, coloniser nematodes with an r-strategy and mostly feeding on bacteria and fungi prevailed, while in later stages, communities hosted more persistent nematodes with a K-strategy and representing more diverse feeding habits. If trait information is available, this approach can be extended to other soil taxa communities to assess whole-ecosystem functional diversity. (B)* Li et al. (2020) *used eDNA to assess functional diversity across a broad range of taxa (invertebrates, fungi, protists, and bacteria) in a Chinese river and showed that ecosystem functionality increases in sites hosting the communities with highest functional diversity.*

Fun-eDNA can reveal whether functional groups respond synchronously to particular drivers and whether these coordinated responses arise from direct effects (shared responses of individual functional groups) or through trophic cascades (Calderón-Sanou et al., 2021; Neyret et al., 2024). For instance, in grassland ecosystems, multiple groups showed consistent and synchronous responses to land-use intensification because of both direct effects on trophic groups and cascading effects across trophic levels (Neyret et al., 2024). Since ecosystem functions are outcomes of ecological processes directly or indirectly influenced by organisms and their interactions (Cardinale, 2011; Cardinale et al., 2002), multi-taxa measures of functional diversity can serve as proxies for ecosystem multifunctionality (Calderón-Sanou et al., 2021; Potapov, 2022). Such measures can reflect key ecosystem functions, such as decomposition and enzyme activities (Li et al., 2020; Figure 1.11B). Specifically, trophic interactions enable the evaluation of how biodiversity affects essential ecosystem functions, including carbon sequestration, nutrient cycling, and pest regulation, as well as overall ecosystem resilience and stability (Potapov et al., 2024; Soliveres et al., 2016). Food-webs integrate interactions and energy fluxes occurring at different trophic levels in a given community and alterations in their structure may reflect impacts on ecosystem functions (Potapov, 2022). However, comprehensive reconstructions of actual trophic interactions are complex, requiring the integration of extensive, heterogeneous information (Fricke et al., 2022), as mere co-occurrences of taxa do not imply trophic relationships (Thuiller et al., 2024). Nevertheless, large-scale, multi-taxa inventories obtained through eDNA can be integrated with available trait information, such as feeding habits, size, and protective strategies, to infer the likelihood of trophic interactions (Fricke et al., 2022; Potapov, 2022; Potapov et al., 2024). Employing machine learning or Molecular Ecological Network Analysis (Meyer et al., 2020) can further increase the accuracy of these reconstructions, using observations of real interactions (Fricke et al., 2022; Pichler et al., 2020). So far, only 11% of the studies using Fun-eDNA considered at least three taxonomic groups (Box 1), often at small spatial scales, and even fewer have attempted to reconstruct food-webs or other interaction networks. Nonetheless, some studies have successfully used Fun-eDNA to identify trophic cascades and trophic niche overlaps (Calderón-Sanou et al., 2021; Meyer et al., 2020), yielding promising results. We anticipate that food-web reconstructions base done DNA will gain popularity in coming years.

1.4.3 Challenges and solutions to promote eDNA use in functional ecology

Despite its successes, the full exploitation of Fun-eDNA is challenged by limitations inherent to both eDNA and trait-based approaches, as well as their integration, particularly when applied across a broad spectrum of taxa and/or at large spatial scales.

1.4.3.1 Challenges inherent to eDNA methods

Taxonomic uncertainty. eDNA inventories frequently contain assignments at taxonomic levels coarser than species (e.g., genus or family). This is typically due to low taxonomic resolution of the markers used – common in generalist markers targeting all eukaryotes – and incomplete local reference databases of sequences.

Incomplete sequence databases pose significant challenges for broad-scale studies and/or assessments in species-rich ecosystems (Marques, Milhau, et al., 2021). Nevertheless, if correctly addressed, these limitations need not preclude robust functional estimates. Recent analyses suggested that taxonomic uncertainty, stemming from incomplete sequence databases, has minimal impact on functional diversity estimates (maximum underestimation compared with actual values of <30%; Condachou et al., 2023). Several methodological strategies can mitigate taxonomic uncertainty when assigning traits to eDNA-based inventories:

- Assigning traits only to taxa identified at the species level (e.g., Aglieri et al., 2021), which is feasible when using comprehensive reference databases and specific markers, because most assignments are at species level.
- Attributing trait values or categories based on a randomly chosen species expected to inhabit the study area within the identified genus or family (Condachou et al., 2023; Marques, Castagné, et al., 2021).
- Condensing information into higher taxonomic ranks: calculating mean values of all available species within the genus/family for continuous traits (e.g., Guerrieri et al., 2024) or assigning general traits conserved at the genus/family level for categorical traits (e.g., Guerrieri et al., 2024). Traits may also be inferred from the phylogeny (Johnson et al., 2021; Kim et al., 2018). These solutions cluster the taxonomic units obtained from eDNA into broader functional groups often closely aligned with taxonomy.

Although these strategies facilitate a functional perspective on communities, the last two points presuppose a phylogenetic signal among species within a given genus or family (i.e., closely related taxa share traits). This assumption does not always hold, as some traits may have evolved independently and different traits can exist within the same clade. Consequently, these strategies may underestimate functional diversity by reducing functional heterogeneity within clades. To enhance Fun-eDNA accuracy, comprehensive sequence databases should be developed (Ruppert et al., 2019; Weigand et al., 2019). Future research should prioritise sequencing efforts for under-represented taxa and regions, as well as taxa within functionally diverse genera/families or bearing extreme traits (Condachou et al., 2023).

eDNA mostly provides presence/absence information. eDNA primarily provides presence/absence data, complicating the ability to ascertain whether an organism is alive, inactive, or dead (Yao et al., 2022). eDNA can also detect the stochastic occurrence of windblown spores of micro-organisms, even though these can have little influence on actual ecosystem dynamics. These limitations pose challenges for testing ecological hypotheses regarding the contribution of species and their traits to ecosystem functioning, particularly when mass ratio effects are expected – that is, when ecosystem functions are primarily determined by the traits of taxa with the highest biomass or abundance (Reiss et al., 2009; M. D. Smith et al., 2020). Nonetheless, taxa with the highest relative abundance or occurrence in eDNA datasets often are the ones with the highest actual abundance (Ariza et al., 2023; Calderón-Sanou et al., 2020; Pansu et al., 2015; L. Wu et al., 2024; Yao et al., 2022). Emerging technologies, such as environmental RNA (eRNA, which degrades more quickly in the environment than eDNA) or shotgun sequencing, may help address these limitations. However, further methodological advances are needed for their wider application (Ficetola & Taberlet, 2023; Peel et al., 2019; Stat et al., 2017; Yao et al., 2022).

1.4.3.2 Challenges inherent to trait-based approaches

Available trait information is dispersed and not exhaustive. Over the past decades, trait-based research has generated extensive but unconnected and heterogeneous datasets across various contexts, resulting in trait information being scattered across disparate repositories. This makes trait assignment time-consuming and cumbersome. Researchers using the Fun-eDNA approach often pool trait data from various sources (Box 1)(Calderón-Sanou et al., 2022; Cantera et al., 2022; Veron et al., 2023) and have to deal with significant database heterogeneity. In addition, available data tend to be biased toward a limited number of taxonomic groups (e.g., plants, vertebrates), regions (e.g., Europe), as well as toward specific trait types, (e.g., feeding habits) (Pereira et al., 2023), which limits the research questions that can be addressed. Finally, for some groups (e.g., tardigrades, rotifers), knowledge about the biology of the species is scarce. To enhance accessibility, integration, and reuse of trait information, we recommend that future trait measurements adhere to established standards for dataset description and structuring (Dawson & Scott, 2013; Giachello et al., 2023; Klimešová et al., 2019; Moretti et al., 2017; Pérez-Harguindeguy et al., 2013), while expanding these frameworks to encompass additional taxa, traits, and ecosystems.

Variability in terminology and concepts. The considerable variability in terminology across studies and databases creates semantic inconsistencies, complicating the effective integration of traits (Pey et al., 2014). For example, in the reviewed articles (Box 1), terms related to feeding exhibited considerable variation (‘nutrition’, ‘diet’, ‘food acquisition’, ‘trophy’, ‘consumption’). Combining traits from very different organisms exacerbates semantic inconsistencies, as preferred terminology differs across taxonomic groups, and can pose conceptual challenges. Indeed, the same trait can exert different functional effects on the ecosystem, depending on the taxon, its specific characteristics, scales of action, and interactions within its respective ecosystems. For instance, for microbes, the term ‘predation’ can refer to phagotrophic protists grazing on bacteria, thereby regulating bacterial populations and local nutrient cycling with rapid and localised effects. The ecological effects of ‘predation’ performed by large vertebrates are not fully comparable with that of microbes. Vertebrate predation involves complex behavioural mechanisms and promotes cascading effects that can persist for long periods, as these animals occur over broad spatial scales and often show long generation times (Ripple et al., 2014). Collaborative initiatives have been developed to promote standardised terminology for traits through consensus among researchers within specific scientific fields and ontology-based applications (Le Guillarme et al., 2023; Pey et al., 2014). Such initiatives should be expanded to include efforts to homogenise terminology across diverse taxa and to use traits as a common currency that respond similarly to a given environmental driver (Neyret et al., 2024; C. Zhang et al., 2024). This will facilitate the identification of key traits to correctly assess the effects of global changes at the ecosystem-level, such as cascading effects on food-webs (Calderón-Sanou et al., 2021) or synchronised versus divergent responses across taxa (Neyret et al., 2024).

1.4.4 Challenges to integrate eDNA with trait-based approaches

Assigning traits to eDNA-based inventories is not straightforward, as traits are collected independently from eDNA data and are described at the species or even individual level. Consequently, trait assignment to inventories is arduous. Trait databases coupling sequencing and detailed taxonomic information with specific guidelines have been recently developed to facilitate and accelerate the functional annotation of eDNA-based inventories (Giachello et al., 2023; N. H. Nguyen et al., 2016). Researchers should consistently upload the assembled trait databases in a freely accessible way with detailed taxonomic and sequencing information (Figure 1.10). Additionally, assigned trait values can derive from individuals inhabiting other habitats and thus likely subjected to very different environmental conditions than those studied. This can overlook local adaptation and intraspecific variation across sites, seasons, and/or life stages (Reiss et al., 2009; Yao et al., 2022), which are crucial aspects shaping species interactions and the ecosystem's ability to cope with environmental changes (Bolnick et al., 2011; Violle et al., 2012). A growing number of studies are exploring eDNA applications to describe within-species genetic variation from short markers (Sigsgaard et al., 2020; Yatsuyanagi et al., 2023), potentially enabling teasing apart lineages with functional differences (Steudel et al., 2016). However, eDNA samples often contain degraded DNA in low quantities, which can lead to erroneous sequence variants, complicating population genetic analyses (Andres et al., 2023). Moreover, not all markers are suitable for population analyses or may not reflect variability in phenotypic traits. Combining eDNA sampling across several sites with a stratified in situ collection of specimens at a strategically chosen subset of the sampling sites (covering key environmental conditions) is an optimal strategy to efficiently measure fine-scale trait variation and better represent local trait measures (Pereira et al., 2023).

1.5 AIMS AND OVERWIEW

The main goal of my PhD project is to describe the functional dynamics of the ecosystems developing after the retreat of glaciers at a large spatial scale. To achieve this goal, we coupled an environmental DNA dataset of soil communities along glacier forelands with trait information to estimate measures of functional diversity and reconstruct soil food webs. Using eDNA data, we obtained an almost exhaustive characterization of soil communities from 46 glacier forelands across four continents. By integrating trait information with data on community composition, we moved beyond taxonomic diversity, revealing functional diversity patterns and inferring biotic interactions.

As illustrated in paragraph 1.4, combining eDNA data and functional traits (Fun-eDNA approach) has great potential but also comes with many challenges. During my PhD, I tried to exploit the potential of the Fun-eDNA approach while overcoming some of the challenges to achieve the ambitious goal of describing the development of ecosystems after glacier retreat from the functional point of view. To do that, I performed three main works that correspond to the three central chapters of this thesis (chapters 2-4). These chapters are followed by a final one where I briefly summarize the overall findings of my PhD project.

The first and second papers focus on soil protists, a neglected yet essential group of organisms playing key roles in below-ground communities for which trait information was scanty and scattered throughout the literature. The third work analyses the whole soil community, estimating the biotic interactions occurring among its components to reconstruct soil food webs.

In chapter 2 (first paper) we define a common set of traits to characterize the functional diversity of soil protists. Through a literature review we propose a trait-based framework with the aim to develop a comprehensive database of functional traits for soil protists designed to: use a consistent terminology, facilitate the retrieval of functional data, and accommodate most of soil protist diversity.

In chapter 3 (second paper), we investigate the changes in functional diversity and composition of soil protist communities developing after glacier retreat combining a broad-scale eDNA dataset from 1251 soil samples along 46 glacier forelands with the common set of traits defined in chapter 2. In particular, we test three main hypotheses: i) functional richness increases over time since glacier retreat, ii) soil protists exhibiting different functional traits follow divergent successional trajectories after glacier retreat, iii) the overall functional composition of soil protist communities changes over time since glacier retreat.

In chapter 4 (third work), we assess the successional dynamics of the food web structure of soil communities developing after glacier retreat. We reconstruct 263 soil food webs along 46 glacier forelands, estimating biotic interactions using eDNA data, functional traits, and food web theory to test three main hypotheses: i) food webs complexity increases over time since glacier retreat, ii) food webs developing after glacier retreat follow a convergent trajectory, iii) the identity of functional groups having a central role in food webs change between early and late communities.

CHAPTER 2

Toward a common set of functional traits for soil protists

Toward a common set of functional traits for soil protist

This chapter is extensively based on the following publication:

Giachello, S., Cantera, I., Carteron, A., Marta, S., Cipriano, C., Guerrieri, A., Bonin, A., Thuiller, W., & Ficetola, G. F. (2023). Toward a common set of functional traits for soil protists. Soil Biology and Biochemistry, 187, 109207.
https://doi.org/10.1016/j.soilbio.2023.109207

2 TOWARD A COMMON SET OF FUNCTIONAL TRAITS FOR SOIL PROTISTS

ABSTRACT

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.

2.1 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 & Van Der Putten, 2014; Delgado-Baquerizo et al., 2020). Each biotic component of the belowground world interacts with the surrounding environment and other soil or ganisms, deeply influencing key ecosystem functions such as organic matter decomposition, soil structure, as well as aboveground productivity (Crowther et al., 2019; Decaëns, 2010; Orgiazzi et al., 2016). 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 & 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 (Burki et al., 2021; Geisen et al., 2019). 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 (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 et al., 2021), and include an unparalleled diversity of eukaryotes estimated in millions of species belonging to highly diverse lineages (Adl et al., 2019; Burki et al., 2021; Orgiazzi et al., 2016). 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 (Bonkowski, 2004; Clarholm, 1981; Coûteaux et al., 1998). 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 under standing 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 (Cardinale et al., 2012; Loreau et al., 2001; Naeem et al., 2012). Thus, refined information on their functionaldiversity 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 (Briones, 2014; Cardinale et al., 2012; Geisen et al., 2023; Naeem et al., 2012). Functional diversity represents the variety of morphological, ecological, behavioral, and physiological traits exhibited by different species (Cadotte et al., 2011; Hooper et al., 2005). 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. 2.1). 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 (Dumack et al., 2020; Fiore-Donno et al., 2019), 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 (Mazel et al., 2022; Seppey et al., 2020; 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, 2008), to family level (Berger, 1999, 2007, 2011; Foissner & 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.

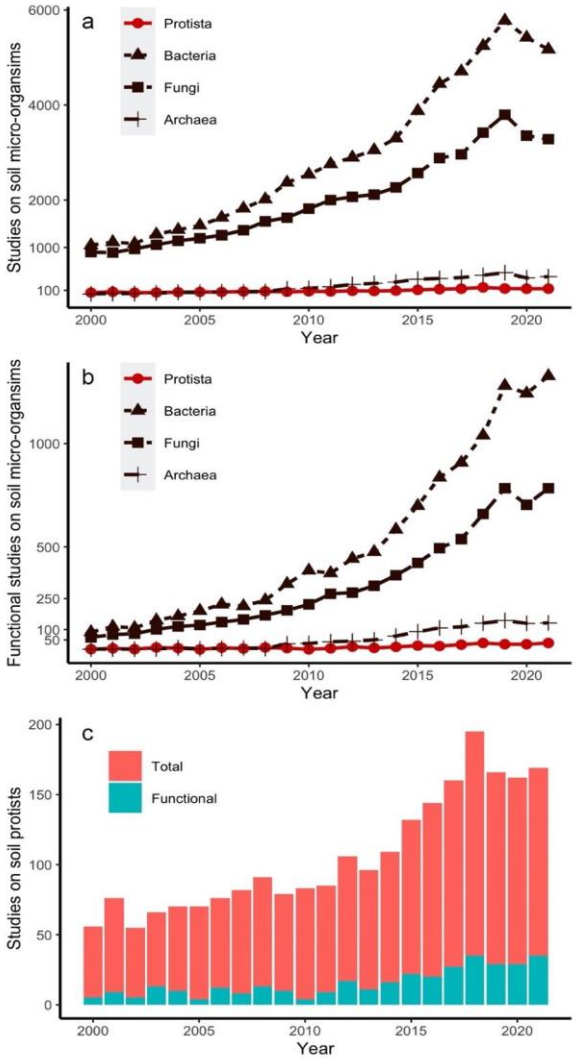

***Figure 2.1**. a) Number of studies on soil microorganisms (bacteria, fungi, archaea and protists); b) number of functional studies for each taxon; c) proportion of functional studies over the total number of studies for protists. See Supplementary Methods 2.1 for details on the literature search.*

2.2 METHODS

2.2.1 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 S2.1). The functional traits found through the review were classified into six broad types (Dumack et al., 2020; Moretti et al., 2017): 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 S2.2). 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 or behaviors depending on features such as the life stage or the environmental conditions (Adl et al., 2019; Archibald et al., 2017), 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.

2.2.2 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. S2.1). 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., 2024). 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 2.2. 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. 2.2). Taxonomy was defined through the NCBI Taxonomy database (Schoch et al., 2020). We then applied our framework to functionally characterize protist 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. 2.2). 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 S2.1). 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 & 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.

2.3 RESULTS

2.3.1 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 identified protists based on their morphological features, 21 used mo lecular 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 2.1). Our review identified 28 distinct traits for the functional characterization of soil protists (Table S2.2), 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 protistdiversity with the data availablein the literature (Table S2.2). 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 2.1).

***Table 2.1**. 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	(B. Chen et al., 2021; Mazel et al., 2022; B.-A. T. Nguyen et al., 2020, 2021; Oliverio et al., 2020; Seppey et al., 2020; Singer et al., 2021; Voss et al., 2019)
	Feeding mode	Group of organisms exploiting analogous food resources	bacterivore; omnivore; eukaryvore; animal parasite; plant parasite; other parasite; saprotroph; histophagous	Cercozoa; Endomyxa	(Dumack et al., 2020; Fiore-Donno et al., 2019, 2020, 2022; Roshan et al., 2021)
				community	(Ritter et al., 2021; Santos et al., 2020; Schulz et al., 2019; Xiong et al., 2018; J. 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 et al., 2020)
				community	(Aslani et al., 2022; Luan et al., 2020)
				amoeboid	(Gulin et al., 2022)
	Locomotion structures	Appendages involved in locomotion	flagella; pseudopodia; pseudopodia and flagella; cilia; none	Cercozoa; Endomyxa	(Dumack et al., 2020; Fiore-Donno et al., 2019, 2020, 2022)
				amoeboid	(Gulin et al., 2022)
Behavior	Lifestyle	Living modality of an organism	free-living; sessile; endoparasite	Cercozoa; Endomyxa	(Dumack et al., 2020; Fiore-Donno et al., 2019, 2020, 2022)
				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)

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 (Bonkowski, M. et al., 2019; Briones, 2014; Potapov, 2022). Considering the high diversity of nutrition modalities within protists, we considered two functional traits with different levels of precision. First, the 'trophic level' (Mazel et al., 2022; Seppey et al., 2020; 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 (Dumack et al., 2020; Fiore-Donno et al., 2019; J. Zhang et al., 2021).

Trophic level

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 furtherdistinguished in phagotrophs (i. e., consumers) and parasites (Bonkowski et al., 2019).

Ecological relevance. The trophic level has been oftenused 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).

Functional categories. Three main trophic categories are generally considered (Mazel et al., 2022; Oliverio et al., 2020; Seppey et al., 2020; 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.

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.

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).

Feeding mode

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

Ecological relevance. Grouping protists in a few welldefined 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 & 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 & Clarholm, 2012) or alternative pathways for nutrient cycling (Geisen et al., 2020; Seppey et al., 2017).

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' (Adl et al., 2019; Lynn, 2008).

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.

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; Calderón-Sanou et al., 2022; Dumack et al., 2020; 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, 2022).

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).

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 (Dumack et al., 2020; Fiore-Donno et al., 2019; 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.

Body size

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.

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 (Fiore-Donno et al., 2022; Geisen & Bonkowski, 2018; Potapov, 2022).

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 Laplace-Treyture et al., 2021; Luan et al., 2020).

Critical aspects. Length and width measurements are often available but highly dispersed in literature, making it timeconsuming to obtain this information, especially if the aim is the func tional 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.

Shell

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; Geisen et al., 2018; Meisterfeld, 2002).

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).

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.

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).

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 & von der Heyden, 2007).

Locomotion structures

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

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 (Dumack et al., 2020; Fiore-Donno et al., 2019, 2022).

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.

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’ (Dumack et al., 2020; Fiore-Donno et al., 2019 for Cercozoa and Endomyxa; Gulin et al., 2022 for amoeboid protists; Laplace-Treyture et al., 2021 for phytoplankton).

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).

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 (Dumack et al., 2020; Gulin et al., 2022; Voss et al., 2019). 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.

Lifestyle

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.

Functional categories

a) Free-living: organism capable of moving throughout the environ ment, 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 cyclein the cells of their host, although they may have ephemeral flagellate stages for dispersal.

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.

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).

Life form

Definition. Life form identifies the level of organizationof 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.

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.

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

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.

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 repro duction and dispersal, while resistant stages enable organisms to survive in unsuitable conditions (Geisen et al., 2018).

Resting cyst

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.

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).

Functional categories.

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

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 for algae (but see Geisen et al., 2018 for other few examples).

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.

Sporulation

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.

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

Functional categories.

Present/Absent: ability to produce spores or not.

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.

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

Habitat

Although habitat preferences are not always considered in common sets of functional traits (Brousseau et al., 2018; Moretti et al., 2017), discriminating protists inhabiting marine, freshwater, or terrestrial environments provides key information on their adaptations. The definition of habitatfor protists is different from that of plantsand 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.

Habitat preferences

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

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.

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).

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).

2.3.2 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. 2.2). 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. S2.2a; Fig. S2.2b). 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. S2.2b*).

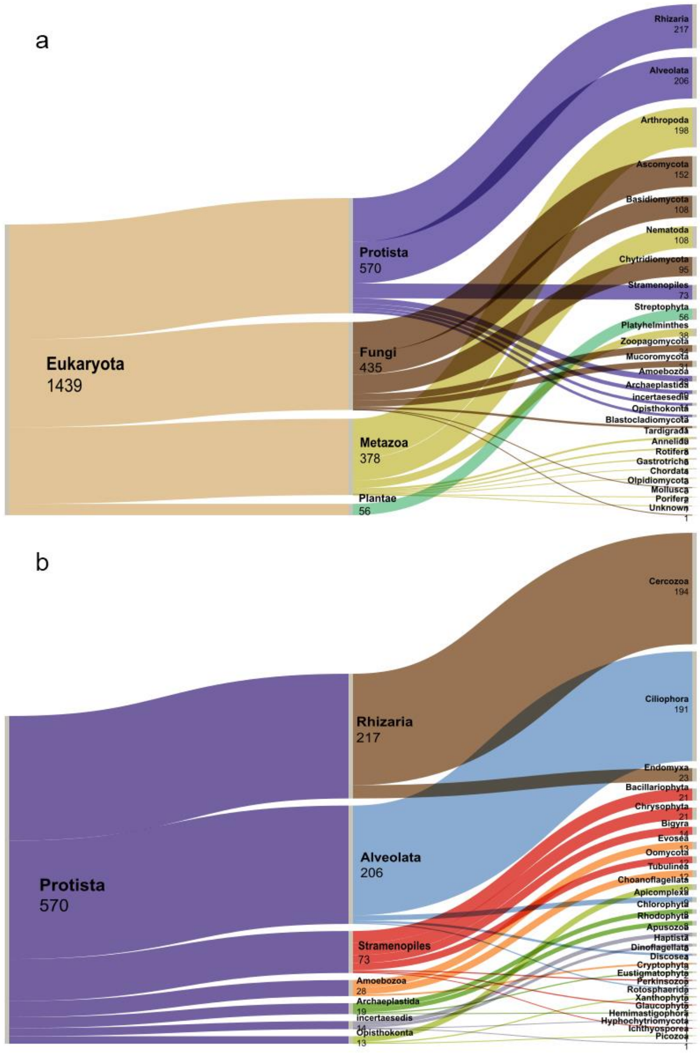

Figure 2.2 *Taxonomic composition of MOTUs detected with the general Eukaryota marker Euka02 (Guardiola et al., 2015) of all soil samples collected for the dataset; numbers indicate the number of MOTUs within each taxonomic group. a) All MOTUs identified at least at the kingdom level divided in the four kingdoms Protista, Fungi, Metazoa and Plantae. b) Taxonomic breakdown of MOTUs belonging to the kingdom Protista.*

The ordination of MOTUs in the functional space showed that communities of proglacial soils are not evenly distributed across the whole functional space (Fig. 2.3) but clusteredin three regions, mostly reflecting the three broad trophic levels: consumers, parasites, and freshwater 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. 2.3). 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.

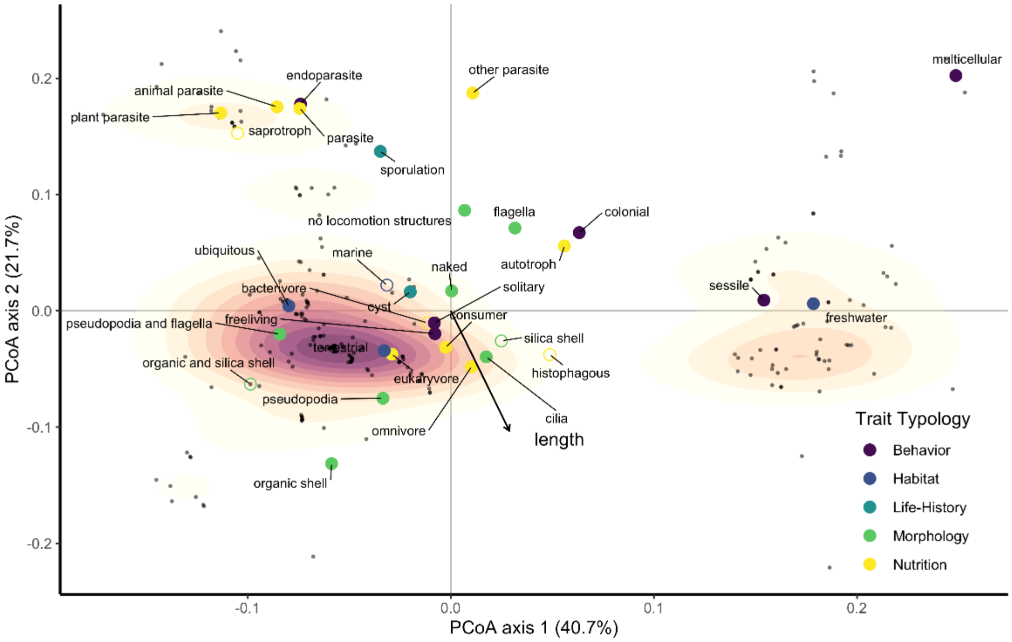

Figure 2.3 *Functional space of protist communities in proglacial soils. Black dots represent MOTUs; centroids of each category of the categorical traits are represented by larger dots colored by trait type; full dots are the categories significantly related to the first two PCoA axes (Table S3). The 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.*

2.4 DISCUSSION

2.4.1 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. 2.1). 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; Gulin et al., 2022; Laplace-Treyture et al., 2021), 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 (Fiore-Donno & Bonkowski, 2021; Oliverio et al., 2020; 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 (S. Krause et al., 2014; Lavorel et al., 2013; Potapov, 2022).

2.4.2 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 2.1). 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 po tential to perform multi-taxa analyses and is not appropriatefor datasets with broad taxonomic scope (i.e., high-throughput sequencing data based on generalist markers). In this case, a commonset of key traitscan 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 belowgroundfood webs (Potapov, 2022). A multi-taxa approach is frequent in metabarcoding studies (69% of reviewed studies; Table S3.1), 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 (Brousseau et al., 2018; de Bello et al., 2010). 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 estimatethe 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 whatwas 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, accountingfor 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 & Verdú, 2016). The application of the framework to a metabarcoding dataset provided a practical example of how these traits can be suitable for highthroughput 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 definedby 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 2.3.1). 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.

2.5 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.

2.6 SUPPLEMENTARY INFORMATION

Supplementary Methods 2.1. *Search strings used to find studies on soil organisms, including protists, fungi, bacteria and archaea (results reported in Figure 1).*

The literature search was carried out on July 8th, 2022, on the ISI Web of Science.

a) SOIL PROTISTS

TS=(soil* AND protist*) OR TS=(soil* AND protozo*) OR TS=(terrestrial AND protist*) OR TS=(terrestrial AND protozo*)

b) SOIL FUNGI

TS=(soil* AND fung*) OR TS=(terrestrial AND fung*)

c) SOIL BACTERIA

TS=(soil* AND bacteria*) OR TS=(terrestrial AND bacteria*)

d) SOIL ARCHAEA

TS=(soil* AND archaea*) OR TS=(terrestrial AND archaea*)

For the search on functional diversity the string 'AND function*' was added to each argument within parenthesis in each of the previous strings (a-d).

Supplementary Methods 2.2. *Sample collection, molecular and bioinformatic analyses*

Soil samples were collected between 2014 and 2020. Soil was collected from 1123 plots along 48 glacier forelands across five continents (Asia, Europe, Oceania, North and South America; Figure S1). Each glacier foreland represents a chronosequence of dated sites (from 3 to 17) covering an interval from 1 to a maximum of 483 years since glacier retreat (Marta et al., 2021). Multiple plots (mean = 4.7, SD = 0.8) were defined within each dated site along the chronosequences. For each plot, five soil subsamples were collected within a distance of 1- m at a depth of 0-20 cm. Immediately after the collection, the five subsamples were homogenized to form a ~ 200 g composite sample representative of the plot. We then removed 15 g of soil from each composite sample and placed them in sterile boxes with 40 g of silica gel to preserve the eDNA (Guerrieri et al., 2022).

We used the 15 g from the composite soils mixed for 15 min with 20 ml of phosphate buffer to extract the eDNA with the NucleoSpin Soil Mini Kit (Macherey- Nagel), with elution in 150 µl. Contamination was controlled performing an extraction control every ~10 samples (Zinger et al., 2019). The eDNA was amplified using the universal primer Euka02 which targets a ~123 bp fragment of the V7 region of the 18S rDNA gene (Guardiola et al., 2015; Taberlet et al., 2018). The obtained DNA was randomized in 96-well plates including blanks, negative/positive PCR control (total across all plates: 291 blanks, 90 negative and 53 positive controls). Blanks were used to identify potential tag-jump issues, while negative PCR controls allowed to check for PCR contamination. Positive PCR controls were used to evaluate cross-contamination and to estimate amplification and sequencing performance (Zinger et al., 2019), and were composed by genomic DNA of two fungal strains at known concentration (ZymoBIOMICS Microbial Community DNA Standard II, Zymo Research; diluted 1:10). The optimal number of amplification cylces was assessed through quantitative PCR (Guerrieri et al., 2022). We used 384-well plates to perform PCR amplification (four replicates per sample) of 1:10 diluted DNA samples. The amplicons were then sent to Fasteris (SA, Geneva, Switzerland) for library preparation and sequencing through the HiSeq 2500 Illumina platforms (Illumina, San Diego, CA, USA; 2 × 150 bp). The average sequence depth was ~10,000 reads per PCR replicate.

Bioinformatic treatment, filtering, clustering and taxonomic assignation were performed using the OBITools software suite (Boyer et al., 2016) and the *sumaclust* program (http//git.metabarcoding.org/obitools/sumaclust/wikis/home) following the procedure proposed by (Guerrieri et al., 2022). Sequences were clustered into Molecular Operational Taxonomic Units (MOTUs) using a 97% similarity threshold (Bonin et al., 2023). Finally, we performed additional filtering in R to remove spurious sequences and contaminants (Calderón-Sanou et al., 2020; Zinger et al., 2019). We discarded MOTUs observed with <12 reads, MOTUs detected in less than two PCR replicates of the same sample (possible false positives; (Ficetola et al., 2015), and MOTUs detected in >1 extraction or PCR negative control (possible contaminants; Zinger et al., 2019). A complete script on bioinformatics and filtering steps is available at https://zenodo.org/record/6620359.

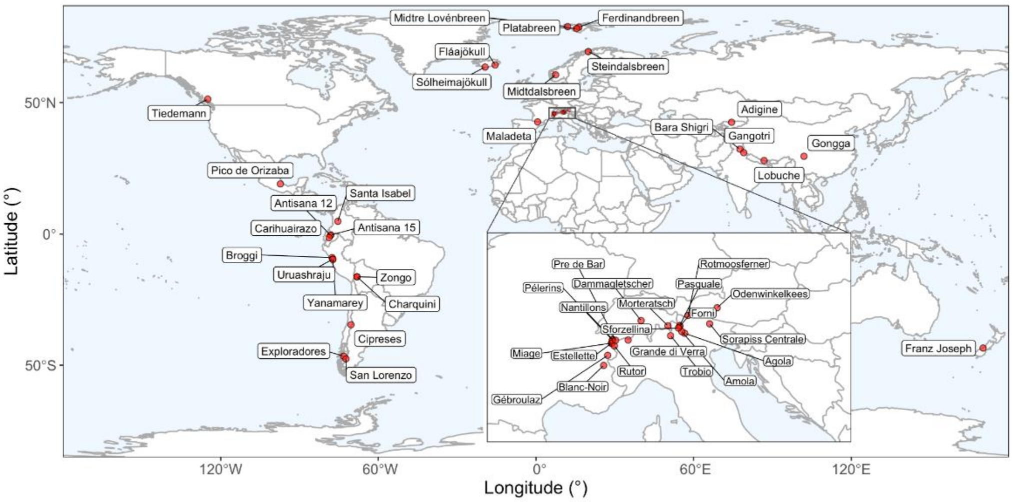

Figure S2.1. *Map of the 48 proglacial forelands sampled. The inset shows forelands located in the European Alps.*

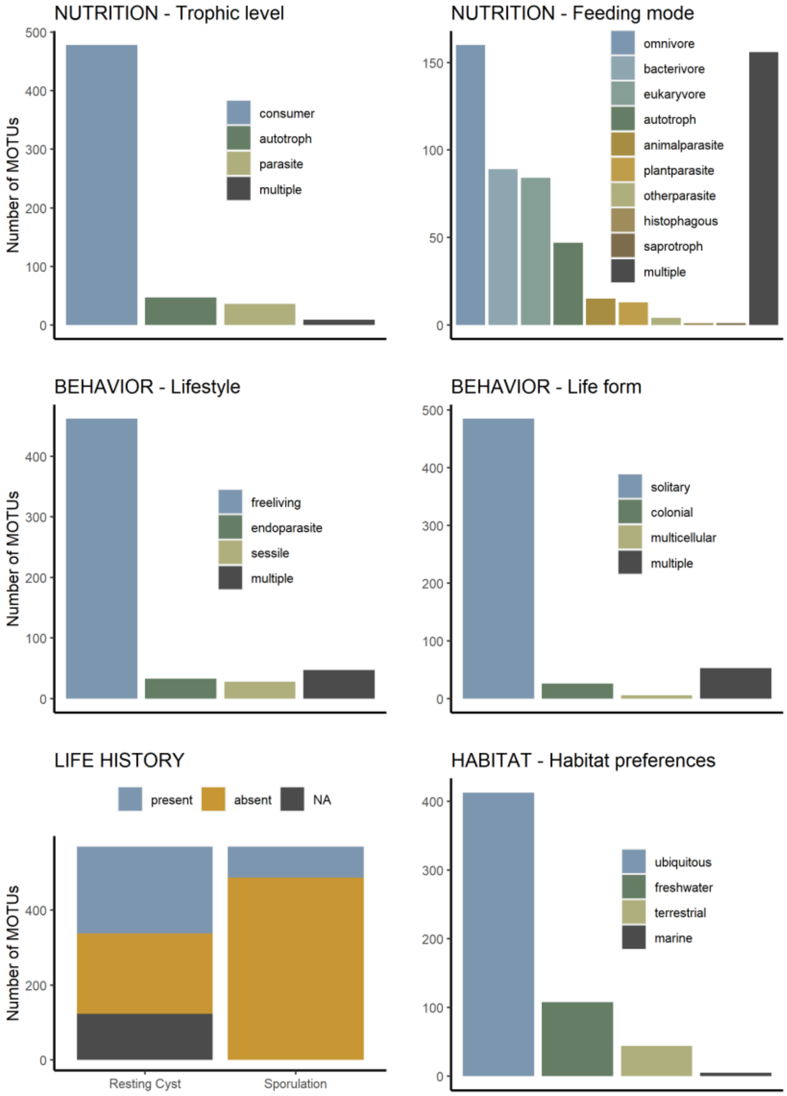

Figure S2.2a. *Number of MOTUs for each functional category of the key traits belonging to types nutrition, behavior, habitat and life-history. The category 'multiple' includes MOTUs that have been assigned to more than one category (e.g. omnivore or bacterivore).*

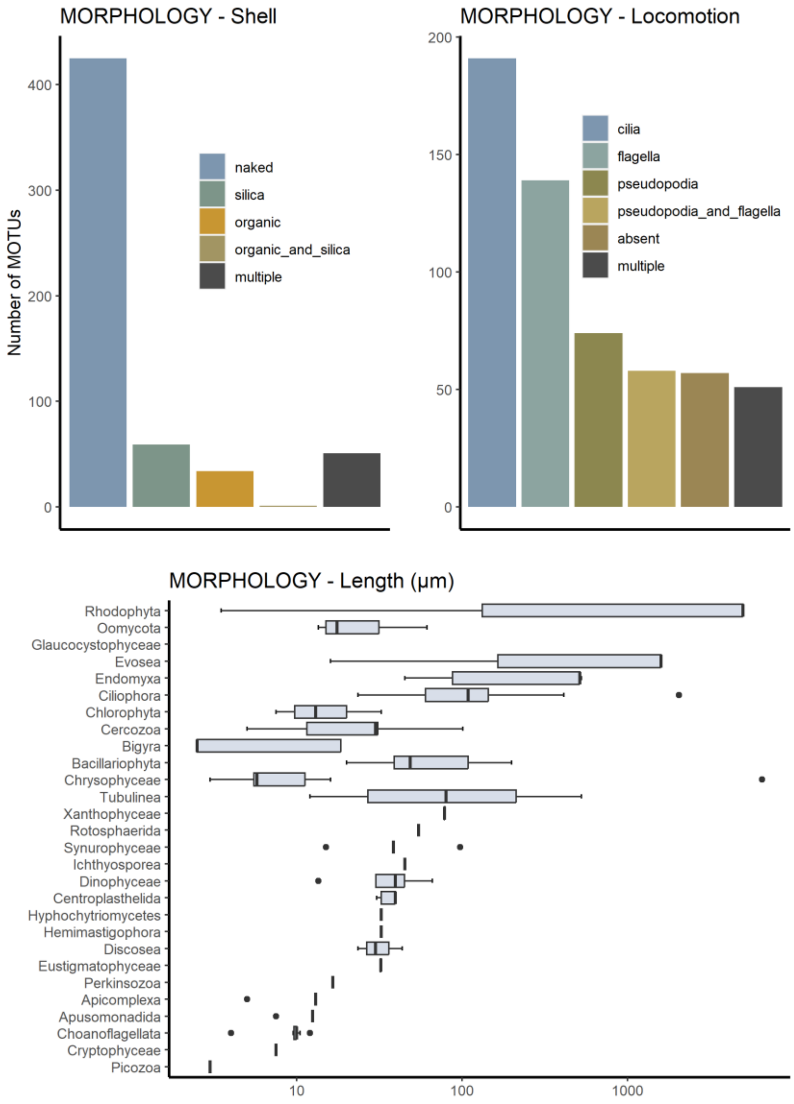

Figure S2.2b. *Number of MOTUs for each functional category of the morphological traits shell and locomotion structures. The category 'multiple' includes MOTUs that have been assigned to more than one category. Length (in micrometers) is plotted on a log10 scale for each phylum/clade.*

Table S2.1. *A) List of articles found with the literature review. For each article we report general article information, scale and location of the study, the research topic, sampling environment, sampling method, habitat sampled, the trait types included, how traits were assigned, the source of trait assignment, and taxa treated. B) Overall dataset of the 570 MOTUs of protists found in the entire survey including 1123 samples from 48 glacier forelands. Reported for each MOTU are: the sequence, the taxonomic assignment (supergroup, phylum, class, order, family, genus, and species; NCBI Taxonomy) with the index of similarity, and the 10 functional traits with references and generalization regarding the trait assignment. The dataset also includes a data key with the information for each column, and information about the literature consulted for the functional trait assignment. The table is included in an Excel file accessible online at the following link https://doi.org/10.1016/j.soilbio.2023.109207 [TableS1].*

Table S2.2. *List of the 28 traits found in the literature review. For each trait we report: the type, the categories composing the trait, whether the trait completely satisfies (Y) or does not satisfy (N) the two selection criteria (AI: availability of information; AP: applicability to most of soil protists), the key trait defined in the framework when the two criteria are satisfied (otherwise 'NA'), and references for the trait.*

Type	Traits	Categories	AI	AP	Key trait	References	Notes
Nutrition	trophic level	consumer; phototroph; parasite	Y	Y	trophic level	Voss et al. 2019; Oliverio et al. 2020; Nguyen et al. 2020; Seppey et al. 2020; Chen et al. 2021; Mazel et al. 2021; Nguyen et al. 2021; Singer et al. 2021	
	feeding mode	bacterivore; eukaryvore; omnivore; plant parasite; animal parasite; not plant parasite	Y	Y	feeding mode	Xiong et al. 2018; Fiore-Donno et al. 2019; Schulz et al. 2019; Fiore-Donno et al. 2020; Santos et al. 2020; Ritter et al. 2021; Roshan et al. 2021; Zhang et al. 2021; Fiore-Donno et al. 2022	
	lifestyle	saprotroph; obligate biotroph; hemibiotroph	Y	N	NA	Fiore-Donno and Bonkowski 2021; Jauss et al. 2021	Not considered because specific to oomycetes and partially overlapping with trophic level and feeding mode.
	food source	algae; bacteria; cyanobacteria; diatoms; fungi; omnivorous; phagotrophic protists; small metazoans	N	Y	NA	Krztoń et al. 2020; Gulin et al. 2022	Not considered because information is not available for many protists.
	feeding strategy	predation; filtration	Y	N	NA	Gulin et al. 2022	Not considered because specific to ciliates.
	mixotrophy	presence of photosynthetic endosymbionts	Y	N	NA	Fournier et al. 2015	Not considered because specific to testate amoebae.
Morphology	body-size (length/ width/ volume)	-	Y	Y	body-size	Fournier et al. 2015; Fournier et al. 2016; Song et al. 2018; Krztoń et al. 2020; Luan et al. 2020; Aslani et	Length is the most often available body size information, thus we used length in our study example. Nevertheless, if available, width and volume are relevant measures.

Type	Traits	Categories	AI	AP	Key trait	References	Notes
						al. 2022; Amacker et al. 2022	
	morphology	shell (testate or naked) + amoeba; flagellate; amoeboflagellate	Y	Y	locomotion; shell	Fiore-Donno et al. 2019; Fiore-Donno et al. 2020; Fiore-Donno et al. 2022; Gulin et al. 2022; Amacker et al. 2022	Information on morphology was splitted in two distinct traits: locomotion structures and shell.
	morphology (algae)	unicellular; colonial; filamentous; siphonous; phylloid	Y	Y	life form	Fang et al. 2021	Information on algae morphology was used to describe a life form trait.
	aperture size	NA	N	N	NA	Fournier et al. 2015	Not considered because specific to testate amoebae.
	aperture position	central; sub-terminal; terminal	Y	N	NA	Fournier et al. 2016	Not considered because specific to testate amoebae.
	pseudopod morphology	filose; lobose	Y	N	NA	Fournier et al. 2016	Not considered because specific to testate amoebae.
	test shape	ovoid; cylindrical-ovoid; saucer-disc shaped; spherical	Y	N	NA	Fournier et al. 2015; Fournier et al. 2016	Not considered because specific to testate amoebae.
	test compression	compressed; not compressed	Y	N	NA	Fournier et al. 2015; Fournier et al. 2016	Not considered because specific to testate amoebae.
	origin of shell material	agglutinate; autogenus	Y	Y	shell	Fournier et al. 2016	Information on shell origin was included in the trait shell.
	trophic position	NA	N	N	NA	Song et al. 2018	Not considered because specific to testate amoebae and information is not easily available in the literature.
	size structure index	NA	N	N	NA	Song et al. 2018	Not considered because specific to testate amoebae and information is not easily available in the literature.
Physiology	feeding pattern	NA	N	N	NA	Amacker et al. 2022	Physiological parameters have only been measured for a few species of protists.
	growth rate	NA	N	N	NA	Amacker et al. 2022	Physiological parameters have only been measured for a few species of protists.
Behaviour	locomotion mode	creeping/gliding on substrate; non-motile endoparasite; freely swimming	Y	Y	lifestyle	Fiore-Donno et al. 2019; Fiore-Donno et al. 2020; Fiore-Donno et al. 2022	Information on locomotion mode was included in the trait lifestyle.

Type	Traits	Categories	AI	AP	Key trait	References	Notes
	mode of locomotion	crawling; jumping; rotating; free-swimming; gliding	Y	Y	lifestyle	Gulin et al. 2022	Information on mode of locomotion was included in the trait lifestyle.
	living mode	free-living; animal parasite/endosymbiont; plant parasite/endophyte	Y	Y	lifestyle	Voss et al. 2019	Information on living mode was included in the trait lifestyle.
	life form	solitary; colonial	Y	Y	life form	Gulin et al. 2022	
	motility	motile; semi-sessile; sessile	Y	Y	lifestyle	Gulin et al. 2022	Information on motility was included in the trait lifestyle.
Habitat	Habitat	freshwater; marine; soil; mosses; ubiquitous	Y	Y	habitat preferences	Dumack et al. 2020; Fang et al. 2021; Gulin et al. 2022	
	ecosystem preference	active sludge; soil; lentic and lotic	N	N	NA	Gulin et al. 2022	Not considered because specific to freshwater species..
	habitat preference	benthos; periphyton; planktonic	N	N	NA	Gulin et al. 2022	Not considered because specific to freshwater species.
	substrate	plantae; metazoans	N	N	NA	Fiore-Donno and Bonkowski 2021	Not considered because specific to oomycetes and partially overlaping with feeding mode.

Table S2.3. *R^2 and p-values for the correlation between each functional category and the two major Principal Coordinates Analysis axes.*

Type	**Trait**	**Categories**	**R^2**	**p**
Nutrition	trophic	trophic_autotroph	0.125	0.001
	trophic	trophic_parasite	0.505	0.001
	trophic	trophic_consumer	0.471	0.001
	feeding	feeding_animalparasite	0.198	0.001
	feeding	feeding_bacterivore	0.005	0.515
	feeding	feeding_eukaryvore	0.035	0.017
	feeding	feeding_omnivore	0.225	0.001
	feeding	feeding_histophagous	0.003	0.755
	feeding	feeding_otherparasite	0.078	0.001
	feeding	feeding_plantparasite	0.234	0.001
	feeding	feeding_saprotroph	0.017	0.155
Morphology	shell	shell_naked	0.142	0.001
	shell	shell_organic	0.204	0.001
	shell	shell_organic_and_silica	0.005	0.505
	shell	shell_silica	0.025	0.05
	locomotion	locomotion_absent	0.171	0.001
	locomotion	locomotion_cilia	0.119	0.001
	locomotion	locomotion_flagella	0.230	0.001
	locomotion	locomotion_pseudopodia	0.181	0.001
	locomotion	locomotion_pseudopodia_and_flagella	0.075	0.001
	length	length_log_s	0.041	0.015
Behavior	lifestyle	lifestyle_endoparasite	0.488	0.001
	lifestyle	lifestyle_freeliving	0.308	0.001
	lifestyle	lifestyle_sessile	0.169	0.001
	lifeform	lifeform_colonial	0.151	0.001
	lifeform	lifeform_multicellular	0.093	0.001
	lifeform	lifeform_solitary	0.204	0.001
Life-History	sporulation	sporulation_present	0.563	0.001
	cyst	cyst_present	0.072	0.001
Habitat	habitat	habitat_freshwater	0.940	0.001
	habitat	habitat_marine	0.001	0.845
	habitat	habitat_terrestrial	0.035	0.023
	habitat	habitat_ubiquitous	0.632	0.001

CHAPTER 3

Functional changes of protist communities in soil after glacier retreat

Functional changes of protist communities in soil after glacier retreat

This chapter is extensively based on the following publication:

Giachello, S., Cantera, I., Carteron, A., Bonin, A., Guerrieri, A., Ambrosini, R., Caccianiga, M., Gobbi, M., Marta, S., & Ficetola, G. F. (2024). Functional changes of protist communities in soil after glacier retreat. Science of the Total Environment, 956(177265). [*https://doi.org/10.1016/j.scitotenv.2024.177265*](https://doi.org/10.1016/j.scitotenv.2024.177265)

3 FUNCTIONAL CHANGES OF PROTIST COMMUNITIES IN SOIL AFTER GLACIER RETREAT

ABSTRACT

Soil hosts key components of terrestrial biodiversity providing essential services to the below- and above-ground ecosystems. The worldwide retreat of glaciers is exposing new deglaciated terrains, offering a unique opportunity to understand the development of soil ecosystems under a changing climate. Many studies have investigated how biotic communities change after deglaciation, but protists have often been overlooked despite their key role in multiple ecosystem functions. Here, we aim to understand how protist communities develop along glacier forelands, describing their successional trajectories. Protist communities were characterized in 1251 soil samples from 46 glacier forelands across four continents. We used environmental DNA metabarcoding to identify the Molecular Operational Taxonomic Units (MOTUs) of protists based on a universal eukaryotic marker. The detected MOTUs were combined with information on multiple traits to assess how the functional diversity and composition of protist communities vary through time. Immediately after glacier retreat, protist communities are like those of polar and high-altitude habitats, with consumers being the dominant trophic group, followed by a relevant presence of phototrophs, while parasites were underrepresented. Over the succession, we detected an increase in taxonomic and functional diversity, but some highly specialized groups (e.g. phototrophic algae) declined. The use of a trait-

based approach allowed us to identify distinct successional patterns depending on functional groups. Through the functional characterization of a crucial but understudied component of soil biotic communities, our study added one of the final pieces needed to predict how soil ecosystems will develop in the rapidly changing environment of glacier forelands.

3.1 INTRODUCTION

The global retreat of glaciers is rapidly transforming high-altitude and high-latitude ecosystems through changes in the local geomorphology, soil, and biological communities (Cauvy-Fraunié & Dangles, 2019; Ficetola et al., 2021; Pothula & Adams, 2022). More than a century of research has provided insights into how organic matter and soil nutrients build up during soil formation, alongside the patterns of biodiversity changes over time following glacier retreat (Cauvy-Fraunié & Dangles, 2019; Ficetola et al., 2021; Khedim et al., 2021; Pothula & Adams, 2022). It is now well established that ecosystem formation along glacier forelands involves an increase of taxonomic and functional richness from recently deglaciated terrains to latesuccessional stages, driven by higher availability of resources and more favourable environmental conditions (Ficetola et al., 2024; Pothula & Adams, 2022). Protists are a vast group comprising all the eukaryotes that do not belong to animals, plants and fungi (Burki et al., 2021). This heterogeneous group mostly includes a vast diversity of unicellular eukaryotes that have a broad variation of functional features and provide essential services for the functioning of belowground ecosystems (Gao et al., 2019; Geisen et al., 2018, 2020). For instance,protists can act as primary producers, regulators of microbial populations, pests or mutualists of plants and animals, and decomposers of organic matter (Bonkowski, 2004; Geisen, 2016; Geisen et al., 2018). Along glacier forelands, protists are among the first colonizers, serving as pioneer primary producers before the appearance of plants, and triggering soil formation by releasing nutrients fundamental for plant growth (Boetius et al., 2015; Hågvar et al., 2020; Rosero et al., 2021). Thus, characterizing the diversity and composition of protist communities along glacier forelands is pivotal to understanding the functional development of ecosystems emerging after deglaciation. Despite efforts to capture the trajectories of several taxonomic groups after glacier retreat (Carteron et al., 2024; Cauvy-Fraunié & Dangles, 2019; Ficetola et al., 2021; Pothula & Adams, 2022), protist communities are seldom included in glacier foreland studies, with assessments of their biodiversity patterns mostly limited to local or regional scales (Lazzaro et al., 2015; H. G. Smith, 1996; Tikhonenkov, 2013). Such scarcity of studies is associated with the absence of a global characterization of the diversity of protist communities in glacier forelands (Oliverio et al., 2020), as well as with the lack of knowledge on their patterns of community assembly over succession (Pothula & Adams, 2022). A recent analysis assessed the overall development of biotic communities after glacier retreat across multiple continents and showed that the taxonomic diversity of protist communities increases over the succession of glacier forelands, as it happens to other components of soil communities (Ficetola et al., 2024). Nonetheless, that study overlooked functional diversity, a key component of biodiversity, and did not consider potential differences between the diverse functional groups of protists. The development of biotic communities along the succession of glacier forelands involves changes in multiple facets of biodiversity (Ficetola et al., 2021). To shedlight on such complex dynamics, analyses of taxonomic diversity must be complemented with a functional approach accounting for the variety of traits exhibited by the species. Traits can describe multiple features of the species such as morphology, ecology, behaviour or physiology, making the connection between biodiversity and ecological processes more explicit (Cardinale et al., 2012; Naeem et al., 2012). For protists, the use of a multi-traitapproach is critical, as protist communities encompass a wide range of evolu tionary lineages belonging to distinct trophic groups that respond differently to environmental gradients and contribute differently to ecosystem functioning (Geisen et al., 2023; Giachello et al., 2023). For instance, the trophic composition of protist communities can show complex responses to latitudinal and elevational gradients (Mazel et al., 2022; Oliverio et al., 2020). Along glacier forelands, phototrophic protists are thought to be among the first colonizers acting as pioneer primary producers and facilitating the establishment of other species, and then their diversity has been suggested to decline with increasing distance from the glacier front (Hågvar et al., 2020; Rosero et al., 2021). By contrast, testate amoebae showed an opposite pattern of increasing diversity over time since deglaciation along an arctic glacier foreland (Hodkinson et al., 2004). Nevertheless, broad-scale analyses of the variation of protist communities after the retreat of glaciers are so far lacking. A joint assessment of the variation of both taxonomic and functional diversity of these organisms is critical to determine how these communities change in the highly dynamic ecosystems that are developing after the retreat of glaciers. Here, we used a broad-scale dataset based on environmental DNA (eDNA) extracted from 1251 soil samples along 46 glacier forelands combined with a multi-trait functional approach (Cantera et al., in press) to characterize protist communities of glacier forelands and test three main hypotheses. First, we assessed the changes in the taxonomic and functional richness of protist communities over the successional stages of glacier forelands, representing longer times since glacier retreat and characterized by different microclimates (temperature). We expect functional richness to exhibit the same pattern of taxonomic diversity, i.e., an increase in diversity over time since glacier retreat (hypothesis 1), paralleling trends observed for other organisms along glacier forelands (Guerrieri et al., 2024). Second, we compared the functional trajectories of different functional categories (e.g., bacterivores, omnivores, phototrophs, etc.) and the overall protist community. Given the high functional variety expressed by protists, we expect that different functional categories would follow divergent successional trajectories after glacier retreat (hypothesis 2). Third, we hypothesize that the overall functional composition of protist communities will shift over time (hypothesis 3), as the predominance of traits will vary with the changes in environ mental conditions.

3.2 MATERIALS AND METHODS

3.2.1 Study area and samping design

Our study took advantage of a unique dataset, using environmental DNA metabarcoding to analyze >1200 soil samples on 46 glacier forelands across four continents spanning from boreal to tropical climate (from 48°S to 79°N: Fig. 3.1). This dataset assessed the overall development of terrestrial ecosystems after glacier retreat (Ficetola et al., 2024) but did not perform detailed analyses of the diversity of protists, nor considered key components of biodiversity such as functional diversity and functional composition. Each glacier foreland constitutes a chronosequence delineated by the dated positions of the glacier over time. Along each chronosequence, we selected multiple dated sites (from three to ten), spanning from 1 to 483 years after glacier retreat (Marta et al., 2021), with a general habitat shift from bare grounds to fully vegetated ecosystems (Cantera et al., 2024). The dated sites were selected to avoid areas affected by geomorphological disturbances, such as river erosion or gravitative processes like debris flows, rockfalls and flooding by glacial river, which can heavily alter the development of biotic communities (Wojcik et al., 2021). Within each dated site, we sampled multiple plots (average: 5 plots per site; range: 2–7) of 1 m 2 each. Overall, we obtained soil samples from 1251 plots within 265 different dated sites and extracted environmental DNA from them (see Cantera et al., 2024; Ficetola et al., 2024; Guerrieri et al., 2024 for additional details on sampling sites and strategies).

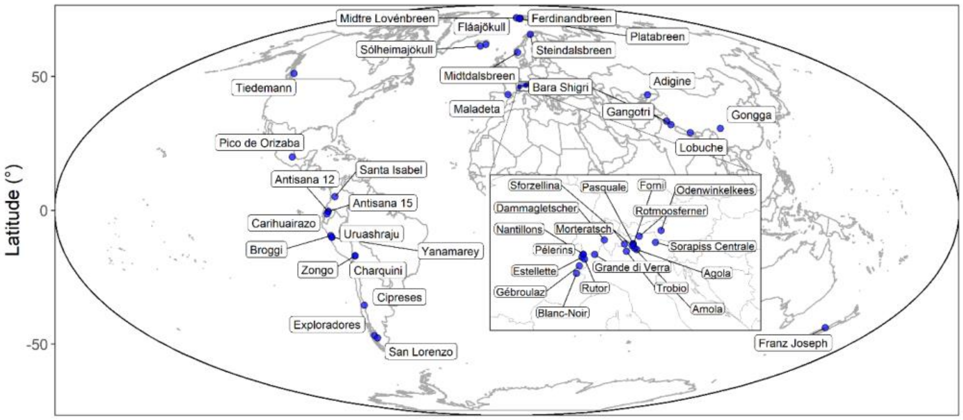

Figure 3.1. *Location of the 46 glacier forelands sampled. The inlet shows the area of the European Alps expanded.*

3.2.2 MOTUs and taxonomic assignment

Molecular Operational Taxonomic Units (MOTUs) of soil protists 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. DNA amplifications were performed in 4 PCR replicates. For details on sample collection, DNA extraction, amplification, sequencing and bioinformatic analyses see Guerrieri et al. (2024) and Ficetola et al. (2024). We obtained MOTUs through the clustering of similar sequences with a threshold set to 97 % similarity (Bonin et al., 2023). Taxonomy was assigned through the NCBI Taxonomy database (Schoch et al., 2020) and soil protist MOTUs were selected as all the MOTUs not belonging to animals, plants, and fungi (Giachello et al., 2023). The taxonomic level of identification of MOTUs was heterogeneous, with some MOTUs identified at fine taxonomic resolution (e.g., *Vorticella sphaeroidalis* or *Euglypha rotunda*), while others identified at coarser resolution (e.g., Ciliophora or Cercozoa). Specifically, 52 % of MOTUs were identified at the genusor species level, 21 % at the family level only, and 9 % at the order level only, with the remaining 18 % identified at very coarse levels (class or phylum level; see Table S3.1 for more details).

3.2.3 Environmental features

For each plot, we considered two key environmental features: time since glacier retreat and mean soil temperature during the growing season, to control for the effect of micro-climatic conditions. Plots within the same dated site are characterized by the same value of time since glacier retreat but can show distinct values of soil temperature. We used time since glacier retreat (years) as it is a key driver of the richness and composition of biotic communities and influences the overall development of ecosystems in proglacial environments (Ficetola et al., 2024). Values of time since glacier retreat were retrieved from an available dataset, which gathers information from historical records, including maps, field data, and remote imaging (Marta et al., 2021). Soil temperature has important effects on the ecosystem dynamics of deglaciated terrains, as warm conditions can accelerate soil development and colonization by some organisms (Guerrieri et al., 2024; Khedim et al., 2021). The average soil temperature of each plot during the growing season was reconstructed using a global microclimatic model focusing on glacier forelands (Marta et al., 2023). The model accounts for topography (aspect, slope) and macro-climatic conditions and was calibrated using data loggers located near the ground to obtain high resolution estimates of soil temperature. The mean soil temperature during the growing season was calculated as the average temperature of months with > 20% days without snow on the ground over the 2015–2019 period. See Marta et al. (2023) for further details and validation of soil temperature. Across the whole dataset, the correlation between soil temperature and time since glacier retreat across all the glacier forelands was moderate (Pearson's r = 0.22).

3.2.4 Functional characterization of protist communities

We used ten traits to obtain a functional characterization of each protist MOTU. Specifically, we considered two categorical traits describing nutrition (trophic level; feeding mode), two categorical and one continuous traits describing morphology (shell presence; locomotion structures, length), two categorical traits describing behaviour (lifestyle; life form), two categorical traits describing life-history (sporulation; ability to produce a resting cyst), and one categorical trait describing habitat preferences. Each categorical trait (e.g., lifestyle) is composed of multiple ‘functional categories’ (e.g., endoparasite, freeliving, sessile) representing the states that the trait can assume (Giachello et al., 2023). Following the Giachello et al. (2023) framework, we were able to assign functional traits to all the MOTUs identified as protists, using the finest taxonomic level available for the MOTU (ranging from species to phylum). MOTUs that could not be characterized by a unique functional category due to low taxonomic resolution (e. g., MOTUs identified as Ciliophora), were described using two or more functional categories (e.g., for Ciliophora, lifestyle: free-living or sessile). Categorical traits were then transformed into fuzzy-coded dummy variables so that each categorical trait was decomposed into multiple variables representing the related functional categories (de Bello et al., 2021; Table S3.1). For instance, for the trait lifestyle, if a MOTU was classified as ‘free-living or sessile ‘ a value of 0.5 was assigned to the dummy variables ‘free-living’ and ‘sessile’ while a 0 was put for the remaining categories of the trait lifestyle (i.e., ‘endoparasite’). See Giachello et al. (2023) for further details on the framework, traits and original sources. Finally, to derive functional diversity measures, we defined unique ‘functional entities’ by grouping the MOTUs sharing the same combination of traits (Mouillot et al., 2014; Table S3.1).

3.2.5 Taxonomic and functional diversity

We applied Hill numbers to calculate the taxonomic and functional alpha diversity for each plot (i.e., community), using, respectively, the number of reads per MOTU and the number of reads per functional entity (i.e., the sum of the number of reads of all the MOTUs belonging to the same functional entity; Table S3.2). To do so, we used the R function ‘hill_taxa’ from the R package *hillR* with values of q parameter equal to 1, indicating a measure of abundance equal to the exponential Shannon entropy (Chao et al., 2014; Mächler et al., 2021; Table S3.2). This is recommended to increase the robustness of biodiversity data obtained through DNA metabarcoding (Calderón-Sanou et al., 2020; Mächler et al., 2021).

3.2.6 Functional composition of communities

To estimate the trajectories of functional categories over time since glacier retreat, we constructed a matrix in which each row represented a plot characterized by the relative abundance of each functional category (columns) based on the functional entities identified in the plot. These values were calculated by computing the relative abundance within plot from the product of two matrices: an ‘abundance matrix’ containing the log-transformed number of reads of functional entities per plot, and a ‘trait matrix’ containing fuzzy-coded values (i.e., 0, 0.5, 1) of each functional category per functional entity (Giachello et al., 2023; Mazel et al., 2022; Table S3.2). To evaluate the variation in the functional composition of protist communities with a multi-trait approach, we first calculated the distances among functional entities using the function ‘daisy’ of the R package *cluster* (Maechler et al., 2022) applying Gower's distance (Gower, 1971). To ensure that each categorical trait composed of N trait categories (dummy variables) had an equal contribution to estimating the distance between functional entities, we weighted each trait category following the ‘gawdis’ function from the *gawdis* R package (de Bello et al., 2021; Giachello et al., 2023). Then, we ran a principal coordinate analysis (PCoA) on the matrix of the trait distances among functional entities (‘pcoa’ function from the *ape* R package; Paradis & Schliep, 2019). The first and second PCoA axes were used to build bidimensional functional spaces representing the functional composition of protist communities. This allowed to build a global functional space representing all the taxa detected in the 1251 sampled communities (i.e., plots). The position of each functional category in the functional space was estimated as the mean of the PCoA coordinates of all the functional entities exhibiting that functional category. Subsequently, we built three functional spaces representing early, mid, and late successional communities. In these functional spaces, we plotted the functional entities found respectively in plots with i) age since deglaciation <30 years (i.e., early communities), ii) age of 30–100 years (mid communities) and iii) age >100 years (late communities). To test if the functional composition of the communities changed over time and with microclimate, we estimated three measures summarizing the position of the communities in the functional spaces: Score along PCoA.1, Score along PCoA.2 and functional specialization. The three measures were estimated only for the 625 plots hosting at least three functional entities using the R function ‘multidimFD’ from the package *mFD* (Magneville et al., 2022). The scores along PCoA.1 and PCoA.2 indicate the position of a given community along the first and second axes of the functional space, calculated as the mean of the PCoA coordinates of all the functional entities in the community. Their joint analysis represents the general patterns of dominant traits supported by the taxa co-occurring in a community. Functional specialization represents the mean distance between the functional entities co-occurring in each community and the average position of all the taxa (i.e. the barycenter) in the bidimensional functional space defined by the PCoAaxis 1 and PCoA axis 2. This index measures the extent of functionally unique taxa present in a community relative to the regional pool of taxa. Functional specialization decreases when a community is dominated by generalist taxa (close to the centre of the functional space) and increases when a community is dominated by specialist taxa (species with extreme trait combinations).

3.2.7 Drivers of community changes

We used Bayesian generalized linear mixed models (GLMMs) to test the effects of time since glacier retreat and soil temperature (independent variables) on i) the taxonomic and functional alpha diversity of protist communities, ii) the relative abundance of functional categories within each plot, iii) the functional composition of protist communities. For the diversity of protist communities, we built two distinct univariate GLMMs, respectively including the taxonomic alpha diversity and functional alpha diversity as dependent variables. Mean soil temperature and time since glacier retreat were the independent variables. Time was log-transformed to reduce skewness, and all the independent variables were scaled (mean = 0, SD = 1) to improve convergence and for a better comparison of their estimated effects. To account for spatial non independence of plots within a site, and of sites within the foreland, we included nested random effects in the model, considering the identity of the glacier foreland, as well as the identity of the dated site (nested within the glacier foreland, see Section 3.2.1 for the sampling design). In these models, we did not detect any collinearity between temperature and time since glacier retreat (

variance inflation factor = 1.2; Dormann et al., 2013). Topography is a relevant driver of succession along glacier forelands. For instance, plots in specific positions might be more subjected to geomorphological disturbance (Wojcik et al., 2021). Thus, we repeated the three GLMMs adding the Topographic Position Index (TPI) as an additional independent variable (Weiss, 2001). TPI is a parameter that is used to differentiate topographic features such as valleys (negative values), ridges (positive values), or flat areas (values close to 0). TPI values were estimated using the R function 'terrain' from the package *raster* (Hijmans, 2023). Nonetheless, adding the TPI to the models (Table S3.3) did not change the overall results of any of the models and confirmed the robustness of models including time since glacier retreat and soil temperature (Table 3.1). For the relative abundance of functional categories, we built one multivariate GLMM including the abundances of all the functional categories as dependent variables assuming a beta distribution. For functional composition, we built a multivariate GLMM including functional specialization and the scores along PCoA axis 1 and PCoA axis 2 as dependent variables. Multivariate GLMMs had the same independent variables and random effects as the univariate GLMMs. All GLMMs were fitted with the R package *brms*, running 3 chains for 10.000 iterations with a 500-iteration burn-in and a thinning rate of 10. Uninformative priors were used as provided in the *brms* package (Bürkner, 2017). These settings ensured the convergence of all the models for each parameter (R-hat <1.01).

3.3 RESULTS

3.3.1 Characterization of protist communities from glacier forelands

The clustering of the sequences from the Euka02 marker led to the identification of 567 MOTUs of protists out of a total of 2008 MOTUs of eukaryotes obtained (Table S3.1). The average number of protist MOTUs detected per plot was 7.3 (range:0–126; at least one MOTU was detected in 76 % of plots). The five most represented clades, i.e. those with the largest number of MOTUs, included: the heterotrophic phyla of Cercozoa (34.0 %) and Ciliophora (33.3 %), the parasitic and consumer clade of Endomyxa (4.0 %), the phototrophic algae Bacillariophyta (3.7 %) and the golden algae Chrysophyceae (2.8 %; Fig. 3.2). Regarding functional diversity, we found 236 distinct functional entities in total, with an average of 6.4 functional entities per plot (range: 0–80). Consumers dominated the protist communities along glacier forelands (84.0 % of MOTUs), followed by phototrophs (8.6 %) and parasites (7.4 %; Fig. S3.1). Among consumers, omnivores represented the most abundant feeding mode followed by bacterivores and eukaryvores. The functional composition of protist communities along glacier forelands was further characterized by the prevalence of solitary, free-living ciliates and flagellates, without shells. Among the detected MOTUs, 40 % were known to form resting cysts, and 15 % were known to produce spores. Protist body length ranged widely from the 2.5 µm of *Siluania monomastiga* to the >6 cm of the multicellular golden alga *Hydrurus foetidus* (Table S3.1).

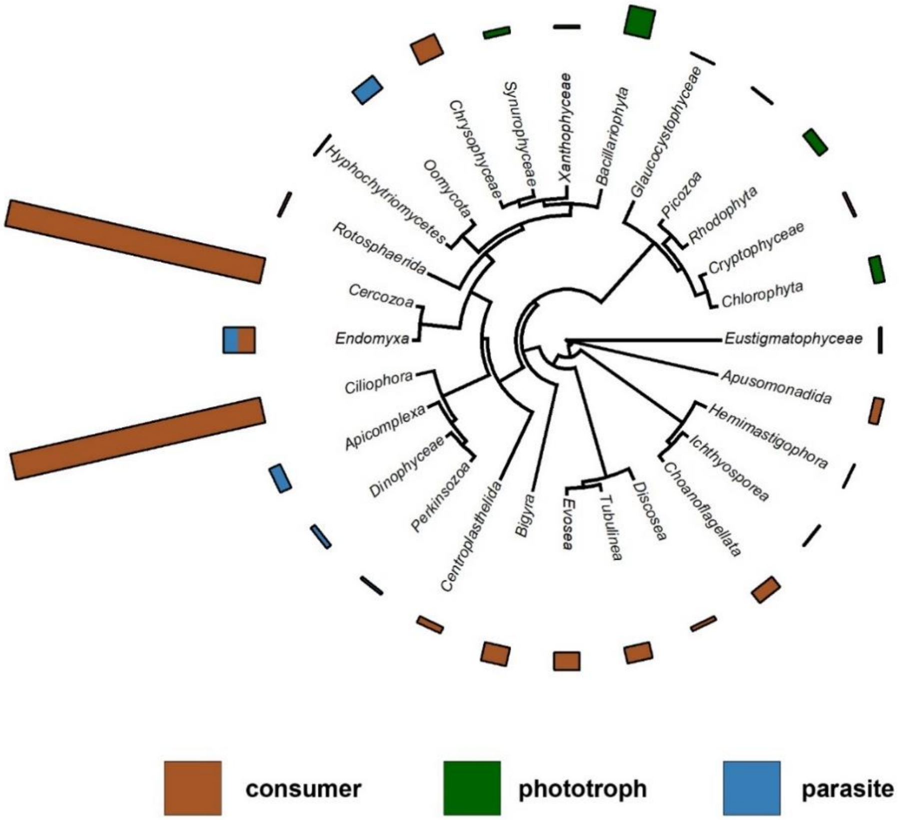

Figure 3.2. *Taxonomic and trophic composition of soil protist communities from 46 glacier forelands. Schematic phylogenetic tree representing the phyla/clades found in all the 1251 soil samples of our study. The height of the bars indicates the proportion of MOTUs within each phylum/clade, while the colors indicate the most representative trophic groups.*

3.3.2 Trajectories of taxonomic and functional diversity

Taxonomic alpha diversity significantly increased over time since glacier retreat but showed no correlation with temperature (Table 3.1a) indicating maximum taxonomic diversity in late communities independently of the microclimate. The same relationship was detected for functional diversity, which also increased over the succession (Table 3.1b).

Table 3.1*. Effect of time and mean soil temperature on the taxonomic alpha diversity (a), functional alpha diversity (b) and functional composition (c) of soil protist communities. Mean estimate and 95% credible interval of the posterior distribution were estimated with Bayesian Generalized Linear Mixed Models. The values in bold indicate a significant effect of the environmental variable on the taxonomic/functional measure. For more information on the measures of functional composition see paragraph 2.6 Functional composition of communities.*

		Independent			
		Time		Mean Temperature	
		B	CI 95%	*B*	CI 95%
a) Univariate analysis. Dependent: taxonomic alpha diversity					
		0.19	**0.11/0.26**	-0.05	-0.18/0.07
b) Univariate analysis. Dependent: functional alpha diversity					
		0.18	**0.11/0.25**	-0.05	-0.17/0.07
c) Multivariate analysis. Dependent: functional composition (3 measures)					
	specialization	**-0.15**	**-0.20/-0.10**	**0.11**	**0.03/0.18**
	score of PCoA axis 1	0.01	-0.02/0.05	0.01	-0.03/0.05
	score of PCoA axis 2	**-0.09**	**-0.12/-0.06**	0.02	-0.01/0.05

Table 3.2. *Effect of time and mean temperature at ground level on the relative abundance of each trait category. Mean estimate and 95% credible interval of the posterior distribution estimated with a multivariate Bayesian Generalized Linear Mixed Model. The values in bold indicate a significant effect of the environmental variable on the trait category.*

Trait	**Category**	**Time**		**Mean Temperature**	
		B	CI 95%	*B*	CI 95%
Trophic	consumer	0.06	-0.04/0.15	-0.03	-0.12/0.07
	phototroph	-0.18	**-0.25/-0.11**	-0.04	-0.12/0.03
	parasite	0.04	-0.03/0.10	0.06	-0.01/0.14
Feeding	animal parasite	0.00	-0.06/0.07	0.01	-0.06/0.08
	plant parasite	0.03	-0.03/0.10	0.06	-0.01/0.13
	bacterivore	0.03	-0.07/0.12	-0.09	-0.20/0.03
	eukaryvore	-0.02	-0.09/0.06	0.04	-0.03/0.12
	omnivore	0.08	**0.00/0.18**	0.08	-0.02/0.18
Locomotion	cilia	0.09	**0.00/0.18**	0.05	-0.06/0.17
	flagella	-0.01	-0.11/0.10	-0.07	-0.18/0.05
	pseudopodia	0.06	-0.02/0.15	0.07	-0.05/0.18
	pseudopodia and	-0.01	-0.09/0.06	0.03	-0.05/0.11
	absent	-0.13	**-0.21/-0.06**	-0.05	-0.12/0.02
Lifestyle	free living	0.03	-0.06/0.12	-0.09	-0.19/0.00
	sessile	-0.14	**-0.21/-0.06**	-0.01	-0.08/0.07
	endoparasite	0.03	-0.04/0.10	0.05	-0.02/0.13
Lifeform	solitary	0.03	-0.06/0.11	-0.05	-0.14/0.05
	colonial	-0.05	-0.11/0.01	0.02	-0.05/0.10
	multicellular	-0.08	**-0.15/-0.01**	0.00	-0.06/0.07
Habitat	terrestrial	0.02	-0.05/0.09	0.04	-0.04/0.12
	freshwater	-0.15	**-0.23/-0.07**	-0.02	-0.11/0.08
	ubiquitous	0.14	**0.04/0.23**	-0.01	-0.11/0.09
Shell		0.01	-0.07/0.08	0.08	-0.02/0.18
Sporulation		-0.09	**-0.17/-0.01**	0.06	-0.02/0.14
Cyst		0.02	-0.09/0.13	0.01	-0.11/0.13
Length		-0.01	-0.03/0.02	0.00	-0.03/0.02

3.3.3 Differences in functional compositions along the ecological succession

The representation of protist communities within the functional space indicated that the average functional composition remained rather stable over the succession as most functional entities were present in all the successional stages (Fig. 3.3). Nevertheless, a few functional entities were frequent in early successional communities, but not in late communities (entities in the upper central part of the functional space; Fig. 3.3). These entities mostly correspond to large phototrophic algae typically living in freshwater environments, such as the multicellularred algae *Kumanoa sp.* The multivariate GLMM assessing the variation of the relative abundance of functional categories supported this pattern (Table 3.2). Through time, we found a significant decrease in the frequency of organisms with traits indicating phototrophy, multicellularity, large body size, the absence of locomotion structures, a colonial lifestyle, the capability of producing spores and the preference for freshwater environments (Table 3.2; Fig. 3.4). Conversely, we detected an increase of omnivores, ciliate protists and organisms able to exploitboth terrestrial and freshwater environments (i.e. the ubiquitous ones) (Table 3.2, Fig. 3.4). None of the functionalcategories was clearly affected by variation in soil temperature (Table 3.2). The GLMM analyzing the functional composition of communities (PCoA axes and specialization) summarized the patterns revealed by the variation of functional categories (Table 3.1c). The PCoA axis 2 (Fig. 3.3) was negatively related to time since glacier retreat (Table 3.1c), indicating the loss of multicellular, large, sessile organisms through time, and the increase of omnivorous and ubiquitous protists in late communities (Table 3.2). Furthermore, functional specialization showed a clear decrease over time (Table 3.1c).

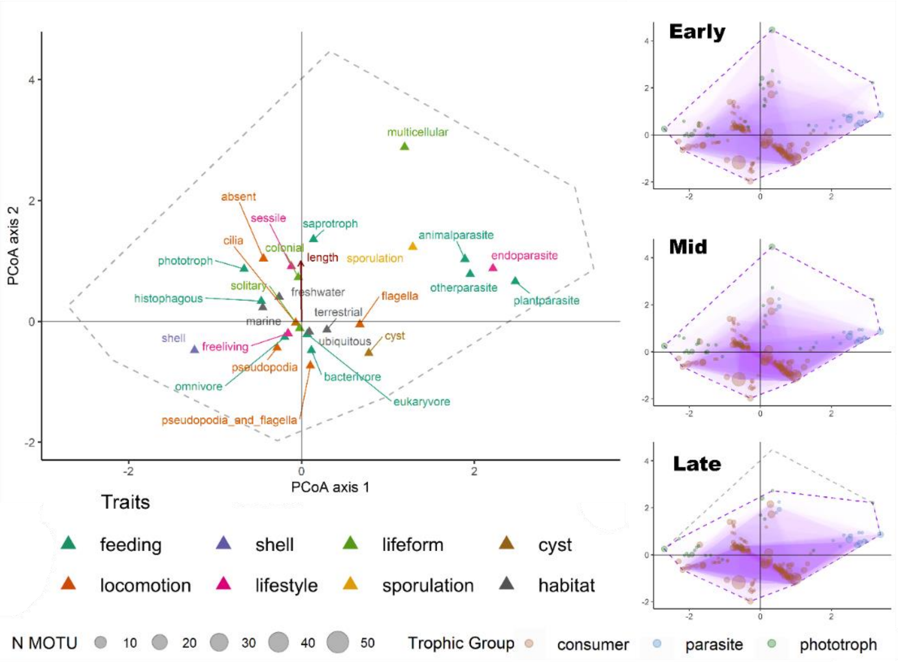

***Figure 3.3**. Functional space of protist communities in different stages of the ecological succession (early, mid, late). Left: functional space of all the protist communities sampled in 1251 plots. Triangles represent the centroid of each trait category estimated as the average coordinates of the functional entities sharing the trait category, while the color indicates the functional trait. Right: functional spaces for early (< 30 years), mid (30-100 years), and late (>100) communities. Circles represent functional entities; their size is proportional to the number of MOTUs within each functional entity and the color identify different trophic groups. Purple shadings represent the functional space of each community (i.e., plot). The dashed polygon lines represent the overall area occupied by the functional entities (i.e., functional richness), the grey polygon line represents the total functional richness across all stages, while the purple line represents the functional richness of each stage of the ecological succession.*

Such a decrease in functional specialization was related to the loss of unique functional entities (such as the large algae at the extremes of the functional space) in the late protist communities (Table 3.1c; Fig. 3.3). After accounting for the effect of time, functional specialization was particularly high in plots characterized by warmer temperatures (Table 3.1c).

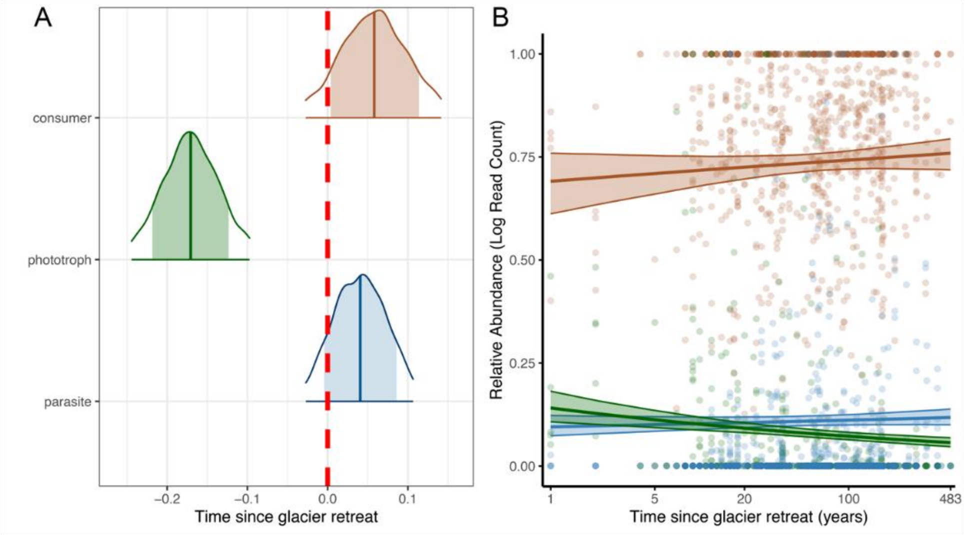

Figure 3.4. *Effect of time since glacier retreat on trophic groups of soil protists. A) Posterior distribution estimated with Bayesian Generalized Linear Mixed Models representing the effect of time since glacier retreat on the relative abundance of each trophic group. The curves indicate the 95% credible interval, shaded areas the 80% credible interval, and the vertical line the mean estimate. B) Trends of the relative abundance of each trophic group over time since glacier retreat in years. The line represents the estimated mean response and the shaded area the 95% credible interval. Colours represent trophic groups (brown: consumers; green: phototrophs; blue: parasites).*

3.4 DISCUSSION

Through an eDNA sampling of 46 glacier forelands across four continents, we were able to characterize the composition and the development of protist communities in the soil ecosystems emerging after glacier retreat. Protist communities inhabiting glacier forelands comprise several taxonomic groups exhibiting a high functional diversity since the earliest stages of succession, with the dominance of consumers. The alpha diversity of protist communities increased along the succession mostly due to the addition of omnivore taxa, but the loss of phototrophs in the mid and late successional stages caused a clear functional shift and a decrease in functional specialization.

3.4.1 Characterization of protist communities from glacier forelands

The taxonomic composition of the analyzed soils showed that, even in the extreme environments of glacier forelands, Cercozoa and Ciliophora remain the two most abundant phyla of soil protists, consistent with what we know for most of the terrestrial ecosystems (Bates et al., 2013; Oliverio et al., 2020; Singer et al., 2021). On the other hand, the diverse parasitic phylum Apicomplexa showed lower representation compared to other habitats (Oliverio et al., 2020; Singer et al., 2021; B. Wu et al., 2022). Such underrepresentation of Apicomplexa can be due to the low density of potential hosts such as animals and plants in these harsh environments (i.e., limited biotic interactions) that generally limits the occurrence of parasitic protists, or to the adverse climatic conditions characterizing glacier forelands (i.e., habitat filtering), since these protists exhibit maximum diversity in warm and humid tropical climates (Bates et al., 2013; Mahé et al., 2017). The multi-trait approach allowed us to infer the functional composition of protist communities from glacier forelands at different stages of the succession. Overall, consumers were the most abundant trophic group (Fig. S3.1), consistent with what has been found in other biomes (Oliverio et al., 2020). The presence of eDNA of multicellular freshwater algae in the forelands (e.g., Chrysophyta: *Hydrurus foetidus* and Rhodophyta: *Kumanoa sp.*) may be linked to the large availability of glacier meltwater (Klaveness, 2017). Consistently with this hypothesis, we detected only a few MOTUs belonging to strictly terrestrial taxa (<10%) while communities were dominated by ubiquitous protists that can exploit both terrestrial and freshwater habitats. The availability of glacier meltwater could also explain the scarcity of MOTUs exhibiting a shell or a resting cyst, which can represent defensive structures against dry conditions (Geisen et al., 2018).

3.4.2 Trajectories of taxonomic and functional diversity

The clear increase in functional diversity of protist communities over time since glacier retreat (Table 3.1) paralleled the overall growth of taxonomic diversity (Ficetola et al., 2024) (hypothesis 1 confirmed). Soil temperature did not show clear effects on either taxonomic or functional diversity. This pattern differed from the one observed for other soil organisms (Guerrieri et al., 2024), and from global analyses which detected a higher protist diversity in warm environments (Oliverio et al., 2020). The lack of a relationship between protist diversity and temperature in our dataset can be related to multiple factors. First, our models focused on variation within forelands, and variation of microclimate within forelands was generally weak. For instance, the range of variation of soil temperatures between plots within the same foreland was, on average, 2.3°C (max = 6.0°C, min = 0.5°C, SD = 1.3°C). Furthermore, within a given foreland, there is some covariation between soil temperature and time after glacier retreat, with recently deglaciated areas being colder due to their proximity to the glacier front at higher altitudes (Marta et al., 2023). Both limited variation within the foreland, and covariation between temperature and time, can reduce the power of analyses assessing the role of temperature. Nevertheless, the effect of time since deglaciation was much stronger than that of microclimate, suggesting that processes such as dispersal limitation may be more relevant than habitat filtering (Carteron et al., 2024; Singer et al., 2019). For instance, the microbial communities found in the earliest stages of the proglacial succession are similar to the microbial supraglacial communities which can represent substantial sources for the colonization of the recently deglaciated terrains (Buda et al., 2020; Cauvy-Fraunié & Dangles, 2019; Hotaling et al., 2017). However, the dispersal mechanisms underlining the colonization dynamics of protists in these environments are still largely unknown.

3.4.3 Phototrophic protists decrease with time since glacier retreat

Despite the overall taxonomic and functional diversity of protist communities increasing over time since glacier retreat, patterns varied among trophic groups (Fig. 3.4; hypothesis 2 confirmed). Such results highlight the complexity of protist communities, which include organisms with enormous functional variation, stressing the importance of multifaceted assessments of their biodiversity (Geisen et al., 2023; Giachello et al., 2023), as key mechanisms can remain undetected by coarse-scale analyses. Analyses pooling all the protists together suggested a growth of their taxonomic diversity (Table 3.1; Ficetola et al., 2024), but key components of communities, such as phototrophic protists, showed the opposite patterns (Fig. 3.4). Early communities hosted a high proportion of phototrophic protists, analogous to that observed in other harsh environments, such as polar grasslands or high-altitude environments (Mazel et al., 2022; Oliverio et al., 2020). The frequency of phototrophic taxa quickly decreased over succession, with mid and late communities exhibiting a trophic composition analogous to that found in temperate forests (Mazel et al., 2022; Oliverio et al., 2020). The abundance of phototrophic protists in the earliest stages of the succession confirms that eukaryotic algae, along with cyanobacteria, are important pioneer species, playing a key role as primary producers in the cold and resource-poor environments emerging at the front of retreating glaciers. The detected decrease of phototrophic protists over time since glacier retreat mimics the decrease observed for cyanobacteria (Kwon et al., 2015) and is aligned with the trajectories found in studies on single glacier foreland (Rosero et al., 2021), confirming this pattern at the global scale.

3.4.4 Functional composition of protist communities along glacier forelands

The overall functional composition of protist communities remained quite stable along the succession of glacier forelands, as most of the traits showed up soon after glacier retreat and were maintained till the latest stages (Fig. 3.3; hypothesis 3 rejected). This suggests that soil protist communities are characterized by a high functional diversity soon after the retreat of glaciers, with moderate changes in the following years, and that the increase in alpha diversity often occurs through the addition of functionally redundant taxa. Along with algae, early colonizers include phagotrophic protists (i.e., consumers) that are capable of surviving even in extremely cold environments (Bamforth et al., 2005; Lazzaro et al., 2015; van Leeuwen et al., 2018). The presence of a highly functionally diverse community already at the beginning of the succession may be important for the development of more complex biotic communities through time since protists can accelerate nutrient cycling and the buildup of organic matter (Bonkowski, 2004; Connell & Slatyer, 1977; Gao et al., 2019; Geisen et al., 2020). The decrease of functional specialization over time indicates the replacement of specialized groups such as phototrophs by more generalist organisms such as omnivores. Such a loss of phototrophs caused a shift in the functional structure of the community. While phototrophic protists represent an important source of primary productivity in the early stages of the succession, likely their role as primary producers is overtaken by plants as the communities develop (Yoshitake et al., 2010). The implications of the shift in the actors involved in the primary productivity are still poorly understood but may represent a turning point in the development of the soil biota, with food webs switching from simplified algal-based systems to more structured plant-based systems (Hågvar et al., 2024; van Leeuwen et al., 2018). The reconstruction of soil food webs of glacier forelands is a promising avenue that would enhance our understanding of ecosystem formation along ecological succession, clarifying the role played by micro-eukaryotes before, during, and after the arrival of plants.

3.4.5 Limitations

Our study represents the first attempt to describe the functional composition of protist communities along glacier forelands at an intercontinental scale, still, it has some limitations. First, the majority of samples belong to glaciers located in temperate regions, while subpolar and tropical forelands only represent 22% and 9% of the samples, respectively. Such geographical bias is related to the small number of tropical glaciers (Rounce et al., 2023) and to the accessibility issues of subpolar regions (Martin et al., 2012). Future studies should provide a better coverage of these areas, particularly in subpolar regions where the largest deglaciated areas will emerge in the next decades (Bosson et al., 2023). Second, we used a single universal eukaryotic marker to target protist sequences. Although using universal markers can provide a good overall representation of protist communities, the use of a singlemarker limits the detection of important soil protist taxa, such as Amoebozoa or Heterolobosea (Geisen et al., 2023). Future studies using a combination of universal and specific markers can allow a better characterization of the whole communities, eventually targeting taxa with specific functional roles (Burki et al., 2021; Ficetola & Taberlet, 2023). Additionally, glacier forelands remain poorly explored environments and are likely to host many species of protists for which there are no sequences deposited in the online databases challenging accurate taxonomic identification and functional characterization. Accurate biodiversity assessments are best achieved when eDNA data are coupled with sequencing of target organisms to produce accurate reference databases (White et al., 2020). Further limitations are related to the processof trait assignment. When traits are obtained from the literature, values can derive from the observation of individuals collected from a different habitat, while in proglacial environments the same species could show specific adaptations or phenotypic plasticity, such as smaller size or a different feeding mode. Again, efforts to collect the traits of organisms observed in a range of reference sites can be a key resource for a better functional characterization of protist communities and can providegreat benefits to broad-scale metabarcoding analyses (Pereira et al., 2023).

3.5 CONCLUSION

Our study provided the first broad-scale overview of protist communities developing in the ecosystems emerging from glacier retreat, considering both functional and taxonomic diversity. The multi-trait approach allowed us to distinguish the trajectories followed by diverse functional groups, detecting distinct successional patterns depending on the trophic level. While we observed a general increase in functional alpha diversity, this was mostly driven by a higher number of omnivore taxa, and the functional specialization of protist communities decreased due to the loss of phototrophic algae. The scarcity of studies on protists in the changing environments of glacier forelands calls for additional field campaigns combining both traditional and eDNA samplings to better understand the dynamics happening along these fast-developing ecosystems.

3.6 SUPPLEMENTARY INFORMATION

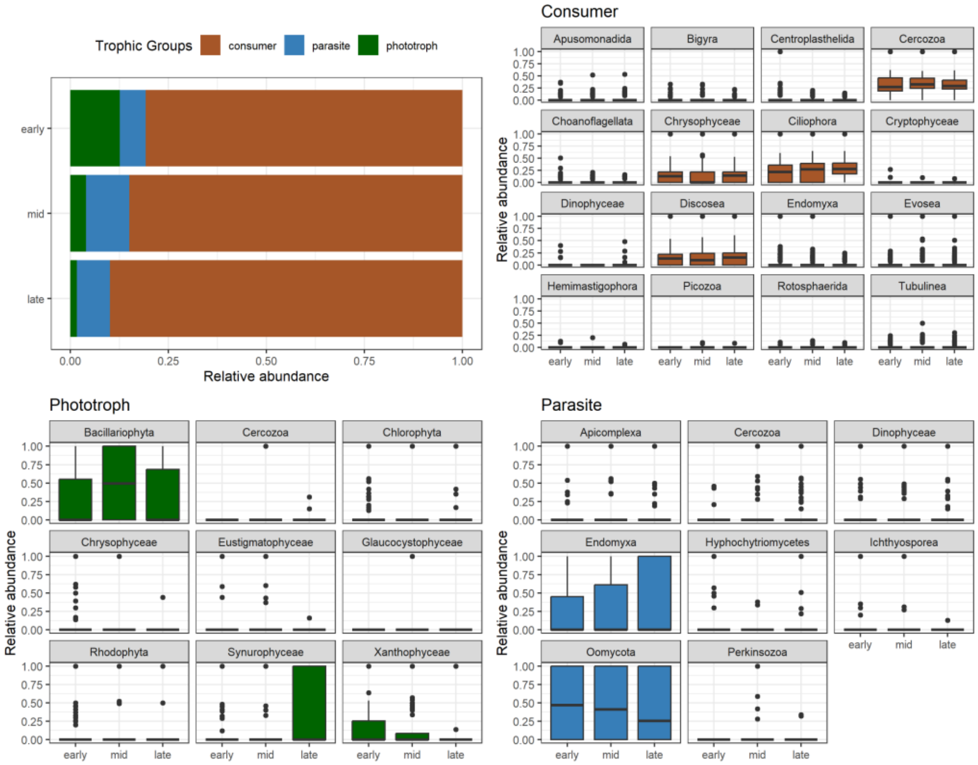

Figure S3.1. *Bar plots showing the relative abundance for each phylum/clade of protists detected in different successional stages (early < 30 years, 30 years < mid <100 years, late > 100 years). Clades are divided and colored based on trophic groups.*

Table S3.1. *Dataset of protist MOTUs and functional entities with three sheets related to taxonomy (A), traits (B), number of reads per plot (i.e., community matrix; C). A) Taxonomic information including the taxonomic id code assigned by NCBI (taxid), the best identity index, the sequence and the taxonomic assignment (supergroup, phylum, class, order, family, genus, and species; NCBI Taxonomy). B) Trait information including 10 functional traits reported as fuzzy-coded dummy variable, so that columns represent the functional categories within each trait. C) Community matrix reporting the number of reads per plot. The table is included in an Excel file accessible online at https://doi.org/10.1016/j.scitotenv.2024.177265 [TableS1].*

Table S3.2. *Dataset including the 1251 plots from all the 46 glacier forelands analyzed in the study. For each plot, we reported the name of the glacier foreland, the name of the dated site, age (early, mid, late), mean temperature, time since glacier retreat log-transformed, measures of taxonomic and functional alpha diversity (Hill numbers q1), score of PCoA.1, score of PCoA.2, functional specialization and the average value of all the functional categories for each of the ten functional traits weighted for the relative abundance of the functional entities expressing that functional category. The table is included in an Excel file accessible online at https://doi.org/10.1016/j.scitotenv.2024.177265 [TableS2].*

Table S3.3. *Effect of time, mean soil temperature and Topographic Position Index (TPI) on the taxonomic alpha diversity (a), functional alpha diversity (b), and functional composition (c) of soil protist communities. Mean estimate and 95% credible interval of the posterior distribution were estimated with Bayesian Generalized Linear Mixed Models. The values in bold indicate credible intervals not overlapping zero.*

	Independent					
	Time		Mean Temperature		TPI	
	B	CI 95%	*B*	CI 95%	*B*	CI 95%
a) Univariate analysis. Dependent: taxonomic alpha diversity						
	0.19	**0.11/0.26**	-0.05	-0.18/0.07	0.02	-0.03/0.07
b) Univariate analysis. Dependent: functional alpha diversity						
	0.18	**0.11/0.25**	-0.05	-0.17/0.07	0.02	-0.03/0.07
c) Multivariate analysis. Dependent: functional composition (3 measures)						
specialization	**-0.14**	**-0.21/-0.08**	**0.08**	**0.00/0.17**	**-0.05**	**-0.11/0.00**
score of PCoA axis 1	**-0.06**	**-0.11/-0.02**	-0.02	-0.07/0.04	0.01	-0.02/0.05
score of PCoA axis 2	**0.10**	**0.07/0.14**	-0.02	-0.05/0.02	0.01	-0.02/0.04

CHAPTER 4

Functional diversity stabilizes the structure of soil food webs developing after glacier retreat

Functional diversity stabilizes the structure of soil food webs developing after glacier retreat

This chapter is extensively based on the following publication:

Giachello, S., Cantera, I., Carteron, A., Bonin, A., Guerrieri, A., Ambrosini, R., Caccianiga, M., Gobbi, M., Marta, S., Thuiller, W., & Ficetola, G. F. (in preparation). Functional diversity stabilizes the structure of soil food webs developing after glacier retreat.

4 FUNCTIONAL DIVERSITY STABILIZES THE STRUCTURE OF SOIL FOOD WEBS DEVELOPING AFTER GLACIER RETREAT

ABSTRACT

The global retreat of glaciers is exposing large surfaces of deglaciated terrains. Predicting how ecosystems will develop on those terrains is required to plan effective management strategies. To predict future ecosystems, a clear understanding of the ecosystem dynamics occurring in deglaciated areas is needed. Here, we built food webs of soil communities along glacier forelands to reveal the dynamics shaping the development of ecosystems in deglaciated terrains. We combined an environmental DNA dataset of 263 soil communities along 46 forelands with trait information to estimate biotic interactions and reconstruct food webs. We calculated food web properties to assess changes in the structure of the communities developing along the forelands using multivariate models, β diversity measures, and null models. We found that the complexity and connectance of food webs were promoted by high functional diversity more than by time since glacier retreat or local temperature. Food web size showed high levels of dissimilarity in early communities depending on the local temperature, but after a hundred years since deglaciation, most of the sites sustained a similarly complex food web, indicating a convergent development of food webs toward late successional stages. Microorganisms like protists and nematodes appeared to be at the foundation of all food webs. In early communities, springtails emerged as important connectors, later

replaced by bigger primary consumers like insects and gastropods. By providing a first assessment of the dynamics shaping the food web structure along glacier forelands at a broad spatial scale, our study contributed to increasing the knowledge on the development of ecosystems in deglaciated terrains.

4.1 INTRODUCTION

The emergence of new land after glacier retreat is a dramatic consequence of climate change. Recent scenarios suggest that this process will accelerate in the next decades, with vast surfaces of new terrestrial ecosystems developing by the end of the century (Bosson et al., 2023). What will happen to this new land is a key question that still needs to be addressed. Answering this question is required to implement effective management strategies to guide the development of these ecosystems.

The loss of permanent ice coverage exposes deglaciated terrains to rapid colonization by multiple micro-organisms (bacteria, fungi, algae, and protists) but also cold-adapted arthropods like collembola and carabid beetles (Ficetola et al., 2024; Hågvar et al., 2024; Pothula & Adams, 2022). Successful colonizers start interacting with each other, organizing in biological communities that rapidly modify the environment, building up organic matter in developing soils, and increasing nutrient cycling (Khedim et al., 2021). Such processes facilitate the establishment of more taxonomically and functionally rich communities, which are critical to the formation of complex ecosystems (Ficetola et al., 2021, 2024; Giachello et al., 2024). Glacier forelands are thus highly dynamic environments with highly contrasting conditions. Recently deglaciated areas are characterized by harsh environmental conditions and low availability of nutrients, whereas the latest stages are far from the glacier, often experience milder climatic conditions, and can sustain more mature ecosystems with higher biodiversity (Ficetola et al., 2024; Pothula & Adams, 2022).

Although glacier forelands have been vastly investigated for more than a century, we are still far from achieving a complete understanding of how soil organisms assemble, contributing to the formation of ecosystems (Esperschütz et al., 2011). Whereas mechanisms such as environmental filtering and dispersal abilities influence the composition of communities along glacier forelands, understanding community assembly requires unravelling the intricate biotic interactions between its components (Ficetola et al., 2021). Predicting the structure of future ecosystems developing on deglaciated terrains thus requires going beyond the analysis of taxonomic diversity and investigating the dynamics of community assembly through a network approach that can detect changes in the structure of the whole community while accounting for the ecological role of each component and their interactions (Poisot et al., 2013).

Food webs are ecological networks that integrate measures of community composition (e.g., taxonomic or functional diversity) and trophic interactions, providing a representation of community structure and functioning (Delmas et al., 2019; Pellissier et al., 2018; Poisot et al., 2016). Modelling biotic interactions allows to consider the diverse ecological role played by different components of communities and provides measures to quantify the importance of each component on the overall food web structure (e.g., centrality measures for the identification of keystone species) (Delmas et al., 2019). The number of interactions (i.e., link diversity) and how they are distributed determines the properties and structure of the food web and provides key information on the function of ecosystems (Bastolla et al., 2009; Thébault & Fontaine, 2010). For instance, the connectance of food webs is linked to functional redundancy in nutrient and energy flows, favouring the stability of the ecosystems (Thébault & Loreau, 2005), while modularity promotes ecosystem resilience through the food web compartmentalization, thereby limiting the spread of disturbances across the community (Olesen et al., 2007; Stouffer & Bascompte, 2010). The analysis of the changes in food web structure along glacier forelands has thus the potential to provide helpful insights into how communities assemble, shaping the functions and the stability of the ecosystems developing after glacier retreat.

In our study, we reconstructed 263 soil food webs from 46 glacier forelands by coupling taxonomic information obtained through environmental DNA (eDNA) metabarcoding data to functional traits in order to estimate the pairwise probability of interaction between functional groups. Through this approach we were able to analyse food webs from four continents to investigate the variation of soil communities after glacier retreat, testing three main hypotheses. First, we assessed the trends of food web properties as link diversity, connectance, and modularity over time since glacier retreat and different microclimates (temperature). We expect an increase in link diversity (i.e., food web size) and connectance over time since glacier retreat (hypothesis 1). In particular, we expect smaller and less connected (low functional redundancy) food webs in recently deglaciated terrains and under harsher environmental conditions (low temperatures). Second, we tested if food webs develop following divergent or convergent trajectories. The divergent-trajectory hypothesis predicts that all early communities start with very similar food webs due to the strong selection imposed by ecological processes like habitat filtering and dispersal limitations (D'Amen et al., 2018; Makoto & Wilson, 2019), while in mid/late stages food webs diversify depending on local environmental conditions or the occurrence of keystone groups. Conversely, the convergent-trajectory hypothesis suggests that early communities are mostly determined by stochastic processes governing colonization (Makoto & Wilson, 2016) or by differences in local environmental conditions (Rydgren et al., 2014); thus, early food webs can show high dissimilarity but then, as communities become more diverse and complex, food webs converge to a similar structure. Early communities exhibiting a low beta diversity (high similarity) in food web properties that increase in the late stages indicate a divergent trajectory, and vice versa; early communities exhibiting a high beta diversity (low similarity) in food web properties that decrease in the late stages indicate a convergent trajectory. We expect that the harsher environmental conditions near the front of the glacier can strongly influence biotic communities, and thus, we think that the food web structure will become more similar in the latest stages (convergent-trajectory hypothesis). Third, we calculated centrality measures to identify potential keystone groups that can shape the structure of food webs (Jordán, 2009; Schwarzmüller et al., 2015). We expect keystone groups to differ between the early and late stages of the succession (hypothesis 3). Specifically, we expect microfauna to have a more central role in the simplified early food webs while bigger animals are likely to become more abundant and important in later food webs.

4.2 METHODS

Testing our three hypotheses required six main steps: i) use eDNA data to identify the functional groups, estimate biotic interactions, and reconstruct the overall metaweb; ii) use information on the occurrence of functional groups to derive site-specific food webs from the metaweb; iii) estimate network metrics to describe the structure of the food webs; iv) perform multivariate models to assess the effect of time since glacier retreat on the food web metrics (hypothesis 1); v) measure dissimilarities between communities (β diversity) and test if food webs developing along glacier forelands follow a convergent or divergent trajectory (hypothesis 2); vi) reconstruct submetawebs of early and late communities to visualize the changes in presence or abundance of functional groups between the two different successional stages (hypothesis 3); vii) estimate centrality indexes to identify keystone functional groups (hypothesis 3).

4.2.1 Study system and data collection

We investigated the development of food webs after glacier retreat, building upon an eDNA dataset describing the composition of soil biotic communities in 265 sites from 46 glacier forelands covering a wide latitudinal gradient (from 48°S to 79°), four continents and different climates (tropical, temperate, boreal). Such eDNA data proved to be a valuable resource to describe the dynamics and drivers of multiple groups of organisms after the retreat of glaciers (Cantera *et al.* 2024 for plants; Carteron *et al.* 2024 for mycorrhizal fungi; Giachello *et al.* 2024 for protists; Guerrieri *et al.* 2024 for nematodes) and to assess the overall development of terrestrial ecosystems (Ficetola et al., 2024). However, most of the previous studies considered only the composition of communities (taxonomic diversity and, for a few taxa, functional diversity) without accounting for biological interactions.

For our study, we sampled 46 glacier forelands. Each foreland represented a chronosequence of multiple dated sites, dated using values of time since glacier retreat (Marta et al., 2021). Therefore, each series of dated sites can be considered as an ecological succession characterized by a gradient of time since glacier retreat ranging from 1 to 483 years after the retreat of glaciers (Marta et al., 2021). We sampled from 3 to 17 dated sites (mean = 5.8, SD = 2.5) per foreland that were selected based on the availability of information about the glacier position in the past, accessibility for the sampling, and absence of evident sources of disturbance that could affect the development of the succession (e.g., landslide, stream erosion or geomorphological instability). We defined ~ 5 plots (mean = 4.7, SD = 0.8) of 1 m2 per site located approximately 20 meters of distance between each other that were considered spatial replicates of the same age. Five soil samples per plot were collected during the mildest months in the period 2014-2020 from a total of 1251 plots. For each plot, the five soil samples were aggregated and then environmental DNA was extracted and analyzed to characterize the taxonomic composition of the plot (see Ficetola et al. 2024 for a more detailed description of the sampling strategy).

In our study, we used the dated site (n = 265) as the minimum spatial unit for food web reconstruction and statistical analyses because very small food webs are usually not representative of the true food webs (Dormann et al., 2017). Furthermore, the plots located within the same site were sampled at a short distance from one another

(~20 meters); thus, ‘plot communities’ are likely to be connected. For these reasons, we aggregated the information on the taxonomic composition of all the plots sampled within the same site to work at the broader spatial unit of the site.

4.2.2 Taxonomic units and functional groups

The taxonomic composition of soil samples was retrieved through DNA analyses using a combination of five specific and two generalist primers to obtain a broad characterization, of the whole biotic community. The specific primers were used to target five key eukaryotic soil taxa: fungi (Fung02; Epp *et al.* 2012), seed plants (Sper01; Taberlet *et al.* 2007), insects (Inse01; Taberlet *et al.* 2018), springtails (Coll01; Janssen *et al.* 2018) and earthworms (Olig01; Bienert *et al.* 2012). The generalist primers Bact02 (Taberlet et al., 2018) and Euka02 (Guardiola et al., 2015) were used to target prokaryotes and the eukaryotes not targeted by specific primers (protists and some animal taxa, particularly rotifers, nematodes, tardigrades and, within arthropods, arachnids, and crustaceans). DNA was amplified performing four PCR replicates per soil sample. The retrieved DNA sequences were then filtered to remove contaminants and spurious sequences (Guerrieri et al., 2023). After the bioinformatic treatments and filtering, the remaining sequences were clustered based on their similarity to form Molecular Operational Taxonomic Units (MOTUs). For each primer, a specific similarity threshold was set (see Bonin et al., 2023 for more details). Taxonomic classification of MOTUs was performed using the NCBI Taxonomy database (Schoch et al., 2020). For more details on DNA analyses and bioinformatic processes refer to Guerrieri *et al.* (2023) and Ficetola et al. (2024).

Following the classification proposed by Potapov (2022), we assigned MOTUs to tropho-functional groups and, from now on, functional groups based on their taxonomy. Since Potapov (2022) considered bacteria and fungi as 'resources' without distinguishing functional groups, we integrated Potapov classification with trophic information for bacteria and fungi from additional sources (Calderón-Sanou et al., 2024; N. H. Nguyen et al., 2016). In this approach, functional groups are defined by combining taxonomy and trait information (e.g., bacteria zooparasite, collembola algivore, or lumbricina endogeic) which are both useful to infer pairwise interactions between groups to reconstruct food webs (Laigle et al., 2018; Potapov, 2022). Including taxonomical information is helpful because traits are often highly conserved within taxa, and thus, taxonomy represents a good proxy for trait information, which is typically difficult to collect (Cantera et al., in press; Laigle et al., 2018). To estimate the probability of interactions between functional groups, each functional group was characterized by information on resource preferences (plants, algae, leaf litter, wood litter, soil organic matter, bacteria, fungi, fauna), mass (mean and standard deviation), protection traits, and position in the soil layers (above, hepi, hemi, eu).

4.2.3 Reconstructing the soil metaweb of glacier forelands

The metaweb represents the set of all the potential interactions (i.e., links) happening between the functional groups (i.e., nodes) of the study system, regardless of their actual presence/absence in a specific site (Dunne, 2006). The functional groups of a metaweb can be generally distinguished into consumers and resources. Resources represent all the primary producers, such as plants or algae, and also include other non-living resources like dead wood, leaf litter, or soil organic matter, while consumers are typically all the heterotrophic organisms that interact with each other or consume resources. To estimate the type and probability of interactions between functional groups, we reconstructed the soil metaweb of glacier forelands following four main assumptions based on food-web theory (Potapov, 2022; Potapov et al., 2024): I) interactions are strictly dependent on the feeding preferences, II) a predator is usually larger than its prey (optimum Predator-Prey Mass Ratio, PPMR), III) the probability of interaction can be limited if they prey exhibit protective traits (preys exhibiting shells or chemical defenses are less preyed), IV) the probability of interaction is dependent on the overlap in the spatial niches of predators and preys (e.g., a predator living above the soil is less likely to prey upon an animal living only in deepest soil horizons). According to these assumptions, we used the trait information of functional groups to estimate a weighted trophic interaction matrix (adjacency matrix) derived from the product of four matrices representing, resource preferences (assumption I), size-based interactions (assumption II), protection traits (assumption III), spatial-based interactions (assumption IV).

All the data were formatted and manipulated in the R environment (R v. 4.2.2) following a food web reconstruction approach for soil organisms (Potapov, 2022).

4.2.4 Building local food webs

Local food webs are networks of interactions happening in a local area or site where co-occurring functional groups interact as defined in the metaweb. Therefore, local food webs are a subset of the metaweb, which integrates abundance data of the functional groups occurring in a specific site (Ohlmann et al., 2019). eDNA data do not provide measures of abundance. However, taxa having higher local abundance generally show a higher detection rate, as can be detected in more plots within site and/or more PCR replicates within the plots where they are detected (W. Chen & Ficetola, 2020; Ficetola et al., 2016, 2018; Furlan et al., 2016). Therefore, the number of detections of each MOTU in the 4 PCR replicates per plot was taken as a proxy of MOTU abundance in the plot (W. Chen & Ficetola, 2020; Furlan et al., 2016). The abundance of functional groups was calculated by summing the abundance of each MOTU belonging to the same functional group. Then, we summed the abundance of all the functional groups present in the plots located in the same site to build site-specific food webs using the 'build_metanet' function from the R package *metanetwork* (Ohlmann, Garnier, et al., 2023). For non-living resources as dead wood or leaf litter we estimated their abundance using respectively the MOTUs of woody plants only and all plant MOTUs. Measures of soil organic matter were not available at all the sites. However, a small amount of organic matter is generally available even at very recently deglaciated sites (Ficetola et al., 2024; Khedim et al., 2021), so we assumed that it was always available (i.e., soil organic matter =1 for each site).

4.2.5 Food web metrics

A popular approach to identifying changes in the structure of food webs along environmental gradients is comparing the properties of the networks (Pellissier et al., 2018). We selected five network properties to describe the food web structure: node diversity, link diversity, connectance, modularity, and nestedness. Node and link diversity represent, respectively, the diversity of functional groups (i.e., a measure of functional diversity) and the size of the food web. Both properties were estimated with the function 'compute diversity' from the R package *metanetwork* (Ohlmann, Garnier, et al., 2023), which uses the Hill number approach to calculate diversity from abundances based on the value of the q parameter that we set to 1 to obtain a measure equal to the exponential Shannon entropy (Chao et al., 2014; Ohlmann et al., 2019). Connectance is a measure of network complexity varying from 0 to 1, representing the number of predicted interactions (number of links) divided by the total potential interactions (number of nodes * number of nodes – 1). Connectance is often associated with network stability and the presence of redundant nodes, i.e., functional redundancy (Delmas et al., 2019). Modularity indicates the presence of distinct 'modules,' i.e., subnetworks containing nodes that are internally highly interlinked but less connected to the other nodes outside the module (Dalsgaard et al., 2013; Montoya et al., 2015). We calculated modularity using the R package *igraph* (Csardi & Nepusz, 2006), estimating first the membership of nodes in modules with the R function 'cluster_walktrap,' which identifies modules using the random walks algorithm (nodes connected by short random walks are likely to belong to the same module; Pons & Latapy 2005), second we included the estimated membership values in the function 'modularity' to obtain the modularity index. Nestedness describes the hierarchical organization of nodes and interactions. A food web shows high nestedness when it is composed of groups of nodes with few interactions (specialists) that are a subset of bigger groups of nodes with more interactions (generalists; Dalsgaard *et al.* 2013). We estimated nestedness using the function ‘nestednodf’ from the R package *vegan* (Almeida-Neto et al., 2008; Oksanen et al., 2022).

4.2.6 Environmental predictors

To assess the influence of environmental drivers on food web structure, each site was characterised by values of time since glacier retreat (in years; Marta *et al.* 2021) and mean soil temperature at level (Marta et al., 2023). Time since glacier retreat was selected because the age of a site is often a main driver of changes in diversity and ecosystem development along glacier forelands (Ficetola et al., 2024; Pothula & Adams, 2022). The date of the sites derives from a spatial explicit dataset containing information on the positions of glacier fronts since the Little Ice Age maxima (Marta et al., 2021). Along with time since glacier retreat, local environmental conditions can be important drivers of glacier foreland succession (Rydgren et al., 2014). For example, local soil temperature influenced the development of soil nematodes communities after glacier retreat (Guerrieri et al., 2024). Thus, we estimated the mean soil temperature during the growing season for each site, averaging the values of soil temperature at high resolution in the snow-free months from 2015 to 2019 (Marta et al., 2023). The high-resolution temperature was calculated through a global microclimatic model calibrated using 175 data loggers located near the soil surface (see Marta *et al.* 2023 for more information).

4.2.7 Food web analyses

Correlation and Principal Coordinates Analysis. Since network properties are often collinear, we performed a correlation analysis, to estimate the pairwise

Pearson correlation coefficient (r_{Pearson}) for all the food web properties. We performed an ordination analysis to visualize the differences among sites according to food web properties in a bi-dimensional space. Through the function 'daisy' of the R package *cluster* (Maechler et al., 2022), we estimated the Euclidean distance between sites based on link diversity, modularity, connectance, and nestedness. We used the distance matrix to run a Principal Coordinates Analysis (PCoA) with the function 'pcoa' from the R package *ape* (Paradis & Schliep, 2019). We evaluated the significance of the contribution of each food web metric to the first and the second PCoA axis using the 'envfit' R function, package *vegan* (Oksanen et al., 2022).

Multivariate Generalized Linear Mixed Models. We evaluated the effect of the environmental predictors on food web properties through a multivariate Bayesian generalized linear mixed model. Most food web properties showed a high correlation (Fig. S1), so we decided to exclude node diversity and nestedness ($r_{\text{Pearson}} = 0.83$) from the multivariate model. Thus, the model was built using the diversity of links, connectance, and modularity as dependent variables, while time since glacier retreat (log-transformed) and mean temperature were included as independent variables. All variables were scaled (mean = 0, SD = 1) to enable the comparison of their estimated effects and improve convergence. We accounted for the spatial non-independence of our study system, i.e., multiple sites within the same foreland, introducing the identity of the glacier foreland as a random effect. The model was fitted with the R package *brms* (Bürkner, 2017) applying uninformative priors and Gaussian distributions. Markov Chain Monte Carlo sampling was performed running 3 chains for 10.000 iterations with a 500-iteration burn-in and a thinning rate equal to 10. These parameters allowed the model to attain convergence (Rhat < 1.1). Because node diversity is often correlated with several food web metrics, we ran a GLMM with the same parameters of the multivariate GLMM to determine the changes in node diversity over time since glacier retreat and mean temperature, and then we used null models to compare the observed values to values generated from random communities (Delmas et al., 2019; Dormann et al., 2017).

Null model alpha diversity. We built null models to compare the observed values of alpha diversity for link diversity, connectance, and modularity, with values estimated by randomly permuting the abundance of functional groups within each food web (1000 replicates), keeping the number of functional groups constant (same node diversity; Pellissier *et al.* 2018). For alpha diversity, the 1000 permutations were used to estimate the standardized effect size (SES) for link diversity, connectance, and modularity. The standardized effect size is calculated by subtracting the observed values from the mean of permuted values and dividing the result by the standard deviation of the permuted values (Gotelli & McCabe, 2002). The SES for the three food web metrics were then used to run a multivariate Bayesian generalized linear model to assess if the changes over time since glacier retreat, mean temperature, and plant diversity were higher (SES > 0) or lower (SES < 0) than expected by chance.

Beta diversity. To verify if food webs developing after glacier retreat followed a convergent or divergent trajectory, we estimated the β diversity (i.e., dissimilarity) of the food web size (link diversity) within different successional stages. First, we estimated the quartiles of the distribution of the values of time since glacier retreat. Then, we selected the food webs belonging to the first (25%) and the last (75%) quartiles and built ‘submetawebs’ for early (<18 years) and for late (>117 years) communities to quantify the similarity in the food web structure of the sites exhibiting the highest differences in values of time since glacier retreat. We estimated the β diversity (q1) of nodes and links for the overall metaweb and the two submetawebs using the function ‘divPartition’ from the R package *econetwork* (Ohlmann, Matias, et al., 2023).

Null models beta diversity. To determine if the dissimilarity in link diversity among food webs within the same successional stage was mainly driven by differences in node diversity, i.e., the diversity of functional groups, or by the identity of specific functional groups, we built null models randomly permuting for 1000 times the abundance of functional groups within the same site, keeping constant the node diversity. Random permutations were performed for all the sites and link beta diversity of early and late stages was estimated from the randomly generated communities. In this way, if early or late communities hosted functional groups playing a disproportionately important role in shaping the structure of the food web, the observed values of link beta diversity would significantly differ from the values generated by the null models.

Aggregating communities. To visualize pairwise differences in the type of nodes or interactions between the submetawebs of different successional stages, we aggregated all the food webs within each submetaweb (e.g., all the food webs in the submetaweb of the early communities, sites < 18 years since glacier retreat), averaging abundance of all the functional groups present in those food webs and finally obtaining an averaged pair of submetaweb for early and late communities. To plot the differences between stages we further aggregated the pair of submetawebs creating a single aggregated metaweb for early + late communities. We then plotted the averaged metaweb using the abundances of the submetaweb to differentiate the functional groups based on their lower or higher abundance in one stage or another (e.g., a specific functional group is more abundant in late communities than in early communities).

4.2.8 Keystone functional groups

Amongst the metrics provided by the analysis of network structure, measures of 'centrality' quantify the importance of a node in a network based on the type of centrality metric used. In food webs, various centrality measures have been applied to identify potential keystone species (Gouveia et al., 2021), even though the actual ability of centrality indexes to detect keystone species remains controversial (Delmas et al., 2019). In the attempt to identify a list of potential keystone functional groups, we considered three centrality measures to obtain multi-faceted information on the importance of a functional group in the food web. For each functional group within each food web, we estimated degree centrality corresponding to number of interactions per functional group, closeness centrality representing the proximity of a functional group to all the other groups in the food web based on pairwise shortest path length, and betweenness centrality indicating the number of time a functional group (connector) is found between two other interacting functional groups (Delmas et al., 2019). We calculated all the centrality indexes with the R package *igraph*, using the function ‘degree’, ‘closeness’, ‘betweenness’ (Csardi & Nepusz, 2006). The degree centrality index was normalized by the maximum degree of the food web; the betweenness centrality index was normalized by the number of pairs of functional groups in the food web, excluding the functional group of interest (Delmas et al., 2019). After estimating all the centrality indexes for all the food webs, we ranked the top five functional groups occurring in more than 10% of the food webs according to the type of centrality index. For the ranking we retained only the functional groups containing fine functional information, e.g., Collembola: detritivores or Nematoda: omnivores, thus excluding the functional groups considered ‘too broad’ (i.e., Acari, Bacteria, Fungi, Collembola, Nematoda, Tardigrada, Heterotrophic protists) because were defined from MOTUs with a broad taxonomic level (e.g., phylum or class) that impeded the assignment of finer functional information.

4.3 RESULTS

4.3.1 The metaweb of glacier forelands

By assigning functional groups to eDNA metabarcoding data from 265 sites across 46 glacier forelands, we identified 87 functional groups. Functional groups included five basal resources (plants, algae, leaf litter, dead wood, and soil organic matter) and 82 groups belonging to taxa like bacteria (6), fungi (12), protists (5), and animals (59) (Fig. 4.1). The metaweb of glacier foreland soils included organisms ranging from a mass in the order of the nanograms (lower part of Fig. 4.1) to big arthropods or earthworms reaching a weight of a gram (higher part of Fig. 4.1). The trophic role of the organisms composing the metaweb varied from tiny chemolithoautotroph proteobacteria (B-chem) to equally small animal parasites of the protistan phylum Apicomplexa (Pr-AP) until reaching bigger detritivore metazoans like Diplopoda (Dpod) or soil apex predators such as spiders (Ar or Ar-La-Wa), Lithobiomorpha centipedes (Chi-Li) or carabid beetles (Cpt-P-Car) (Fig. 4.1). Trophic roles were not evenly distributed across different body masses. Predators tended to exhibit a higher body mass, being relegated to the higher right part of the metaweb (Fig. 4.1), except for nematode predators (Ne-P). Also, most of the detritivores were animals with high body mass, with the only exception of detritivores collembola (Cla-D). All the parasites were characterized by a very low mass apart from the parasitoid wasp (Hym). Omnivores instead covered all the body mass, ranging from small Phagotrophic protists (Pr-P) to Coleoptera (Cpt-O) and Dermaptera (Der). The overall metaweb, developed on the basis of the occurrence of these 87 functional groups (nodes), comprised 1992 potential interactions (links).

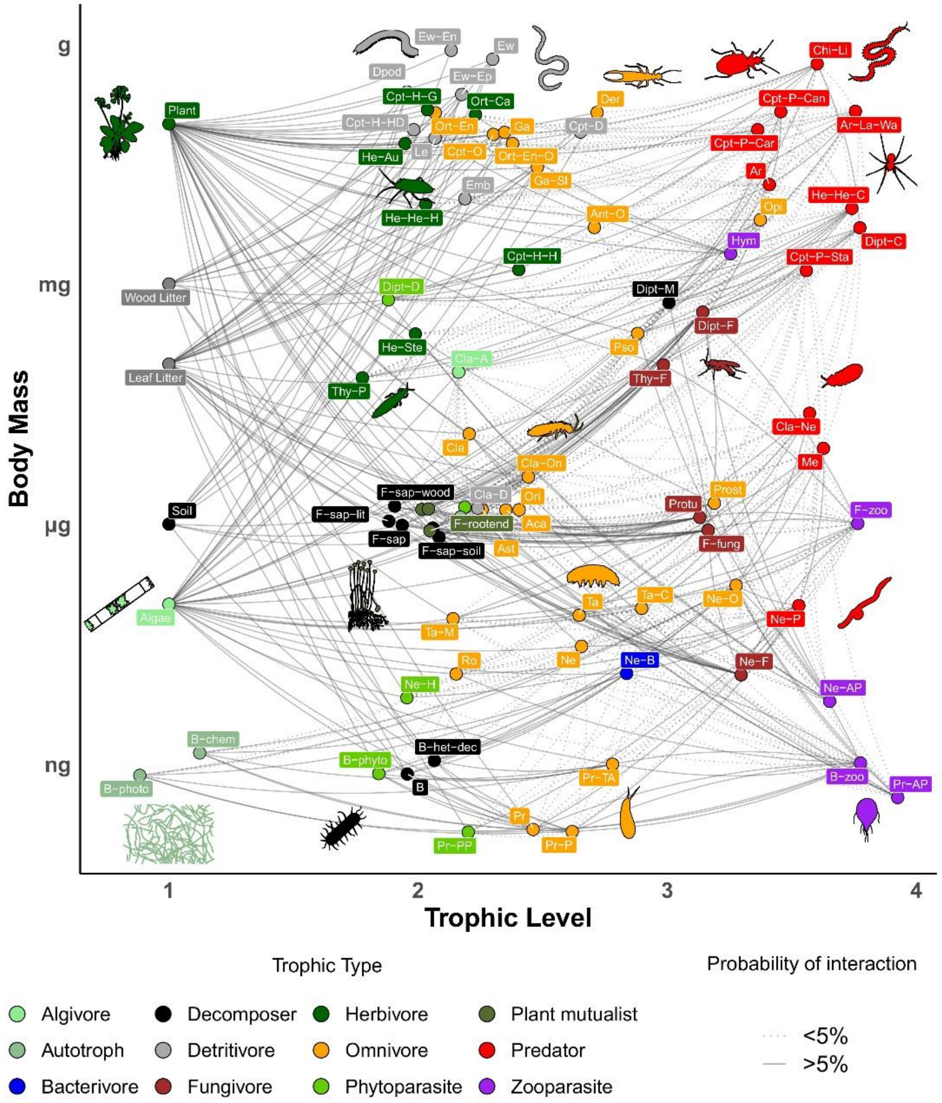

***Figure 4.1**. Metaweb of all the functional groups (nodes represented with circles) found in the 1251 soil samples collected from 265 sites along 46 glacier forelands. Colors indicate the trophic type. Lines connecting the nodes indicate trophic interactions (links). Dashed lines indicate a low probability of interaction (<5%).*

4.3.2 Food web structure

We integrated the metaweb with estimates of the occurrence of functional groups to reconstruct 263 site-specific food webs. The number of functional groups per food web ranged from 6 to 51 (mean = 31), while the number of predicted interactions varied from 2 to 667 (mean = 270), with a total of 1597 predicted interactions considering all the food webs. Two recently deglaciated sites from the Verra Glacier foreland (Northern Italy; < 10 years after glacier retreat) presented less than three functional groups and no interactions. Thus, no food webs were estimated for those sites.

The size of the food webs varied considerably, with values of link diversity ranging from 1.53 to 100.16 (mean = 42.62, SD = 21.03; Fig. S2). Most of the food webs showed very low values of modularity (mean = 0.08, SD = 0.05) and moderate levels of nestedness (mean = 30.89, SD = 8.51) and connectance (mean = 0.25, SD = 0.05; Fig. S2). All the food web metrics were highly collinear (Fig. S4.1). Connectance was positively related to nestedness (Pearson's r = 0.83) and negatively to modularity (r = -0.75), while node diversity was strongly related to link diversity (r = 0.83). In the Principal Coordinates Analysis, most of the total variance of food web structure was explained by the first PCoA axis (77.8%), which was significantly related to all the food web properties ($\text{pvalue}_{\text{envfit}} = 0.001$), while the second PCoA axis explained 11.3% of the and was related to modularity and link diversity (Fig. 4.2). The pairwise relationships between food web metrics showed that small food webs (low link diversity) exhibited higher values of modularity but lower values of connectance and nestedness (Fig. 4.2). As the size of the food web increased (higher link diversity) modularity decreased whereas connectance and nestedness increased (Fig. 4.2).

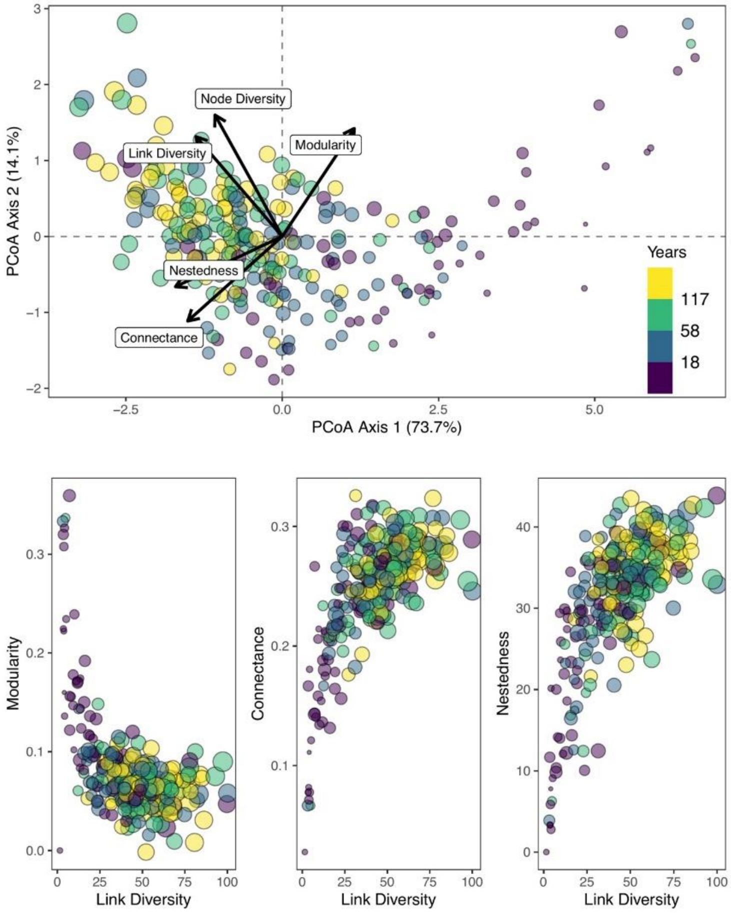

***Figure 4.2**. Relationships between the food web metrics, node diversity, link diversity, modularity, connectance, and nestedness. Top) Food web structure summarized by the first and the second PCoA axis estimated using the four food web metrics (black arrows). Circles represent sites, size and color are proportional to time since glacier retreat (expressed in years). Bottom) Pairwise relationship between link diversity (x axis) and the other food web metrics.*

4.3.3 Trajectories of food web metrics

The variation of all the food web metrics was strongly related to time since glacier retreat. At increasing values of time since glacier retreat, link diversity (i.e., food web size) and connectance increased, while modularity decreased (Table 4.1; Fig. 4.3). Soil temperature showed a strong direct relationship with link diversity, as sites characterized by milder temperature sustained bigger food webs (Table 4.1; Fig. 4.3). Furthermore, the relationship between time and food web properties was strongly modulated by temperature. Immediately after glacier retreat, sites with cold temperatures showed lower link diversity and modularity than sites with similar ages but with mild temperatures (smallest food webs in the coldest sites).

***Table 4.1**. Estimated effects of time since glacier retreat and mean temperature on link diversity, connectance, and modularity calculated with multivariate Bayesian generalized linear mixed models.*

Food web metric	Environmental predictor	Estimate	Est. Error	l-95% CI	u-95% CI
Link diversity	Time	**0.45**	**0.05**	**0.35**	**0.54**
	Temperature	**0.22**	**0.07**	**0.09**	**0.36**
	Time : Temperature	**-0.12**	**0.05**	**-0.22**	**-0.02**
Connectance	Time	**0.40**	**0.06**	**0.29**	**0.51**
	Temperature	0.07	0.06	-0.06	0.18
	Time : Temperature	**-0.18**	**0.06**	**-0.30**	**-0.06**
Modularity	Time	**-0.36**	**0.06**	**-0.47**	**-0.24**
	Temperature	-0.02	0.06	-0.14	0.10
	Time : Temperature	0.12	0.07	-0.02	0.25

However, these differences decreased through time and, approximately one century after glacier retreat, sites with different temperatures showed similar values of link diversity and connectance (Table 4.1; Fig. 4.3).

These changes in network metrics were determined mainly by the variation through time of node diversity (Fig. S4.3), i.e., |Standardized Effect Size| < 1.96 in null models (Fig. S4.4). This happened for 85% of sites for link diversity, 90% of sites for connectance, and 100% of sites for modularity.

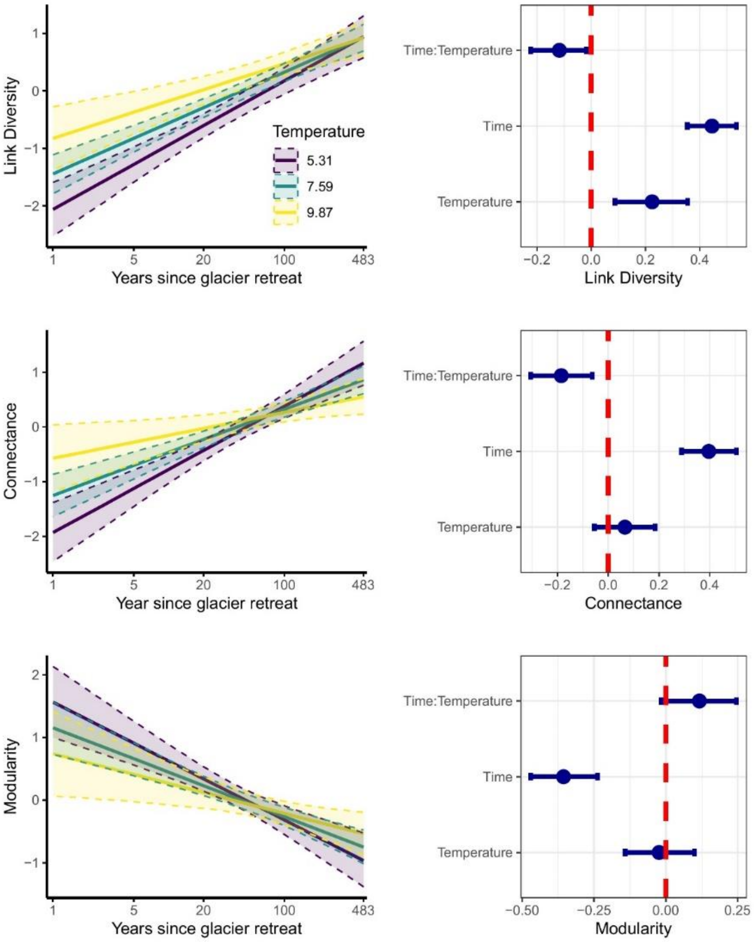

Figure 4.3. *Effect of time since glacier retreat, mean temperature, and their interaction (Time:Temperature) on the food web metrics based on the results of the multivariate Bayesian generalized linear models. Left: trajectories of the food web metrics for the interaction between time since glacier retreat (on the x axis) and mean temperature represented by lines of different colors. Right: strength of the effect of each environmental predictor on the food web metrics, circles represent the mean effect size, and the blue lines the 95% credible intervals of the posterior distribution.*

4.3.4 Differences between early and late communities

Comparing the aggregated submetaweb of the early communities (sites younger than 18 years) with one of the late communities (sites older than 117 years) highlighted that, although most functional groups were more abundant in late communities, some specific groups like algae, tardigrades, plant feeding Thysanoptera and even some large predators like centipedes or Coleoptera Cantharidae decreased in abundance (Fig. 4.4). However, all the functional groups found in early communities were also present in the late food webs but not vice versa. Late food webs were enriched by many new functional groups of arthropods such as detritivores millipedes, parasitoid Hymenoptera, and predators like spiders and assassin bugs but also Gasteropoda or much smaller animals like parasitic nematodes (Fig. 4.4).

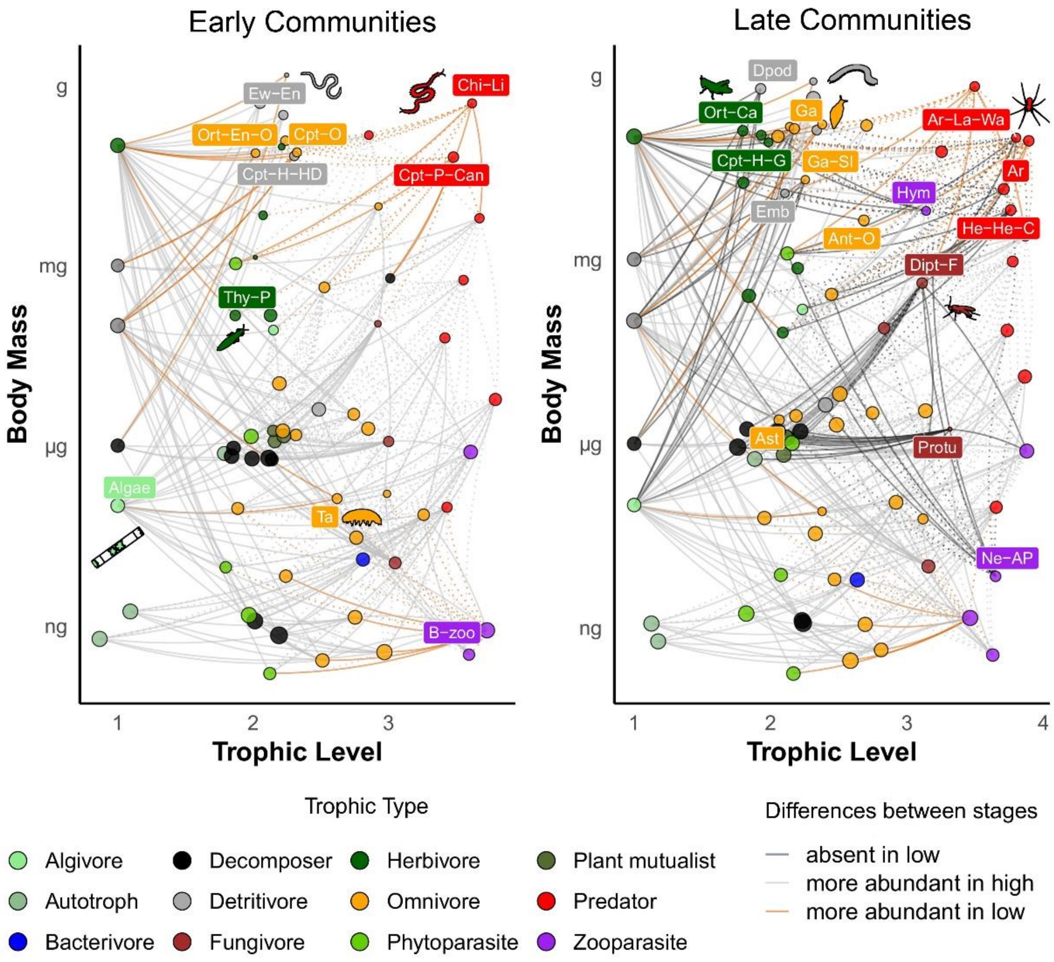

***Figure 4.4**. Submetawebs of food webs of recently deglaciated sites (< 18 years since deglaciation) and older sites (> 117 years since deglaciation). Circles are functional groups (i.e., nodes) colored by trophic type. Lines are interactions (i.e., links) colored by the difference in abundance of functional groups between the early and late communities submetaweb. Labels are included only for more abundant groups in early communities and for exclusive groups in late communities.*

4.3.5 Food web dissimilarities

The indexes of β diversity showed that food webs of early communities (sites deglaciated since less than 18 years) were more diversified in terms of node diversity (functional richness; $\beta\ div_{early} = 1.35$ vs $\beta\ div_{late} = 1.18$) and link diversity (size of the food web; $\beta\ div_{early} = 1.89$ vs $\beta\ div_{late} = 1.22$) compared to the food webs of late communities (sites deglaciated since more than 117 years; Table 4.2).

Table 4.2. *Beta diversity of the metaweb and aggregated food webs based on the first and last quartiles of the distribution of time since glacier retreat and mean temperature.*

Predictor	**Aggregated food webs**	**Values**	**B nodes**	**B links**
	All		1.27	1.55
Time (years)	Early	< 18	1.35	1.89
Time (years)	Late	> 117	1.18	1.22

Null models for link beta diversity showed that early communities did not exhibit a higher dissimilarity in link diversity than the one predicted by chance (Fig. 4.5). The food webs in late communities (>117 years since glacier retreat) instead, showed a very low value of link beta diversity, which was significantly smaller (< 0.025 percentile) than the values estimated for randomly generated communities with the same node diversity (Fig. 4.5), indicating that food webs in late communities were more similar to each other than what we would expect by chance.

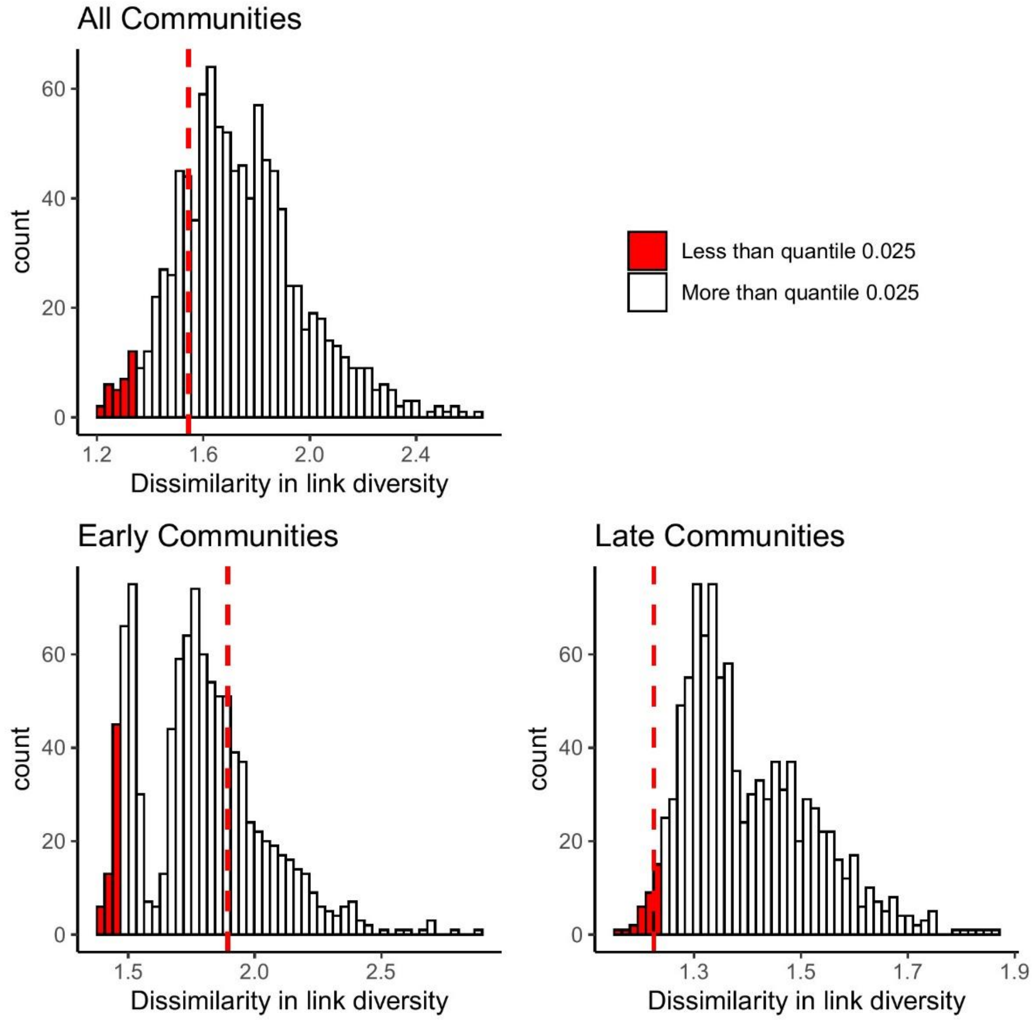

Figure 4.5. *Distribution of the values of link beta diversity (dissimilarity in interactions) predicted for 1000 simulated food webs built randomly, permuting the abundances of functional groups while maintaining the same node diversity. Red bars represent the values of link beta diversity < 0.025 quantile, the red line indicates the observed values of link beta diversity for the metaweb and the submetawebs of early and late communities.*

4.3.6 Keystone groups

The three centrality indexes provided different lists of the top five functional groups (Fig. 4.6). The five groups showing the highest number of interactions (degree centrality) were mostly omnivores and belonged to phagotroph protists, testate amoeba and microfauna as acarid Prostigmata and nematodes omnivores (Fig. 4.6). For degree centrality, the only difference between early and late communities was that collembola detritivores were among the most central groups in early communities but not in late communities, while predator nematodes had a central role in late communities but not in the early ones. Closeness centrality indicated that the functional groups that were more proximate to all the other functional groups of the food web, i.e., that influence more effectively the overall food web, differed between early and late communities. Early communities were characterized by functional groups with a low body size having a high closeness centrality like the omnivore springtails of the family Onychiuroidea, the plant-feeding nematodes or omnivores tardigrades with the exception of earthworms (Fig. 4.6). On the other hand, in late communities the list of top five functional groups for closeness centrality changed toward groups of bigger body size like gastropod and insects as Dermaptera and predator Diptera. Betweenness centrality showed a similar pattern, with early communities hosting functional groups with a low body size being the best ‘connectors’ (i.e., having a more relevant role in connecting various modules of the food webs) like detritivores springtails, phagotrophic protists, oribatid mites, or nematodes plant feeders, while in late communities the role of connectors was taken by bigger omnivore animals like Dermaptera and gasteropoda (Fig. 6).

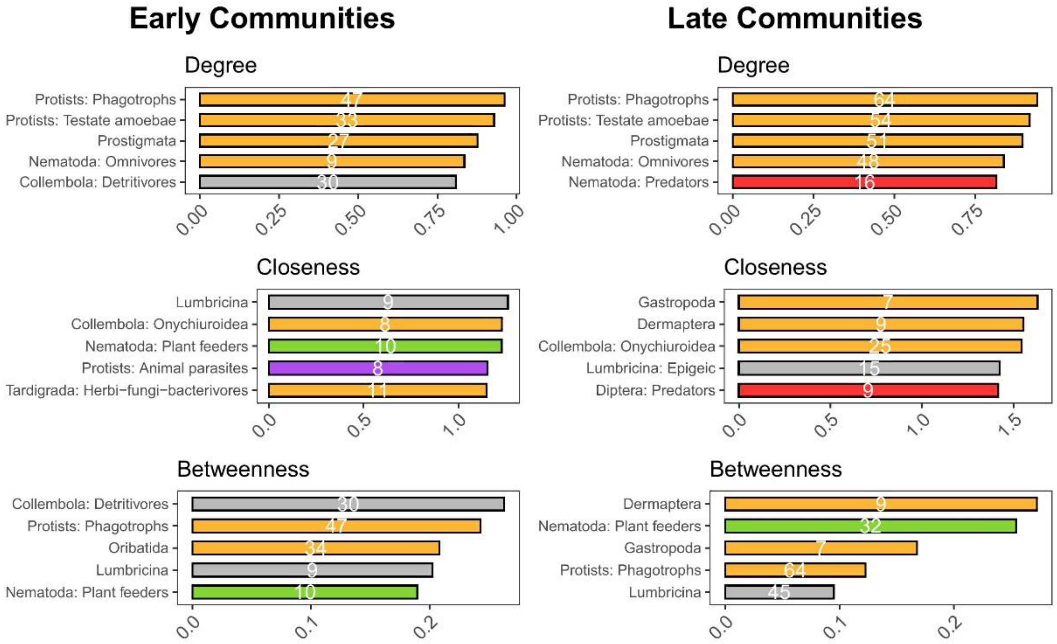

Figure 4.6. *Keystone functional groups according to three centrality metrics for early and late communities. Barplots show the top 5 functional groups with a higher score of the centrality metric that were present at least in 10% of the sites (i.e., > 6 sites). The color of the bar represents trophic type, orange = omnivores, grey = detritivores, red = predators, green = plant parasites, purple = zooparasites The number inside the bar indicates how many networks the functional group was present.*

4.4 DISCUSSION

Combining eDNA data from a large-scale dataset and trait information enabled us to predict trophic interactions between functional groups for 263 dated sites along 46 glacier forelands, building a metaweb representative of the soil community developing after glacier retreat across four continents. We used data on community composition for each site to reconstruct site-specific food webs, describing their structure through the estimation of food web metrics. We then assessed the trajectories of food web metrics along the glacier foreland succession, finding that link diversity and connectance increased over time since glacier retreat, with the rate of increase being dependent on the local mean temperature (hypothesis 1). Null models of link beta diversity of aggregated communities revealed that the structure of the food webs in late communities was much more similar than expected by chance, indicating a convergent trajectory of food web structure from early to late stages, driven by the arrival of functional groups that stabilizes link diversity (hypothesis 2). The estimation of centrality indexes suggested a shift in keystone functional groups from early to late communities, with microorganisms like protists, nematodes, and collembola having a central role in early food webs, replaced by bigger animals like gastropods and insects in late food webs (hypothesis 3).

4.4.1 The architecture of soil food web along glacier forelands

The structure of the metaweb representing soil communities developing along glacier foreland exhibited a high diversity of functional groups (Fig. 4.1) with a composition comparable to the one detected in more stable ecosystems (Calderón-Sanou et al., 2024). Comparing the submetawebs of different successional stages highlighted the presence of many trophic groups with diversified ecological roles and trophic levels just after a few years since glacier retreat (early communities < 18 years), indicating that even the most recently deglaciated sites can sustain complex food web structure (Fig. 4.4). The submetaweb of early communities contained all the functional groups found in a supraglacial food web from an intensively studied glacier in Northern Italy (Crosta et al., 2024) and closely resembled a hypothesized food web for communities living in proximity of a retreating glacier (Hågvar et al., 2024), with algae being the main primary producers (Giachello et al., 2024), tardigrades and collembola being key primary consumers, and bigger arthropods like coleoptera occupying the highest trophic level of the food web as pioneer apex predators (Hågvar et al., 2024). In late communities, the increased diversity of plants and litter seems to facilitate the arrival of functional groups typical of alpine meadows as detritivores millipedes and herbivore insects like grasshoppers (e.g., Gomphocerus living in alpine meadows) or the widespread leaf feeding carabid Amara communis (node Cpt-H-G in Fig. 4.4).

4.4.2 Food web dynamics along glacier forelands

We assessed food web dynamics along glacier forelands, estimating food web properties, which are frequently used to detect changes in food web structure across environmental gradients (Pellissier et al., 2018; Tylianakis & Morris, 2017). However, ecological interpretations need to take into account the high collinearity among food web properties since properties such as connectance and modularity are often very correlated to the size of the food web (Delmas et al., 2019; Vermaat et al., 2009). We performed null models to account for the high correlation that we found for most of the food web metrics (Fig. S4.1) and identified variation that is not determined by the overall patterns of increasing functional alpha-diversity (node diversity; Fig. S3). Multivariate models showed that link diversity and connectance increased over time since glacier retreat depending on the local mean temperature (Table 4.1; Fig. 4.3), supporting the hypothesis that food webs increased in size and became more connected over time since glacier retreat (hypothesis 1 confirmed). Colder sites sustained smaller and less connected food web, but with increasing time since glacier retreat, link diversity and connectance reached similar values independently on the temperature (Fig. 4.3). These results suggested that not all the successions along glacier forelands developed in the same way. The harsh conditions of some forelands, particularly in subpolar regions or at extreme altitudes, represent strong constraint for the formation of complex food webs. Such findings are in agreement with multiple studies along glacier forelands which found that early stages of colder forelands sustained less diversity than early stages with milder conditions (Devetter et al., 2021; Valle et al., 2022). Indeed, in early sites where conditions were milder (i.e., higher temperatures) food webs were bigger and much more connected (Fig. 4.3). Null models showed that these changes in food web metrics were mainly due to the addition of functional groups independently on the identity of the functional groups added (Fig. 4.4). This suggests that functional diversity (i.e., node diversity) was the most important driver shaping the food web structure along glacier forelands, with food webs with higher functional diversity being bigger, more connected and less modular (Fig. 4.3). A decrease in modularity with increasing functional diversity represents an opposite pattern to what has been found in multiple studies analysing complex food web (Lin et al., 2024; Montoya et al., 2015). The observed decrease in modularity along glacier foreland could be related to the type of functional groups that were added through time, which mostly represent generalist animals. Furthermore, since modularity identifies subgroups of taxa that are highly interlinked with each other but less with the rest of the network, we argue that, in the harsh environments of the most recently deglaciated terrains (early stages of the succession), the presence of specialist taxa adapted to extreme conditions and limited resources (Crosta et al., 2024; Hågvar et al., 2024) can confer higher modularity to the food web structure in respect of the latest successional stage where more generalist taxa are likely to interact with much more other taxa (Fig. 4.3).

4.4.3 The convergent development of food webs

The estimation of the link beta diversity for early vs late communities (Tab. 4.2) and the null models on link beta diversity (Fig. 4.5) supported the hypothesis that late communities are more similar than expected by chance, indicating a convergent trajectory from early to late stages (hypothesis 2 confirmed) toward more stable communities. Integrating this information with the one obtained by the multivariate model (Fig. 4.3), we can conclude that the stability in the food web structure along glacier forelands appeared to be promoted by larger and more complex food webs (i.e., higher node and link diversity) exhibiting higher levels of connectance and thus a higher functional redundancy (Fig. 4.4). Such stability could also be provided by the arrival of some keystone functional groups that facilitate the establishment of more complex (Fig. 4.3) and stable (Fig. 4.4; Tab. 4.2) food webs.

4.4.4 More consumers, more predators

Centrality indexes suggested that microorganisms like omnivore protists and microfauna such as mites and nematodes supported most of the link diversity and connectance of the food webs from early to late stages (high value of centrality degree Fig. 4.6). Such organisms are likely to be the most abundant consumers in soil communities and we found them in many of the sites sampled (white number within bar in Fig 4.6). Their wide occurrence and high abundance are probably the factors that enabled even the recently deglaciated sites (i.e., early communities, < 18 years since glacier retreat) to sustain complex food webs with many functional groups exhibiting diverse ecological roles and trophic levels (Fig. 4.4).

Centrality betweenness identified detritivores collembola as the key 'connectors' (i.e., high values betweenness) for the early communities (Fig. 4.6). This indicated that, soon after deglaciation, springtails are relevant consumers but also important prey for apex predators like Coleoptera and centipedes. A comparable role of springtails as food web connectors was described for supraglacial food webs (Crosta et al., 2024). Additionally, the high values of centrality closeness for omnivore springtails (Collembola; Onychiuroidea) highlighted again the key role of these organisms in the early food webs as they closely interacted with many functional groups (Fig. 4.6). In late communities, instead, the highest values of closeness and betweenness were associated to much bigger organisms like omnivore consumers such as gastropods and Dermaptera (hypothesis 3 confirmed, Fig. 4.6), suggesting that the convergence and stabilization of the food web structure could be driven by the increasing abundance of functional groups of bigger consumers which favour the arrival of more predators and more animal parasites (Fig. 4.6). Future research on realized food webs could improve our understanding on the role of keystone functional groups in shaping the organization of communities developing after glacier retreat.

4.4.5 Limitations

The reconstruction of the metaweb through the estimation of pairwise interactions between functional groups built by combining eDNA data and trait information represented a powerful approach to describe food dynamics along several glacier forelands on a large spatial scale, overcoming issues related to the complexity of identification of all the organisms using traditional approaches. However, such approach has important limitations related to multiple levels of uncertainty inherent to the often low taxonomic resolution of eDNA metabarcoding data, which prevented the assignment of many MOTUs to specific functional groups (Cantera et al., in press) and the quality of the trait information used to predict biotic interactions (Pereira et al., 2023). Furthermore, it is not clear to what extent the realized food webs can be approximated by food webs predicted under the assumption that co-occurring nodes with matching traits always interact with a fixed probability of interaction (Delmas et al., 2019; Tylianakis & Morris, 2017). Another limitation of eDNA data is that they do not provide reliable estimates of abundance or biomass (Calderón-Sanou et al., 2020) which are critical information required to obtain more accurate estimates of biotic interactions especially when the analyses of food webs are based on predicted interactions (Vázquez et al., 2007). Missing values of true abundance or biomass also limited the ability to identify keystone groups since, in ecological communities, the importance of a taxon or a functional group is often determined by its quantity, i.e., common taxa are more likely to have a strong influence on the food web structure than rare one (Delmas et al., 2019). Despite these limitations, the prediction of food webs combining eDNA data and the trait matching approach offered a unique opportunity to investigate food web dynamics at a large spatial scale. Including the collection of data on abundance and traits during the eDNA sampling will highly improve the power of this approach, reducing most of the limitations (Pereira et al., 2023). Future research must focus on validating this approach by comparing the predicted food webs to the realized ones.

4.5 CONCLUSION

The emergence of large surfaces of deglaciated terrains is already, and will be even more, an important phenomenon that will bring opportunities and challenges. Identifying the dynamics behind the organization of soil communities along glacier foreland represents an essential first step to predicting what type of ecosystems will develop on deglaciated terrains in a warming climate. Good predictions are necessary for better planning and management of the emerging territories, addressing the challenges to maximize the opportunities. In our study, we found that functional diversity was a more important driver of food web complexity than time since glacier retreat or mean temperature. Communities in recently deglaciated terrains showed a high dissimilarity in food web size depending on the local temperature, but after a hundred years since deglaciation, most of the sites could sustain a similarly complex food web. Microorganisms appeared to be at the foundation of all food webs with springtails being important connectors in early communities, later replaced by bigger primary consumers as insects and gastropods. Predicting food webs combining eDNA data and trait information enabled us to describe the general dynamics of community organization along glacier forelands at a large spatial scale. However, the important limitations of this approach require further investigation to validate our results and better shed light on the role of keystone groups in shaping the structure of soil communities along glacier forelands.

4.6 SUPPLEMENTARY INFORMATION

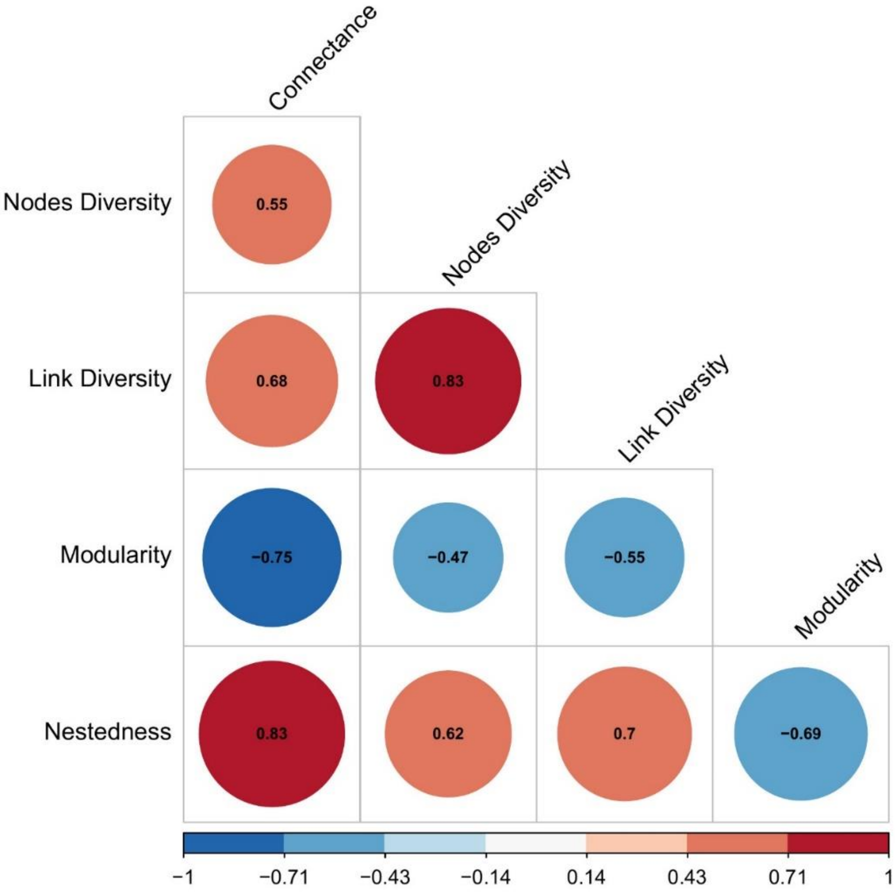

Figure S1. *Correlation matrix of the site-specific food web properties. The size of the circles represents the strength of the correlation, the colors in the direction (blue = negative, red = positive), and the values within the circles are the correlation values based on the Pearson coefficient.*

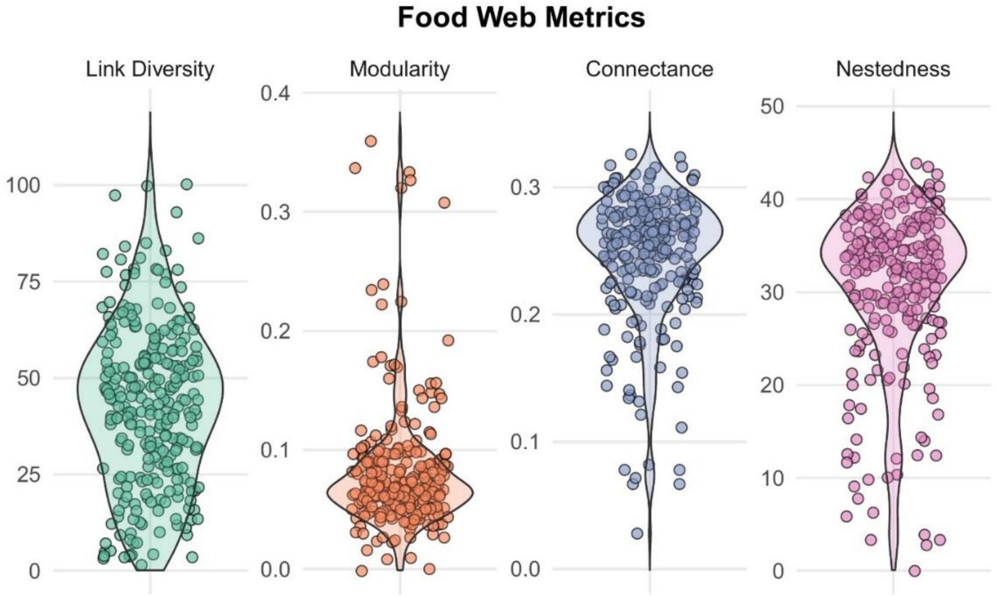

Figure S2. *Distribution of the values for the food web properties of all 263 sites from 46 glacier forelands.*

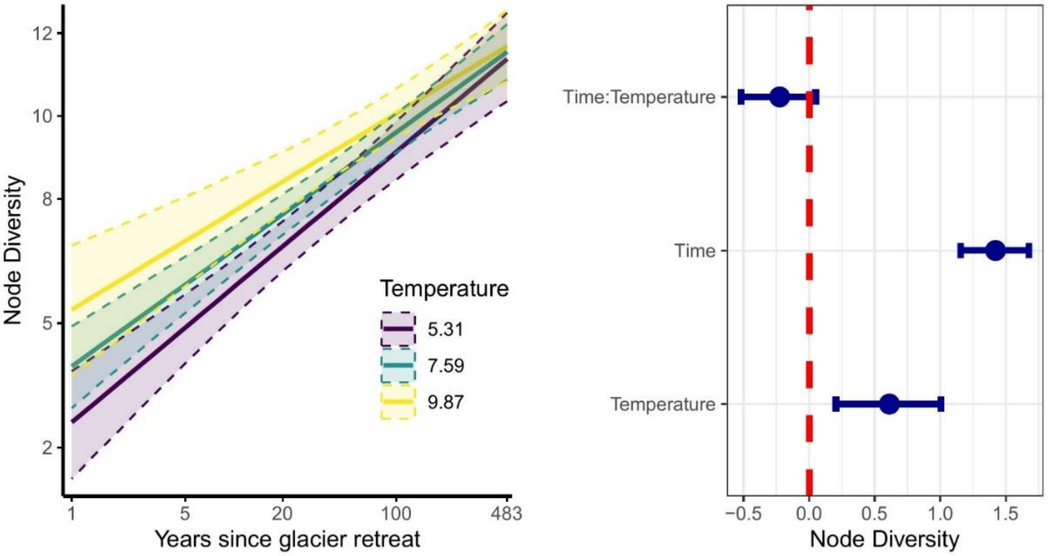

Figure S3. *Effect of time since glacier retreat, mean temperature, and their interaction (Time:Temperature) on node diversity based on the results of Bayesian generalized linear models. Left: trajectories of node diversity for the interaction between time since glacier retreat (on the x axis) and mean temperature represented by lines of different colors. Right: strength of the effect of each environmental predictor on node diversity, circles represent the mean effect size, and the blue lines the 95% credible intervals of the posterior distribution.*

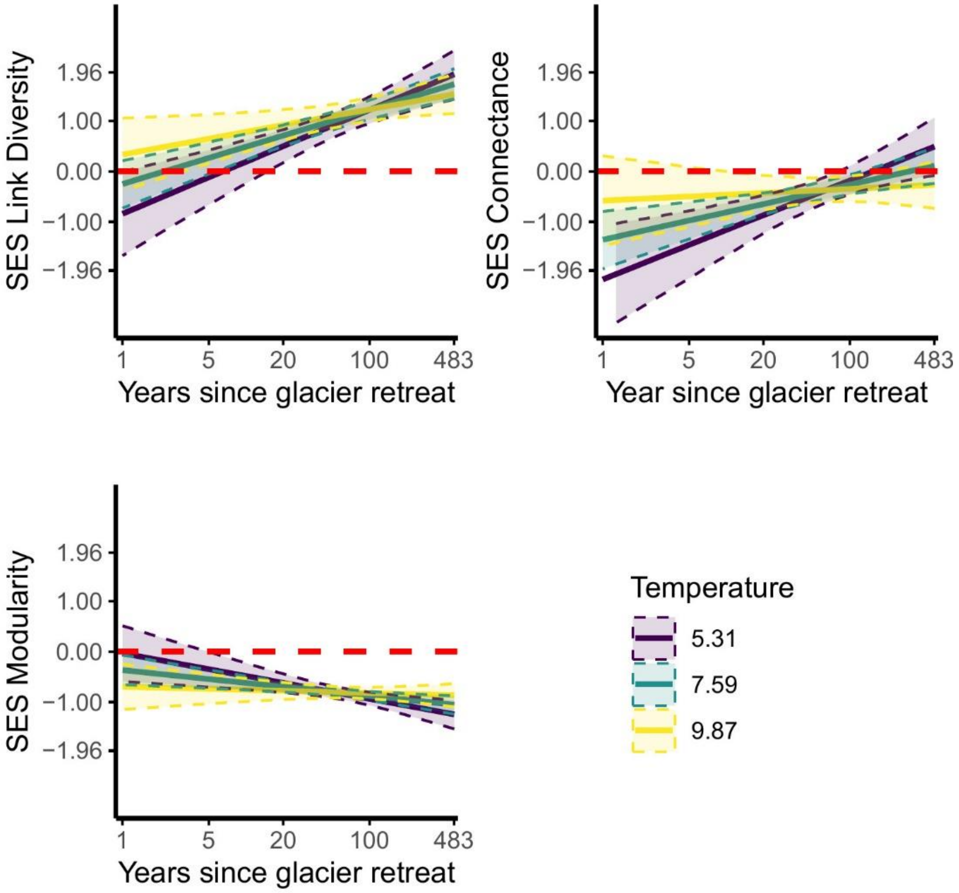

Figure S4. *Relationship between standard effect sizes (SES) of food web metric and environmental predictors. The red line represents SES = 0. Positive values of SES indicate that the values of the food web metric are higher than expected by chance, given the observed node diversity, and vice versa.*

CHAPTER 5

Conclusion

Conclusion

5 CONCLUSION

In times of drastic environmental changes, fast and effective broad-scale studies are essential to rapidly assess the ongoing ecosystem responses. Such studies are crucial for generating predictive large-scale biogeochemical models on future ecosystems that can be used to inform robust mitigation and adaptation strategies (Intergovernmental Panel On Climate Change, 2023b). To achieve this goal, ecologists have been forced to expand their research focus by integrating interdisciplinary fields of ecology that have traditionally operated as independent domains, developing new approaches and techniques (Cantera et al., in press; Pereira et al., 2023; Potapov et al., 2023).

Through the application of innovative approaches coupling high-throughput sequencing techniques such as environmental DNA metabarcoding with functional information, my thesis aimed to investigate the functional responses of biotic communities after glacier retreat. Characterizing the functional diversity of biotic communities using environmental DNA offers great opportunities but also poses substantial challenges that may hinder the improvement as well as the widespread adoption of this Fun-eDNA approach (paragraph 1.4. Cantera et al., in press). Exploiting the opportunities and tackling the challenges is essential to future-proofing the 'Fun-eDNA' approach, facilitating its diffusion while maximizing its potential (paragraph 1.4. Cantera et al., in press).

To this end, in pursuit of understanding the development of biotic communities following glacier retreat, my thesis contributed to four key research topics bridging community ecology and functional ecology:

1) **Integrating environmental DNA and functional traits.** I consolidated the use of environmental DNA metabarcoding data integrated with a trait-based approach to describe the functional diversity of communities, estimate biotic interactions, and reconstruct food webs.
2) **Enhancing accessibility of functional information.** I addressed a major limitation in functional ecology by increasing the accessibility and usability of trait information for ecological studies.
3) **Describing successional dynamics in proglacial ecosystems.** I provided insights into how the functional diversity of crucial components of soil communities changes along the succession of glacier forelands.
4) **Revealing the development of biotic interactions and food webs after glacier retreat.** I inferred biotic interactions and food web structure to investigate their development over time since glacier retreat, quantifying the importance of functional diversity and functional groups.

In chapter 2, we addressed the scarcity of accessible, functional information (research topic 2), developing a trait-based framework for soil protists, a key component of soil ecosystems that is particularly understudied compared to other soil microorganisms (Fig 2.1; Gao et al., 2019; Geisen et al., 2020; Oliverio et al., 2020). The framework is composed of a set of 10 key functional traits that can be applied to high-throughput sequencing data to obtain a comprehensive functional characterization of soil protist communities. The validation of the framework through the assignment of the 10 traits to environmental DNA data of soil protist communities enhanced the potential of integrating these two approaches ('Fun-eDNA' paragraph 1.4; research topic 1), leading to the production of the first trait dataset of soil protists from glacier forelands, further increasing the availability of trait information (research topic 2). The validation of the framework and the construction of the functional dataset paved the road for investigating the changes in the functional diversity of soil protist communities after glacier retreat (research topic 3).

In chapter 3, we used the common set of traits defined in chapter 2 to investigate the functional changes of soil protist communities along the ecological succession of 46 glacier forelands across four continents. Despite a great body of literature focused on determining the trajectory of several taxonomic groups after glacier retreat (Cauvy-Fraunié & Dangles, 2019; Ficetola et al., 2024; Pothula & Adams, 2022), a global characterization of the functional diversity of protist communities in glacier forelands was missing (Oliverio et al., 2020). This paper represented the first taxonomic and functional broad-scale overview of protist communities developing in soil ecosystems after glacier retreat, enhancing our understanding of the successional dynamics of these crucial components of soil biodiversity (research topic 3). Our study showed that although the overall functional alpha diversity increased through time following a trajectory similar to the one of taxonomic diversity (Table 3.1), phototrophic protists declined (Fig. 3.4), highlighting a shift in the functional composition of protist communities from recently deglaciated sites to older sites (Fig. 3.3). This reduction along the succession of important early primary producers such as phototrophic protists is likely to affect the entire community composition, with food webs switching from simplified networks based on algae and cyanobacteria to more complex plant-based networks (Hågvar et al., 2024; van Leeuwen et al., 2018). To evaluate the potential cascading effects of such a shift at the community level, we inferred the biotic interactions between the different components of soil communities reconstructing soil food webs (chapter 4).

In chapter 4, we delved deeper into the organization of the whole soil communities along glacier forelands, investigating how biotic interactions and food web structure developed over time since glacier retreat (research topic 4). To achieve our ambitious goal of predicting the structure of future ecosystems that will develop on the large surface that will deglaciate in the next decades (Fig. 1.2; Bosson et al., 2023), we moved beyond the analysis of taxonomic and functional diversity. Thus, we used a network approach that accounts for the ecological role of each community component but also considers the interactions among all the components of the community, enabling a more exhaustive understanding of the dynamics of community assembly (Poisot et al., 2013). Once again, the integration of eDNA data and trait information proved valuable in inferring biotic interactions based on shared traits and food web theory (Fig.1.8; research topic 1). Through the reconstruction of 263 food webs along 46 glacier forelands and the analysis of their structure we found that food webs increased in complexity and connectance over time since glacier retreat, with cold local temperature being an important constraint to the development of early food webs (Fig. 4.3). However after, one hundred years most of the food webs reached a similar level of complexity regardless of local temperature, indicating a convergent development of food web structure toward later successional stages (Fig. 4.3, Table 4. 2). Such increase in complexity over time since glacier retreat was likely driven by an equal increase in functional diversity that conferred more stability to the food web structure through an increase in functional redundancy and connectance. These findings suggest that ecological successions happening after glacier retreat tended to develop similar food webs worldwide, providing crucial information for the prediction of future proglacial ecosystems (research topic 4). However, the role of specific functional groups in sustaining food webs (keystone groups) is still unclear. We found that microorganisms like protists and springtails were central in early food webs, whereas in later communities, soil macrofauna like omnivore insects and gastropods became more important (Fig. 4. 6). Nonetheless, the dynamics involving keystone groups and their role in the organization of soil communities require further investigation, including the estimation of energy fluxes (Potapov et al., 2024) which incorporate biomass measurements that were not considered in this study.

The three works presented in this thesis further demonstrated that the integration of environmental DNA data with multi-trait information represents an invaluable approach to performing broad-scale studies to detect macro patterns of taxonomic and functional diversity (chapter 3), infer trait-based biotic interactions to reconstruct food webs (chapter 4) and investigate the successional dynamics driving ecosystem functioning (chapter 3-4). Nonetheless, major limitations emerged from our three works. These limitations included the scarcity of functional information for soil organisms (Guerra et al., 2020) that we contributed to reducing with our first paper (chapter 2), the low taxonomic resolution of eDNA data that hampered the detection of functional differences between closely related taxa potentially underestimating functional diversity, the lack of measures of true abundance and biomass that impeded the reconstruction of energy fluxes between the components of the food webs, further hindering the identification of keystone taxa.

To obtain an exhaustive and comprehensive understanding of the ecosystem dynamics and functioning of the communities emerging from deglaciated lands, future research should focus on addressing such limitations by combining broad-scale eDNA metabarcoding samplings with targeted local samplings performed with more precise approaches like integrative taxonomy or population genomics. The collection of taxonomic and trait data at the species or even individual level is urgently needed to better characterize the diversity of these rapidly changing ecosystems, especially in understudied tropical countries (Cameron et al., 2018; Guerra et al., 2020), which are also likely to lose most of their glaciers by the end of the century (Rounce et al., 2023). Cooperation with taxonomists and the use of integrative taxonomy approaches that couple morphological identification with DNA sequencing provide precise information to better discriminate between taxa accounting for intraspecific trait variability (Hågvar et al., 2024; Potapov et al., 2020), thus enabling the identification of ecosystem dynamics that require a much finer taxonomic and functional resolution than the one provided by eDNA metabarcoding. In this regard, global monitoring programs using common methodologies are critical initiatives for advancing our understanding of soil ecosystem dynamics (Potapov, Sun, et al., 2022). Such programs include the measurement of abundances and biomasses of taxonomic and functional groups, which are essential for building more realistic food webs, estimating energy fluxes, and determining keystone species (Potapov et al., 2024). Finally, genomic approaches can be used to identify subtle genetic differences between closely related populations (Colella et al., 2020; Sherpa & Després, 2021), providing insights into the dynamics of colonization of the terrains emerging after glacier retreat (see Annex 7.1 for an ongoing project on this topic).

To conclude, this thesis demonstrated the potential of the Fun-eDNA approach to describe large-scale functional dynamics of biotic communities, solidifying the role of environmental DNA as a critical tool to perform fast and effective samplings of multiple components of biotic communities. However, despite the strengths of the Fun-eDNA approach, a comprehensive understanding of the ecosystem dynamics that govern the functioning of soil communities developing after glacier retreat requires the integration of diverse methodologies embracing multiple fields of ecology.

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7 ANNEXES

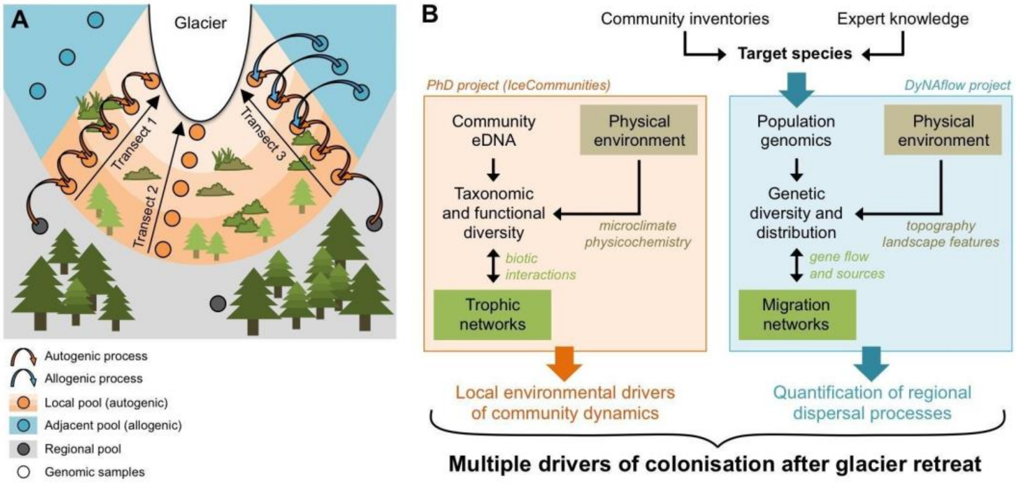
7.1 DYNAFLOW: COLONISATION DYNAMICS OF ARCTIC ENDEMICS ACROSS GLACIAL LANDSCAPES

Project Awarded the Arctic Field Grant 2024

Project manager: Simone Giachello, University of Milan, IUSS Pavia

Project owner: Stephen Coulson, UNIS, the university centre in Svalbard

Project member: Stéphanie Sherpa, University of Milan



The DyNAflow project in a nutshell. *A) Schematic of the two alternative hypotheses for the origin of colonising organisms after glacier retreat: autogenic (Transect 1) versus autogenic and allogenic (Transect 3). The sources will be explored using genomic data using the represented sampling design. B) Approaches and data used to characterise the effects of local and regional processes on colonisation dynamics.*

Project objectives

The project aims to combine community ecology (both traditional and eDNA-based) and population genomics approaches to better understand the colonization and evolutionary dynamics of arthropod communities in recently deglaciated terrains exposed by the retreat of glaciers. The two main objectives are: (1) determining the origin of pro-glacial communities, by quantifying the relative importance of regional dispersal (i.e., colonization from ice-free areas adjacent to the glacier foreland) and local dispersal (i.e., within foreland site-site colonization) processes; (2) testing for adaptive evolution of populations in multiple species along environmental gradient at very local scale (succession patterns).

Main achievements

To achieve the project goals, two main fieldwork tasks have been set. The first task consisted in an extensive sampling of terrestrial arthropods (e.g., springtails, spiders, mites) in multiple sites along the foreland of the Midtre Lovénbreen and in the adjacent areas for community inventory and genomic analysis. The second task focused on the collection of soil samples in the same sites, for eDNA metabarcoding analysis. Both fieldwork tasks have been successfully achieved. The implementation of two distinct sampling methodologies for the collection of arthropods, pitfall traps collection and Berlese-funnel extraction, lead to the acquisition of specimens from all the taxonomic groups of interest.

Fieldwork summary

The field campaign was performed in the area of Kongsfjorden, Ny-Ålesund, by two people, Simone Giachello and Stéphanie Sherpa, from the 8th to the 22nd of July 2024. Fieldwork activities focused on the Midtre Lovénbreen glacier (Fig. A.1).

***Figure A.2**. The foreland of the Midtre Lovénbreen with the pioneer flower Silene uralensis growing on the loose debris of a 20 years old moraine left by the retreat of the glacier (in the background).*

We included a total of 18 sites: 12 sites were placed in two distinct transects along the foreland of Midtre Lovénbreen following the coordinates of two previous sampling campaigns (Fig. A.2; in yellow); 3 sites were located on rocky ridges in front of the terminal moraine of Midtre Lovénbreen (Fig. A.2; in green); and 3 other sites were identified in the two adjacent glacier forelands of the Austre and Vestre Lovénbreen (Fig. A.2 2; in purple and orange).

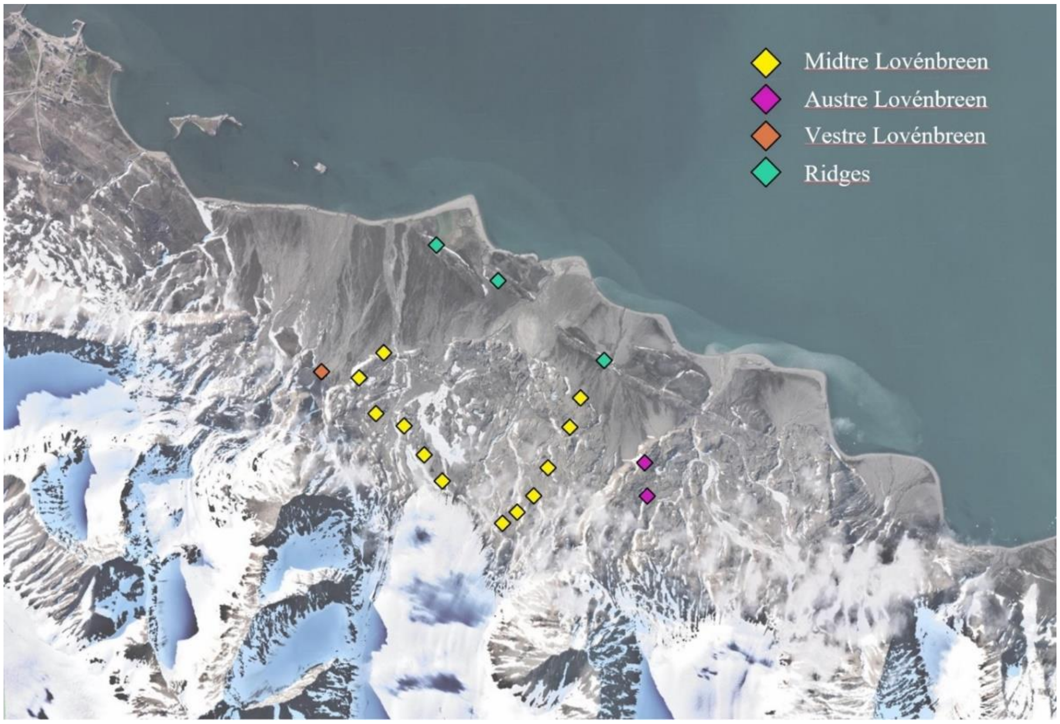


Figure A.2*. Distribution map of the 18 sampling sites in the area of the Midtre Lovénbreen.*

Seven days were spent on the field to identify the sites, collect data on vegetation cover, place and collect pitfall traps, collect soil samples for arthropod extraction through Berlese-funnel, and collect soil samples for eDNA metabarcoding analyses (Fig. A.3). The remaining days were spent at the terrestrial laboratory in Ny-Ålesund to perform the Berlese-funnel extraction, collect and store the specimens (Fig. A.4).

***Figure A.3**. Collection of soil samples from one of the sample sites along the foreland of the Midtre Lovénbreen.*

The field campaign resulted in the collection of a total of 514 springtails, 77 spiders, and 565 mites across the 18 sites sampled. Specifically, pitfall traps collected 244 collembola, 77 spiders and 344 mites while Berlese-funnel extracted 180 collembola and 221 mites. This includes at least four species with wide distribution over the sampled area, representing good candidates for the objectives of this study.

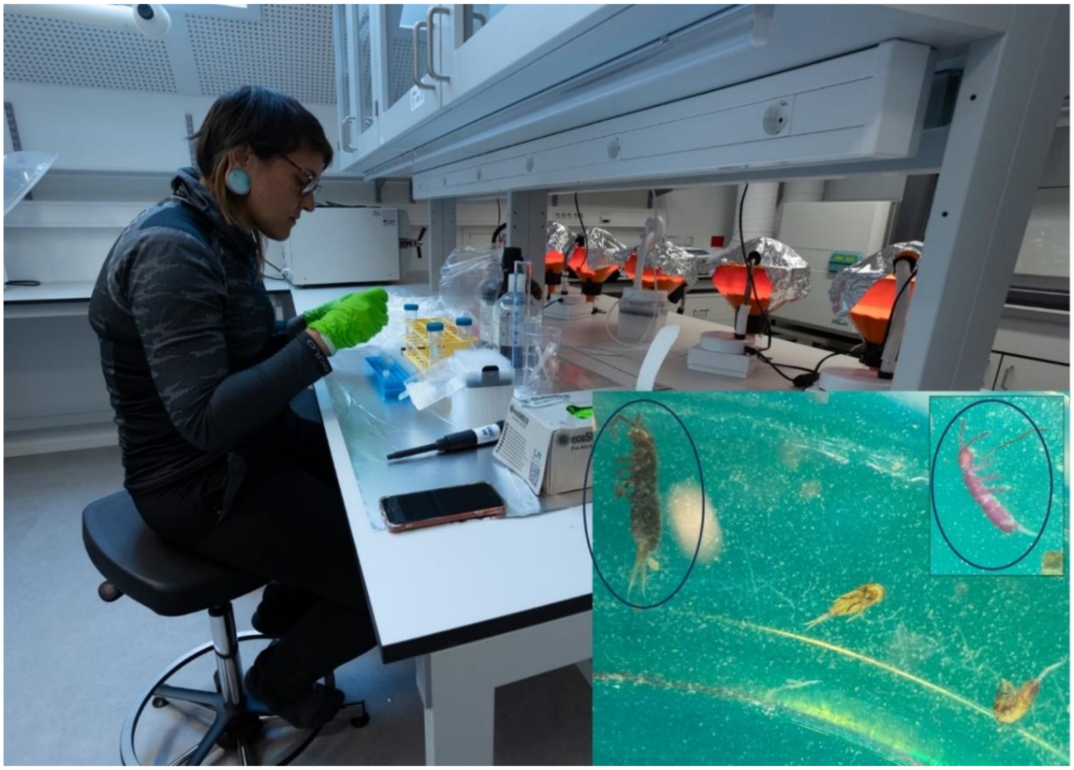


Figure A.4*. Preparation of collected specimens of arthropods extracted using the Berlese-funnel method.*