

Niche conservatism drives the elevational diversity gradient in major groups of free-living soil unicellular eukaryotes

Leonardo D. Fernández (✉ limnoleo@gmail.com)

Universidad Bernardo O'Higgins and University of Neuchâtel <https://orcid.org/0000-0001-9550-1921>

Christophe V. W. Seppey

University of Neuchâtel and UiT The Arctic University of Norway

David Singer

University of Neuchâtel, University of São Paulo and Université d'Angers

Bertrand Fournier

University of Potsdam

Dylan Tatti

Bern University of Applied Sciences BFH

Edward A. D. Mitchell

University of Neuchâtel

Enrique Lara

University of Neuchâtel

Research Article

Keywords: microbial biogeography, microbial macroecology, mid-domain effect, protists, Rapoport effect, species–energy effect

DOI: <https://doi.org/10.21203/rs.3.rs-133244/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Ancestral adaptations to warm and humid climates drive the biogeographical and macroecological patterns of numerous multicellular organisms. Recent evidence suggests that this niche conservatism may also be shaping broad-scale diversity patterns of soil unicellular organisms, although empirical evidence is limited to Acidobacteria and testate amoebae. Herein, we tested the predictions of this hypothesis for five major soil protist groups (Bacillariophyta, Cercomonadida, Ciliophora, Euglyphida and Kinetoplastida), separately, as well as combined, along an elevational gradient in Switzerland. We found support for the predictions of this hypothesis in all protist groups, including decreasing diversity and increasing geographical ranges towards high and cold elevations (Rapoport effect); correlations between diversity and temperature (species-energy effect); and communities phylogenetically structured by competition (phylogenetic overdispersion) at warm-humid sites and habitat filtering (phylogenetic clustering) at cold-humid sites. Mid-domain null models confirmed that these findings were not the result of stochastic processes. Our results therefore suggest that soil protists exhibit evolutionary constraints to warm and humid climates, probably linked to an ancestral adaptation to (sub)tropical-like environments, which limits their survival in exceedingly cold sites. This niche conservatism possibly drives their biogeographical and macroecological patterns both at the local (e.g., temperature, humidity gradients along elevation gradients) and more global (e.g., latitudinal gradients) spatial scales.

Introduction

Biological diversity varies over spatial gradients, giving rise to biogeographical and macroecological diversity patterns [1]. Diversity patterns are considered to result from a combination of ecological processes (e.g., habitat filtering, competition) and historical contingencies (e.g., climatic stability, diversification), which together influence the spatial variation in speciation, extinction and dispersal rates [2].

Phylogenetic niche conservatism (PNC) is a holistic, parsimonious and increasingly accepted hypothesis that integrates the processes involved in the formation of diversity patterns. This hypothesis proposes that taxa retain their ecophysiological traits (e.g., thermal tolerance) over evolutionary time and that, therefore, they tend to remain in environments that exhibit the abiotic conditions within which they evolved [3]. This evolutionary trend has been proposed to explain why most modern taxa fail to disperse into new environments, offering an underlying explanation to the diversity patterns that we observe today in nature [4–6].

The PNC offers testable predictions that together can shed light on the relative role played by ecological processes and historical contingencies in shaping diversity patterns. The basic prediction of this hypothesis is that diversity will peak in warm and moist (optimal) sites [7, 8], because they mirror the tropical-like environmental conditions within which most modern plants and animals originated [9]. Accordingly, this hypothesis also predicts that diversity will decline towards exceedingly hot or cold (suboptimal) sites because the ancestral adaptation of modern taxa to moist (sub)tropical-like

environments imposes a strong constraint on their ability to tolerate extremely high or low temperatures [3]. Others predictions of the PNC include a positive correlation between diversity and temperature [4, 5, 7], an increase in the size of taxa distribution ranges towards the colder end of an environmental gradient [10, 11], giving place to a macroecological pattern known as the Rapoport effect [12]; and local communities structured by competition in warm sites and structured by habitat filtering in cold sites [3, 13].

These predictions have been extensively tested in multicellular organisms, proving useful to explain their diversity patterns in terms of both ecological processes and historical contingencies (e.g., [4–7]). By contrast, these predictions have been investigated in few studies dealing with unicellular organisms, including bacteria (e.g., [8, 14, 15]) and protists such as testate amoebae [11]. Thus, many biogeographical and macroecological generalizations proposed for plants and animals have not yet been sufficiently tested for microorganisms. Accordingly, we still do not know whether multicellular and unicellular diversity patterns are produced and maintained by similar processes [16, 17]. This gap in knowledge is even more serious for free-living soil eukaryotic microorganisms (soil protists), since most research on microbial biogeography and macroecology has been traditionally conducted on prokaryotes, fungi or aquatic protists [18]. Unravelling the processes that originate and maintain the spatial distribution of soil protist diversity could therefore contribute to filling gaps in knowledge and to the construction of ecological and evolutionary generalization for all forms of life on Earth.

Soil protists represent a good model to test the predictions of the PNC hypothesis. Research suggests that, just like several groups of plants and animals, they also seem to retain their ecophysiological traits over evolutionary time [11, 19]. Particularly, it appears that their current need for warm and humid climates is well-rooted in their evolutionary history. While the origin of the first eukaryotic microorganisms probably dates back to the Proterozoic (e.g., [20]), fossil evidence available (e.g., [21, 22]), and time estimates from molecular clock studies (e.g., [23, 24], suggest that at least some extant terrestrial (soil) protist taxa evolved under the warm and humid terrestrial environments that characterized much of the Mesozoic [9]. The climatic conditions under which they possibly evolved seem to have imposed evolutionary constraints on their ability to adapt to regions with exceedingly hot or cold temperatures, thus influencing their current diversity patterns, performance and fitness. Indeed, nowadays, soil protists exhibit peaks of diversity in warm and humid environments, such as temperate and tropical rain forests [25, 26] and declines in diversity towards hot [27] and cold [28] deserts. Soil protist biogeography is usually predicted either by temperature [26], water [29] or both variables (a water-energy balance, [11]). Experimental evidence also shows that soil protists exhibit higher performance and fitness (e.g., they have high survival, growth and reproductive rates) at warm (ca. 18 and 24 °C) than at exceedingly cold or hot temperatures (e.g., [30, 31]). Taken together, these facts suggest that most of them lack the necessary ecophysiological adaptations to survive and reproduce in exceedingly cold or hot environments. Probably, the retention of their ancestral adaptation to warm and humid climates imposes strong constraints on their ability to colonize sites with extreme temperatures and drives their current biogeographical and macroecological patterns on Earth.

Herein, we tested the predictions of the PNC hypothesis for the elevational diversity gradient (see Table 1) on five major soil protist groups: Bacillariophyta, Cercomonadida, Ciliophora, Euglyphida, Kinetoplastida and a group combining all these taxa (i.e., “the soil protist group”). To do so, we used a high-throughput sequencing approach to test for correlations between their richness, elevation and temperature (plus other environmental variables); as well as to investigate their elevational patterns in range distribution and phylogenetic community structure along a humid but increasingly cold elevational gradient of beech forests in Switzerland. We selected the above-mentioned soil protist taxa because (1) they are frequent and diverse in soils, (2) they have different life history strategies and functional roles, and (3) they are well distributed across the eukaryotic tree (Geisen et al. 2018). Our rationale behind that choice was to be able to generalize conclusions to as many soil eukaryotes as possible.

Material And Methods

Study Site and Sampling Strategy

We collected soil samples in ten permanent plots of the Swiss Biodiversity Monitoring program BDM (<http://www.biodiversitymonitoring.ch/en/home.html>). The plots were selected in beech forests (*Fagus sylvatica* L.) which are among the few natural beech-dominated forests remaining in Europe [32]. The plots are distributed across Western Switzerland and range from 458 to 1,308 m a.s.l. (Fig. 1). The climate of the study area is maritime temperate with an average annual precipitation and temperature of 1,200 mm and 8.3 °C, respectively [32].

At each forest, we randomly collected three soil cores at a depth and diameter of 5 cm at the periphery of the area used for repeated vegetation survey as part of the BDM monitoring program. The three cores were then pooled to obtain a representative soil sample of the site. The samples were kept cool during transport and DNA was extracted in the laboratory immediately after sampling.

Characterization of Soil Protist Communities

DNA extraction, PCR, sequencing (Illumina, targeting the SSU rRNA gene V9 region of eukaryotes) and subsequent taxonomic assignment of the obtained reads followed [17] (as described in Appendix S1). We randomly subsampled 50,000 reads from each site to account for unequal sample sizes between soil protist communities. From these subsamples, we selected those assigned to Bacillariophyta, Cercomonadida, Ciliophora, Euglyphida and Kinetoplastida. We also combined all the reads assigned to the abovementioned taxa into a group herein referred to as the “soil protist group”. Species accumulation curves confirmed that sampling effort and sequencing covering were enough to record a significant proportion of the total OTU richness found at each sampling site (Appendix S1: Fig. S1). We defined a community as all OTUs originating from a single beech forest sample. The taxonomic richness within each community was calculated as the total number of OTUs within each community. This diversity metric was correlated to phylogenetic diversity (Appendix S1: Fig. S2).

Assessment of the Predictions of the PNC Hypothesis

Table 1 summarises predictions that can be drawn and test from the PNC, which proposes that climatic conditions within which a taxon evolved (i.e. warm and humid conditions for most modern taxa) should be conserved in the present [3]. These predictions stem from biogeographical and macroecological mechanisms that once were independently proposed to explain the occurrence of biodiversity patterns. These predictions are valid for temperate elevational gradients, which often exhibit increasing precipitation and decreasing temperature with elevation [33].

Species-energy Effect

This mechanism predicts correlations between temperature and biological diversity [34]. At the study site temperature decreases and precipitation increases with elevation [31, 35]. It is thus expected that soil protist diversity will decrease with elevation (Prediction 1.1.1.) and show a positive relationship with temperature (Prediction 1.1.2.). To test these predictions, we standardised OTU richness (as described in Appendix 2) to avoid sampling biases introduced here by the unequal distribution shown by the beech forests sampled along the elevational gradient investigated. Then, we estimated the average annual temperature based on the average annual temperature reported for the lowest sampling site (10 °C, [35]) and assuming a moist adiabatic lapse rate of 0.6 °C for each 100-m increase in elevation [33]. Finally, we constructed linear and quadratic models using richness as response variable and both the elevation and the average annual temperature as descriptor variables. The best-fitting model was selected based on the Akaike's information criterion (AIC) for each model. We also assessed the role played by other environmental variables, such as humidity, pH, humus content, etc. (as measured by Landolt's indicator values). However, we did not record any correlation between these variables and richness (Appendix S3).

Elevational Ranges of Distribution

Climate becomes harsher with altitude, and thus habitat filtering plays a major role in the distribution of diversity on mountainsides [7]. If soil protists have evolutionary constraints to adapt to cold climates, we predict that they will exhibit a Rapoport effect [12]; i.e., their distribution ranges will become broader with elevation because colder temperatures at higher sites will select a few eurythermal taxa with high dispersal capability towards the mountaintop (Prediction 2.1.1.). By contrast, if thermal constraints (and thus habitat filtering) do not drive soil protists distribution in the humid but increasingly cold elevational gradient studied, we predict that they will exhibit a mid-domain effect [36]; i.e., their distribution ranges will be randomly distributed within the lower and upper (hard) limits of the elevational gradient studied resulting in a unimodal diversity gradient (Prediction 2.1.2.). The Rapoport effect was tested using Pearson's correlations to assess the relationship between the mean elevational range size of all OTUs co-occurring in the same forest site and elevation. The unimodal diversity curve predicted by the mid-domain effect and its confidence intervals were computed using the Mid-Domain Null program [7] based on 50,000 Monte Carlo simulations sampled without replacement from empirical taxa-range-size distributions.

Niche-based Processes

We also explored the existence of a constrained evolutionary response to temperature by assessing the role of biotic interactions and habitat filtering in community structuring. If soil protists do not have a constrained evolutionary response to cold temperatures, then their communities will be structured by biotic interactions and composed by distantly related taxa in the study site (phylogenetic overdispersion, Prediction 3.1.1.). By contrast, if they cannot cope with decreasing temperature, then their communities will be structured by habitat filtering, since cold temperatures will only favour the occurrence of closely related taxa with broad thermal tolerances (phylogenetic clustering, Prediction 3.2.1.). The existence of phylogenetic overdispersion/clustering was tested using the standardised version of both the mean pairwise distance and the mean nearest taxon distance indices (i.e., $-NRI$ and $-NTI$, respectively) [37]. Negative values of these indices denote phylogenetic clustering, whereas positive values indicate phylogenetic overdispersion. Both indices were computed using the R package *picante* [37] against a trial swap null model (999,000 swaps).

Results

Cercomonadida (373 OTUs: 12,437 reads) and Ciliophora (582 OTUs: 15,599 reads) were the most diverse and abundant heterotrophic taxa at each forest site, followed by Kinetoplastida (223 OTUs: 10,377 reads) and Euglyphida (157 OTUs: 8,058 reads). The phototrophic taxon Bacillariophyta was the less diverse and abundant (78 OTUs: 923 reads) (Appendix S1: Fig. S3). So, the taxon combining all these taxa (i.e., the soil protist group) represented 1,413 OTUs and 47,394 reads along the elevational gradient (Appendix S4).

Species-energy Effect

The diversity of all taxa showed a negative correlation with elevation and a positive correlation with average annual temperature (Table 2), supporting the expectations of the species-energy effect (Predictions 1.1.1. and 1.1.2.).

Elevational Ranges of Distribution

The size of the mean elevational range of all taxa exhibited a positive correlation with elevation, conforming to a Rapoport effect (Fig. 2, Prediction 2.1.1.). We did not find support for a mid-domain effect (Fig. 3, Prediction 2.2.1.). This outcome confirmed the role of habitat filtering in structuring soil protist communities along the elevational gradient investigated.

Niche-based Processes

The assessment of the phylogenetic structure within communities yielded different results among taxa (Fig. 4). The soil protist group exhibited local communities of both distantly and closely related taxa over the elevational gradient. This outcome did not support any of the predictions proposed (see Table 1). However, Bacillariophyta only exhibited local communities of distantly related taxa, which agreed with

prediction 3.1.1. The remaining taxa were represented by local communities of closely related taxa in the study site. This result supported prediction 3.2.1.

Discussion

Previous research (e.g., [26, 29, 38] and the present study support the idea that soil protist diversity increases at warm (and humid) sites. However, the novelty of our study is that we also provide evidence supporting the idea that this temperature-diversity relationship (or species-energy effect) might have an evolutionary origin rooted in the conservatism of ancestral regimes rather than on present-day thermal conditions. Here, we explored the phylogenetic signal for this thermal niche conservatism among distantly related soil protists by also assessing their elevational patterns in range-size distributions and their within-community phylogenetic structure over an elevational gradient.

In our study, average annual temperature was identified as the main driver of the decline in soil protist diversity with increasing elevation. This result was consistent with a species-energy effect, a climatically based mechanism proposed to explain the occurrence of diversity patterns in nature [34]. The species-energy effect often proposes temperature as a proxy of ambient energy and as the main driver of biodiversity patterns [34]. That prediction is true in places where temperature is at or below the tolerance range for life [39], such as on the way to mountaintops [7]. In mountain slopes, temperature decreases ~ 0.6 °C for each 100-m increase in elevation [33], a trend that also applies to the elevational gradient studied herein. Since our lowest and warmest sampling site exhibits an average annual temperature of 10 °C [35], a temperature value that is already below the thermal optimum for most protists [30, 31, 40], it is obvious that environmental conditions, including temperature, become harsher for soil protists towards the top of the elevational gradient investigated despite the increase in precipitation along the gradient.

The abovementioned species-energy effect (as measured by the average annual temperature) does not prevent site-specific (local) abiotic factors to shape soil protist diversity. Indeed, soil protist diversity has been shown to vary in relation to nutrient availability, pH and conductivity, among others local abiotic factors [18]. However, these factors seem to be more relevant at fine spatial scales, or microbial cultures [41, 42]. By contrast, climatic conditions seem to explain better the diversity patterns at broad-spatial scales, which are relevant for soil protist biogeography and macroecology [11, 17, 26, 29]. In fact, local abiotic factors often lose power to predict diversity patterns at increasing spatial scales [1]. This is mainly because the factors influencing diversity gradients are scale dependent, and thus, explanations vary with the spatial scope of the analysis (extent) and with sample resolution (grain) [43]. Also, climatic conditions ultimately regulate local abiotic conditions, explaining why climate often arises as the main driver of biogeographical and macroecological patterns of eukaryotic organisms [1], including soil protists [17, 29]. Nevertheless, some studies also highlight the joint role of local abiotic factors and climate in shaping elevational gradients in soil protist diversity (e.g., [44, 45]. However, as these studies did not control for confounding effects of local abiotic conditions, the relative contribution of local and climatic drivers in shaping soil protist diversity could not be determined.

In this study, we did control for confounding effects of local abiotic conditions since we always sampled along an elevational gradient of beech-dominated forests. Beech and other dominant trees homogenize soil properties and change their physicochemical characteristics (pH, conductivity, organic matter, etc.) [46, 47]. This contributes to reducing the effect of site-specific abiotic factors in driving soil protist diversity along elevational gradients [38, 48]. This could explain why local abiotic factors had little, or no predictive power as compared to temperature (species-energy effect) to explain protist diversity patterns.

Water, either alone [29] or in conjunction with temperature [11], is another driver of soil protist diversity across broad spatial scales. However, the role of water as a predictor of biodiversity varies spatially, becoming more important in sites where it is scarce [39]. In fact, water does not predict soil protist diversity in wet environments [49]. We did not record any relationship between humidity and soil protist diversity. So, as we expected, water was not a limiting factor at the study site. Indeed, precipitation (another proxy of water availability) increases along the elevation gradient investigated [50], while our results show that protist diversity tends to decrease. So, while temperature is really putting the fitness of protists to the test, water availability seems to be high enough (at least on average despite the regular occurrence of drought periods in summer) to not limit their physiological functions in the study site.

The assessment of the elevational patterns in soil protist range-size distributions suggested that the species-energy effect observed might be ultimately driven by a thermal niche conservatism. Indeed, all soil protist taxa investigated exhibited a Rapoport effect depicted by a progressive increase in their distribution ranges from lower and warmer sites to higher and colder sites. The occurrence of a Rapoport effect has been related to the existence of thermal evolutionary constraints that prevent multicellular organisms [10, 51] and soil protists [11] from adapting to and eventually colonizing areas with severe, often exceedingly cold (or hot) climates. So, the observation of a Rapoport effect suggests that, in general, soil protists lack ecophysiological traits to overcome the low temperatures that predominate at higher elevational sites over the year. Indeed, the low diversity recorded at higher and colder sites, coupled with the occurrence of a Rapoport effect supports the idea that few soil protist taxa can physiologically overcome the lower temperatures of higher sites. Probably at higher and colder elevations, habitat filtering processes select comparatively few eurythermal protists, which, in turn, represent cases of recent adaptation to exceedingly cold temperatures.

In addition to assessing the elevational patterns in soil protist range-sizes distributions, we also investigated their within-community phylogenetic structure to track the existence of a thermal niche conservatism. The soil protist group that combines all the taxa investigated exhibited both phylogenetically clustered and phylogenetically over-dispersed communities over the elevational gradient. This suggests that, overall, the soil protist group is represented both by taxa with broad and narrow thermal tolerances. The analyses performed on individual taxonomic groups, confirmed the above-mentioned outcome. Bacillariophyta, the only phototrophic taxon included in our study, exhibited phylogenetic overdispersion among co-occurring representatives and, therefore, communities structured by biotic processes [13, 37]. This outcome suggests that Bacillariophyta have broader thermal tolerances than the heterotrophic soil protist taxa investigated. Indeed, phototrophic protists exhibit higher

reproductive rates than heterotrophic protists at cold temperatures [30, 31, 40]. Given that the temperature range recorded at our study sites seems not to play an important role on the structuring of Bacillariophyta communities, we hypothesize that habitat filtering (as measured by the average annual temperature) possibly plays a pivotal role in the assembly of Bacillariophyta communities at higher and colder elevations than those surveyed at our study site (and thus not in beech forests). Probably, at our study site, competition for sunlight is the most important biotic process in the assembly of their local communities, since spotlights are scarce and unevenly distributed in forests. Future research performed over broader elevational ranges are needed to test our hypothesis. By contrast, Cercomonadida, Ciliophora, Euglyphida and Kinetoplastida (all of them heterotrophic taxa), exhibited phylogenetic clustering among their co-occurring representatives. This result confirmed that these taxa exhibit local communities structured by habitat filtering over the elevational gradient investigated [13, 37]. Therefore, heterotrophic soil protists exhibit a narrower thermal tolerance than Bacillariophyta to the monotonic decrease in temperature observed in the study site.

Conclusion

Herein, we have shown that diversity patterns in soil protists such as Bacillariophyta, Cercomonadida, Ciliophora, Euglyphida and Kinetoplastida, could be explained through the phylogenetic niche conservatism. In particular, the evidence suggests that most soil protists exhibit evolutionary constraints to temperature, which imposes strong restrictions on their ability to colonize sites with extreme temperatures. This thermal constraint might also be driving their current biogeographical and macroecological patterns on Earth and might be the reason why temperature often arises as an important predictor of soil protist diversity over latitudinal and elevational gradients. Considering that the phylogenetic niche conservatism has contributed to successfully explain the occurrence of diversity patterns in plants and animals, we could also state that our study contributes with additional evidence to demonstrate that eukaryotic multicellular and unicellular diversity patterns might be produced and maintained by similar processes. Our findings contribute thus to generalizing broad evolutionary mechanisms to the whole domain Eukarya and, arguably, to all life on Earth.

Declarations

Funding: L.D.F. was supported by ANID (grants 21110037 and 78130011) and ANID-FONDECYT (project 11170927); C.V.W.S by the University of Neuchâtel and the Norwegian Research Council (projects 256132 and 270252), D.S. by the Swiss National Science Foundation (P2NEP3 178543) and E.A.D.M. by the Swiss Federal Office for the Environment (FOEN) to cover sampling and sequencing costs.

Conflicts of interest/Competing interests: The authors declare that there is no conflict of interest or competing interests

Availability of data and material: We provide all complementary and relevant information as supplementary material

Code availability: Not applicable

Authors' contributions: L.D.F. conceived the idea and wrote a first draft. L.D.F, C.V.W.S, D.S., B.F., E.L., D.T. performed the statistical and/or molecular analyses. E.A.D.M. provided funding. All authors contributed to write the final version of this manuscript

References

1. Gaston K, Blackburn T (2008) Pattern and process in macroecology. John Wiley & Sons
2. Mittelbach GG, Schemske DW, Cornell HV et al (2007) Evolution and the latitudinal diversity gradient: speciation, extinction and biogeography. *Ecol Lett* 10:315–331. <https://doi.org/10.1111/j.1461-0248.2007.01020.x>
3. Wiens JJ, Ackerly DD, Allen AP et al (2010) Niche conservatism as an emerging principle in ecology and conservation biology. *Ecol Lett* 13:1310–1324. <https://doi.org/10.1111/j.1461-0248.2010.01515.x>
4. Dorey JB, Groom SVC, Freedman EH et al (2020) Radiation of tropical island bees and the role of phylogenetic niche conservatism as an important driver of biodiversity. *Proceedings of the Royal Society B: Biological Sciences* 287:20200045. <https://doi.org/10.1098/rspb.2020.0045>
5. Li F, Shao L, Li S (2020) Tropical Niche Conservatism Explains the Eocene Migration from India to Southeast Asia in Ochyroceratid Spiders. *Syst Biol* 69:987–998. <https://doi.org/10.1093/sysbio/syaa006>
6. Su X, Shrestha N, Xu X et al (2020) Phylogenetic conservatism and biogeographic affinity influence woody plant species richness–climate relationships in eastern Eurasia. *Ecography* 43:1027–1040. <https://doi.org/10.1111/ecog.04839>
7. McCain CM (2009) Global analysis of bird elevational diversity. *Glob Ecol Biogeogr* 18:346–360. <https://doi.org/10.1111/j.1466-8238.2008.00443.x>
8. Choudoir MJ, Buckley DH (2018) Phylogenetic conservatism of thermal traits explains dispersal limitation and genomic differentiation of *Streptomyces* sister-taxa. *The ISME Journal* 12:2176–2186. <https://doi.org/10.1038/s41396-018-0180-3>
9. Evolution of Terrestrial Ecosystems Consortium, Behrensmeyer AK, Damuth JD et al (1992) *Terrestrial Ecosystems Through Time: Evolutionary Paleoecology of Terrestrial Plants and Animals*. University of Chicago Press
10. Moreno RA, Rivadeneira MM, Hernández CE et al (2008) Do Rapoport's rule, the mid-domain effect or the source–sink hypotheses predict bathymetric patterns of polychaete richness on the Pacific coast of South America? *Glob Ecol Biogeogr* 17:415–423. <https://doi.org/10.1111/j.1466-8238.2007.00372.x>
11. Fernández LD, Fournier B, Rivera R et al (2016) Water–energy balance, past ecological perturbations and evolutionary constraints shape the latitudinal diversity gradient of soil testate amoebae in south-western South America. *Glob Ecol Biogeogr* 25:1216–1227. <https://doi.org/10.1111/geb.12478>

12. Stevens GC (1989) The Latitudinal Gradient in Geographical Range: How so Many Species Coexist in the Tropics. *Am Nat* 133:240–256. <https://doi.org/10.1086/284913>
13. Webb CO, Ackerly DD, McPeck MA, Donoghue MJ (2002) Phylogenies and Community Ecology. *Annu Rev Ecol Syst* 33:475–505. <https://doi.org/10.1146/annurev.ecolsys.33.010802.150448>
14. Bryant JA, Lamanna C, Morlon H et al (2008) Microbes on mountainsides: Contrasting elevational patterns of bacterial and plant diversity. *Proc Natl Acad Sci USA* 105:11505. <https://doi.org/10.1073/pnas.0801920105>
15. Andam CP, Doroghazi JR, Campbell AN et al (2016) A Latitudinal Diversity Gradient in Terrestrial Bacteria of the Genus *Streptomyces*. *mBio* 7:e02200–e02215. <https://doi.org/10.1128/mBio.02200-15>
16. Geisen S, Mitchell EAD, Wilkinson DM et al (2017) Soil protistology rebooted: 30 fundamental questions to start with. *Soil Biol Biochem* 111:94–103. <https://doi.org/10.1016/j.soilbio.2017.04.001>
17. Seppely CVW, Broennimann O, Buri A et al (2020) Soil protist diversity in the Swiss western Alps is better predicted by topo-climatic than by edaphic variables. *J Biogeogr* 47:866–878. <https://doi.org/10.1111/jbi.13755>
18. Geisen S, Mitchell EAD, Adl S et al (2018) Soil protists: a fertile frontier in soil biology research. *FEMS Microbiol Rev* 42:293–323. <https://doi.org/10.1093/femsre/fuy006>
19. Singer D, Mitchell EAD, Payne RJ et al (2019) Dispersal limitations and historical factors determine the biogeography of specialized terrestrial protists. *Mol Ecol* 0: <https://doi.org/10.1111/mec.15117>
20. Lahr DJG, Kosakyan A, Lara E et al (2019) Phylogenomics and morphological reconstruction of Arcellinida testate amoebae highlight diversity of microbial eukaryotes in the Neoproterozoic. *Curr Biol*. <https://doi.org/10.1016/j.cub.2019.01.078>
21. Harwood DM, Nikolaev VA, Winter DM (2007) Cretaceous records of diatom evolution, radiation, and expansion. *The Paleontological Society Papers* 13:33–59
22. Martín-González A, Wierzchos J, Gutiérrez JC et al (2008) Morphological Stasis of Protists in Lower Cretaceous Amber. *Protist* 159:251–257. <https://doi.org/10.1016/j.protis.2007.08.006>
23. Lahr DJG, Bosak T, Lara E, Mitchell EAD (2015) The Phanerozoic diversification of silica-cycling testate amoebae and its possible links to changes in terrestrial ecosystems. *PeerJ* 3:e1234. <https://doi.org/10.7717/peerj.1234>
24. Fernandes NM, Schrago CG (2019) A multigene timescale and diversification dynamics of Ciliophora evolution. *Mol Phylogenet Evol* 139:106521. <https://doi.org/10.1016/j.ympev.2019.106521>
25. Fernández LD, Lara E, Mitchell EAD (2015) Checklist, diversity and distribution of testate amoebae in Chile. *Eur J Protistol* 51:409–424. <https://doi.org/10.1016/j.ejop.2015.07.001>
26. Lara E, Roussel-Delif L, Fournier B et al (2015) Soil microorganisms behave like macroscopic organisms: patterns in the global distribution of soil euglyphid testate amoebae. *J Biogeogr* 43:520–532. <https://doi.org/10.1111/jbi.12660>

27. Pérez-Juárez H, Serrano-Vázquez A, Lara E et al (2019) Population dynamics of amoeboid protists in a tropical desert: seasonal changes and effects of vegetation and soil conditions. *Acta Protozoologica*; Volume 57 Issue 4
28. Thompson AR, Powell GS, Adams BJ (2019) Provisional checklist of terrestrial heterotrophic protists from Antarctica. *Antarct Sci* 31:287–303. <https://doi.org/10.1017/S0954102019000361>
29. Bates ST, Clemente JC, Flores GE et al (2013) Global biogeography of highly diverse protistan communities in soil. *ISME J* 7:652. <https://doi.org/10.1038/ismej.2012.147>
30. Souffreau C, Vanormelingen P, Verleyen E et al (2010) Tolerance of benthic diatoms from temperate aquatic and terrestrial habitats to experimental desiccation and temperature stress. *Limnol Oceanogr* 55:309–324. <https://doi.org/10.2216/09-30.1>
31. Li C, Xu K, Lei Y (2011) Growth and grazing responses to temperature and prey concentration of *Condylostoma spatiosum*, a large benthic ciliate, feeding on *Oxyrrhis marina*. *Aquatic microbial ecology* 64:97–104
32. Heiri C, Wolf A, Rohrer L, Bugmann H (2009) Forty years of natural dynamics in Swiss beech forests: structure, composition, and the influence of former management. *Ecol Appl* 19:1920–1934. <https://doi.org/10.1890/08-0516.1>
33. Burt PJA (2010) Roger G, Barry, 2008. *Mountain Weather and Climate*, Cambridge University Press, Cambridge, UK. ISBN 978-0-521-86295-0. xxiv + 506 pp. *Meteorological Applications* 17:382–382. <https://doi.org/10.1002/met.222>
34. Evans KL, Warren PH, Gaston KJ (2005) Species-energy relationships at the macroecological scale: a review of the mechanisms. *Biol Rev* 80:1–25
35. Swiss Federal Statistical Department (2014) *Territory and environment: Panorama*. BFS
36. Colwell RobertK, Lees DC (2000) The mid-domain effect: geometric constraints on the geography of species richness. *Trends Ecol Evol* 15:70–76. [https://doi.org/10.1016/S0169-5347\(99\)01767-X](https://doi.org/10.1016/S0169-5347(99)01767-X)
37. Kembel SW, Cowan PD, Helmus MR et al (2010) Picante: R tools for integrating phylogenies and ecology. *Bioinformatics* 26:1463–1464. <https://doi.org/10.1093/bioinformatics/btq166>
38. Heger TJ, Derungs N, Theurillat J-P, Mitchell EAD (2016) Testate amoebae like it hot: species richness decreases along a subalpine-alpine altitudinal gradient in both natural *Calluna vulgaris* litter and transplanted *Minuartia sedoides* cushions. *Microbial ecology* 71:725–734. <https://doi.org/10.1007/s00248-015-0687-3>
39. Eiserhardt WL, Bjorholm S, Svenning J-C et al (2011) Testing the Water–Energy Theory on American Palms (Arecaceae) Using Geographically Weighted Regression. *PLOS ONE* 6:e27027. <https://doi.org/10.1371/journal.pone.0027027>
40. Rose JM, Caron DA (2007) Does low temperature constrain the growth rates of heterotrophic protists? Evidence and implications for algal blooms in cold waters. *Limnol Oceanogr* 52:886–895. <https://doi.org/10.4319/lo.2007.52.2.0886>
41. Ehrmann O, Puppe D, Wanner M et al (2012) Testate amoebae in 31 mature forest ecosystems – Densities and micro-distribution in soils. *European Journal of Protistology* 48:161–168.

- <https://doi.org/10.1016/j.ejop.2012.01.003>
42. Krashevskaya V, Sandmann D, Maraun M, Scheu S (2014) Moderate changes in nutrient input alter tropical microbial and protist communities and belowground linkages. *The ISME Journal* 8:1126–1134. <https://doi.org/10.1038/ismej.2013.209>
 43. Willis KJ, Whittaker RJ (2002) Species Diversity–Scale Matters. *Science* 295:1245. <https://doi.org/10.1126/science.1067335>
 44. Shen C, Liang W, Shi Y et al (2014) Contrasting elevational diversity patterns between eukaryotic soil microbes and plants. *Ecology* 95:3190–3202. <https://doi.org/10.1890/14-0310.1>
 45. Lanzén A, Epelde L, Blanco F et al (2016) Multi-targeted metagenetic analysis of the influence of climate and environmental parameters on soil microbial communities along an elevational gradient. *Sci Rep* 6:28257. <https://doi.org/10.1038/srep28257>
 46. Ayres E, Steltzer H, Berg S et al (2009) Tree Species Traits Influence Soil Physical, Chemical, and Biological Properties in High Elevation Forests. *Plos One* 4:e5964. <https://doi.org/10.1371/journal.pone.0005964>
 47. Chandra LR, Gupta S, Pande V, Singh N (2016) Impact of forest vegetation on soil characteristics: a correlation between soil biological and physico-chemical properties. *3 Biotech* 6:188. <https://doi.org/10.1007/s13205-016-0510-y>
 48. Mitchell EAD, Bragazza L, Gerdol R (2004) Testate amoebae (Protista) communities in *Hylocomium splendens* (Hedw.) BSG (Bryophyta): relationships with altitude, and moss elemental chemistry. *Protist* 155:423–436. <https://doi.org/10.1078/1434461042650334>
 49. Fournier B, Coffey EED, van der Knaap WO et al (2016) A legacy of human-induced ecosystem changes: spatial processes drive the taxonomic and functional diversities of testate amoebae in *Sphagnum* peatlands of the Galápagos. *J Biogeogr* 43:533–543. <https://doi.org/10.1111/jbi.12655>
 50. Derungs N (2007) Étude sur l'écologie des thécamobiens des sols le long d'un gradient altitudinal: influence des facteurs environnementaux sur la distribution des espèces. MSc thesis, Université de Neuchâtel–Laboratoire Sol & Végétation and Swiss Federal Research Institute WSL-Laboratoire ECOS (EPFL) 135
 51. Veter NM, DeSantis LRG, Yann LT et al (2013) Is Rapoport's rule a recent phenomenon? A deep time perspective on potential causal mechanisms. *Biol Lett* 9:20130398. <https://doi.org/10.1098/rsbl.2013.0398>

Tables

Table 1

Predictions that can be drawn from the phylogenetic niche conservatism hypothesis.

Name of the mechanism	Theory	Testable prediction
1. Species-energy effect (e.g., Evans et al. 2005)	1.1. Richness is positively related to temperature rather than to any other environmental factor	1.1.1. Positive correlation between elevation and diversity
		1.1.2. Positive correlation between temperature and diversity
2. Taxa-range-size distributions (Stevens 1989, Colwell and Lees 2000)	2.1. Temperature decreases with elevation and progressively filters (selects) taxa with broader thermal tolerances and elevational ranges	2.1.1. Positive correlation between elevation and taxa-range-size distributions (i.e., a Rapoport effect)
	2.2. Geometric constraints drive taxa-range-size distributions over the elevational gradient	2.2.1. Taxa-range-size distributions match the predictions of a null model (i.e., a mid-domain effect)
3. Niche-based processes (e.g., Webb et al. 2002)	3.1. Community assembly is mediated by biotic interactions along the elevational gradient	3.1.1. Communities are phylogenetically overdispersed along the gradient
	3.2. Community assembly is mediated by habitat filtering along the elevational gradient	3.2.1. Communities are phylogenetically clustered along the gradient

Table 2

Relations between (a) richness and elevation, and (b) richness and average annual temperature. Relationships were modelled with both linear (L) and quadratic (Q) models. The better model was selected using the Akaike's information criterion.

Relations	Eukaryotic microbial group	Linear model		Quadratic model		Better Model
		R ²	P	R ²	P	
(a) Richness vs. elevation	Soil protist group	-0.699	0.002	0.699	0.353	L
	Bacillariophyta	-0.886	0.000	0.896	0.222	L
	Cercomonadida	-0.853	0.000	0.847	0.425	L
	Ciliophora	-0.579	0.006	0.785	0.021	Q
	Euglyphida	-0.776	0.000	0.842	0.077	L
	Kinetoplastida	-0.593	0.006	0.877	0.003	Q
(b) Richness vs. average annual temperature	Soil protist group	0.733	0.002	0.766	0.353	L
	Bacillariophyta	0.898	0.000	0.919	0.222	L
	Cercomonadida	0.869	0.000	0.881	0.425	L
	Ciliophora	0.625	0.006	0.833	0.021	Q
	Euglyphida	0.801	0.000	0.877	0.077	L
	Kinetoplastida	0.638	0.006	0.904	0.003	Q

Figures

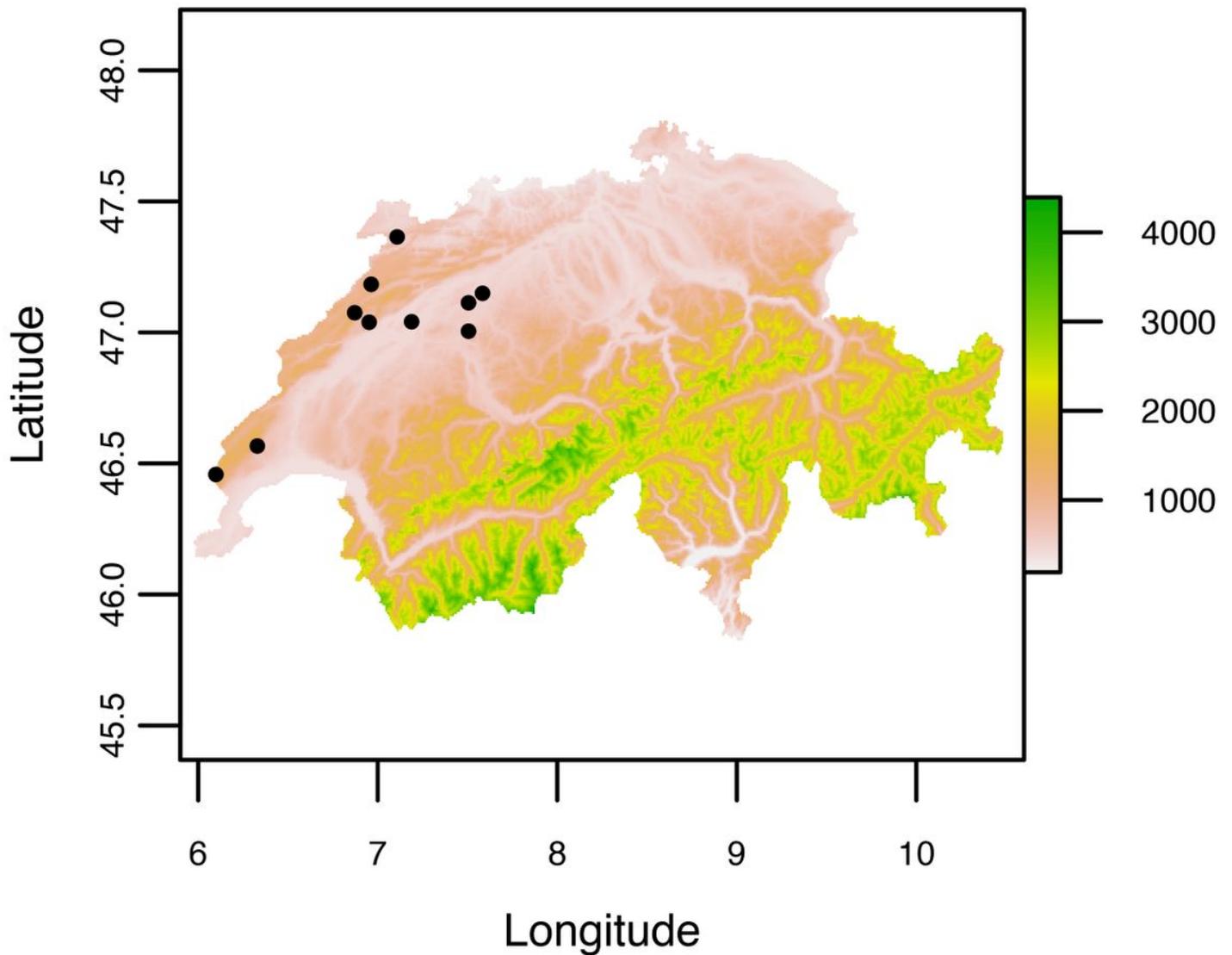


Figure 1

Distribution and elevation in m a.s.l. (color-coded bar) of each of the prospected plots in beech dominated forests (*Fagus sylvatica* L.) in western Switzerland. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

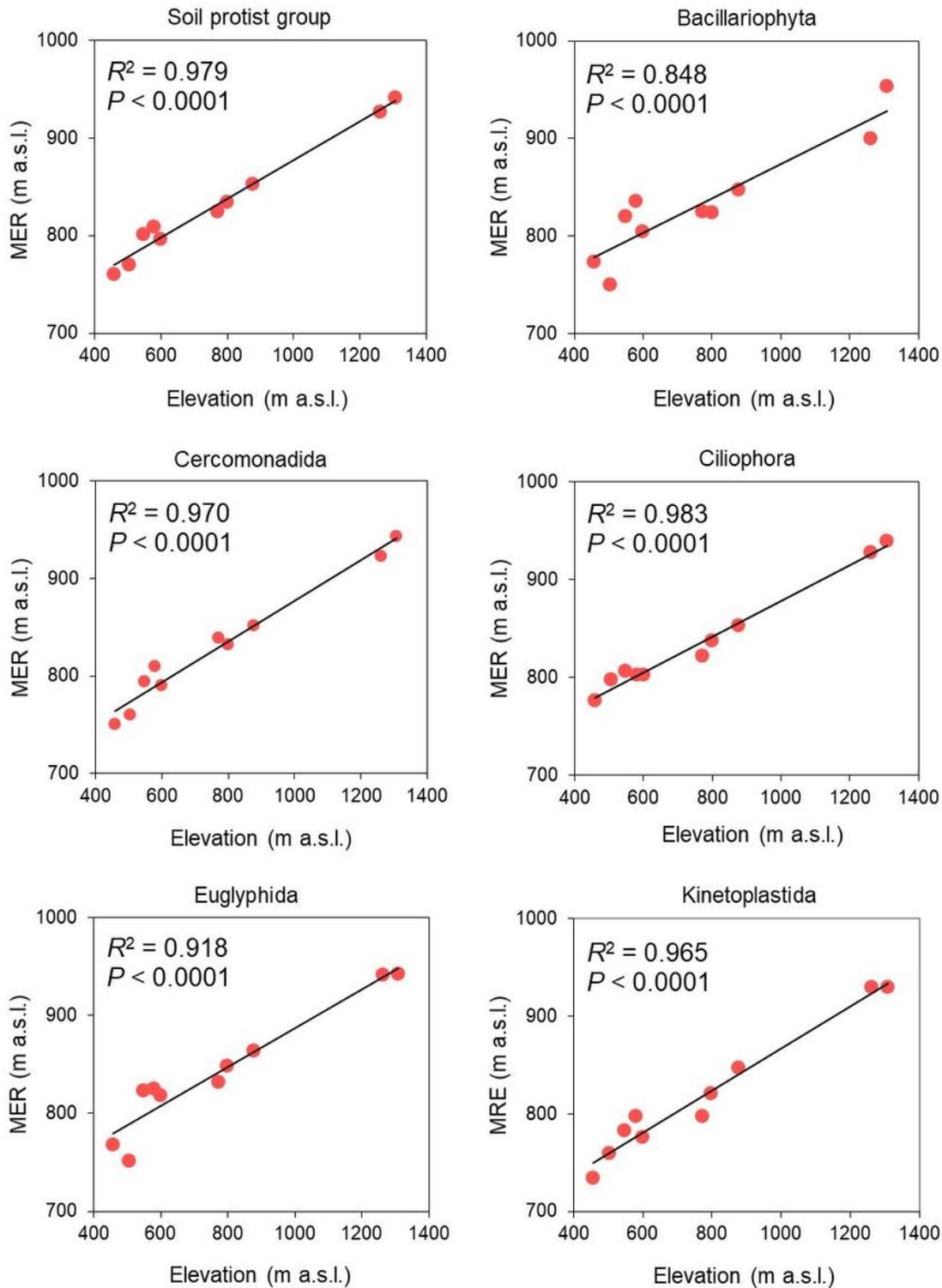


Figure 2

Relationship between the mean elevational range size (MER) of all eukaryotic microbial groups studied and elevation. All eukaryotic microbial groups exhibited a significant increase of their elevational ranges with elevation, conforming to the macroecological pattern of the Rapoport effect.

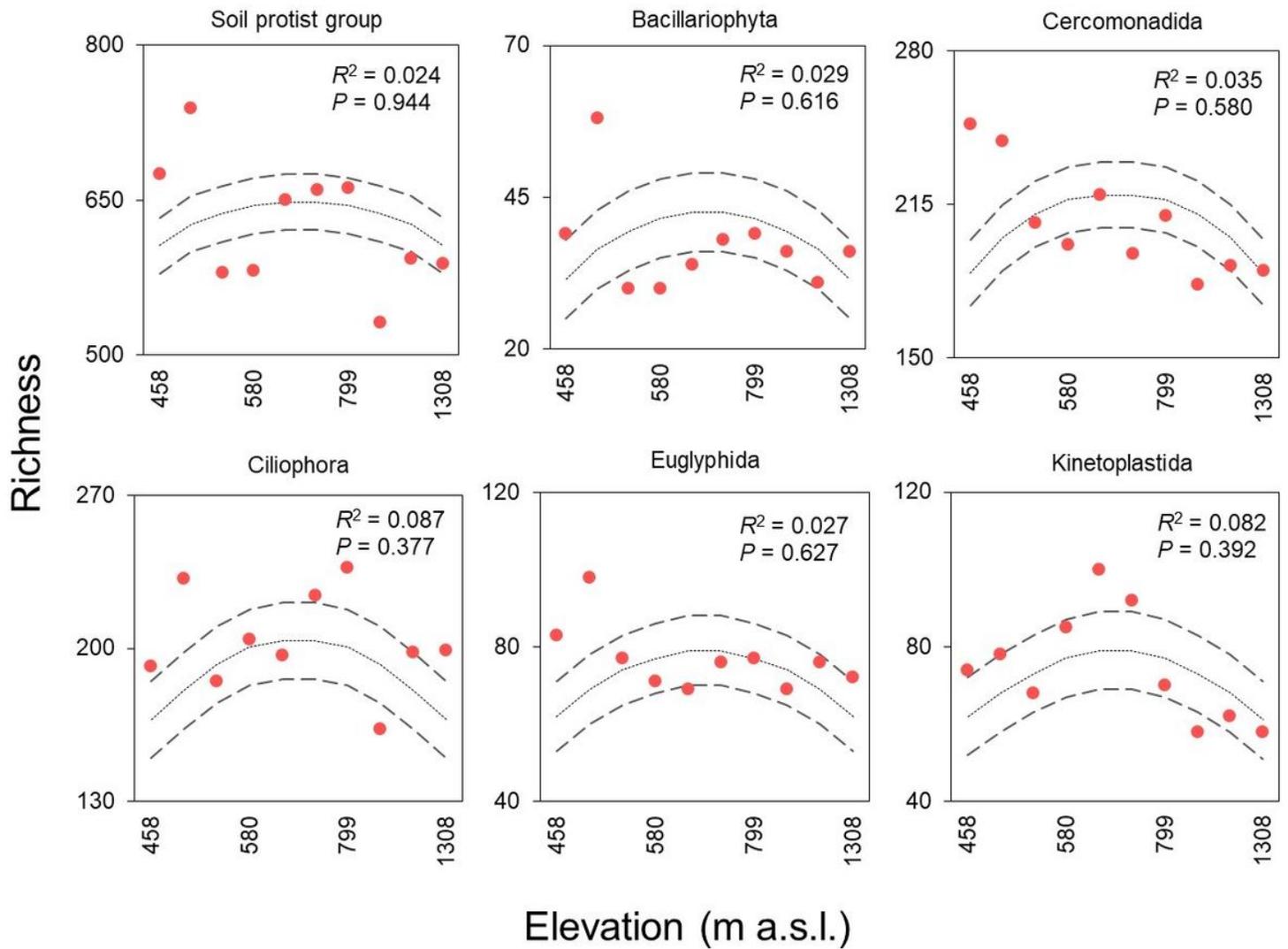


Figure 3

Richness (solid circles), upper and lower 95% confidence limits (broken lines) and average null model predictions (dotted line) computed with the Mid-Domain Null program (50,000 Monte Carlo simulations sampled without replacement from empirical taxa-range-size distributions). In all cases, the observed richness did not show significant relationship ($P > 0.05$) with null model predictions, indicating that the observed patterns were not significantly influenced by random effects.

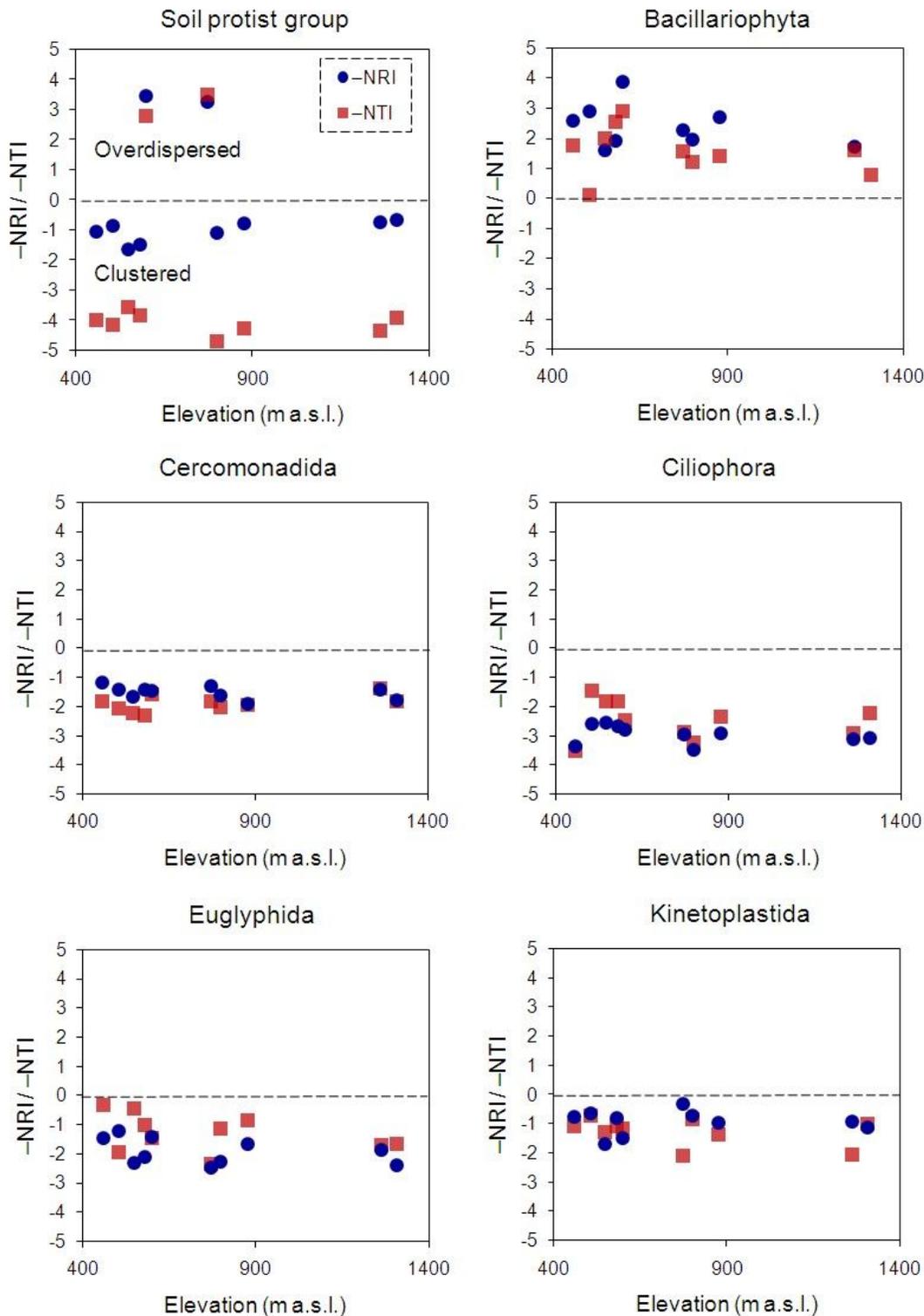


Figure 4

Variation in community phylogenetic relatedness along the gradient studied as measured with both $-NRI$ and $-NTI$ indices. Above zero values indicate phylogenetic overdispersion, while below zero values indicate phylogenetic clustering. The $-NRI$ and $-NTI$ values were significant in all cases ($P < 0.05$).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [AppendixS1.docx](#)
- [AppendixS2.docx](#)
- [AppendixS3.docx](#)
- [AppendixS4.docx](#)