

RESEARCH PAPER



Soil protist diversity in the Swiss western Alps is better predicted by topo-climatic than by edaphic variables

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Funding information

Norges Forskningsr d, Grant/Award Number: 256132 and 270252; Pro-Femmes Fellowship program from the Faculty of Biology and Medicine of the University

Abstract

Aim: Trends in spatial patterns of diversity in macroscopic organisms can be well predicted from correlative models, using topo-climatic variables for plants and animals allowing inference over large scales. By contrast, diversity in soil microorganisms is generally considered as mostly driven by edaphic variables and, therefore, difficult to extrapolate on a large spatial scale based on predictive models. Here, we compared the power of topo-climatic versus edaphic variables for predicting the diversity of various soil protist groups at the regional scale.

Location: Swiss western Alps.

Taxa: Full protist community and nine clades belonging respectively to three functional groups: parasites (Apicomplexa, Peronosporomycetes and Phytomyxea), phagotrophs (Sarcomonadea, Tubulinea and Spirotrichea) and phototrophs (Chlorophyta, Trebouxiophyceae and Diatomeae).

Methods: We extracted soil DNA from 178 sites along a wide range of elevations with a random-stratified sampling design. We defined protist Operational Taxonomic Units assemblages by metabarcoding of the V4 region of the rRNA small subunit gene. We assessed and modelled the diversity (Shannon index) patterns of all above-mentioned taxonomic groups based on topo-climatic (topography, slope southness, slope steepness and average summer temperature) and edaphic (soil temperature, relative humidity, pH, electroconductivity, phosphorus percentage, carbon/nitrogen, loss on ignition and shale percentage) variables in Generalized Additive Models (GAM).

Results: The respective significance of topo-climatic and edaphic variables varied among taxonomic and—to a certain extent—functional groups: while many variables explained significantly the diversity of the three phototrophs this was less the case for the three parasites. Topo-climatic variables had a better predictive power than edaphic variables, yet predictive power varied among taxonomic groups.

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of Lausanne; University of Neuchâtel; Swiss National Science Foundation, Grant/Award Number: 310003A 143960, 31003A-152866, CR2312-162754, P2NEP3-178543 and PDFMP3-135129; University of Lausanne; European Community FP7-PEOPLE-2010-IIF program, Grant/Award Number: 273965; Atracción de talentos: Community of Madrid, Grant/Award Number: 2017-T1/AMB-5210; Agassiz Foundation; Spanish Government, Grant/Award Number: PGC2018-094660-B-I00

Handling Editor: Vincent Merckx

Main conclusions: Topo-climatic variables (particularly slope steepness and summer temperature if we consider their significance in the GAMs) were, on average, better predictors of protist diversity at the landscape scale than edaphic variables. However, the predictive power of these variables on diversity differed considerably among taxonomic groups; such relationships may be due to direct and/or indirect (e.g. biotic) influences (like with parasitic taxa, where low predictive power is most likely explained by the absence of information on the hosts' distribution). Future prospects include using such spatial models to predict hotspots of diversity and disease outbreaks.

KEYWORDS

algae, functional groups, macroecology, meadow soils, microbial ecology, parasites, predictive modelling, protozoa, spatial modelling

1 | INTRODUCTION

Protists, that is, all eukaryotes with the exception of fungi, plants and animals are hyper-diverse in soil systems (Geisen et al., 2018; Mahé et al., 2017), where they play many ecological roles as primary producers, saprotrophs, predators or parasites (Adl & Gupta, 2006; Geisen et al., 2016), and, thus, play a key role in ecosystem functioning. Photosynthetic groups are essential components of cryptogamic crusts (Elbert et al., 2012; Pushkareva, Johansen, & Elste, 2016) and constitute a significant source of organic carbon for soil organisms (Schmidt, Dyckmans, & Schrader, 2016; Seppey et al., 2017). Predatory protists occupy different levels of the microbial food web, as primary consumers of algae (cyanobacteria or eukaryotic), fungi and bacteria (Bonkowski & Clarholm, 2012; Dumack, Mueller, & Bonkowski, 2016; Hess & Melkonian, 2014) but also occupy higher trophic levels by preying on phagotrophic protists or even micro-Metazoa (e.g. nematodes; Geisen et al., 2015; Gilbert, Amblard, Bourdier, Francez, & Mitchell, 2000). Parasites are thought to regulate natural populations, notably of animals (Mahé et al., 2017) and can be either very specific such as between the parasitic Gregarines and their animal hosts (Clopton, 2009), or generalist as for *Phytomyxa* species which can infect hosts from different eukaryotic kingdoms (Neuhauser, Kirchmair, Bulman, & Bass, 2014). Characterizing such complex communities is essential to understand the main ongoing ecological processes in soil, and represents a first step towards predicting the effects of environmental changes on communities and, consequently, on ecosystem functioning.

As a whole, soil protist communities have been shown to respond to edaphic conditions, such as gradients of pH (Dupont, Griffiths, Bell, & Bass, 2016), nutrients and moisture (Singer et al., 2018), as well as pesticide amounts (Ekelund, 1999; Foissner, 1999; Nesbitt & Adl, 2014) and other perturbations (Foissner, 1997). These variables are rarely integrated in spatial modelling of biodiversity in general (Mod, Scherrer, Luoto, and Guisan, (2016) for plant communities), especially at broad spatial scales, because they are most often not available at the sites of species observations and not easily generalizable in a spatially explicit way (Buri et al., 2017; Cianfrani, Buri, Verrecchia, & Guisan, 2018;

Dubuis et al., 2013). On the other hand, topo-climatic variables (such as slope steepness or air temperature) can be more easily modelled at large spatial scales using digital elevation models based on interpolations of weather stations and/or remote sensing methods. These variables have already proved themselves to be useful to model the spatial distribution of plants and animals (Franklin, 2010; Guisan, Thuiller, & Zimmermann, 2017; Peterson et al., 2011) but much more rarely applied to microorganisms. As a consequence, spatial modelling of the distribution of microorganisms has been restricted to small areas or aquatic environments (Bulit, 2014; Fraile, Schulz, Mulitza, & Kucera, 2008; King et al., 2010; Langer, Weinmann, Loetters, Bernhard, & Roedder, 2013; Mitchell et al., 2000; Zaric, Schulz, & Mulitza, 2006; Zinger, Shahnavaz, Baptist, Geremia, & Choler, 2009). Nevertheless, soil protists show broad spatial patterns in their distributions from very different environments and spatial scales (Fernández, 2015; Lara, Roussel-Delif, Fournier, Wilkinson, & Mitchell, 2016; Lentendu et al., 2018; Schiaffino et al., 2016) and the understanding of their eco-geographic requirements could benefit from spatial modelling as much as it benefited macroorganisms. The development of such models at the landscape scale would, if repeated across many regions, allow assessing at a much broader scale the processes driven by microorganisms, such as nutrient cycling or greenhouse gases fluxes and help improve climatic models. In addition, economic and sanitary management could benefit from microbes spatial modelling, for instance by predicting zones at risk of disease outbreaks and therefore make the use of a potential treatment more parsimonious. A third outcome of spatial modelling of soil microbes could also focus on their conservation by identifying microbe diversity hotspots or refine distribution zones of endemic microorganisms (Cotterill, Al-Rasheid, & Foissner, 2008).

Here, we built spatial predictive models of protist diversity, focusing on general communities as well as on nine broad protist taxa chosen within three functional groups—phototrophs, phagotrophs and parasites—along a wide elevational gradient in the western Swiss Alps. We assessed the diversity of protists in 178 meadow soil samples, resulting from a robust random-stratified field survey by metabarcoding of the V4 regions of the small subunit rRNA gene. This study assessed the extent of protist diversity in mountainous

meadows and determined to what extent two sets of environmental variables (edaphic and topo-climatic) can predict this diversity over the whole Swiss western Alps of the Vaud state. In addition, we brought an interpretation of the patterns observed based on knowledge of the lifestyles of the different groups surveyed.

2 | MATERIALS AND METHODS

2.1 | Sampling

Meadow soils were sampled from 194 plots distributed across the Swiss western Alps; of these plots, 178 samples successfully yielded sequencing data and were used in the current study (see Appendix S1.1). Sampling was performed from July 4th to September 1st 2013 according to a random-stratified sampling design. From each plot, five soil cores (100 g per core between the depths of 0–5 cm after removing plants, mosses and insects) were taken from the four corners and the centre of a 2 m² plot. The five cores, were then pooled in a sterile plastic bag and kept in an icebox or at 4°C until DNA extraction and soil analyses were done. A subsample of the pooled soil was also flash frozen at each sampling site and kept frozen until further soil analyses. For more details, see Yashiro et al. (2016).

2.2 | Edaphic variables

We selected eight edaphic variables, one measured directly on site—the soil temperature at a depth of 5 cm (Soil_temp)—and seven in the laboratory from the soil samples collected. The soil relative humidity (rh) was assessed by weighing the mass of the soil sample before and after drying at 105°C during 2 days. Soil organic carbon content was determined by loss of ignition (LOI) at 1,050°C. The percentage of shale was determined by laser granulometry. The pH and electroconductivity (EC) were measured from a soil and Milli-Q water slurry in a 1:2.5 and 1:5 (wt/vol) ratio, respectively. Total phosphorus amount (P) was determined by colorimetric analysis after a mineralization at 550°C with Mg(NO₃)₂. The C/N ratio was calculated from the total organic carbon and nitrogen percentages measured by ROCK EVAL pyrolysis (Vinci Technologies, Ruell-Malmaison, France) and combustion infrared spectroscopy (Carlo Erba CNS2500 CHN), respectively. All methods were described in detail in Yashiro et al. (2016) and Buri et al. (2017).

2.3 | Topo-climatic variables

Values for seven topo-climatic variables were retrieved from maps of 25 square metre resolution for each sample location. We used the number of growing degree-days above 0°C (gdd), potential evapotranspiration (etp), topography (topo), slope southness (asp) and slope steepness (slp) (Zimmermann, Edwards, Moisen, Frescino, & Blackard, 2007; Zimmermann & Kienast, 1999). In addition, we calculated the summer temperature average (tmean678) and precipitation sum (psum678) for the months of June to August with values of monthly temperature means and precipitation sums from 1981 to 2010. See Buri et al. (2017) for more details.

2.4 | Molecular analysis

DNA was extracted from the soil samples using the MoBio PowerSoil DNA extraction kit following the manufacturer instructions. The V4 region of the 18S rRNA gene was then amplified using the general eukaryotic primers TAREuk454FWD1 and TAREukREV3 (Stoeck et al., 2010). The PCR mix was composed of 3 µl DNA extract, 0.4 µl of 10 mg/ml BSA, 4 µl of PCR buffer (Promega GoTaq M7845), 0.2 µl of Taq polymerase (Promega GoTaq M7845), 0.6 µl of dNTPs (Promega kit U1420), 0.6 µl of each primer (MicroSynth, Balgach, Switzerland) and 10.6 µl of ultra-pure water. The PCR reactions started with a denaturation step at 95°C for 5 min followed by 45 cycles of 94°C for 30 s, 47°C for 45 s and 72°C for 1 min, and terminated with an elongation step of 72°C for 10 min. For each DNA sample, the amplifications were performed in triplicate with a PTC-200 Peltier Thermo Cycler (BioConcept, Allschwil, Switzerland). DNA was then quantified with a Qubit[®] 2.0 Fluorometer (Invitrogen) and 20 ng of each triplicate was pooled. A DNA library was prepared from the pools using the TruSeq Nano PCR-free Library Preparation kit and the paired-end 2 × 300 bp sequencing was done on an Illumina[®] MiSeq at the University of Geneva (Molecular Systematics & Environmental Genomics Laboratory). Sequences are available on European Nucleotide Archive via the project number PRJEB30010 (ERP112373).

2.5 | Bioinformatics pipeline

Good quality sequences were selected based on their nucleotides Phred scores. Every sequence with a Phred score average below 20 for a 50 nucleotides window was discarded. The chimeras were then removed using the program *vSEARCH* 1.11.1 (Rognes, Flouri, Nichols, Quince, & Mahé, 2016) by comparing the environmental sequences (a) with each other for each replicate and (b) against the PR² database trimmed according to the V4 primers (downloaded on the 12 September 2016; Guillou et al., 2013). To reduce the noise caused by very rare sequences, we then discarded every singleton. Triplicates were then pooled according to their respective samples and OTUs were built with the program *SWARM* 2.1.8 (Mahé, Rognes, Quince, de Vargas, & Dunthorn, 2015) with the default options (d=1). The dominant sequence of each OTU was taxonomically assigned by aligning it to the trimmed PR² database using the global pairwise alignment program *GGSEARCH* 36.3.6 (Pearson, 2000).

We removed every OTU that did not belong to protists, namely Metazoa, Embryophyceae and Fungi. We also discarded OTUs with a percentage of identity (PID) below 65% with the database PR² as sequences with such low PID are usually of prokaryotic origin (threshold verified manually by aligning low PID environmental sequences on GenBank database). From the 178 plots, 4 were sampled twice and 13 were sampled three times during the sampling period. For each of these 17 plots, we took the average (2 samples) or median (3 samples) sequence abundance of each OTU for the samples from the same plot. In addition to the total protist community matrix, we also selected nine broad taxonomic groups (i.e. clades, low taxonomic

resolution: Adl et al., 2019) from three functional groups (a) parasites: Apicomplexa, Peronosporomycetes and Phytomyxea; (b) phagotrophs: Sarcomonadea (sensu Howe et al., 2011), Tubulinea and Spirotrichea, and (c) phototrophs: Chlorophyceae, Trebouxiophyceae and Diatomeae). These taxa were selected because they are abundant and diverse in soils and are functionally homogeneous. For each of these taxa, we established a PID threshold verified manually on GenBank to discard potential misidentification (Apicomplexa: 80%, Peronosporomycetes: 80%, Phytomyxea: 75%, Sarcomonadea: 80%, Tubulinea: 75%, Spirotrichea: 90%, Chlorophyceae: 90%, Trebouxiophyceae: 85%, Diatomeae: 77%).

2.6 | Richness and diversity analyses

For each of the 10 taxonomic datasets (all protists plus nine broad groups), OTU richness and Shannon diversity (H) were calculated, and the differences between their statistical distributions tested by a multiple comparisons of mean rank sums test (Nemenyi test; Hollander, Wolfe, & Chicken, 2015, `posthoc.kruskal.nemenyi.test` function, 'PMCMR' package 4.1; Pohlert, 2014). The relation between H and the proportion of sequences kept (non-Metazoa/Embryophyceae/Fungi) was also measured to verify if the percentage of non-wanted taxa were biasing the diversity estimate (Spearman correlation tests).

To assess how much predictors impact the protist diversity and distribution, a nonmetric multidimensional scaling was calculated on the 10 Bray–Curtis distance matrices and environmental predictors were fitted to the ordinations (`envfit` function, 'vegan' package 2.5–2; Oksanen et al., 2018). We ran the analyses on all samples with at least two OTUs.

For each of the 10 datasets, H was modelled as a function of the environmental variables using a Generalized Additive Model (GAM; assuming Gaussian residuals and identity link function). For each dataset, three models were calibrated; the first with topo-climatic variables only, the second with edaphic variables only and the third with both sets of variables. All models were iterated 100 times based on bootstraps composed of 80% of the 178 original samples. In total, $10 \times 3 \times 100$ models were fitted. For each model, the predictive power was estimated as the root mean square error (RMSE) calculated on the independent samples not included to build the model (20% left-out samples). The effect of taxonomic group and the set of predictors on predictive power (RMSE) was tested by a Nemenyi test. Finally, the diversity values of the nine broad taxa and total protist diversity were extrapolated across the full area of the western Swiss Alps based on a GAM including the topo-climatic variables (i.e. the only spatially explicit variables).

3 | RESULTS

3.1 | Observed diversity patterns

We retrieved a total of 24,322,487 good quality sequences of which 97% were not chimeric and 71% were not singletons. The 17,110,114 remaining sequences were clustered into 41,048 OTUs of which

19,260 were assigned to protists (see Appendix S2.4). Protist diversity was dominated (proportion of sequences) by Cercozoa (principally Sarcomonadea and Thecofilosea) and Alveolata of which more than half were assigned to Apicomplexa and ca. 45% to Ciliophora (mostly from classes Spirotrichea, Oligohymenophorea, Litostomatea and Colpodea; see Appendix S2.6). The three other dominant groups were the Stramenopiles (including Peronosporomycetes and Diatomeae), Amoebozoa (including Tubulinea) and Archaeplastida (with Chlorophyceae and Trebouxiophyceae; see Appendix S2.6).

The nine taxa selected jointly contributed to more than half (54%) of all retained sequences and represented over 35% of the total OTU richness (see Appendix S2.4). The average richness per sample of these clades varied from 7 (Phytomyxea) to 249 (Sarcomonadea). Richness was on average lowest for phototrophs (15 OTUs/sample) and highest for phagotrophs (122 OTUs/sample; Figure 1). Shannon diversity indices followed the same trend, varying from an average value of 1.1 (Phytomyxea) to 4.3 (Sarcomonadea). The

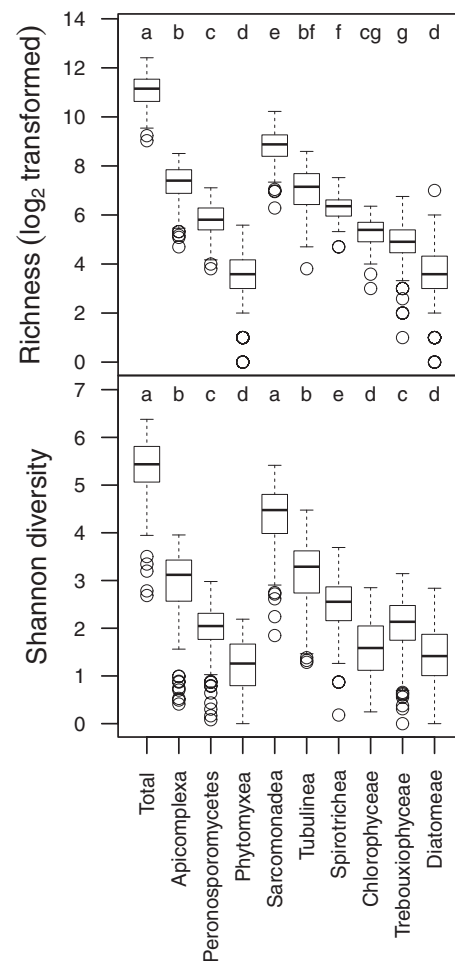


FIGURE 1 Shannon diversity and richness (log transformed) distributions of protist operational taxonomic units communities retrieved from 178 plots in the Swiss western Alps. The distributions are shown for the total community as well as for nine broad taxa. The letters above the boxplots represent groups according to a multiple comparison mean rank sums test (Nemenyi test $p < .05$)

correlation tests revealed that only Spirotrichea, Trebouxiophyceae and Diatomeae were showing a relation between diversity and the proportion of sequences kept in a sample (see Appendix S2.5).

3.2 | Environmental models of diversity

A total of 15 edaphic and topo-climatic parameters were determined for each site. Correlation analysis of topo-climatic and edaphic variables indicated clustering and interdependence for tmean678, gdd, etp and psum678 ($|r| > .7$; Dormann et al., 2013). Consequently, only tmean678 was kept from these four topo-climatic variables for further analyses (see Appendix S1.2 and Appendix S1.3).

We found that the distribution and diversity of the overall protist community and of each taxon were explained by different environmental factors. The overall protists and nine taxa community distribution were structured mostly by pH and rh as well as, on a lesser extent, by Soil_temp, shale, topo and tmean678 (see Appendix S2.8 and Appendix S2.9). In contrast, distinct taxonomic groups were structured by specific profiles of environmental predictors when diversity was modelled by GLM (Table 1, see Appendix S3.10). One example was the Diatomeae where the topo-climatic predictors (slp and tmean678) seemed to be as important in explaining diversity as edaphic predictors (pH and rh) for the overall community (Figure 2, see Appendix S3.10). Similarly, the diversity was much significantly explained by slp for Tubulinea, Sarcomonadea and Phytomyxea, tmean678 for Spirotrichea or pH for Sarcomonadea. The significance of certain environmental predictors was even more accentuated when only topo-climatic predictors were taken into account as for the tmean678 (see Appendix S4.11 and Appendix S4.12).

The predictive power showed lower RMSE values (i.e. a better power) for the topo-climatic than for the edaphic variables for all taxa except for the Chlorophyceae, Trebouxiophyceae and Sarcomonadea for which the values were higher or similar (Figure 2). In addition, the RMSE of the models calculated on the edaphic and topo-climatic variables together were never significantly lower than the RMSE calculated for the topo-climatic variable alone. The RMSE also varied among taxonomic groups when a given set of variables was considered and the diversity of certain taxa were significantly better predicted (e.g. Peronosporomycetes) than others (e.g. Apicomplexa) (Figure 3). The predictive power of the overall community was in general lower in comparison to the specific taxonomic groups for all sets of variables even if some taxa were less adequately predicted (e.g. Apicomplexa with the topo-climatic predictors or Diatomeae when all predictors).

4 | DISCUSSION

4.1 | General patterns of protist communities in soils

Our study revealed several important findings on patterns of protist communities across temperate mountain landscapes.

TABLE 1 Significance of edaphic (soil temperature: Soil temp, relative humidity: rh, pH, electroconductivity: EC, total phosphorus amount: P, carbon/nitrogen ratio: C/N, loss of ignition: LOI, shale percentage) and topo-climatic (topography: topo, slope steepness: slp, summer temperature average: tmean678) predictors on the diversity modelled (Generalized Additive Model) from overall protist community and nine broad taxonomic groups from operational taxonomic units gathered from 178 meadow soils in the Swiss western Alps.

	Edaphic										Topo-climatic					adj_R2
	Soil_temp (°C)	Rh (1/10000)	pH	EC (µS/cm)	P(%)	C/N	LOI (%)	Shale (%)	topos	Asp (%)	Slp (%)	tmean678 (°C * 100)				
Total	(+)	(+)	(-)												0.181	
Apicomplexa	(+)						+-								0.269	
Peronosporomycetes						+	(+)								0.113	
Phytomyxea					(+)	(+--)				++					0.164	
Sarcomonadea			--					(-)	(-)	++					0.161	
Tubulinea						-				+++					0.189	
Spirotrichea			-			(-)					---				0.133	
Chlorophyceae			+	+-	+										0.329	
Trebouxiophyceae				(+-)	++					+					0.252	
Diatomeae										(-)					0.057	

Note: The + and - signs show if the diversity is positively or negatively associated to the predictor and the number of signs inform on the strength of the association (between parenthesis: $p < .1$, one sign: $p < .05$, two signs: $p < .01$, three signs: $p < .001$). The ++ and +- indicate minimum and maximum of diversity at mid-predictor value respectively. Details of the response of each taxonomic group to the different variables can be found in Appendix S10.10.

Phagotrophs (e.g. Sarcomonadea & Tubulinea) and parasites (Apicomplexa) were the most abundant functional groups in terms of read abundance. Apicomplexan sequences, albeit numerous, were proportionally much less abundant and diversified than in Neotropical soils: as arthropods are less abundant and diversified in temperate regions, this brings further support to the hypothesis that soil apicomplexan communities mirror that of arthropods in the ecosystem (Mahé et al., 2017). Another abundant parasitic group is the Peronosporomycetes (until recently referred as Oomycota: Stramenopiles), which contains many plant parasites but also animal pathogens and a few free-living, saprotrophic forms (Beakes, Glockling, & Sekimoto, 2012; Lara & Belbahri, 2011). Peronosporomycetes are shown to be common and diverse in temperate soil systems (Seppey et al., 2017; Singer et al., 2016). By contrast, they are less abundant and diverse in neotropical forest soil ecosystems, where they comprise mostly animal parasites (Mahé et al., 2017).

Within phagotrophs, the high proportion of sequences from Cercozoa (mostly to Sarcomonadea) was in line with previous soil eukaryotic DNA surveys (Bates et al., 2013; Harder et al., 2016; Seppey et al., 2017). Furthermore, earlier studies based on microscopy observations showed the prevalence of these groups in soils (Adl & Gupta, 2006). Ciliates were also a well-represented phagotrophic group, and were dominated by Spirotrichea, which corroborates also other findings on soil protist molecular diversity (Lara, Berney, Ekelund, Harms, & Chatzinotas, 2007). In summary, the protist communities found in the Swiss western Alps were typical for temperate soil ecosystems and the findings can likely be extrapolated to other climatically similar regions. However, soil communities have been shown to differ in their composition in contrasted climates such as neotropical rainforests (Mahé et al., 2017); therefore, it can be expected that communities from desert, hypersaline soils and other such extreme ecosystems may differ in their structure and may also be controlled by other sets of predictors.

Our data are in accordance with previous studies related to the impact of edaphic variables on protist communities (Dupont et al., 2016; Ekelund, 1999; Foissner, 1997, 1999; Nesbitt & Adl, 2014; Singer et al., 2018) but we also show that topo-climatic predictors explain equally well soil protists distributions. Therefore, the method of measurement of the predictors (in situ for edaphic variables or remote sensing/modelling for topo-climatic) did not seem to affect our capacity to explain protist community distribution.

4.2 | Model fit and predictive power of topo-climatic and edaphic variables on protist diversity

Slope steepness and pH were the two variables most often found to significantly contribute to the fit of our different protist diversity models. Slope steepness affects drainage and leaching of nutrients and is generally inversely correlated to soil depth. Nevertheless, an enhanced drainage reduces the likelihood of water-logging, which would select for very specialized protists tolerating anoxia and generally would lead to lower diversity. Soil pH is well known as a major driver of microbial diversity, including protists (Bates et al., 2013;

Dupont et al., 2016; Shen et al., 2014) but also bacteria (Santoyo, Hernández-Pacheco, Hernández-Salmeron, & Hernández-Leon, 2017; Yashiro et al., 2016) and fungi (Noyce et al., 2016; Pellissier et al., 2014; Zhang, Jia, & Yu, 2016). The relationship between pH and protist diversity was significant only for three groups, being negative for two groups of phagotrophs (Spirotrichea and Sarcomonadida) and positive for Chlorophyceae. It is unclear whether these relationships reflect a direct effect of pH or rather indirect effects such as biotic effects (e.g. impact on bacterial or fungal food sources), the availability of nutrients for the growth of autotrophs (correlation coefficient between pH and EC = -0.14 ; $p = .055$), shifts in plant-microbial interactions and root exudate composition (Yashiro et al., 2018), or other drivers.

Predictability varies also to a large extent among taxonomic groups. Indeed, while many variables explained significantly the diversity of the three groups of phototrophs and phagotrophs, it was less so for parasites (Apicomplexa and Peronosporomycetes) particularly when only topo-climatic variables were taken into account (see Appendix S4.11). The latter functional group depends directly on the availability of host species and only indirectly on environmental values for which the influence will be indirect and the fraction of variance explained by these variables and their significance will thus be lower. Additionally, the contrasted predictive power among taxonomic groups may be due to the fact that certain taxa are better discriminated by metabarcoding than others because of differences in taxonomic resolution of the 18S rRNA gene. In our dataset, many OTUs assigned to Apicomplexa and Tubulinea were considered as undetermined as their identity with the best match in the database did not reach 80% (see Appendix S2.7). Nevertheless, while the diversity of Apicomplexa was poorly predicted in comparison with other taxa, the models predicted the diversity of Tubulinea with an accuracy that was comparable with other taxa. For 9 out of the 10 taxonomic group tested, the predictive power of the topo-climatic variables was either significantly better, or at least not different than the ones including the edaphic variables. Moreover, it was never lower than the predictive power of the models including both sets of variables. This suggests that, within the levels of predictability achieved, predictive models built solely on topo-climatic variables are as accurate, or possibly even better, than the models built with the addition of edaphic variables. These variables are available at large scales and are already largely used for modelling the spatial distribution of macroorganisms (Guisan & Zimmermann, 2000), to the contrary of local edaphic values that are always tedious and costly to measure in the landscape across large regions and environmental gradients. These findings open the way to larger sampling designs that could further increase the performance of models.

4.3 | Interpretation of the spatial patterns of protist diversity modelled with topo-climatic variables

As for macroorganisms (D'Amen, Pradervand, & Guisan, 2015; Dubuis et al., 2011; McCain, 2005; Reymond, Purcell, Cherix, Guisan, & Pellissier, 2013), and increasingly reported for other

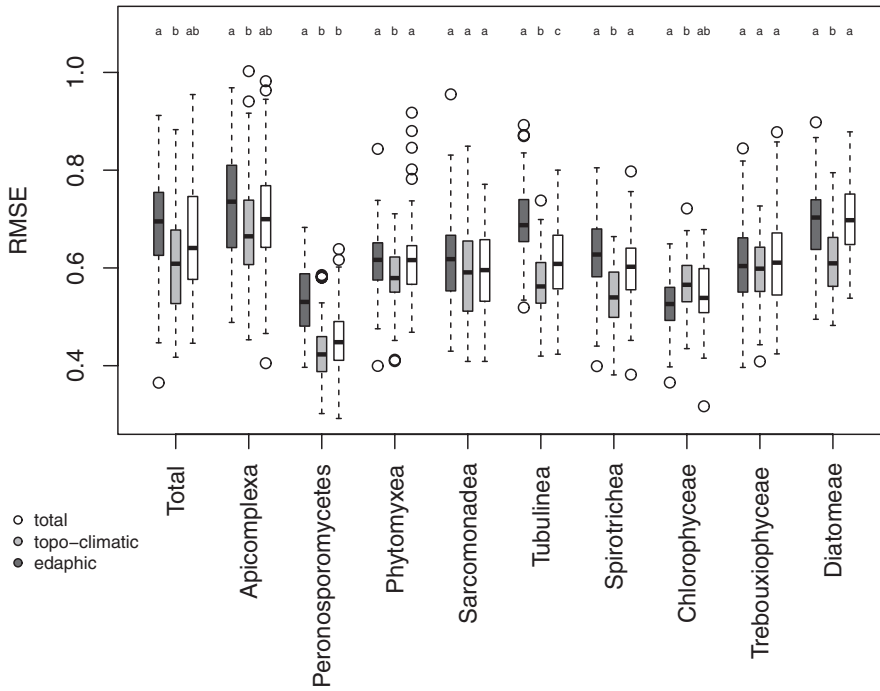


FIGURE 2 Predictive power (root mean square error: RMSE) of edaphic (dark grey), topo-climatic (pale grey) and overall (white) predictors calculated on the diversity of protist operational taxonomic units from the total community and nine broad taxa retrieved from 178 meadow soils in the Swiss western Alps. The RMSE were calculated on 100 cross validation of Generalized Additive Models performed with 20% of the samples as test dataset. The letters on the top of the barplot represent significantly different groups according to a multiple comparison mean rank sums test (Nemenyi test $p < .05$) for each of the total communities and nine broad taxa

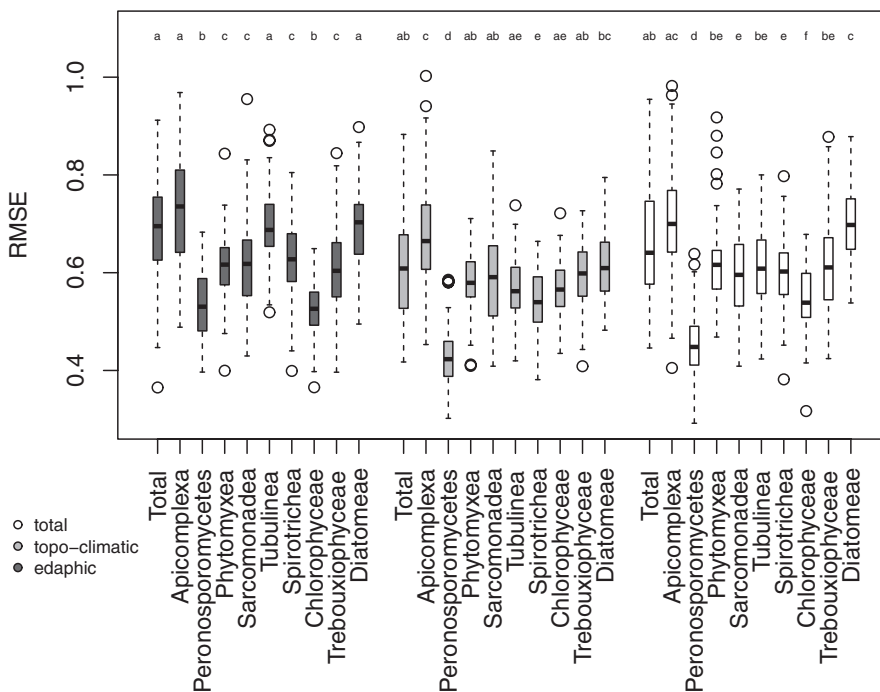


FIGURE 3 Predictive power (root mean square error: RMSE) of edaphic (dark grey), topo-climatic (pale grey) and overall (white) predictors calculated on the diversity of protist operational taxonomic units from the overall community and nine broad taxa retrieved from 178 meadow soils in the Swiss western Alps. The RMSE were calculated on 100 cross validation of Generalized Additive Models performed with 20% of the samples as test dataset. The letters on the top of the boxplots represent significantly different groups according to a multiple comparison mean rank sums test (Nemenyi test $p < .05$) for each of the edaphic, topo-climatic and overall variables

soil microorganisms (Geml, Morgado, Semenova-Nelsen, & Schilthuizen, 2017; Pellissier et al., 2014; Shen et al., 2019), protists diversity showed clear spatial and elevational patterns when only topo-climatic variables were taken into account to build the model (Figure 4). This pattern seemed to be driven by summer temperature in most cases (see Appendix S4.11 and Appendix S4.12), either in a positive (Diatomeae, Phytomyxea and Tubulinea), unimodal (Apicomplexa, Sarcomonadea and Spirotrichea) or negative way (Chlorophyceae, Peronosporomycetes). A positive correlation of diversity with temperature (and, thus, productivity) is a typical

pattern in macroecology that can be related to the species-energy hypothesis as long as moisture is not a limiting factor. This pattern has already been demonstrated for protist communities, more exactly testate amoebae (Fernández et al., 2016; Lara et al., 2016), a paraphyletic group which comprises, interestingly, many Tubulinea (Adl et al., 2019). Other related models for diversity patterns, like elevational gradients (Huston, 1994; see Spehn and Körner, (2009)) have been also shown in testate amoebae (Heger, Derungs, Theurillat, & Mitchell, 2016). On the other hand, if moisture is limiting, unimodal patterns are to be expected, and diversity

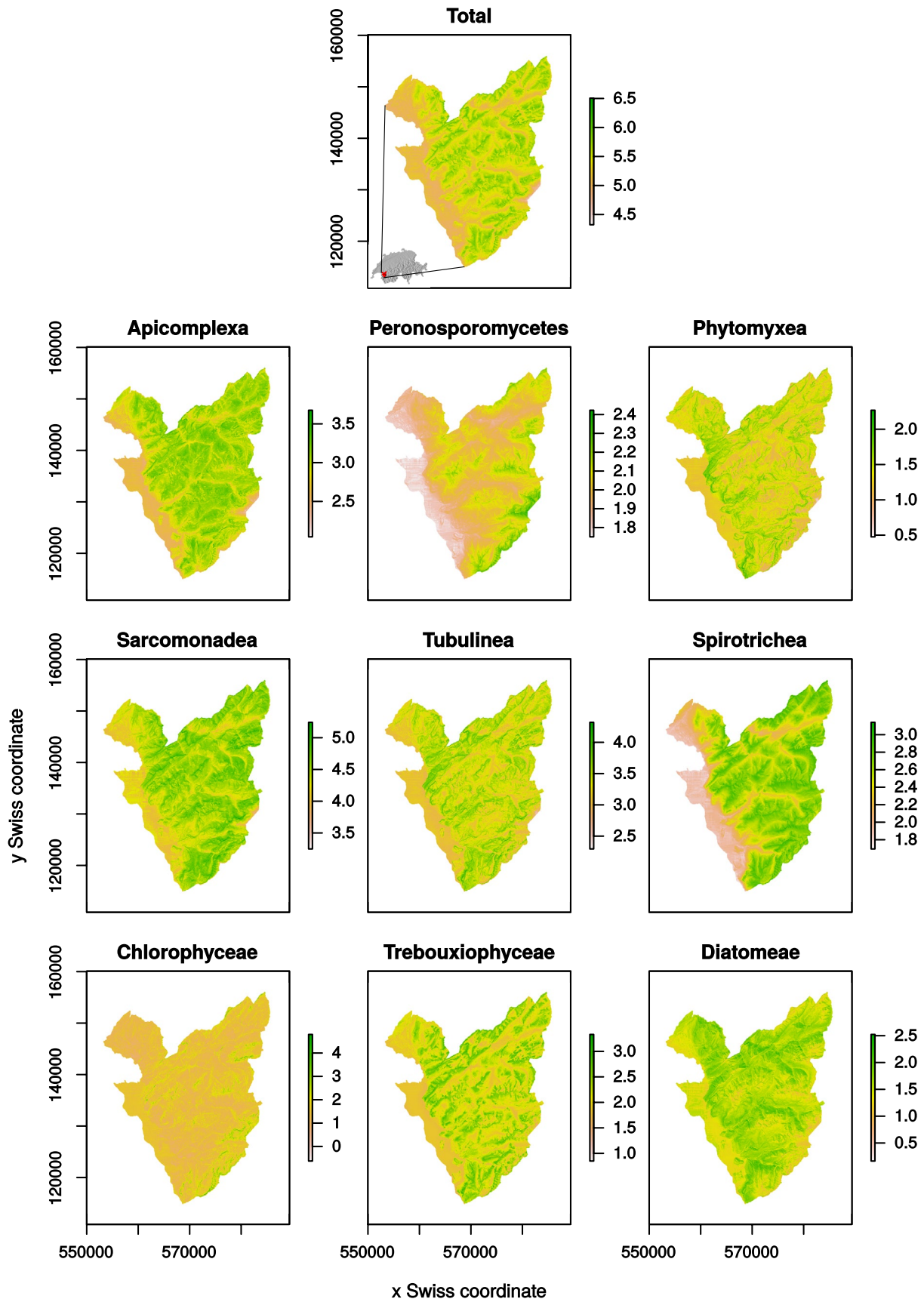


FIGURE 4 Diversity of the total protist community and nine broad taxa predicted from Generalized Additive Model through the Swiss western Alps based on the topography, slope southness, slope steepness and average temperature from June to August 2013 [Colour figure can be viewed at wileyonlinelibrary.com]

peaks where both moisture and energy are optimal (water energy model: Fernández et al. (2016)) intermediate disturbance hypothesis or mid-domain effect (discussed for the same area in Dubuis et al. (2011)). Finally, Chlorophyceae and Peronosporomycetes are typically sensitive to high temperatures and desiccation, both including often flagellated life stages for dispersal that needs at least a thin water film to disperse (Jeger & Pautasso, 2008). In addition, high diversity in Chlorophyceae in the lowest temperature zone (Figure 4, see Appendix S4.12) could be explained by the fact that micro-eukaryotic algae have a higher growth rate at low temperatures, favouring diversification in cold environments (Rose & Caron, 2007) or possibly reduced competition from vascular plants. However, while these patterns can be observed in some groups, they cannot be extended to the whole protist community; indeed, another study showed no significant correlation between elevation and diversity when considering entire microbial eukaryotic communities (Shen et al., 2014).

4.4 | Technical and methodological issues

The correspondence between OTUs and biological species has always been a hot topic in eukaryotic environmental microbiology. The V4 region of the gene coding for the RNA molecule of the small subunit of the RNA (SSU rRNA or SSU for short) has been listed among a handful DNA fragment for protist barcoding (Pawlowski et al., 2012). However, a single SSU rRNA gene sequence may include, in certain groups, a wide diversity of species with different lifestyles and ecological preferences. This has been shown for different soil protists such as ciliates (Lara & Acosta-Mercado, 2012). In contrast, in Myxomycetes (Amoebozoa), SSU sequences are truly hypervariable and discriminate relatively accurately between species; intragenomic polymorphism of SSU sequences has been even detected (Dahl et al., 2018), which may artificially inflate interpretations on environmental diversity. However, the accuracy of the estimation can be expected to increase with the narrowing of the taxonomic range of the investigated organisms, as evolutionary drivers become more homogeneous. In other words, comparing the diversity of, for example, Apicomplexans between two localities can be reasonably expected to be more accurate than comparing the whole eukaryotic diversity.

Computation of H indices includes quantitative data, classically the proportion of a given species in a given sample, which can be reasonably inferred by numbers of reads in high-throughput sequencing data. Indeed, there is a correspondence between this number of reads and the biovolume (calculated from simple measurements of the cell and assuming geometrical shapes; Charrière et al., 2006) of individual organisms that has been shown for many groups of protists (Giner et al., 2016; Kosakyan, Mulot, Mitchell, & Lara, 2015). de Vargas et al. (2015) showed a linear relationship between the logarithm of organisms' length versus the logarithm of 18S rRNA copy numbers (see Supplementary figure W4 in that article). Thus, H indices provide a satisfactory cell quantification based on sequence data in species that display a stable rDNA

copy number (Rodríguez-Martínez et al., 2009). Nevertheless, if an organism violates the correspondence by inducing more reads per biovolume, its presence in a sample would be translated by OTUs covering a large proportion of the community, which would result in lower diversity. For example, Foraminifera are particularly prone to biases in inferring the abundance from rDNA sequences due to alternation of generation, variation in ploidy and variation in number of nuclei (Weber & Pawlowski, 2013). Similar biases have been shown for ciliates, which are known for having highly polyploid macronuclei, and sometimes smaller species may have higher rDNA copy number than larger cells (Dunthorn, Stoeck, Clamp, Warren, & Mahe, 2014). To avoid such biases, the sequence abundance of each species needs to be normalized by rDNA copy number. However, such an approach requires a previous characterization of the species rRNA genes, and therefore cannot be applied to unknown biodiversity. It is therefore important to keep this in mind when assessing the diversity of groups with a heterogeneous number of reads per biovolume. Future studies should assess if new normalization approaches could be applied to such data, and how it could impact the type of findings reported here.

In some cases, even when avoiding collinearity, it is possible that some edaphic and topo-climatic predictors still depend on each other (e.g. soil temperature and $t_{mean678}$; Yashiro et al., 2016 see Appendix S1.2). Nevertheless, the comparison between two dependent variables coming from different measurement methods (in situ for edaphic and remote sensing for topo-climatic predictors respectively) is still interesting because we aim to assess if topo-climatic predictors achieve at least as accurate models as edaphic ones.

5 | CONCLUSION

We showed that the diversity of some taxa belonging to major functional groups in the Swiss western Alps was explained up to >30% by topo-climatic and edaphic conditions. A somewhat surprising result was that topography and climate predicted protist diversity as well or better than the edaphic variables more commonly used in soil microbial studies. This implies that soil protist diversity patterns could be at least partly inferred, for some groups (e.g. Chlorophyceae) and to some extent (22%), based on topo-climatic spatial models only. The applicability of spatial modelling of protists diversity to soil under other climates than temperate is still to be established. Nevertheless, considering that spatial patterns of microorganisms is increasingly recognized, it is likely that spatial modelling will become a powerful tool in microbial ecology in the near future.

Such an approach could be applied at finer taxonomic levels to predict the distribution of individual species, which would be of high socio-economic relevance in the case of invasive agricultural or forestry pests of economic importance such as certain Peronosporomycetes. The models could be improved by refining the taxonomic groups, as taxa responding more homogeneously to environmental conditions may show stronger correlation with

abiotic variables than the broad group classification we used. For instance, the Peronosporomycetes contain organisms belonging to other functional groups than parasites (e.g. saprotroph; Beakes et al., 2012; Lara & Belbahri, 2011) or able to target a wide range of hosts (e.g. *Phytophthora cinnamomi*; Hardham, 2005). Therefore, other modelling techniques, such as calculating the diversity after modelling the abundance of individual OTUs and stacking then (i.e. stacked-SDMs; Guisan & Rahbek, 2011), could enhance the predictive power on certain taxa. These improvements would pave the way towards extrapolation of protists diversity across large spatial scales and provide useful tools to identify biodiversity hotspots, predict spatially the risk of pathogen infection or model soil protist diversity according to future environmental change scenarios.

ACKNOWLEDGEMENTS

The authors would like to thank all fields and laboratory assistants and technicians who participate in the alpine soil project, with a particular thank to Amandine Pillonel, Laura Desponds and Dessislava Savova Bianchi for the laboratory work. Special thanks to the Transports Publics du Chablais and Glacier 3000 (<https://www.glacier3000.ch/en>) who provide their installations for free to carry the soils from the sampling sites. We would also like to thank all anonymous farmers who allowed sampling on their lands. The study was funded by the Swiss National Science Foundation under the projects 310003A 143960 to E.L., 31003A-152866 (SESAM'ALP), PDFMP3-135129 (MICROBIAL BIOGEOGRAPHY) and CR2312-162754 (INTEGRALP) to A.G. and P2NEP3-178543 to D.S., as well as the internal funding of the Universities of Neuchâtel and Lausanne. E.L. would also like to thank the program 'Atracción de talentos' from the Community of Madrid project 2017-T1/AMB-5210 and the project MYXOTROPIC VI (PGC2018-094660-B-I00) from the Spanish Government. E.Y. also thanks the European Community FP7-PEOPLE-2010-IIF program (MP-Alps, grant agreement 273965), the Agassiz Foundation, and the Pro-Femmes Fellowship program from the Faculty of Biology and Medicine of the University of Lausanne. C.V.W.S. also thanks the Norwegian Research Council projects 270252 (BiodivERSA-Climatic change impacts on Arctic soil and lake microbiomes) and 256132 (ERAnet-LAC, METHAnogenic Biodiversity and activity in Arctic and Sub-Antarctic Ecosystems affected by climate change). A significant part of the computations was also performed on resources provided by the Calculations Center of the Faculty of Science of the University of Neuchâtel and by UNINETT Sigma2 – the National Infrastructure for High Performance Computing and Data Storage in Norway project NN9579K.

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BIOSKETCH

The Laboratory of Soil Biodiversity (<https://www.unine.ch/biol-sol>), led by Prof. Edward A.D. Mitchell, is interested in the diversity, biogeography and ecology of soil organisms with a strong focus on protists and links to other soil organisms and ecosystem ecology. The lab combines observational and experimental studies leading to applications in biomonitoring, palaeoecology, ecotoxicology and forensic sciences. The Spatial Ecology Group (<http://www.unil.ch/ecospat>), led by Prof. Antoine Guisan, is specialized in spatial modelling of biodiversity at the levels of species, communities and ecosystems. Models are applied to the conservation of endangered species, the management of biological invasions and the assessment of global change impact on biodiversity, with a special and long-term focus on above- and below-ground biota in the Western Swiss Alps.

Author contributions: E.Y., E.A.D.M., H.N.H., A.G. and E.L. conceived the idea; E.Y., E.A.D.M., A.G. and E.L. provided the funding; A.B., E.Y., E.P.F. and A.G. collected the data; A.B., E.Y., E.P.F., D.S., Q.B. and E.L. performed the laboratory work; C.V.W.S., O.B., A.B., A.G. and E.L. analysed the data and C.V.W.S., O.B., E.Y., D.S., Q.B., E.A.D.M., A.G. and E.L. wrote the manuscript. All authors gave final approval for publication.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Seppey CVW, Broennimann O, Buri A, et al. Soil protist diversity in the Swiss western Alps is better predicted by topo-climatic than by edaphic variables. *J Biogeogr.* 2020;47:866–878. <https://doi.org/10.1111/jbi.13755>