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Functional changes of protist communities in soil after glacier retreat

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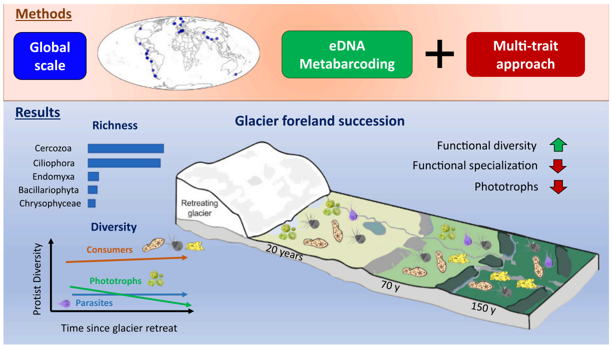
HIGHLIGHTS

- eDNA provided a global, multi-trait overview of protists along glacier forelands.
- Protist diversity increases through time but functional specialization decreases.
- Functional groups show distinct successional patterns after deglaciation.
- Successional patterns of protists are crucial to grasp changes in soil ecosystems.

GRAPHICAL ABSTRACT



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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

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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.

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 and Dangles, 2019; Ficetola et al., 2021; Pothula and 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 and Dangles, 2019; Ficetola et al., 2021; Khedim et al., 2021; Pothula and 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 late-successional stages, driven by higher availability of resources and more favourable environmental conditions (Ficetola et al., 2024; Pothula and 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., 2020, 2018). 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 et al., 2018, 2016). 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 and Dangles, 2019; Ficetola et al., 2021; Pothula and 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; 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 and 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 shed light 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-trait approach is critical, as protist communities encompass a wide range of evolutionary 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., 2021; 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 environmental conditions.

2. Materials and methods

2.1. Study area and sampling 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. 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., 2024a). 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., 2024a; Ficetola et al., 2024; Guerrieri et al., 2024 for additional details on sampling sites and strategies).

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 genus or 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 S1 for more details).

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 $r = 0.22$).

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, free-living, 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

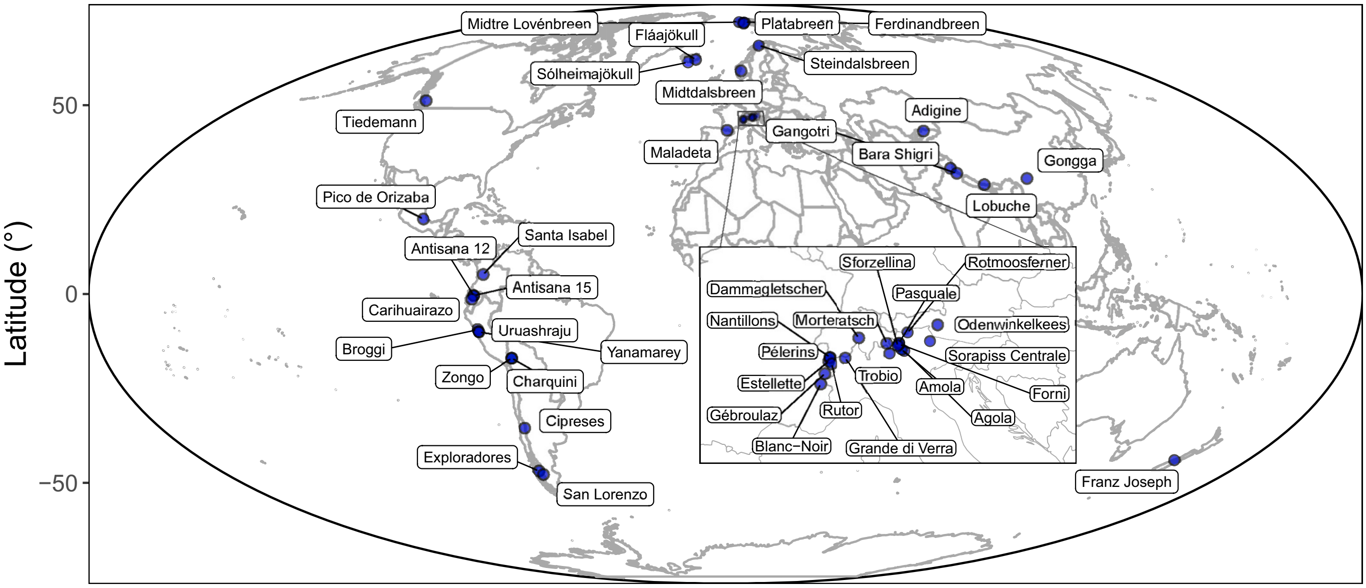

Fig. 1. Locations of the 46 glacier forelands sampled. The inset shows the area of the European Alps expanded.

Bello et al., 2021; Table S1). 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 S1).

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 S2). 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 S2). This is recommended to increase the robustness of biodiversity data obtained through DNA metabarcoding (Calderón-Sanou et al., 2019; Mächler et al., 2021).

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., 2021; Table S2).

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 and 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 PCoA axis 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).

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 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) 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 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. Results

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

Table 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 credible intervals non overlapping zero a significant effect of the environmental variable on the taxonomic/functional measure. For more information on the measures of functional composition see Section 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

eukaryotes obtained (Table S1). 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. 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. S1). 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 S1).

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 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 1b).

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). 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). These entities mostly correspond to large phototrophic algae

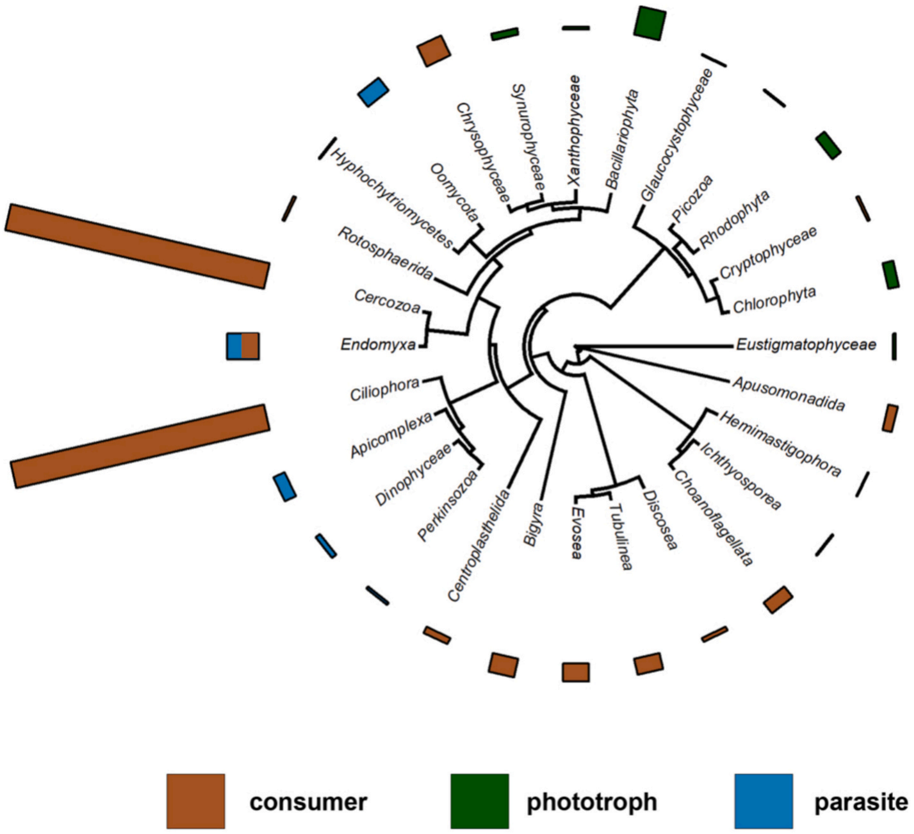

Fig. 2. Taxonomic and trophic composition of soil protist communities from 46 glacier forelands. Schematic phylogenetic tree representing the phyla/clades found in all the 1251 soil samples of our study. The height of the bars indicates the proportion of MOTUs within each phylum/clade, while the colors indicate the most representative trophic groups.

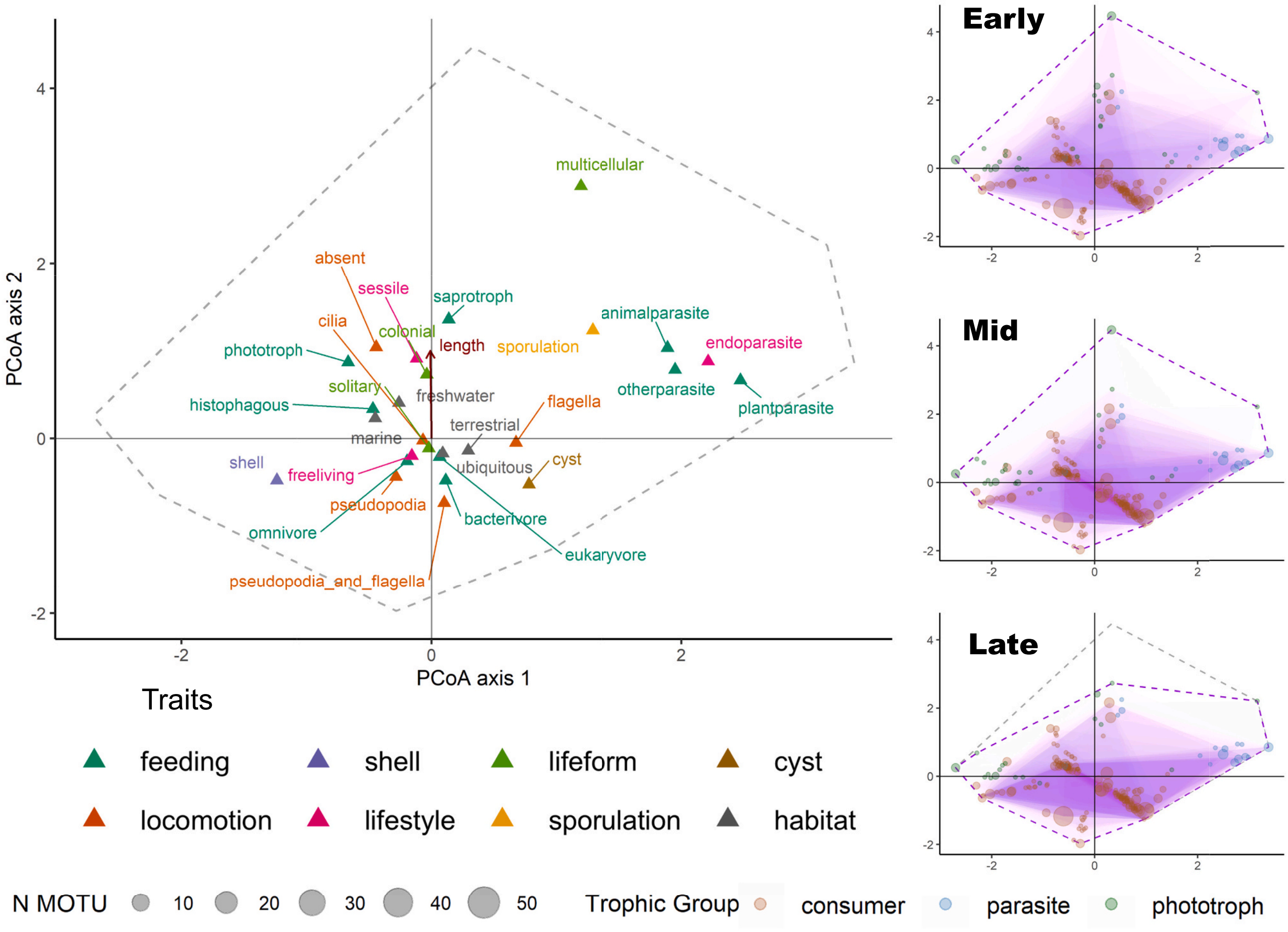

Fig. 3. Functional space of protist communities at different stages of the ecological succession (early, mid, late). Left: functional space of all the protist communities sampled in 1251 plots. Triangles represent the centroid of each trait category estimated as the average coordinates of the functional 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.

typically living in freshwater environments, such as the multicellular red algae *Kumanoa* sp.

The multivariate GLMM assessing the variation of the relative abundance of functional categories supported this pattern (Table 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 2; Fig. 4). Conversely, we detected an increase of omnivores, ciliate protists and organisms able to exploit both terrestrial and freshwater environments (i.e. the ubiquitous ones) (Table 2, Fig. 4). None of the functional categories was clearly affected by variation in soil temperature (Table 2).

The GLMM analyzing the functional composition of communities (PCoA axes and specialization) summarized the patterns revealed by the variation of functional categories (Table 1c). The PCoA axis 2 (Fig. 3) was negatively related to time since glacier retreat (Table 1c), indicating the loss of multicellular, large, sessile organisms through time, and the increase of omnivorous and ubiquitous protists in late communities (Table 2). Furthermore, functional specialization showed a clear decrease over time (Table 1c). 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 1c; Fig. 3). After accounting for the effect of time, functional specialization was particularly high in plots characterized by warmer temperatures (Table 1c).

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.

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

Table 2

Effect of time and mean soil temperature 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 95 % credible intervals non overlapping zero.

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 flagella	−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

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; 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. S1), 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).

4.2. Trajectories of taxonomic and functional diversity

The clear increase in functional diversity of protist communities over time since glacier retreat (Table 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, min = 0.5, 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 and Dangles, 2019; Hotaling et al., 2017). However, the dispersal mechanisms underlining the colonization dynamics of protists in these environments are still largely unknown.

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. 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 1; Ficetola et al., 2024), but key components of communities, such as phototrophic protists, showed the opposite patterns (Fig. 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., 2021; 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., 2021; Oliverio et al., 2020). The abundance of phototrophic protists in the earliest stages of the succession confirms that eukaryotic algae, along with cyanobacteria, are

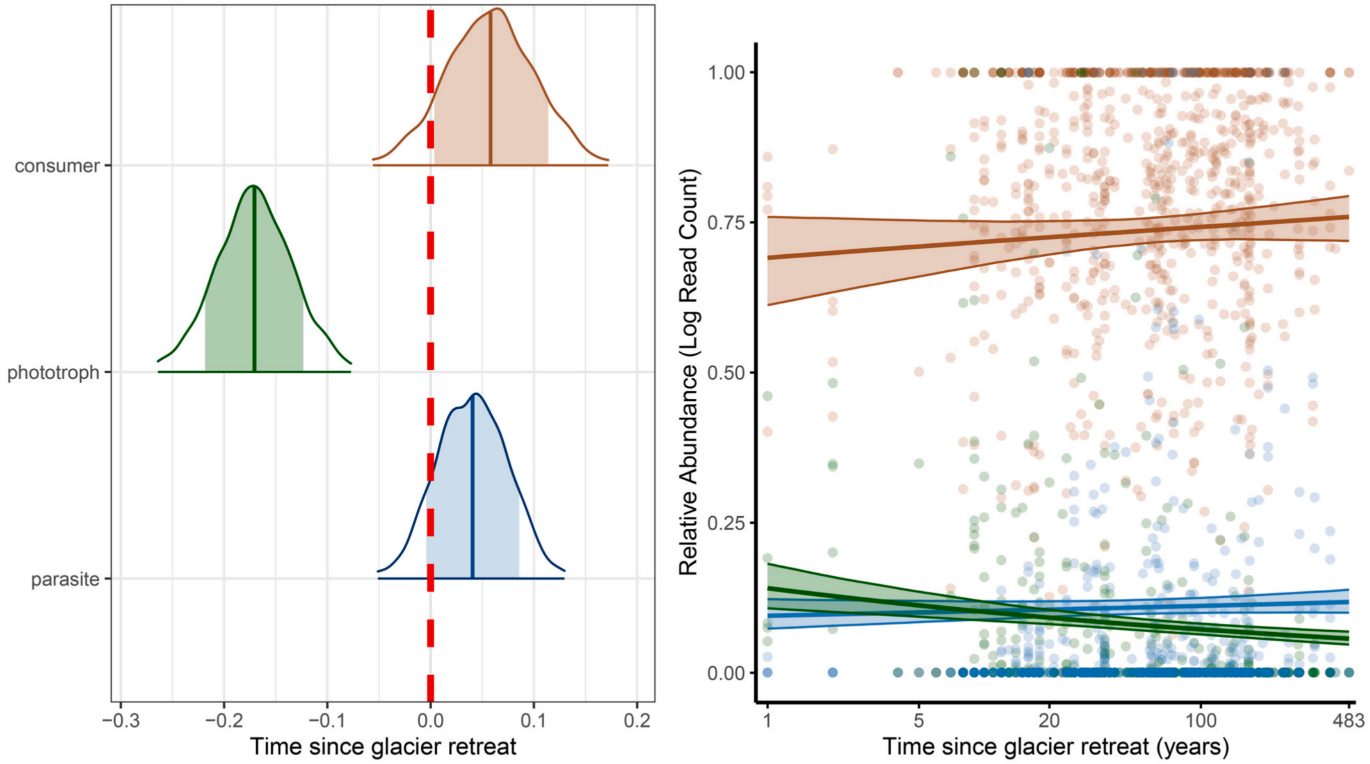

Fig. 4. Effect of time since glacier retreat on trophic groups of soil protists. A) Posterior distribution estimated with Bayesian Generalized Linear Mixed Models representing the effect of time since glacier retreat on the relative abundance of each trophic group. The curves indicate the 95 % credible 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).

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.

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; 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 and 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.

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 single marker 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 and 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 process of 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 provide great benefits to broad-scale metabarcoding analyses (Pereira et al., 2023).

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.

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CRediT authorship contribution statement

Simone Giachello: Writing – review & editing, Writing – original draft, Visualization, Software, Project administration, Methodology, Formal analysis, Data curation, Conceptualization. **Isabel Cantera:** Writing – review & editing, Supervision, Methodology, Formal analysis. **Alexis Carteron:** Writing – review & editing, Visualization, Methodology, Conceptualization. **Aurelie Bonin:** Writing – review & editing, Data curation. **Alessia Guerrieri:** Writing – review & editing, Data curation. **Roberto Ambrosini:** Writing – review & editing, Data curation. **Marco Caccianiga:** Writing – review & editing, Data curation. **Mauro Gobbi:** Writing – review & editing, Data curation. **Silvio Marta:** Writing – review & editing, Data curation. **Gentile Francesco Ficetola:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

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

Data availability

Raw sequencing data is deposited in Zenodo (https://doi.org/10.5281/zenodo.6620359). The supplementary tables and the R script used to run the analyses are available as external files named respectively; “TableS1.xslx”, “TableS2.xslx”, “FunGroups_SP_script.R”.

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