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RESEARCH ARTICLE

Higher spatial than seasonal beta diversity of soil protists along elevation gradients

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Abstract

Biodiversity patterns along elevation gradients have long been studied for plants and animals, but only quite recently for soil microorganisms, especially protists (eukaryotes excluding plants, animals, and fungi). Microorganisms have shorter generation times than macroorganisms, and their abundance, diversity, and community structure are known to vary rapidly in response to abiotic and biotic factors. If microbial diversity varies more seasonally than spatially, a single sampling campaign along an elevation gradient, with contrasted phenologies, could introduce bias into biodiversity studies comparing multiple elevation gradients across different seasons, habitats, regions or latitudes. To address this question, we investigated the relative magnitude of spatial versus temporal diversity (alpha diversity) and community turnover (beta diversity) of soil protist communities along elevation gradients in two distant European mountain ranges. We collected soil samples in forests and grasslands below the treeline along five elevation gradients in two consecutive seasons (spring and summer) in the Spanish Sierra Nevada and the Swiss Alps, covering two distinct biogeographic regions. Using general eukaryotic primers and amplicon sequencing of soil environmental DNA, we decomposed total protist amplicon sequence variants diversity into local alpha- and beta diversity components and identified climatic and edaphic predictors of biodiversity patterns using redundancy analyses. Soil protist communities varied spatially within and among transects but temporal turnover was comparatively low. The best edaphic predictors of community variations were the same in spring and summer, but their explanatory power differed among seasons. The dominant spatial component of beta diversity suggests that patterns of soil protist communities along elevation gradients are more strongly driven by spatial heterogeneity than inter-seasonal turnover. Thus, in temperate climates, our results suggest that sampling only once between the end of spring and late summer across an elevation gradient does not introduce bias due to phenological differences when comparing beta diversity across multiple gradients.

Highlights

- Spatio-temporal dynamics of soil protists communities were studied in forests and grasslands below the tree line along five elevation gradients in the Spanish Sierra Nevada and the Swiss Alps during two consecutive seasons (spring and summer).
- The total diversity of soil protist communities was predominantly shaped by beta-diversity components with spatial heterogeneity rather than temporal turnover as the main driver of soil protist community composition.
- Community dissimilarity of soil protists did not differ in response to temporal changes between habitats (i.e., forests *versus* grasslands)
- The significant edaphic predictors of protist community composition were highly similar in the Swiss Alps and identical in the Spanish Sierra Nevada between both seasons, but their explanatory power varied between spring and summer.
- Soil protist beta diversity patterns along different

elevation gradients remained constant between seasons. This suggests that, in temperate climates, sampling at one time across an elevation gradient will not bias results stemming from phenological contrasts, allowing comparison of beta diversity patterns along such gradients between regions even if sampling is not simultaneous.

Keywords

beta diversity, DNA metabarcoding, elevation gradients, microbial community, protists, Spanish Sierra Nevada, sampling strategy, soil biodiversity, spatio-temporal dynamics, Swiss Alps

Introduction

Protists, which include all eukaryotes excluding plants, metazoans, and fungi, are highly diverse microorganisms in terms of morphology, phylogeny, and function (Adl et al. 2019; Burki et al. 2021). They represent the bulk of eukaryotic diversity and are abundant in the soil, playing crucial roles in soil ecosystem functioning (Geisen et al. 2018), notably through nutrient cycling via the microbial food web (Bonkowski 2004; Adl and Gupta 2006), and biogeochemical cycles (Aoki et al. 2007; Jassey et al. 2022). The development of high-throughput sequencing of environmental DNA (eDNA) has substantially broadened our understanding of soil protist biogeography (Bates et al. 2013; Lara et al. 2016; Lentendu et al. 2018; Oliverio et al. 2020). Due to highly uneven sampling among different taxonomic groups and geographic regions (Foissner 2007; Beyens and Bobrov 2016; Geisen et al. 2018; Burdman et al. 2021), the total diversity of protists remains largely unknown and most species undescribed (Mahé et al. 2017; Singer et al. 2020). This lack of representation is particularly concerning, as most studies on soil microbial diversity and biogeography do not include protists (e.g., Bryant et al. 2008; Fierer et al. 2011; Hendershot et al. 2017; Nottingham et al. 2018), *de facto* neglecting a significant part of soil biodiversity and contributing to the Linnean (taxonomic) and Wallacean (distribution) shortfalls that characterise protists (Hortal et al. 2015). Consequently, the study of their diversity and distribution at different spatial and temporal scales remains an unexplored frontier of research (Geisen et al. 2020), especially in mountain areas (Praeg et al. 2023).

Assessments of soil protist richness (i.e., alpha diversity) and compositional turnover (i.e., beta diversity) along elevation gradients are key to understanding global distribution patterns of soil protists. Additionally, these natural environmental gradients are perfect settings to answer a central question in microbial biogeography: do large-scale spatial diversity patterns and drivers differ between macro- and microorganisms (i.e., bacteria, archaea, fungi, protists, and micrometazoa) (Delgado-Baquerizo et al. 2019). Indeed, elevation gradients are ideal settings to address alpha and beta diversity patterns of species in response to a combination of natural environmental gradients varying with the elevation and condensed over short distances (Lomolino 2001; Körner 2007; Sundqvist et al. 2013). Temperature, atmospheric pressure, UV radiations, precipitations, soil conditions (e.g., pH, carbon content and soil moisture), and season length are among the main abiotic drivers of both micro- and macro-organism community composition changes along these gradients (Körner 2007). However, elevation gradients are characterised by contrasted environmental conditions not only in space (e.g., between low, mid, and high elevation sites) but also over time (e.g., due to natural seasonal variations of abiotic factors).

Several relatively well-studied taxonomic groups of soil protists, particularly testate amoebae, display distinct diversity patterns along different elevation gradients. Indeed, these patterns include a peak of diversity at mid-elevation (Krashevska et al. 2007, 2010; Lamentowicz et al. 2013), a linear decrease with elevation (Heger et al. 2016), a U-shaped relationship with elevation (Tsyganov et al. 2022), or no significant relationship at all (Mitchell et al. 2004). Furthermore, a study conducted in the Swiss Alps revealed that the richness patterns of protist functional groups varied with elevation, but these patterns differed between groups (Mazel et al. 2022). Thus, soil protists respond in contrasting ways to spatial heterogeneity along elevation gradients, notably due to variations in climate (Fernández et al. 2016), topography (Seppey et al. 2020), vegetation (Heger et al. 2018) and litter quality (Krashevska et al. 2017).

In addition to spatial heterogeneity, temporal variation was shown to be an important driver of community patterns along environmental gradients but is only rarely investigated in surveys on soil protists (Fournier et al. 2020; Mazei et al. 2020). In soils, the temporal variability of soil protists is function of the organisms' size, being highest (i.e., a few days) for the smaller bacterivorous flagellates (Clarholm 1981), and lowest (i.e., more than two weeks) for the larger testate amoebae (Lousier 1974). Temporal changes over longer timeframes (e.g., sampling at monthly intervals or once per season) were also reported for testate amoebae (Lara et al. 2011; Lamentowicz et al. 2013; Marcisz et al. 2014; Koenig et al. 2017; Mazei et al. 2020), ciliates (Zhao et al. 2013), and cercozoans and endomyxans (Fiore-Donno et al. 2019; Walden et al. 2021). Additionally, (Couteaux 1976) demonstrated that, at finer taxonomic level, the seasonal cycle was species-dependent in testate amoebae. Turnover rates of soil protist communities were shown to be lower than those of bacteria and fungi in alpine grasslands (Kang et al. 2022), and lower than those of bacteria in floodplains (Fournier et al. 2020). But, in the latter study, protist temporal changes were much lower than spatial ones. A study conducted in China reported that soil protist alpha diversity primarily varied seasonally, while beta diversity had a stronger association with elevation (Shen et al. 2021). Similarly, this pattern of beta diversity was also observed for testate amoebae

along an elevation gradient of *Sphagnum*-dominated peatlands in Switzerland (Lamentowicz et al. 2013).

As soil protist diversity along elevation gradients is rarely investigated across multiple seasons (Shen et al. 2021), it is unclear to what extent seasonality influences community composition along these gradients. However, environmental conditions along the gradient are also influenced by seasonal variations in abiotic and biotic factors influencing the community, and the magnitude of these variations may change among elevations. Thus, significant changes in environmental conditions over space and time could potentially lead soil protist communities to present varying phenological stages along a single elevation gradient. For instance, the decrease in temperature with increasing elevation, coupled with natural seasonal temperature fluctuations, leads to variations in the conditions in which microbial activity takes place across different elevations since ecosystem respiration is temperature-dependent (Merbold et al. 2012). Consequently, lower-elevation sites offer more favourable conditions for microbial activity earlier in the year compared to their higher-elevation counterparts (in other words, higher-elevation sites "lag behind" the lower ones), because the window of similar environmental conditions will vary in relationship to i) the elevation, and ii) the season. Simultaneously, low-elevation sites may experience extended periods of hot and dry conditions in summer, potentially causing a strong reduction in the abundance or activity of soil protists compared to high elevation sites (Hu et al. 2022), while the insulating effect of snow allows for microbial activity, e.g., in winter (Warren and Taranto 2011; Saccone et al. 2013). Thus, the protist communities vary between the lower and upper sections of the gradient, but also over the year in response to seasonal dynamics of environmental conditions. Hence, when comparing beta diversity across multiple elevation gradients between different seasons, habitats, regions or latitudes, the results could be potentially biased if temporal turnover (i.e., temporal beta diversity) is comparable to or exceed the spatial variations (i.e., spatial beta diversity).

In this study, we address the question of the relative importance of spatial variations (i.e., spatial beta diversity) *versus* temporal turnover (i.e., temporal beta diversity) of soil protist communities along multiple elevation gradients to improve our understanding of community assembly processes. Also, characterizing spatio-temporal dynamics of soil protist communities is an essential prerequisite for designing sound sampling protocols for soil microbial macroecology and biogeography studies. Indeed, if temporal turnover in soil protist community composition is similar or higher than the spatial variation along the elevation gradient, then sampling should be done at multiple times during the year to ensure i) capturing a higher proportion of protist diversity within each plot, and ii) accurately describing the general patterns of compositional variations. Here, we present results from a comprehensive field survey involving the collection of 104 soil samples along five elevation gradients in two distant regions (the Spanish Sierra Nevada and the Swiss Alps). These samples were gathered from two contrasted habitats, namely forests and grasslands below the treeline, from two different seasons, i.e., spring and summer. Our goal was to estimate richness and diversity of soil protists using amplicon sequencing of soil environmental DNA and quantify spatio-temporal variations among regions, habitats, and seasons. Finally, we related soil protist diversity to edaphic factors likely driving communities along elevation gradients. We hypothesized that soil protist beta diversity would be higher between regions and habitats than between seasons. Moreover, we expected that grasslands would demonstrate higher temporal variations compared to forests due to the contrasting soil exposure and related abiotic conditions, especially moisture. Additionally, we expected that the same set of edaphic factors would explain soil protist beta diversity in the two habitats, regions, and seasons. By encompassing all components of diversity and quantifying the spatio-temporal turnover of soil protist communities, our approach aimed to determine whether sampling at a single time point could introduce bias in soil protists diversity and biogeography studies.

Materials and methods

Study sites

We collected a total of 104 soil samples in 52 sites along five elevation gradients, three in the Swiss Alps (Pellissier et al. 2010), and two in the Spanish Sierra Nevada (Table 1). The main rationale behind choosing these two mountain ranges was the representation of distinct geologic and bio-climatic conditions within relatively close proximity, facilitating sampling within short time intervals between regions and accurate sample processing. Moreover, the criteria for selecting these five gradients included: i) an elevation range of at least 1000 m between the lowest site and the tree line, ii) the presence of homogeneous forest and grassland sites, characterized by similar slope and exposure, separated by an elevation of approximately 200 meters, iii) accessibility and logistical manageability, ensuring rapid sample processing. Furthermore, the gradient selection in the Swiss Alps was determined by the existence of pre-defined elevation gradient sites as outlined in Pellissier et al. (2010), who gave a detailed description of vegetation. In contrast, the elevation gradients in the Spanish Sierra Nevada were specifically established for the purposes of the current study with vegetation description based on Lorite (2002) and Lorite et al. (2020). Along these gradients, perennial steppe-like pasturelands in lowland areas (thermo- and mesomediterranean belts) are covered by alfa grass (*Stipa tenacissima*) together with matorral shrubs and sub-shrubs (*Genista umbellata*, *Anthyllis cytisoides*, *Artemisia barrelieri*). The mid-mountain area (Supramediterranean) is mostly *Festuca scariosa* pasturelands with some sub-shrubs such as *Thymus* spp., *Cistus* spp. *Lavandula lanata* or *Salvia lavandulifolia*, all on

Table 1. Metadata summary of the five elevation gradients in the Swiss Alps and the Spanish Sierra Navada: location, bedrock, climate, treeline, habitat type, number of plots, elevation range, mean annual temperature range and annual precipitation range. Mean annual temperature and annual precipitation ranges are based on CHELSA bio1, and bio12, respectively, for the period 1981–2010 (Karger et al. 2017, 2021).

Gradient	Country	Mountain range	Bedrock	Climate	Approx. tree line (m.a.s.l.)	Habitat	Number of sites	Elevation range (m.a.s.l)	Mean annual temperature range (°C)	Annual precipitation range (kg · m^{-2})
Vallon de Nant	Switzerland	Outer Alps	Calcareous (limestone)	Sub-oceanic	1740	Forests	6	510–1630	2,45–9,35	1045,5–1688,6
						Grasslands	5	770–1560	3,25–7,75	1175,9–1549,2
Salgesch	Switzerland	Inner Alps	Calcareous (limestone)	Sub-continental	2130	Forests	5	590–1690	2,05–9,45	708,3–1410,9
						Grasslands	6	650–1550	1,85–8,35	947,9–1427,4
Mont Rogneux	Switzerland	Inner Alps	Siliceous (moraine)	Sub-continental	2050	Forests	5	1190–1810	1,25–6,05	1134,1–1426,5
						Grasslands	5	920–1750	0,65–7,85	1133,6–1397,4
Sierra Nevada North	Spain	Sierra Nevada	Calcareous (limestone), siliceous (micaschists)	Mediterranean	2400	Forests	5	826–2180	7,25–15,85	697,7–897,5
						Grasslands	6	900–2475	5,35–15,45	681,4–904,5
Sierra Nevara South	Spain	Sierra Nevada	Calcareous (limestone), siliceous (micaschists)	Mediterranean	2500	Forests	4	918–1824	9,65–15,15	578,4–677,3
						Grasslands	5	919–2100	8,45–15,15	578,4–677,3

limestone, while on micaschist *Festuca scariosa* also dominates, together with *Halimium umbellatum* and *Cistus* spp. The lowland forest (thermo- and mesomediterranean belts) presents *Pinus halepensis* and *Quercus ilex* subsp. *ballota*. Mid-mountain area (Supramediterranean) is dominated also by *Quercus ilex* subsp. *ballota*, but in northern and more humid aspects, *Q. pyrenaica* and spotted *Q. faginea* appear frequently. *Pinus pinaster* is abundant only on dolomite, whereas stands of *Pinus sylvestris* var. *nevadensis* and *Pinus nigra* are very scarce. The natural treeline, presumably formed by *Pinus nigra* subsp. *salzmannii* and *P. sylvestris*, is completely gone and the existing stands are afforestations with *P. sylvestris* and more rarely with *P. uncinata*, having an artificial structure and composition.

Sampling approach

Samples were collected in two distinct habitats, i.e., forests and grasslands below the tree line. Four to six sites per habitat were selected along each elevation gradient and each site were approximately equidistant in elevation. Sampling took place in 2019 and each site was sampled once in spring and once in summer. The sampling plot area was ca. 5 × 20 m in forests and 5 × 10 m in grasslands. At least ten sub-samples of ca. 100 g of litter, mosses and the upper 5 cm of topsoil were collected using a clean and disinfected trowel. To cover the widest possible range of micro-habitats presents within the sampling site, sub-samples of litter at different stages of decomposition and of soil and litter under the different dominant plant species were taken. We took account of the structural complexity of the sites by taking representative sub-samples of the different micro-topographies, i.e., by sampling flat, hollow, and hummocky areas equally. The sub-samples were subsequently pooled into one composite sample of ca. 1 kg. This pooling strategy allows for assessing the full microbial diversity from a relatively small area by minimizing the effects of local heterogeneity (Staley and Sadowsky 2018). Soil samples were kept at 4 °C for a maximum of three days before further processing. After sieving (5-mm followed by 2-mm mesh size), about 1 g of soil was fixed in 1 mL LifeGuard Soil Preservation Solution (Qiagen, Hilden, Germany) for eDNA analyses and stored at -20 °C until DNA extraction. The remaining soil was kept at room temperature for later soil parameters analyses.

Environmental variables

Soil physical and chemical analyses

Sieved soil was dried for at least 48 hours at 40 °C and sieved again at 2 mm to break up clumps. The pH was determined after diluting 5 g of soil in distilled water in a 1:2,5 (wt/vol) ratio using a pH meter (Metrohm pH 621, Metrohm, Herisau, Switzerland). Residual humidity (Res_hum) was calculated by weighting the mass of soil before and after drying the sample at 105 °C for 24 hours. The percentage of soil organic matter (Org_mat) was determined performing a loss-on-ignition, i.e., the 105 °C dried subsamples was heated at 450 °C for 4 hours using a muffle-furnace (Nabertherm, Lilienthal, Germany). Bioavailable phosphorus (P_bio) was measured by colorimetry (Olsen et al. 1954). After additional grinding of the soil, organic carbon (C_org) and organic nitrogen (N_org) were measured by a CHN analyser (FLASH 2000, Thermo Fischer Scientific, Waltham, USA). The general biological activity was evaluated using the org. carbon / org. nitrogen ratio (C_N_ratio) and the org. nitrogen / bioavailable phosphorus ratio (N_P_ratio) from CHN and colorimetric analyses results.

Monthly bioclimatic variables

To characterise the climatic conditions of the sampling sites, we retrieved the 10 monthly high-resolution (resolution: 30 arcsec) variables from the Climatic Research Unit Timeseries – CRU-TS v4.05 (Harris et al. 2020): cld, cloud cover; dtr, diurnal 2m temperature range; frs, frost days; pet,

potential evapo-transpiration; pre, precipitation rate; tmn, minimum 2m temperature; tmx, maximum 2m temperature; vap, vapour pressure; wet, number of wet days. CRU-TS data were downloaded over three months at and before each sampling time point: (a) data of the sampling month (M, "month"), and (b) data of the two months preceding the sampling (Mm1: "month minus 1"; Mm2: "month minus 2").

Transformation and selection of environmental variables

The 8 soil and 10 CRU-TS climatic variables were normalized by applying a Tukey ladder of power transformation using the transformTukey2 function with possible transformation exponent limited between 0 and 2 (GuiBioDiv R package v1.1, Lentendu 2023; Suppl. material 1: table S1). Transformed values were then scaled between -1 and 1. For CRU-TS variables, the average of the transformed values (i.e., the average of transformed M, Mm1, and Mm2 values) was calculated and used for further analyses. Pearson correlations were then measured among transformed variables within the two categories of environmental variables (i.e., soil and average climatic variables; Suppl. material 1: figs S1, S2). Within each category, variables with Pearson correlation values ≥ 0.9 or ≤ -0.9 were used to create composite variables to reduce collinearity for further statistical analyses. To do so, we included the highly correlated variables as input data to a principal component analysis (PCA; Suppl. material 1: figs S1, S2), and then determined the composite variables by selecting the significant principal component axes based on their eigenvalue scores and broken-stick model results (Suppl. material 1: figs S1, S2). In addition, non-correlated variables (i.e., variables with a Pearson correlation < 0.9 or > -0.9) and the elevation were included as independent variables. Composite and independent variables were calculated for (1) the whole dataset (i.e., all samples together) using both soil and climatic variables (Suppl. material 1: fig. S1, table S2), and (2) four subsets using soil variables only: Spanish Sierra Nevada in spring, Spanish Sierra Nevada in summer, Swiss Alps in spring, and Swiss Alps in summer (Suppl. material 1: fig. S2, table S2).

DNA extraction, V4 18S rRNA amplification, and Illumina sequencing

The LifeGuard Soil Preservation Solution was removed by centrifugation (2500 g, 5 min) before eDNA extraction from 0.25 g of soil using the DNeasy PowerSoil Pro Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. The 18S rDNA hyper-variable V4 region was amplified by PCR using the universal eukaryotic primer pair TAReuk454FWD1 and TAReukREV3 (Stoeck et al. 2010) to which an 8-nucleotide barcoding sequence and a 0 to 4-nucleotide heterogeneity spacer were attached to the 5'-end. The PCR mix contained 0.5 U μl^{-1} of GoTaq® hot start polymerase (Promega, Madison, USA), 0.3 mM of DNTPs, 1X of buffer, 2.5 mM of $MgCl_2$, 0.3 µM of each primer and 1 µL of template DNA (concentration: 5 to 10 ng/µL; quantified by Nanodrop (Thermo Fischer Scientific, Waltham, USA) and was adjusted to a final volume of 20 µL with ultra-pure water. Amplification steps consisted of an initial denaturation at 95 °C for 5 min, 35 cycles at 94 °C for 30 s, 47 °C for 45 s, and 72 °C for 1 min; and a final elongation at 72 °C for 10 min. Positive and negative controls were included following Taberlet et al. (2018).

All PCR were run in triplicate and amplicons were pooled after control of amplification success on an agarose gel. DNA quantification and purification were carried out using, respectively, a fluorometer (Qubit 1x dsDNA HS Assay, Thermo Fisher Scientific, Waltham, USA) and the Wizard® SV Gel and PCR Clean-Up System kits (Promega, Madison, USA). Up to 160 cleaned PCR products of samples or controls were pooled equimolarly and sent to a sequencing facility (ID-Gene, Plan-les-Ouates, Switzerland). After library preparation using the TruSeq DNA PCR-Free kit (Illumina Inc. San Diego, USA), sequencing was carried out with a MiSeq v3 Reagent kit of 600 cycles on a MiSeq sequencer (Illumina Inc., San Diego, USA) according to the manufacturer's instructions. The resulting pair-end reads (2 × 300 bp) used in this study originated from three distinct sequencing runs.

Sequence processing, taxonomic assignment, and tag-jump control

The bioinformatic processing was performed as described in Lentendu et al. (2023). Briefly, using the HPC metabarcoding automatic pipeline DeltaMP v0.5 (Lentendu 2022) raw reads were demultiplexed and barcodes were trimmed at the 5'-end allowing up to two mismatches using Cutadapt v.2.10 (Martin 2011). Maximum expected error (EE) rates of up to four EE were then estimated using VSEARCH v2.13.6 (Rognes et al. 2016) and filtered for sequences of a minimum of 230 nucleotides long and at least 500 nucleotides cumulative for both libraries. Reads with smaller length and/or higher EE over the truncated length as well as any ambiguous nucleotide or homopolymer longer than eight nucleotides were discarded. Amplicon sequence variants (ASV) were inferred in a two-step approach in R (v4.2.2; R Core Team 2022), using an error model-based correction algorithm in the Dada2 package v1.14.1 (Callahan et al. 2016). Chimeras were controlled and subsequently removed from the set of ASV using the UCHIME algorithm as implemented in VSEARCH (Edgar et al. 2011). All ASV were taxonomically assigned by comparing them against the Protist Ribosomal Reference database (PR^2 v4.12.0; Guillou et al. 2013) using global pairwise alignment in VSEARCH. The reference sequences were reduced to the amplified region before the assignment using cutadapt with the same variable as for the raw read primer trimming. For ASV with multiple best matches, taxonomy was reduced to a taxonomic path with a consensus of 60% over all best matches. The control of tag-jumps (i.e., reads with wrong combinations of barcoding tag(s) produced during the Illumina library construction,

thus incorrectly assigned to a sample (Schnell et al. 2015) was described in (Lentendu et al. 2023). DNA positive controls of cultivated freshwater algae species were included in each sequencing run. As these algae are not expected to be found in soil samples, any ASV assignment to one of these species in a soil sample was likely originating from tag-jump. A linear model was used for each library to compute a regression between the maximum number of reads assigned to each positive control ASV in any soil sample and the total number of reads of this positive control ASV. This allowed for setting a threshold based on the 99.9% confidence interval of the model predicted values below which reads of any given ASV in any sample can be considered as tag-jump based on the ASV total read count. For a given soil sample, read counts of each ASV below the predicted threshold were removed (i.e., set to zero) and redistributed to the other samples based on a probability distribution. Only reads and ASVs taxonomically assigned to protists were used for the diversity analyses.

Diversity analyses

All diversity analyses were performed in the R environment (R v4.2.2, R Core Team 2022) with the RStudio interface (v2022.11.999, RStudio Team 2022) and using packages Vegan v2.6-4 (Oksanen et al. 2022), Tidyverse v1.3.2 (Wickham et al. 2019), and ggplot2 v3.3.2 (Wickham 2016). The link for the full R code and data is provided in the Data Accessibility Statement section.

Taxonomic composition and alpha diversity: whole soil protist community

Rarefaction curves were computed based on the total number of ASV to determine if sufficient sequencing depth was achieved. All other analyses were performed after Hellinger transformation (square root of reads ratio) of the ASV matrix, hereafter called the normalized data. The taxonomic composition between regions, habitats, and seasons was calculated using per sample percentages of the normalized data. The normalized data was then used to compute the alpha diversity indices (i.e., ASV richness, Shannon, Simpson, and evenness). ASV richness and Shannon index were strongly correlated to log-transformed total read counts per sample, indicating a sequencing depth bias (Suppl. material 1: fig. S3, table S3). To account for this bias, we used the residuals of the respective linear models, i.e., only the residual variance of alpha diversity that is not due to sequencing depth bias was analysed (Hiiesalu et al. 2014). For indices showing no correlation with read counts (i.e., Simpson and evenness), raw values were kept. All alpha diversity indices were then scaled between -1 and 1 for comparison. Statistical differences between regions and seasons for each habitat were calculated using a Tukey's HSD test.

Beta diversity: whole soil protist community

We assessed the variation in soil protist assemblages between regions, habitats, and seasons using a non-metric multidimensional scaling (NMDS) ordination based on Bray-Curtis dissimilarity calculated on the normalized data. The significance of region, habitat, season, elevation, and their interactions as explanatory variables of community composition was assessed by a permutational analysis of variance (PERMANOVA). To estimate the proportion of beta diversity due to spatial and temporal turnover, we performed a total diversity partitioning by decomposing total ASV diversity (gamma) into alpha diversity, temporal beta diversity, and spatial beta diversity components (Veech et al. 2002; Tylianakis et al. 2005; Fournier et al. 2020). The total diversity can be described as $\gamma = \alpha + \beta_T + \beta_S$ with (i) α being defined as the alpha diversity (i.e., the mean number of ASVs per plot per season), (ii) β_T is the temporal turnover of ASVs between seasons and is calculated as the mean of all β_{TPlot}, i.e., the temporal turnover of ASVs of a given plot (i.e., β_{TPlot} is the total number of ASVs found within a given plot over the two seasons minus α), and (iii) β_S representing the spatial beta diversity of ASVs (i.e., the total ASVs in spring and summer per habitat type minus the mean number of ASVs per plot of that habitat type over spring and summer). We explored the correlation between ASV community composition and spatial, temporal, soil, and climatic variables using variance partitioning analysis (Suppl. material 1: table S4). Differences between habitat and season were investigated by applying a Tukey's HSD test. We performed redundancy analyses (RDA) with forward selection to identify models explaining at best changes in community structure based on edaphic variables and elevation. Four subsets of the ASV matrix separated according to regions and seasons were explored independently using soil variables (i.e., both composite and independent variables, Suppl. material 1: fig. S2, table S2) and the elevation. The significance of variable selected in the model was tested with an analysis of variance (ANOVA).

Diversity analyses for four major protist groups

To assess the robustness of our findings when considering individual groups of protists, we conducted separate analyses for major protist groups exhibiting the highest total ASV richness. Specifically, we focused on four groups with a total richness exceeding 1,000 ASVs, i.e., Cercozoa, Apicomplexa, Ciliophora, and Lobosa. While the Lobosa is no-longer a formally recognised group (it includes mainly the Tubulinea and Discosea), we retained it here as it includes groups with relatively similar morphology and life habits, i.e., mostly predatory amoeboid protists. The selected ASVs threshold ensured comprehensive coverage across all sampling sites for each phylum and maintained comparable group sizes for statistical analyses across sites. Our approach for these selected groups followed

the methodology applied to the total soil protist community analysis. Briefly, we initially addressed potential sequencing depth bias within each phylum independently (data not shown). Then, we calculated alpha diversity indices, i.e., ASV richness, Shannon, Simpson, and evenness. To assess variations in community composition (i.e., beta diversity) across different regions, habitats, and seasons, we calculated Bray-Curtis dissimilarity matrices on Hellinger transformed data for each phylum. These matrices served as the basis for NMDS, PERMANOVA, variance partitioning, and total diversity partitioning analyses.

Results

Metabarcoding of soil protists' environmental DNA

The final amplicon matrix contained 6'527'552 cleaned reads that represented 19'781 Eukaryote ASV according to the PR^2 database (Guillou et al. 2013). About 46% of the total reads (i.e., 2'999'620 reads) and more than 59% of the total ASVs (i.e., 11'706 ASVs) were assigned to protists. Rarefaction curves showed that ASVs richness reached a saturation plateau for all samples, independently of the region, habitat, or season (Suppl. material 1: fig. S4).

Taxonomic composition, and alpha diversity

The phyla with the highest total ASV diversity were Cercozoa (4'445 ASVs), Ciliophora (1'725 ASVs), Apicomplexa (1'176 ASVs), "Lobosa" (1'131 ASVs), Conosa (968 ASVs) and Chlorophyta (488 ASVs). Cercozoa dominated the protist community both in terms of reads number and ASV richness. In the Spanish Sierra Nevada forests, Ciliophora and Apicomplexa ranked as the second and third most abundant phyla, while in the Swiss forests, their positions were reversed (Suppl. material 1: fig. S5). Chlorophyta reads and ASV numbers were higher in open habitats compared to forests. In these open habitats, Chlorophyta was the third most abundant phylum after Cercozoa and Ciliophora. At the whole soil protist community level, analyses of alpha diversity revealed a decrease in ASV richness from spring to summer in the Swiss Alps grasslands, but no significant change between seasons for all other categories (Fig. 1). In forests, the Shannon indices were significantly lower in the Spanish Sierra Nevada than in the Swiss Alps but did not differ between seasons. In grasslands, Shannon indices were not significantly different between seasons, but significantly lower in spring in the Spanish Sierra Nevada compared to the Swiss Alps. The Simpson indices did not differ significantly between seasons and regions. In contrast, both seasons in the Swiss Alps forests showed significantly higher evenness values compared to the Spanish Sierra Nevada spring. The evenness in grasslands did not differ significantly between seasons and regions.

Spatial and temporal beta diversity in soil protist assemblages

At the whole soil protist community level, the NMDS ordination identified four distinct clusters of samples, separating the two habitats along the first axis and the two regions along the second axis (Fig. 2). By contrast, there was no

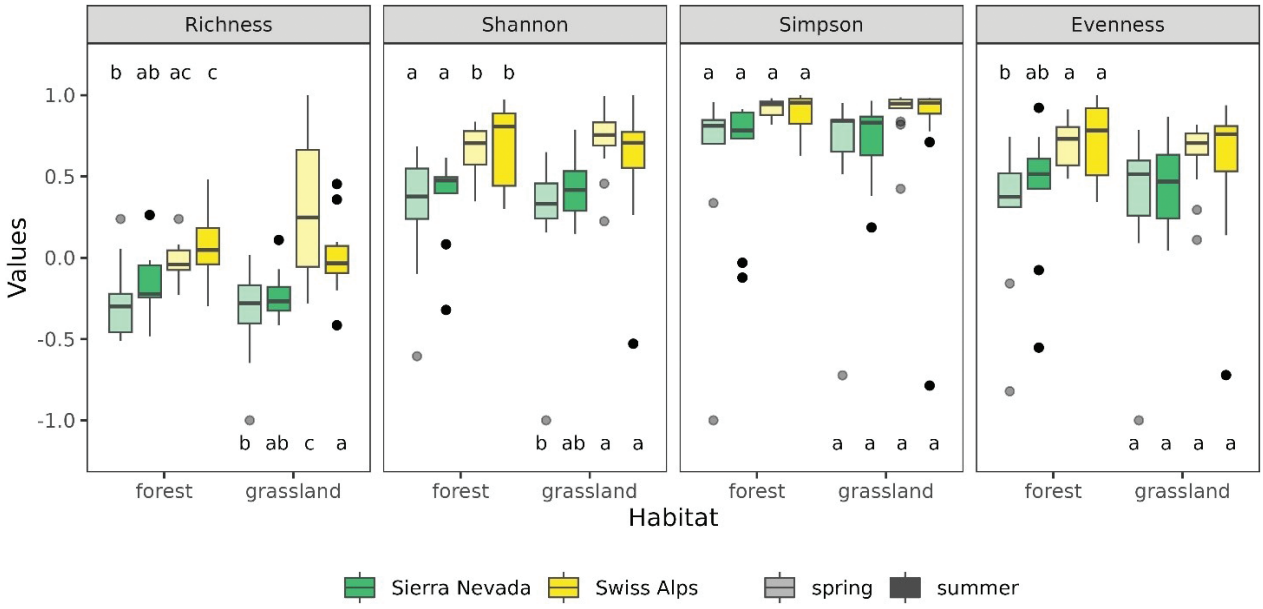

Figure 1. Alpha diversity of soil protists based on amplicon sequence variants (ASV) grouped by habitat (x-axis), region (Spanish Sierra Nevada: green; Swiss Alps: yellow), and season (spring: lighter hue; summer: darker hue). Indices were normalized and scaled between -1 and 1 (see also Suppl. material 1: table S4). Outliers are shown as dots. Significant differences between treatments were calculated with a Tukey's HSD test ($p < 0.05$). Statistical tests were carried out across the region × season interactions, thus letters cannot be compared across habitats.

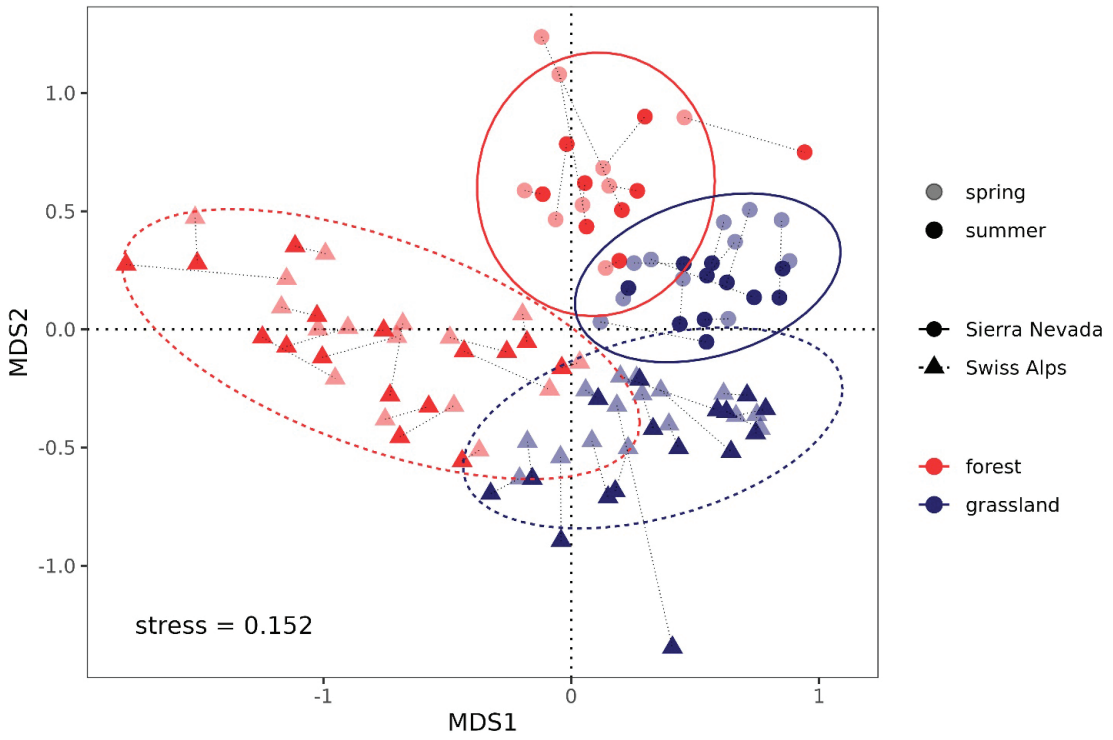

Figure 2. Non-metric multidimensional scaling (NMDS) biplot of the complete data set (n = 104) based on the Bray-Curtis dissimilarity index of soil protist amplicon sequence variants (ASV) in the Spanish Sierra Nevada (circles) and the Swiss Alps (triangles), in forest (red) and grassland (blue) habitats. Pairs of samples from the same site (spring: lighter hue; summer: darker hue) are linked with black dashed lines. Ellipses were drawn at a 90% confidence level.

evidence for temporal variation. This observation was statistically supported by a PERMANOVA in which the temporal variation as well as its interactions with the other variables were not significant (Suppl. material 1: table S5). The results of the PERMANOVA also showed that community composition differed significantly between the two regions and habitats, and, to a lesser degree, across elevations. The additive partitioning of total ASV diversity allowed for quantifying the relative contribution of alpha and beta diversity components to gamma diversity at the local level (i.e., along elevation gradients within the same region and habitat) and showed that soil protist community composition varied more spatially than seasonally (Fig. 3a). Indeed, spatial beta diversity (β_S) within regions and habitats (Spanish Sierra Nevada: average β_S = 70.40% ±0.76; Swiss Alps: average β_S = 85.06% ±1.29) was higher than temporal turnover (β_T) between spring and summer (Spanish Sierra Nevada: average β_T = 14.17% ±1.19; Swiss Alps: average β_T = 3.84% ±0.38). The percentage of total diversity explained by local diversity (α) was higher than the temporal turnover, except in the grasslands of the Spanish Sierra Nevada (α = 13.48% and β_T = 15.36%). Furthermore, the average proportion of total diversity due to both alpha diversity (α) and temporal turnover (β_T) were higher in the Spanish Sierra Nevada than in the Swiss Alps. When analysing both local and regional spatial scales together, the partitioning of variance revealed that the interaction of space (i.e., elevation, habitat, region), soil variables, and CRU-TS bioclimatic variables ("climate") significantly explained 29% of the total soil protist community variations (Fig. 3b, Suppl. material 1: table S4). On the contrary, season alone did not explain any of the community composition, but explained 3 % in combination with space, soil, and climatic variables.

Inter-seasonal variations in soil protist assemblages

The similarity in the total ASV composition [1 – Bray-Curtis dissimilarity] was highest between spring and summer samples from the same site (Fig. 4a). The slightly higher inter-seasonal similarity in ASV composition between seasons (i.e., comparing pairs of samples from the same site) for grasslands as compared to forests in both regions (Fig. 4b) was not statistically supported by the Tukey's HSD test (p-value > 0.05).

Edaphic predictors of soil protist community beta diversity

In redundancy analyses with edaphic factors as explanatory variables, forest and grassland samples were clearly separated in both the Spanish Sierra Nevada and the Swiss Alps (Fig. 5, Suppl. material 1: table S6). In each region, the same set of edaphic variables were identified as significant predictors of the whole soil protist community variations in spring and summer, but their explanatory power varied between seasons, as shown by the ANOVA (Suppl. material 1: table S6). The composite variable

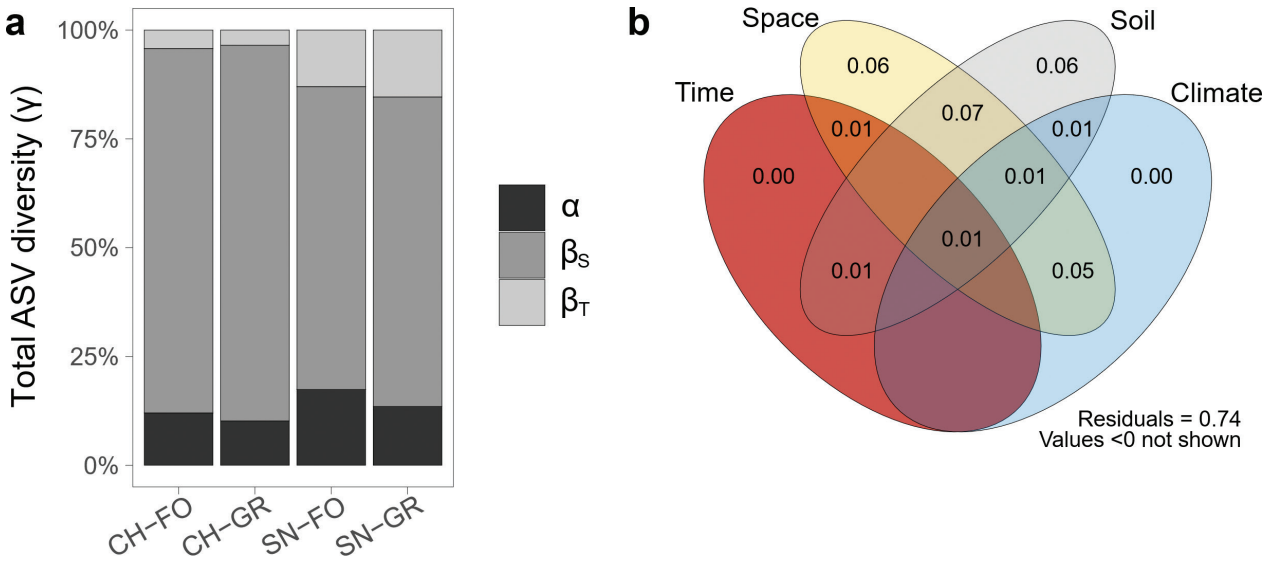

Figure 3. (**a**) Partitioning of total diversity (γ) into alpha diversity (α), spatial beta diversity (β_S), and temporal beta diversity (β_T) per region and habitat ($\gamma = \alpha + \beta_T + \beta_S$). SN: Spanish Sierra Nevada, CH: Swiss Alps, FO: forest, GR: grassland. (**b**) Partitioning of the variation in ASV community composition of the entire dataset among temporal ("time", 3 %), spatial ("space", 21 %), soil (17 %), and climatic ("climate", 8 %) components. See also Suppl. material 1: table S5.

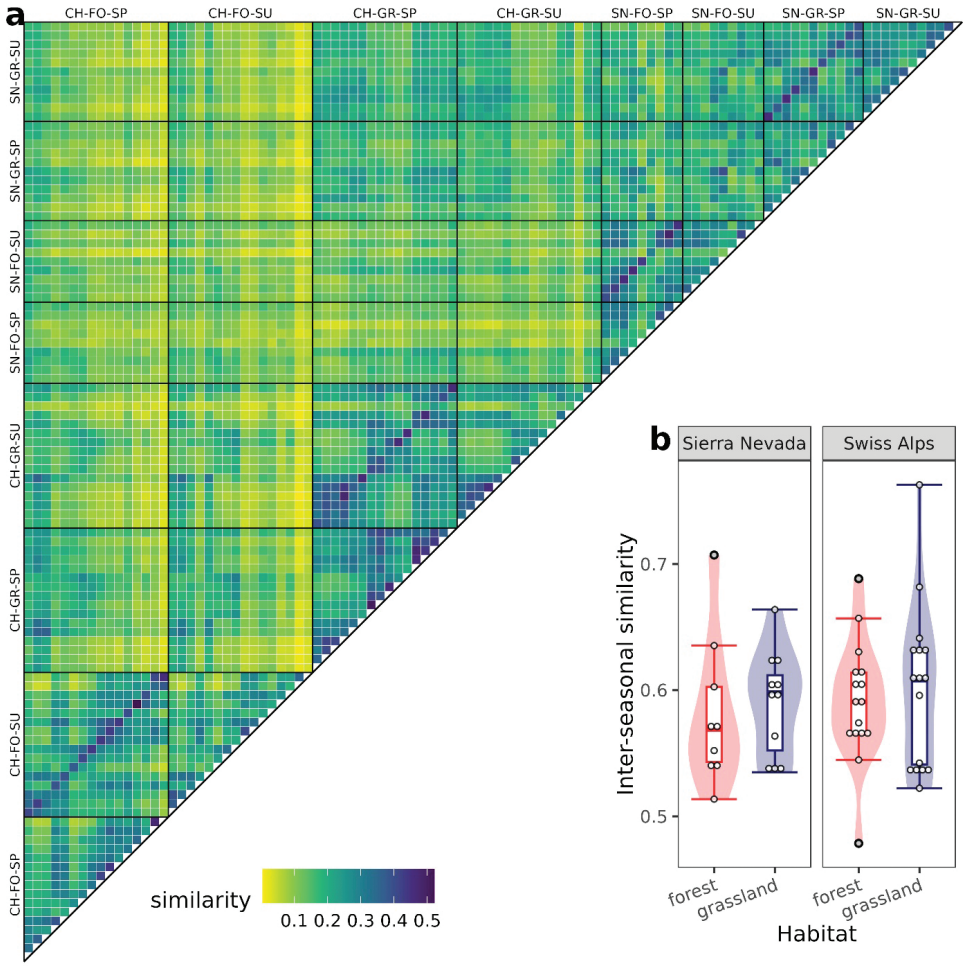

Figure 4. Similarity (1 - Bray-Curtis dissimilarity index) in soil protist community composition based on amplicon sequence variants (ASV). (**a**) Similarity of protist ASV composition inside and between region, habitat, and season. SN: Spanish Sierra Nevada, CH: Swiss Alps, FO: forest, GR: grassland, SP: spring, SU: summer. (**b**) Inter-seasonal Bray-Curtis similarity of sample pairs (i.e., spring versus summer pair of samples from the same site) in each region. Pairs are in red for forest and in blue for grassland. The Tukey's HSD test indicated no significant difference between forest and grasslands.

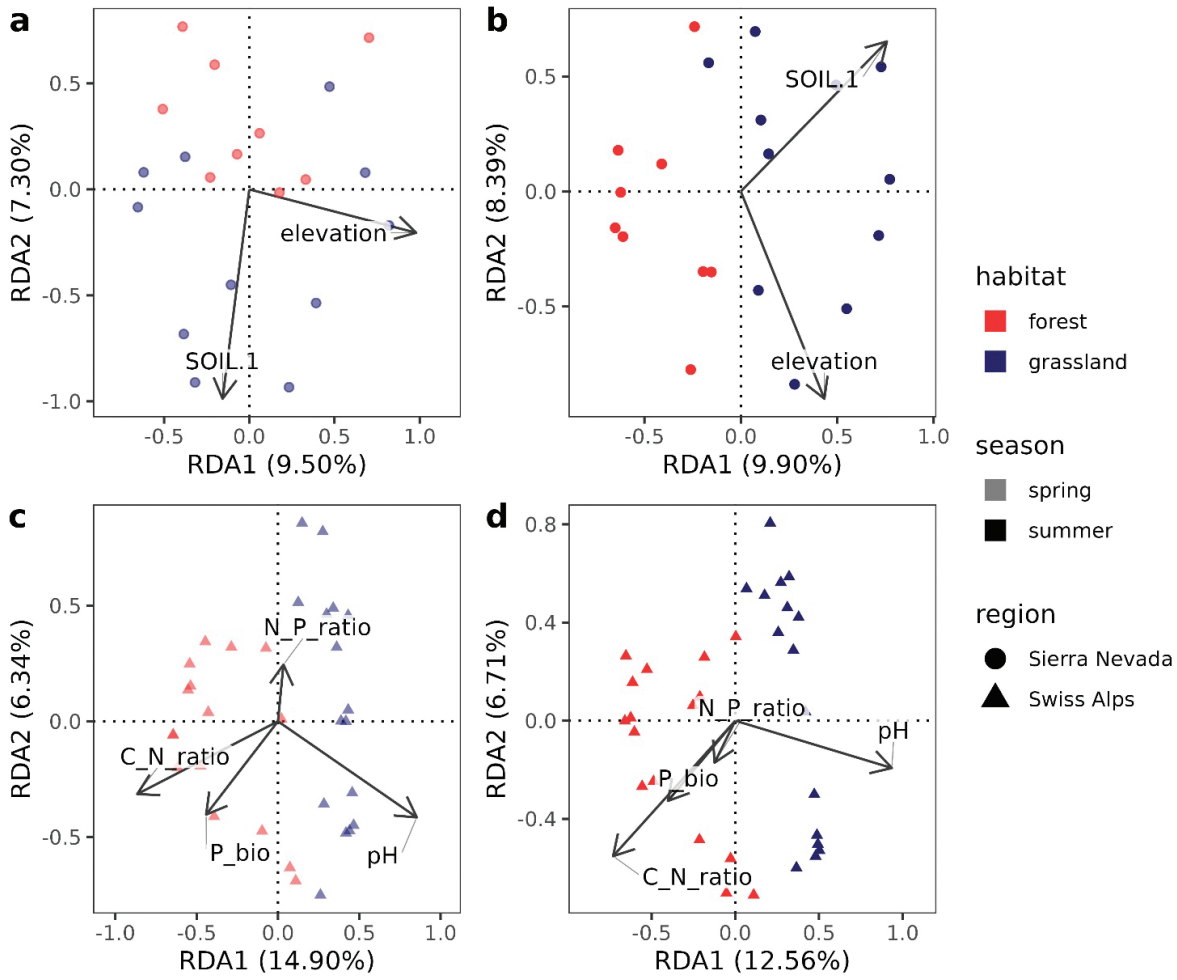

Figure 5. Redundancy analyses (RDA) biplots of soil protists community subsets (based on amplicon sequence variants – ASV): (**a**) Spanish Sierra Nevada in spring, (**b**) Spanish Sierra Nevada in summer, (**c**) Swiss Alps in spring, and (**d**) and Swiss Alps in summer. Vectors represent soil variables best shaping protist community and were determined by forward selection (P value ≤ 0.05). ANOVA permutation test results in Suppl. material 1: table S7. C_N_ratio, organic carbon / organic nitrogen ratio; N_P_ratio: organic nitrogen / bioavailable phosphorus ratio; P_bio, bioavailable phosphorus; SOIL.1, composite variable, i.e., the first axis of a PCA based on soil organic matter, organic carbon, organic nitrogen, and residual humidity variables.

SOIL.1 (i.e., the first axis of a PCA based on soil organic matter, organic carbon, organic nitrogen, and residual humidity variables; Suppl. material 1: fig. S2, table S2) was the only significant edaphic variable for both seasons in the Spanish Sierra Nevada, while this variable had no explanatory power in the Swiss Alps. In the latter region, soil pH, C/N ratio, N/P ratio and bioavailable phosphorus were the significant edaphic predictors of differences in soil protists communities for both seasons. Elevation emerged as a factor explaining the variation in the protist community for the Sierra Nevada only.

Alpha and beta diversity patterns of four major protist groups

None of the four major protist groups analysed individually showed significant variations of alpha diversity indices between spring and summer (Suppl. material 1: fig. S6). NMDS ordinations allowed to separate the two habitats and the two regions in distinct clusters, but no effect of the season was observed between the spring and summer samples (Suppl. material 1: fig. S7). PERMANOVA analyses supported this observation for each phylum as the season, along with its interactions with other explanatory factors, was not statistically significant in explaining variations within the communities (Suppl. material 1: table S7). The only exception was for Ciliophora where the season explained a minor portion of the community composition. Irrespective of the phylum considered, the partitioning of total ASV diversity was in line with the results of the total soil protist community analysis: community compositions exhibited 1) a higher proportion of total diversity attributed to beta diversity as compared to local diversity (i.e., alpha diversity), and 2) a larger proportion of total diversity was attributed to spatial beta diversity as compared to temporal beta diversity (i.e., seasonality; Suppl. material 1: fig. S8a). At the regional scale, the partitioning of variance of each phylum indicated that the combined effect of spatial factors (i.e., elevation, habitat, region),

soil characteristics, and climatic conditions accounted for the predominant portion of the observed community variations (Suppl. material 1: fig. S8b). Depending on the phylum considered, the season alone explained between 0 and 1 % of the community composition variations, and combined with the three other explanatory factors, accounted for approximately 2 % to 3 % of the variance.

Discussion

Soil protists are one of the least known components of biodiversity (Geisen et al. 2020). To address this knowledge gap, broad-scale comparisons of soil protist diversity and meta-analyses combining multiple datasets are especially useful. This makes it crucial to understand the possible limitations and sources of bias due to methodological differences among studies. Here we addressed the relative importance of seasonal *versus* spatial variability in soil protist diversity and community assembly processes, which are poorly understood (Fournier et al. 2020), especially in mountains (Shen et al. 2021). Using high throughput environmental DNA sequencing, we characterized soil protist diversity (i) along elevation gradients in two mountain ranges (the Swiss Alps and the Spanish Sierra Nevada), (ii) between two contrasted habitats (forests and grasslands below the treeline), and (iii) between spring and summer of the same year. To our best knowledge, this is the first comparison of beta diversity patterns of soil protists along multiple elevation gradients and comparing the effect of the season between two regions and two habitats.

We recorded a high diversity of protists in soils, with a total of 11'706 ASVs and the dominance of Cercozoa and Ciliophora sequences, in line with previous soil protist diversity studies (Bates et al. 2013; Chen et al. 2015; Dupont et al. 2016; Lentendu et al. 2018; Mundra et al. 2021; Singer et al. 2021). The diversity of Apicomplexa, which were either the second or third most dominant group in our samples, most likely reflects soil animal parasitism and agrees with recent findings (Geisen 2016; Mahé et al. 2017; Singer et al. 2020). We found no significant differences in alpha diversity indices between spring and summer sites within the same region and habitat, except for the richness in the Swiss Alps grassland. Furthermore, the lower average site richness in the Spanish Sierra Nevada compared to the Swiss Alps could be explained by higher temperature and lower precipitation during summer (Lamprecht et al. 2021) that were shown to reduce protist diversity (Hu et al. 2022).

Regardless of the region or the habitat, the contribution of alpha diversity to total diversity was always smaller than that of the beta diversity, i.e., the combination of spatial and temporal components of beta diversity, which is consistent with another study on soil protists along an elevation gradient in China (Shen et al. 2021). By comparing multiple elevation gradients in the Swiss Alps and the Spanish Sierra Nevada, our study demonstrates the recurrency of these patterns along such gradients in temperate climates.

Protist community composition was best explained by geographic location (i.e., the region), followed by habitat and elevation. This observation highlights the dominance of the spatial beta- diversity component in explaining the overall soil protist diversity. Our findings are in line with Shen et al. (2021) who found that elevation more than seasonality was the main driver of microeukaryote community composition along an elevation gradient in China. In a floodplain, Fournier et al. (2020) also highlighted higher beta than alpha diversity, and higher spatial than seasonal variations as main drivers of diversity. However, in contrast to the findings of the two latter studies, we observed no significant temporal effects for the whole soil protist community, regardless of the spatial scale considered – whether at the local level (i.e., along elevation gradients within the same region and habitat) or at the regional level (i.e., encompassing both the region and the habitat). Additionally, the analysis of alpha and beta diversity of the four major protist groups, i.e., Cercozoa, Apicomplexa, Ciliophora, and Lobosa, highlighted the consistency of our findings at a finer taxonomic level. While the PERMANOVA results revealed a significant seasonal effect for Ciliophora, the fundamental spatio-temporal patterns remained similar to those observed in the total soil protist communities, with greater spatial variations than temporal changes influencing community composition.

Consequently, our results suggest that, in temperate climate mountain zones, sampling at a single time point along elevation gradients is unlikely to introduce bias when comparing beta diversity patterns across several regions. Similarly, samples collected in spring can be compared with those collected in summer, and vice versa. Therefore, we recommend conducting sampling anytime between the end of spring and late summer. Additionally, we advise to follow new protocols that were recently published on ways to increase soil protist diversity estimates along elevation gradients or for biogeography studies by: 1) collecting soil at sites spanning the broadest possible elevation range, in contrast to collecting multiple replicates at fewer elevations (Huang et al. 2023), and 2) applying a filtration-sedimentation method to improve the coverage of shelled protist, e.g., testate amoebae, in metabarcoding studies (Lentendu et al. 2023). Likewise, our results hold significant methodological importance for soil protist studies along elevation gradients because, in practice, logistical or financial constraints often do not allow for repeating soil sampling more than once a year, especially in the case of large- or global-scale studies conducted across multiple elevation gradients. Thus, we specifically chose to compare spring and summer in the present study to avoid elevation gradient truncation until the tree line (e.g., due to higher elevation sites being snow-covered) and because autumn and winter sampling are frequently impractical (e.g., due to snow cover, restricted road access), or for safety concerns, such as the risk of avalanches. Nevertheless, the insulating effect of snow allows for microbial activity in winter too (Warren and Taranto 2011; Saccone et al. 2013).

Our results are in line with one of the few publications with a sampling design spanning multiple years, which addressed Cercozoa and Endomyxa seasonality in tree canopy over a two-year period and revealed consistent recurrent seasonal patterns (Walden et al. 2021). We therefore predict that consistent annual recurrence in seasonal patterns of soil protist diversity and community composition should be observed. However, our assumption will remain true only if no significant environmental changes disrupt the seasonal patterns of soil protists. Indeed, hot and dry conditions can cause a substantial reduction in the abundance or activity of soil protists (Hu et al. 2022). Also, the effect of drier conditions was shown to vary among protist groups, with larger protist species responding more strongly to drought than smaller species (Geisen et al. 2014; Jassey et al. 2016; Koenig et al. 2017, 2018). Thus, climate change could significantly alter protists community composition and seasonality, as observed in an experiment mimicking a +4 °C temperature increase in forests (Li et al. 2023). This also shows the need to document spatial and temporal patterns of soil protist communities as such baseline data will be essential to assess to what degree ongoing climate change is having an impact on soil biodiversity.

Our exploration at a finer taxonomic resolution sheds light on spatio-temporal dynamics of soil protist functional groups along elevation gradients. Indeed, the selected groups encompass a significant portion of consumers (Cercozoa, Ciliophora, and Lobosa), while parasites are represented by Apicomplexa (Geisen et al. 2018). Hence, our results suggest that functional groups diversity is not affected by seasonality. However, functional group composition varies across ecosystems with consumers dominating across most soil types, parasites being more abundant in tropical forests, and phototrophs being more present in arid ecosystems (Mahé et al. 2017; Oliverio et al. 2020; Singer et al. 2020). Furthermore, a study in the Swiss Alps covering a 3’000 m elevation gradient across multiple habitats, Mazel et al. (2022) observed significant effect of elevation on consumer and parasite community composition. To date, there is no evidence that these group-specific response remain consistent between seasons, regions or climates, thereby our results on seasonality may not be transposable to other climates or habitats. Moreover, our analyses do not encompass phototrophs, the third functional group present in soil, despite their important roles in grasslands (Seppey et al. 2017). Therefore, further research is needed to expand our understanding of the spatio-temporal dynamics of these functional groups along elevation gradients, considering their proportional representation and ecological roles.

Furthermore, based on the comparison of inter-seasonal similarity between spring and summer sample pairs, the magnitude of temporal changes at the whole soil protist community level did not differ significantly between forests and grasslands, irrespective of the region. Thus, despite the more stable temperatures and soil moisture contents generally observed in forests (Renaud et al. 2011), we found no significant difference in the temporal stability of soil protist community composition between forests and grasslands below the treeline.

Moreover, while the sets of edaphic variables explaining the whole soil protist community assembly differed between the Spanish Sierra Nevada and the Swiss Alps, within each region, the same variables were selected for spring and summer. In the Spanish Sierra Nevada, the main explanatory factors were the elevation and the composite variable SOIL.1 (i.e., the first axis of a PCA based on soil organic matter, organic carbon, organic nitrogen, and residual humidity variables). The presence of the residual humidity in this composite variable likely reflects the capacity of organic matter particles to retain water molecules, given the strong correlation generally obsereved between residual humidity and soil organic matter, organic carbon, and organic nitrogen (Gobat et al. 2010). In the Swiss Alps, the selected drivers were related to organic matter and nutrient cycling, with variables such as the C/N ratio being a proxy of the speed of organic matter turnover and ultimately of primary productivity (Bardgett et al. 2005). Thus, independently of the region, these results highlight the influence of organic matter characteristics as the main factors influencing the composition of soil protists.

Finally, microscopic counts have traditionally been the method of choice for studying soil protist diversity, particularly in cases when taxonomic classification relies on morphological characteristics, such as ciliates or testate amoebae. Despite the rise of molecular tools, microscopy remains a widely used method for groups such as testate amoebae for which identification is primarily based on morphology (e.g., Koenig et al. 2015; Heger et al. 2016; Tsyganov et al. 2022; Wanner et al. 2022). In this context, it would be noteworthy to assess the extent to which our eDNA-based (i.e., metabarcoding of the V4 region of the 18S rDNA gene) conclusions apply to microscopy-based studies. To the best of our knowledge, there is no comprehensive study directly comparing DNA-based methodologies to microscopic counts for seasonal variations of soil protist communities. However, such comparisons have been made for freshwater protists and demonstrated that both methods can effectively detect seasonal shifts in abundant alveolate taxa (primarily ciliates), but that sequence read number does not correlate with cell abundance (e.g., Medinger et al. 2010; Stoeck et al. 2014; Pitsch et al. 2019). In soil, future research should seek in bridging the gap between molecular and morphological diversity assessments to enhance our understanding of spatio-temporal dynamics of soil protists.

Conclusion

Our analysis of soil protist diversity along elevation gradients in the Swiss Alps and the Spanish Sierra Nevada revealed a clear dominance of beta diversity components in explaining the total diversity and identified spatial heterogeneity as the main driver structuring community

composition, while temporal turnover was not significant. This suggests that, in temperate climates, comparisons of beta diversity patterns are possible for samples collected along elevation gradients from different regions and habitats independently of a spring or summer sampling period. Future research is needed to assess the validity of our findings across different climates, latitudes, extended timeframes, and by encompassing more diverse habitats. This will help to design robust sampling designs for large- or global-scale studies on microbial diversity, macroecology, and biogeography in mountains and for sound inter-study comparisons and meta-analyses. Moreover, evaluating the effects of temporality is crucial, as changes in spatio-temporal dynamics, e.g., due to climate change, could significantly influence soil protist communities, and ultimately impact ecosystem functioning.

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Author contributions

EADM, GL, and EPB conceived and designed the study. EADM provided funding. EPB, JL, JP, PV, EADM, and GL collected samples. GL did the bioinformatic processing, and EPB performed all diversity analyses. GL, MM, and BF provided parts of the code for the statistical analyses. EPB, EADM, and GL interpreted the results with inputs from MM and BF. EPB designed the figures and wrote the first draft. All authors discussed and revised the final version of this manuscript.

Data Accessibility Statement

Raw sequence data and associated metadata are available on the Short Read Archives projects PRJEB56206 and PRJEB59986. Sampling sites metadata (including raw reads accession numbers and raw soil parameters values), the R code and the R data used for the statistical analyses are available in the data package at https://doi.org/10.5281/zenodo.11126546.

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Supplementary materials

Supplementary material 1

Supplementary tables S1–S7 and figs S1–S8 (.pdf)
Link: https://doi.org/10.21425/fob.17.132637.suppl1