1 **Running title:** Niche conservatism drives protist biogeography

2 **Niche conservatism drives the elevational diversity gradient in major groups of free-**

3 **living soil unicellular eukaryotes**

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Abstract

Ancestral adaptations to tropical-like climates drive most multicellular biogeography and macroecology. Observational studies suggest that this niche conservatism could also be shaping unicellular biogeography and macroecology, although evidence is limited to Acidobacteria and testate amoebae. We tracked the phylogenetic signal of this niche conservatism in far related and functionally contrasted groups of common soil protists (Bacillariophyta, Cercomonadida, Ciliophora, Euglyphida and Kinetoplastida) along a humid but increasingly cold elevational gradient in Switzerland. Protist diversity decreased and the size of the geographic ranges of taxa increased with elevation and associated decreasing temperature (climate), which is consistent with a macroecological pattern known as Rapoport effect. Bacillariophyta exhibited phylogenetically overdispersed communities assembled by competitive exclusion of closely related taxa with shared (conserved) niches. By contrast, Cercomonadida, Ciliophora, Euglyphida and Kinetoplastida exhibited phylogenetically clustered communities assembled by habitat filtering, revealing the coexistence of closely related taxa with shared (conserved) adaptations to cope with the humid but temperate to cold climate of the study site. Phylobetadiversity revealed that soil protists exhibit a strong phylogenetic turnover among elevational sites, suggesting that most taxa have evolutionary constraints that prevent them from colonising the colder and higher sites of the elevation gradient. Our results suggest that evolutionary constraints determine how soil protists colonise climates departing from warm and humid conditions. We posit that these evolutionary constraints are linked to an ancestral adaptation to tropical-like climates, which limits their survival in exceedingly cold sites. This niche conservatism possibly drives their biogeography and macroecology along latitudinal and altitudinal climatic gradients.

Keywords: biogeography and macroecology; cercomonadids; ciliates; diatoms; kinetoplastids; testate amoebae

Introduction

Niche conservatism is a hypothesis proposed to explain why biological entities exhibit diversity (biogeographic) patterns along climatic gradients [1]. Niche conservatism states that taxa remain in the same climate in which their ancestors evolved because they tend to retain their ancestral niche traits over the evolutionary course [1, 2]. This would explain why most taxa fail to disperse into new climates, providing an underlying mechanism for the biogeographic patterns observed along latitudinal or altitudinal climatic gradients [2, 3].

Since most modern taxa originated in tropical-like climates [4], niche conservatism predicts that biodiversity will peak in warm, humid (optimal) climates because they should have evolutionary constraints that prevent them from thriving at exceedingly hot or cold (suboptimal) climates [1]. Accordingly, biodiversity will be better predicted by air temperature at sites with humid and cold climates and by water availability at sites with hot and dry climates (Prediction 1).

Niche conservatism also predicts that few taxa have evolved new traits (evolutionary novelties) to cope with suboptimal climates [1, 5]. If so, most taxa should have narrow geographic ranges restricted to optimal climates, while few taxa should have broad geographic ranges extending beyond optimal climates [6]. This idea is consistent with the Rapoport effect [6, 7], a macroecological pattern that describes an increase in taxa ranges from optimal to suboptimal climates (Prediction 2).

Due to niche conservatism, closely related taxa share ancestral niche traits (Prediction 3). Therefore, these taxa cannot coexist in places with optimal climates because they compete for similar resources [5, 8]. Optimal climates then promote community assembly via competitive exclusion, resulting in phylogenetic overdispersion or in the coexistence of distantly related taxa with non-overlapping niches [8, 9]. However, closely related taxa that evolved and share traits to survive in cold or hot conditions can coexist in suboptimal climates [8]. This occurs because suboptimal climates filter out taxa that are not adapted to survive in cold or hot conditions [10]. Therefore, closely related taxa adapted to suboptimal climates compete less with each other under cold or hot conditions because they have access to more resources than in optimal climates [8, 10]. Suboptimal climates then promote community assembly via habitat filtering, resulting in phylogenetic clustering or in

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108 the coexistence of closely related taxa sharing evolutionary novelties to cope with

109 exceedingly hot or cold climates [8, 9].

110 Finally, since evolutionary constraints prevent taxa from colonising suboptimal climates

111 [1], habitat filtering will limit the dispersal of less adapted taxa as they approach the hotter

112 or colder end of the climatic gradient [11, 12]. Phylogenetic beta diversity

113 (phylobetadiversity) or phylogenetic variation among communities [13] should therefore

114 reveal changes in phylogenetic composition along any climatic gradient (Prediction 4).

115 While these predictions are consistent with niche conservatism, they are also consistent

116 with the idea that there are abiotic constraints on the conditions that an organism can

117 readily tolerate (i.e., they do not require that these be constrained by the past evolutionary

118 history of the group). Therefore, local physicochemical conditions represent a confounding

119 variable that needs to be controlled for in order to examine a cause-and-effect relationship

120 between niche conservatism and the occurrence of diversity patterns in nature [14, 15].

121 Typically, this is achieved by using a restrictive sampling strategy, which consists of

122 assessing predictions along a climatic gradient of comparable habitats to reduce the

123 confounding effects of local abiotic factors [9, 14, 15].

124 Niche conservatism predictions have been extensively tested in multicellular organisms [1-

125 4], showing the usefulness of niche conservatism in explaining biogeographic patterns. By

126 contrast, they have been seldomly tested in unicellular organisms, including bacteria [5, 9,

127 10] and protists such as testate amoebae [12]. Thus, many biogeographic generalizations

128 proposed for plants and animals have not yet been sufficiently tested for microorganisms,

129 particularly for soil protists [16].

130 Soil protists are good model organisms for testing whether niche conservatism applies to

131 unicellular organisms. Soil protists appear to retain their traits over their evolutionary

132 course, including a need for warm, humid climates [12, 17]. Evidence suggests that at least

133 some soil protist taxa evolved under the tropical-like climate that characterized much of the

134 Mesozoic [18, 19]. These past climatic conditions seem to have imposed evolutionary

135 constraints on their ability to adapt to exceedingly hot or cold climates, influencing their

136 current diversity patterns, performance and fitness [12]. Indeed, soil protists diversity peaks

137 in warm, humid climates [15, 20, 21] and declines towards exceedingly hot [22] and cold

5

138   [23] climates. They also show high survival, growth and reproductive rates at warm

139   temperatures (ca. 18 and 24 °C) than at exceedingly cold or hot temperatures [24-26]. This

140   background suggests that most soil protists lack ecophysiological adaptations to survive

141   and reproduce in exceedingly cold or hot climates. Probably, the retention of their ancestral

142   adaptation to tropical-like climates imposes strong constraints on their ability to colonize

143   sites with suboptimal climates and drives their current biogeographic patterns.

144   Herein, we tested niche conservatism predictions on free-living representatives of five

145   major, phylogenetically and functionally contrasted common soil protist groups:

146   Bacillariophyta (diatoms), Cercomonadida (cercomonadids), Ciliophora (ciliates),

147   Euglyphida (euglyphids or filose testate amoebae) and Kinetoplastida (kinetoplastids) and a

148   group combining all these taxa (herein refer to as the "soil protist group"). We selected

149   these soil protist taxa because (1) they are frequent, abundant and diverse in soils, (2) they

150   have different life history strategies and functional roles, and (3) they are well distributed

151   across the eukaryotic tree [27]. Our rationale behind this choice was to be able to generalize

152   conclusions to as many soil eukaryotes as possible.

153   Niche conservatism predictions were tested along an elevational gradient in Switzerland,

154   which exhibits moderate to high humidity over the year but decreasing temperature with

155   altitude [28]. This elevational gradient is ideal for assessing niche conservatism in soil

156   protists because the forests are dominated by Beech (*Fagus sylvatica*) throughout the

157   gradient [29]. Beech trees require moderate to high precipitation and through shading and

158   litterfall establish similar microclimate and soil properties on sites where they are the

159   dominant plant species [30, 31]. Therefore, beech-dominated forests represent comparable

160   habitats for unicellular organisms [31]. This feature reduces the confounding effects of

161   local abiotic factors [14, 15] and contributes to unravelling the role that niche conservatism

162   plays in shaping soil protist diversity patterns.

163

164   **Material and Methods**

165   Study Site and Sampling Strategy

166   The study was conducted in Western Switzerland, which has a temperate maritime climate

167   [28] (Fig. 1). Within this region, forests are mostly dominated by beech (*F. sylvatica*) trees

168 and, although mostly exploited for timber, their structure and vegetation composition

169 remains similar to pristine forests [29]. The studied sites were selected within the

170 permanent plots of the Swiss Biodiversity Monitoring (BDM) program

171 (https://bit.ly/3puxExC). We chose 10 plots from 458 to 1,308 m a.s.l. to study biodiversity

172 along nearly the entire elevation range of beech-dominated forests in Switzerland. This

173 elevational gradient exhibits high humidity over the year and decreasing temperature with

174 elevation (average annual precipitation and temperature: 1,200 mm and 8.3 °C,

175 respectively) [28, 29].

176 Beech-dominated forests have comparable microclimate/soil properties and constitute

177 similar habitats for microorganisms [30, 31]. We therefore collected samples at the

178 periphery of the BDM plots (Fig. 1) to reduce the confounding effects of abiotic factors [9,

179 14, 15]. At each site we collected three soil cores (5 cm diameter x 5 cm depth) and then

180 mixed them to obtain a composite sample for each sampling site. We kept the soil samples

181 cool at all times and performed DNA extraction within 24h of sampling.

182

183 Characterization of Soil Protist Communities

184 We followed standard protocols published elsewhere [32] for DNA extraction, PCR and

185 sequencing (Illumina, targeting the SSU rRNA gene V9 region of eukaryotes), as well as

186 for taxonomic assignment of the reads obtained. These protocols are summarised in the

187 supplementary material ESM1.

188 We randomly subsampled 50,000 reads from each composite sample to account for unequal

189 sample sizes and defined a community as all operational taxonomic units (OTUs)

190 originating from a single sampling site. From these subsamples, we selected those assigned

191 to Bacillariophyta, Cercomonadida, Ciliophora, Euglyphida and Kinetoplastida. Another

192 group (the soil protist group) was also created combining all the reads assigned to those

193 taxa. We estimated taxonomic richness in each community based on the total number of

194 OTUs, a metric that in turn was correlated to phylogenetic diversity (see ESM1: Fig. S1).

195 We assessed the quality of our sampling effort by building species accumulation curves for

196 each taxon in the package vegan [33] and using R 3.1.2 [34].

197

198   Abiotic Factors and Confounding Effects

199   Abiotic factors (soil temperature, moisture, humus content, continentality, light, nutrients,

200   pH) were estimated at each sampling site using Landolt Indicator Values [35]. This

201   bioindication method estimates soil abiotic factors by averaging the individual indicator

202   values (i.e., the individual ecological tolerances) of plant species present on a site. The

203   average indicator values range between 1 and 5 and provide robust information on the long-

204   term environmental conditions characterizing a site [36]. Abiotic factors were estimated

205   using Landolt Indicator Values because their performance in predicting biodiversity

206   patterns outperforms that of data interpolated from climatic databases popularly used in

207   ecological studies [37]. In part, this is because climate databases still exhibit data gaps and

208   low resolution, particularly in forested regions of Europe such as our study site [38].

209   After reducing the set of abiotic factors to a representative subset to avoid multicollinearity

210   (we retained soil temperature, light, humidity, pH and humus content), we constructed

211   linear models with protist diversity as response variable and the subset of abiotic factors as

212   descriptors and tested the overall model significance as well as the significance of each

213   descriptor using ANOVA in the package vegan [33]. Since our sampling strategy aimed to

214   decrease the confounding effects of abiotic factors to disentangle the role of niche

215   conservatism in shaping protist diversity patterns, we expected to record a lack of

216   correlation between abiotic factors and soil protist diversity.

217

218   Prediction 1

219   The elevational gradient investigated exhibits high humidity but decreasing air temperature

220   with altitude [28, 29]. Therefore, according to niche conservatism, temperature should limit

221   protist survival along this humid but cold altitudinal gradient. To test this prediction, we

222   standardised OTU richness to avoid the unwanted effects of sampling artefacts. This

223   process was conducted using a combination of range interpolation and species richness

224   estimates. Range interpolation consisted in dividing the entire elevational gradient into 100-

225   m elevational bands and assuming that taxa were present at all elevations between the

226   lowest and highest observed elevations [39]. Richness estimates involved the calculation of

227   the expected OTU richness for each 100-m elevational band using the richness estimator

8

228   available in the package iNEXT [40]. Then, we estimated the average annual temperature

229   for each 100-m elevational band based on the average annual air temperature reported for

230   the lowest sampling site (10 °C) [41] and assuming a decrease (lapse rate) of 0.6 °C for

231   each 100-m increase in elevation [39]. While the estimated and the empirical rates at which

232   air temperature cools with altitude may differ, the use of a lapse rate may not pose an issue

233   in our case. Our estimate of air temperature decreases monotonically with altitude,

234   mirroring the empirical trend observed at the study site in a previous study [28]. We then

235   constructed linear and quadratic models using richness as response variable and both the

236   elevation and the average annual air temperature as descriptor variables. The best-fitting

237   model was selected based on the Akaike's information criterion for each model.

238

239   Prediction 2

240   We used the midpoint method [6] to assess the elevational Rapoport effect. This method

241   analyses the correlation between elevation and the mean elevational range size of all taxa

242   present at each sampling site. The correlation between both variables was tested using

243   Pearson's correlations in R 3.1.2 [34]. A positive correlation between both variables would

244   support the occurrence of an elevational Rapoport effect, i.e., an increase in the elevational

245   range sizes of soil protists from lower and warmer sites to higher and colder sites. This

246   outcome would suggest that soil protists are evolutionarily constrained to cope with cold

247   climates.

248

249   Prediction 3

250   Analysis of phylogenetic structure informs whether taxa coexisting within a community are

251   distantly related (or phylogenetically overdispersed) or closely related (or phylogenetically

252   clustered) [8]. We therefore investigated phylogenetic structure within communities to

253   assess the relatedness among taxa coexisting within the same community. For this task we

254   used two indices, namely the mean pairwise distance index (-NRI) and the mean nearest

255   taxon distance index (-NTI) [42]. -NRI estimates the mean phylogenetic distance among all

256   pairs of taxa within a community, whereas -NTI estimates the mean phylogenetic distance

257   between each taxon and its closest relative within a community. Positive values of these

258   indices indicate phylogenetic overdispersion and negative values indicate phylogenetic

259   clustering within communities. If climate is optimal for soil protists along the elevational

260   gradient communities will be phylogenetically overdispersed [8, 9]. Conversely, if climate

261   is suboptimal for soil protists, then communities will be phylogenetically clustered [8, 9].

262   Finally, if soil protists do not conserve their traits over evolution, their within-community

263   phylogenetic structure will be predicted by chance [1, 42]. Both -NRI and -NRI indices

264   were estimated using the package picante [42] and their outcomes were compared against

265   values predicted by a null model computed using a trial swap algorithm (999,000

266   randomizations).

267

268   Prediction 4

269   We investigated phylobetadiversity to assess the elevational phylogenetic variation among

270   communities in the study site. Phylobetadiversity was estimated using the PhyloSor index,

271   which estimates the phylogenetic composition shared by two or more sites [9]. We also

272   decomposed phylobetadiversity in its two additive components, namely phylogenetic

273   turnover and phylogenetic nestedness [13]. Measuring both components allows defining the

274   proportion of phylobetadiversity that is caused by phylogenetic replacement among sites

275   (or true turnover) and the proportion that is caused by loss of phylogenetic diversity among

276   sites, respectively. If soil protists exhibit a phylogenetically conserved response to climate,

277   then the elevational decrease in temperature will progressively limit soil protist colonisation

278   towards colder and higher elevations. If so, phylogenetic composition will vary among

279   communities and phylobetadiversity will deviate from that expected by chance. In turn, if

280   soil protists do not conserve their ancestral niche traits, then their phylogenetic composition

281   will vary randomly along the elevational gradient since all taxa will be equally likely to

282   colonise the entire gradient of comparable (beech-dominated) habitats. The PhyloSor index

283   and its two additive components were estimated in betapart [43] and their outcomes were

284   compared against values predicted by a null model computed using an independent swap

285   algorithm (50,000 randomisations).

286

287

288 **Results**

289 Characterization of Soil Protist Communities

290 We recorded a total of 1,413 OTUs and 47,394 reads along the elevational gradient. The

291 Cercomonadida (373 OTUs: 12,437 reads) and Ciliophora (582 OTUs: 15,599 reads) were

292 the most diverse and abundant heterotrophic taxa at each forest site, followed by

293 Kinetoplastida (223 OTUs: 10,377 reads) and Euglyphida (157 OTUs: 8,058 reads). The

294 phototrophic taxon Bacillariophyta was the less diverse and abundant (78 OTUs: 923

295 reads). Species accumulation curves confirmed that sampling effort and sequencing

296 covering were enough to record a significant proportion of the total OTU richness found at

297 each sampling site (supplementary material ESM2: Fig. S1).

298

299 Abiotic Factors and Confounding Effects

300 Soil abiotic factors as inferred from Landolt Indicator Values varied within narrow ranges

301 among beech-dominated forests (ESM2: Table S1). An ANOVA test revealed that abiotic

302 factors were not significantly correlated to soil protist diversity (Table 1), suggesting that

303 our restrictive sampling strategy succeeded in reducing the confounding effects of local

304 abiotic factors.

305

306 Prediction 1

307 Protist diversity exhibited a negative correlation with elevation and a positive correlation

308 with climate (mean annual air temperature) (Table 2). Therefore, protist diversity peaked at

309 lower and warmer elevations. This was consistent with the idea that most soil protists are

310 subjected to evolutionary constraints which make it challenging for them to cope with

311 colder climates.

312

313 Prediction 2

314 We recorded a positive and significant correlation between elevation and the mean

315 elevational range size of soil protists (Fig. 2). Thus, the elevational range of soil protists

11

316   increased in size from lower and warmer to higher and colder elevations. This increasing

317   pattern in the elevational range size of soil protists is consistent with an elevational

318   Rapoport effect and suggests that most soil protists have ecophysiological constraints to

319   adapt to cold climates.

320

321   Prediction 3

322   -NRI and -NTI indices varied in their absolute values (ESM2: Table S2) but led to the same

323   conclusions in terms of phylogenetic structure within soil protist communities (Fig. 3). The

324   analysis of the phylogenetic structure within the "soil protist group" combining all

325   investigated taxa revealed that, overall, soil protists exhibit both phylogenetically

326   overdispersed communities composed of distantly related taxa (average value: -NRI= 3.37

327   and -NTI: 3.15) and phylogenetically clustered communities composed of closely related

328   taxa (average value: -NRI= -1.03; -NTI= -4.09). On the other hand, an assessment of the

329   phylogenetic structure within monophyletic taxa revealed that the autotrophic taxon

330   Bacillariophyta (diatoms) exhibited phylogenetically overdispersed communities composed

331   of distantly related taxa (average value: -NRI= 2.41; -NTI= 1.59). By contrast, the analysis

332   revealed that the heterotrophic taxa Cercomonadida, Ciliophora, Euglyphida and

333   Kinetoplastida exhibited phylogenetically clustered communities composed of closely

334   related taxa (average value: -NRI= -1.83; -NTI= -1.73).

335

336   Prediction 4

337   Phylobetadiversity as measured by the PhyloSor index revealed that all taxa exhibited a

338   significant dissimilarity in phylogenetic composition between any pair of soil protist

339   communities taken at random from our study site (PhyloSor ranged from 0.57 to 0.66, $P <$

340   0.05; Table 3). Phylobetadiversity was mainly the result of phylogenetic turnover

341   (PhyloSor$_{Turn}$ ranged from 0.5 to 0.60, $P < 0.05$; Table 3) and, to a lesser extent,

342   phylogenetic nestedness (PhyloSor$_{PD}$ ranged from 0.04 to 0.09, $P < 0.05$; Table 3).

343

344

Discussion

Consistent with Prediction 1, soil protist diversity was significantly correlated with climate (mean annual air temperature). This so-called species-energy effect [44] was previously shown to be as an important driver of soil protist biogeography in latitudinal and elevational gradients exhibiting humid climates and a significant thermocline [12, 15, 21, 23]. Other studies have also found that soil protist diversity patterns result from the synergy between climate and local abiotic factors [45, 46]. Indeed, this synergy is important in shaping the biogeography of any taxon, although it is also known that the relative importance of climate and local abiotic factors varies significantly with the spatial scale of the study [47]. One way to disentangle the role of climate as a driver of biodiversity is to use a restrictive sampling strategy to reduce the confounding effects of local factors [9, 14, 15]. In our case, we restricted our sampling to beech-dominated forests, because they have similar microclimate and soil properties and represent comparable habitats for soil microorganisms [30, 31]. This restrictive sampling strategy proved to be effective in our case, as soil protist diversity did not exhibit any correlation with local abiotic factors. Instead, the elevational variation of soil protist diversity was significantly correlated to climate (mean annual air temperature).

The evaluation of Prediction 2 supported the idea that climate is a good predictor of the elevational diversity gradient of soil protists. All taxa exhibited an elevational Rapoport effect or a progressive increase in their distribution ranges from lower and warmer sites to higher and colder sites. The Rapoport effect has been related to the existence of evolutionary constraints that prevent multicellular organisms [6, 7, 11], and soil protists [12] from adapting to and colonizing areas with severe, often exceedingly cold/hot climates. So, the observed elevational Rapoport effect suggests that overall, soil protists lack the adaptations needed to cope with the cold climate of higher elevation.

Following Prediction 3, the assessment of within-community phylogenetic structure in the soil protist group revealed both phylogenetically overdispersed communities assembled by competitive exclusion and phylogenetically clustered communities assembled by habitat filtering. Since phylogenetic overdispersal and phylogenetic clustering are respectively expected to occur in optimal and suboptimal climates [1, 2], we can conclude that the

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375 humid, cold climate prevailing at our study sites represents a nearly optimal climate for

376 some taxa and a harsh climate for others. Soil protist taxa exhibiting phylogenetically

377 overdispersed communities probably have a broader thermal tolerance than those exhibiting

378 phylogenetically clustered communities. This outcome is consistent with niche

379 conservatism [1-5] and suggests that soil protists conserve their niche traits among closely

380 related taxa and over evolutionary time.

381 Assessment of within-community phylogenetic structure in monophyletic taxa revealed that

382 the autotrophic taxon Bacillariophyta (diatoms) exhibited phylogenetically overdispersed

383 communities assembled by competitive exclusion, while heterotrophic taxa

384 Cercomonadida, Ciliophora, Euglyphida and Kinetoplastida exhibited phylogenetically

385 clustered communities assembled by habitat filtering along the humid and cold elevational

386 gradient of beech forests. In these forests, diatoms assembled their communities by

387 competitive exclusion because they probably compete for the scarce sunlight available. In

388 fact, sunlit spots are often sparse and scattered in beech forests because the trees develop a

389 dense canopy that limits the amount of sunlight reaching the ground [29]. Therefore,

390 closely related diatoms with similar niche (light intensity/wavelength) requirements must

391 actively compete for this limiting resource. On the other hand, heterotrophic protists

392 possibly assemble their communities by habitat filtering because the prevailing cold climate

393 restricts most of their representatives. Evidence suggests that heterotrophic protists have

394 lower fitness and performance (reproductive, growth and survival rates) than autotrophic

395 protists when exposed to cold temperatures [24-26]. Some do not survive or cannot

396 reproduce below 10 °C [24]. Thus, our study site must harbour only a subset of the total

397 pool of heterotrophic taxa found in the region. These closely related taxa must share

398 evolutionary novelties that allow them to cope with the elevational decrease in temperature

399 that characterises the study site.

400 Evaluation of phylobetadiversity (Prediction 4) revealed that the phylogenetic composition

401 varies significantly between forests (as shown by PhyloSor). In particular, most OTUs

402 occurred at only one site (as shown by $PhyloSor_{Turn}$), while a comparatively minor part

403 occurred at two or more sites (as shown by $PhyloSor_{PD}$) [13]. Thus, there is a strong

404 phylogenetic turnover between sites [43], suggesting that each taxon (i.e., Bacillariophyta,

405 Cercomonadida, Ciliophora, Euglyphida and Kinetoplastida) is mostly represented by

406 individuals adapted to survive in specific temperature ranges. Therefore, most soil protists
407 exhibit evolutionary constraints that do not allow them to colonise and thrive in sites that
408 exhibit temperatures that exceed their tolerance range. This lends further support to the
409 existence of evolutionary constraints that prevent taxa from adapting to and surviving in
410 new climatic conditions [11, 12]. Thus, niche conservatism represents a potential
411 underlying mechanism for soil protist phylobetadiversity. These results suggest that niche
412 conservatism not only acts at the community level (as revealed by analyses of within-
413 community phylogenetic structure), but also at metacommunity level.

414

Conclusion

416 Previous research and the present study support the idea that soil protist diversity increases
417 towards warm (and humid) climates. However, the novelty of our study lies in the fact that
418 we provide evidence suggesting that this species-energy effect may have an evolutionary
419 origin rooted in the conservatism of ancestral thermal regimes rather than on present-day
420 climatic conditions. The phylogenetic signal of this thermal niche conservatism was herein
421 explicitly detected by assessing the within-community phylogenetic structure (phylogenetic
422 overdispersion/clustering) and phylogenetic beta diversity (phylobetadiversity) along an
423 elevational and climatic gradient of comparable habitats. The outcomes provided by these
424 metrics suggest that soil protists exhibit evolutionary constraints that do not allow them to
425 adapt to climates departing from warm and humid conditions. This thermal constraint might
426 also be driving their current biogeographical and macroecological patterns on Earth and
427 might be the reason why temperature often arises as an important predictor of soil protist
428 diversity over latitudinal and elevational gradients. Considering that the phylogenetic niche
429 conservatism has contributed to successfully explain the occurrence of diversity patterns in
430 plants and animals, we could also state that our study contributes with additional evidence
431 to demonstrate that eukaryotic multicellular and unicellular diversity patterns might be
432 produced and maintained by similar processes. Our findings contribute thus to generalizing
433 broad evolutionary mechanisms to the whole domain Eukarya and, arguably, to all life on
434 Earth.

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Figure Legends

Fig. 1. The upper half of the figure depicts a map of Switzerland: the black dots show the location of the sampling sites (beech-dominated forests). The altitude of each sampling site is reported in m a.s.l. according to the colour coded bar on the right margin of the map. The lower half of the figure portrays some of the sampling sites: (a) Mooseedorf, (b) Zielebach, (c) Berolle, (d) Les Bois.

Fig. 2. Rapoport effect or positive correlation between elevation and mean elevational range size (MER) of all OTUs we found co-occurring at each sampling site (circles). The positive correlation observed between both variables shows that all eukaryotic microbial groups exhibited a significant increase in the size of their elevational ranges with elevation.

Fig. 3. Within-community phylogenetic structure as measured by -NRI (circles) and -NTI (squares) indices at each sampling site. Positive values (above zero) indicate phylogenetic overdispersion or communities of distantly related taxa assembled by competitive exclusion. Negative values (below zero) indicate phylogenetic clustering or communities of closely related taxa assembled by habitat filtering. Within-community phylogenetic structure estimations were significant in all cases ($P < 0.05$).
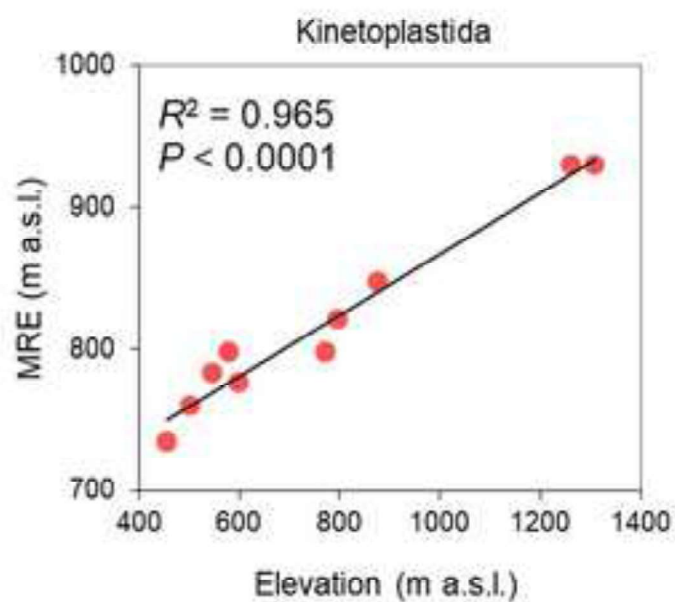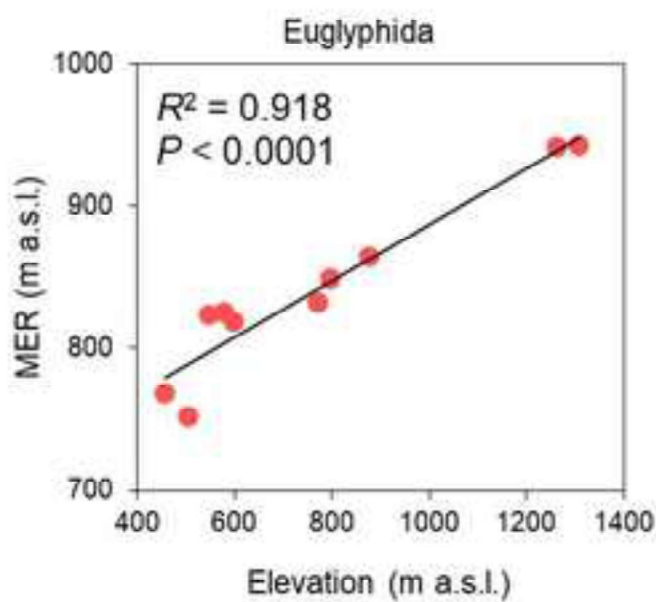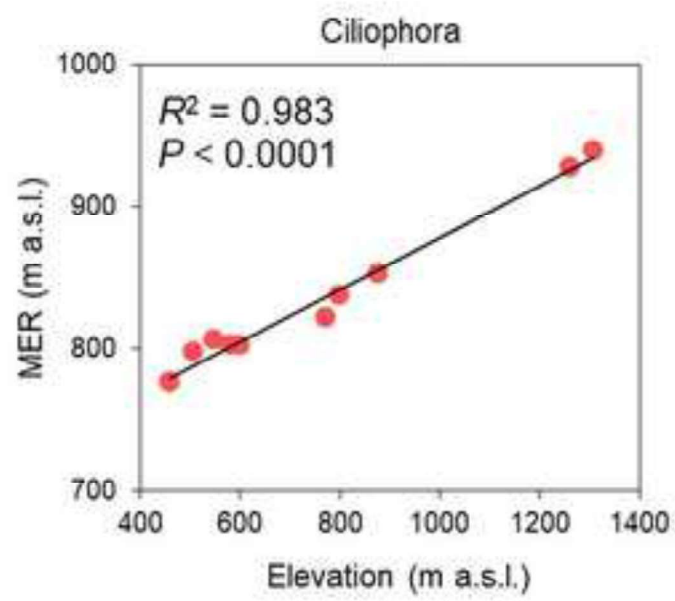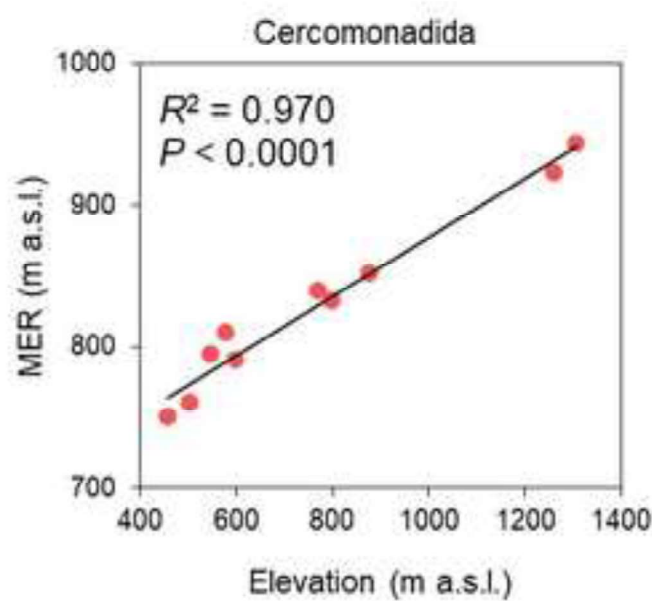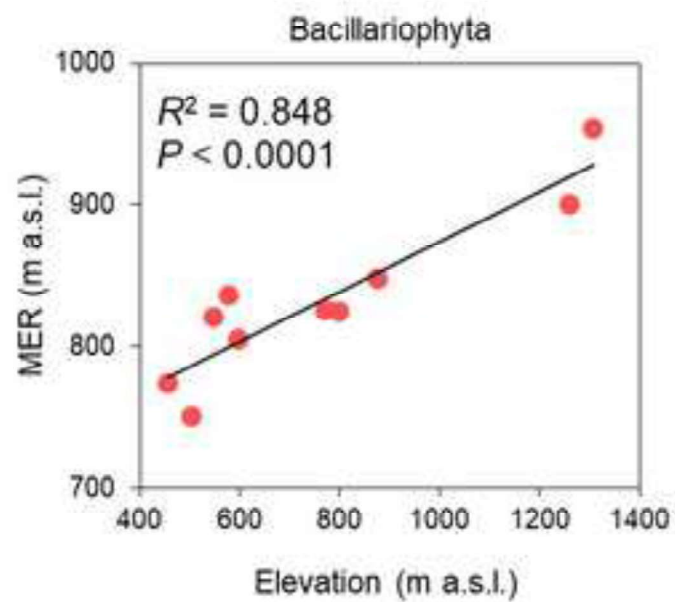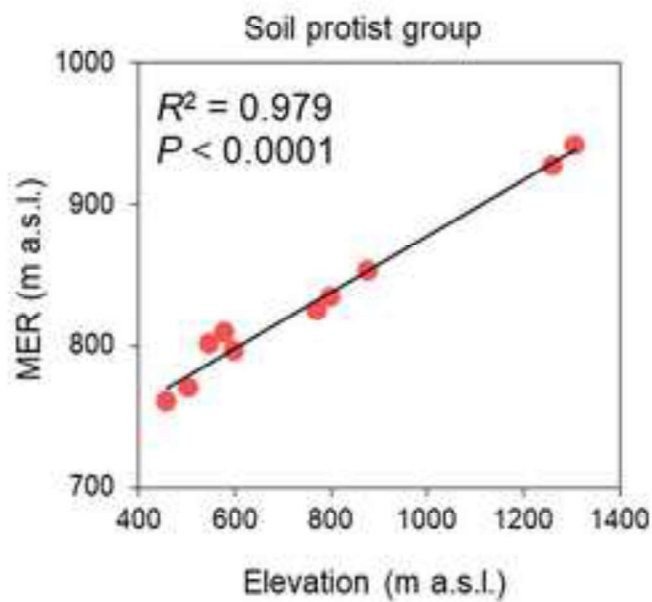
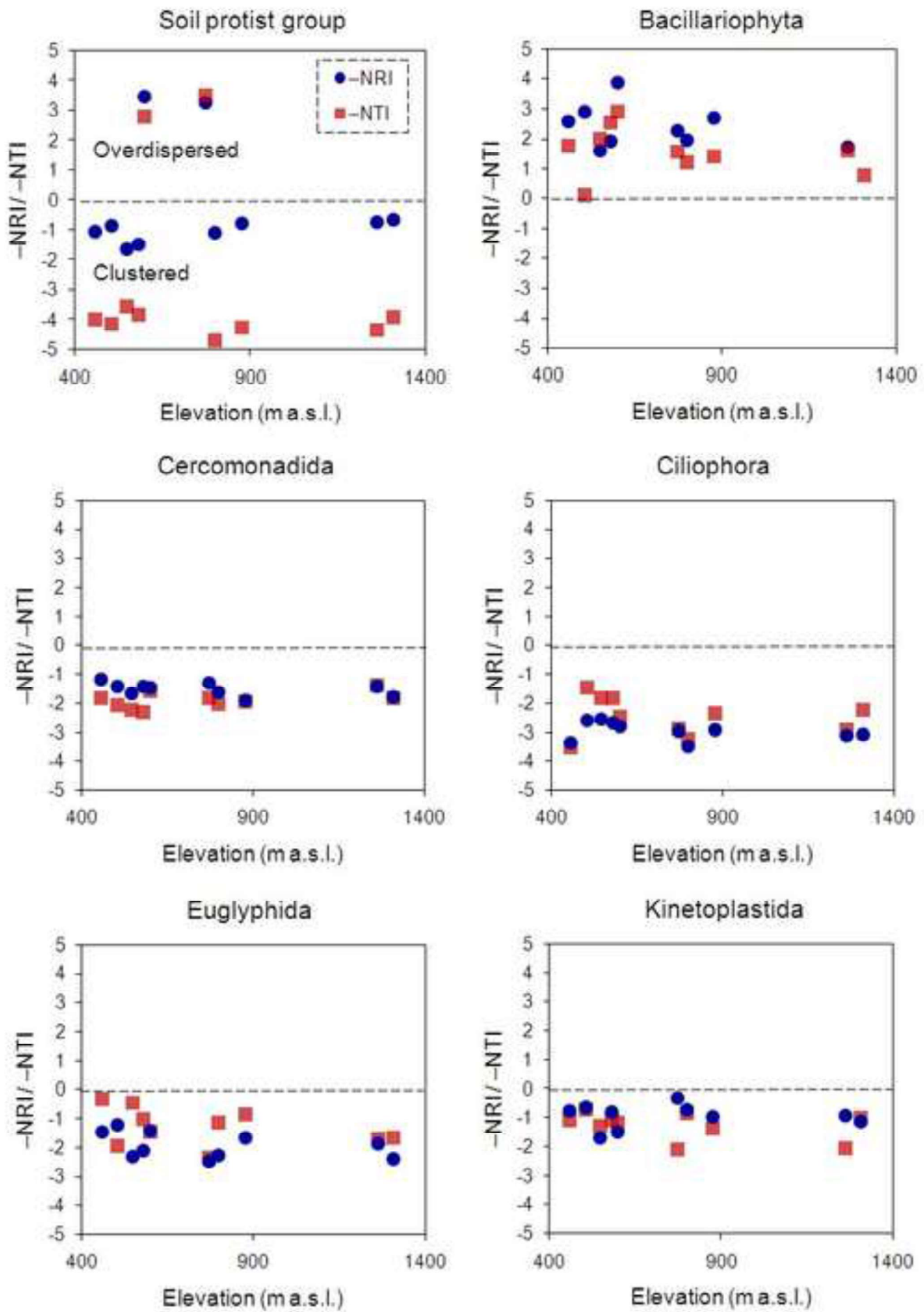Figure 1

Figure 2

Figure 3

Figure 3
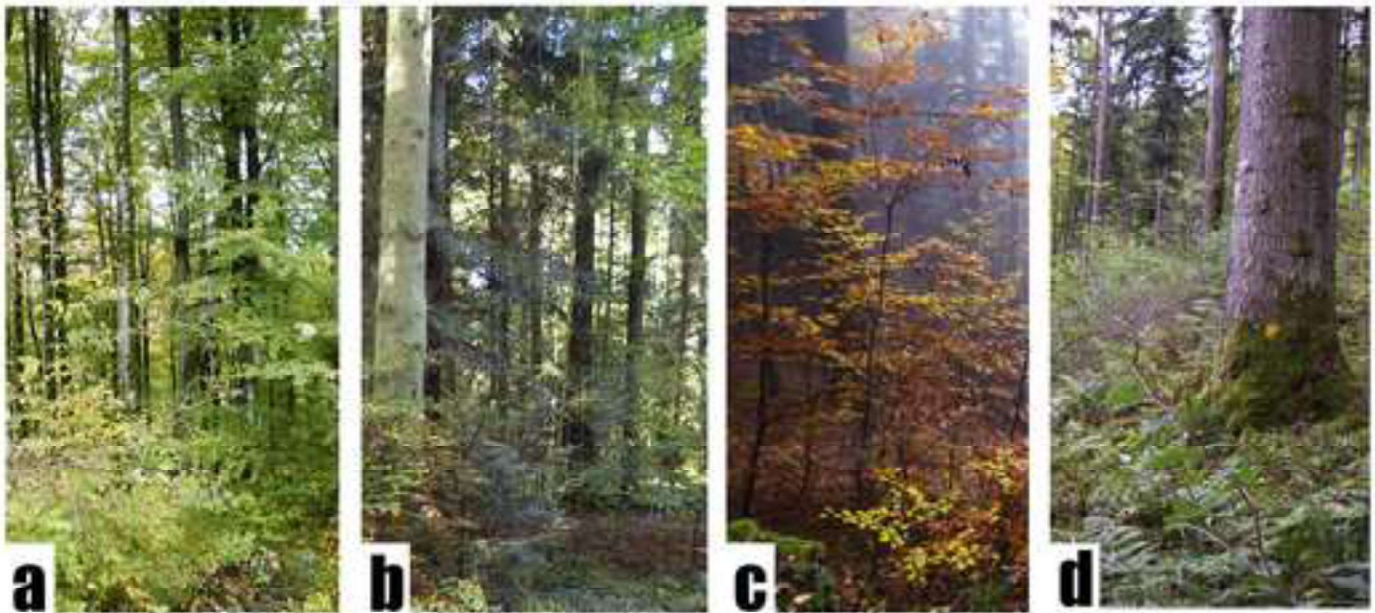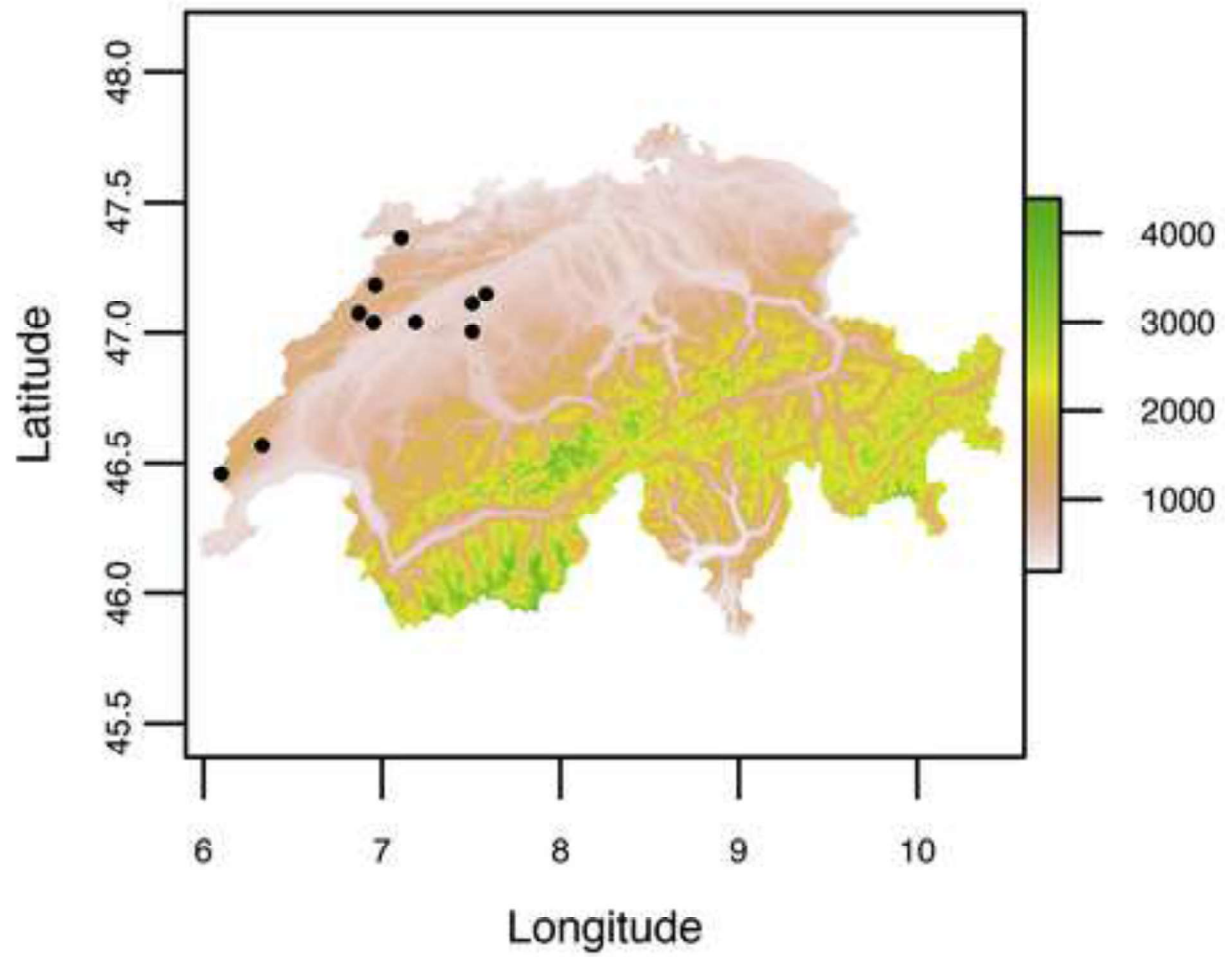
Table 1

Table 1. Correlations between soil protist diversity and local physicochemical parameters estimated at each sampling (beech forest) site. After reducing the set of environmental variables to a representative subset to avoid multicollinearity (we retained soil temperature, light, humidity, pH and humus content), we constructed linear models with richness as response and variables as descriptors and tested the overall model significance as well as the significance of each descriptor using ANOVA in the R package vegan [33]. *P*-values are shown for the whole model and for each physicochemical parameter. The physicochemical parameters have been coded using the nomenclature originally proposed by Landolt [35]. The codes are explained in the lower margin of this table.

	Overall model			Descriptor variables				
	Adjusted R^2	*P*-value	AIC	F	H	L	R	T
Soil protist group	0.09	0.37	124.42	0.60	0.26	0.34	0.13	0.40
Bacillariophyta	-0.03	0.53	82.25	0.35	0.33	0.40	0.11	0.60
Cercomonadida	0.70	0.06	91.86	0.13	0.02	0.22	0.06	0.25
Ciliophora	-0.34	0.69	108.53	0.94	0.96	0.42	0.62	0.59
Euglyphida	0.37	0.55	81.85	0.89	0.63	0.22	0.06	0.29
Kinetoplastida	-0.45	0.94	100.71	0.45	0.63	0.92	0.50	0.80

AIC: Akaike Information Criterion; F: moisture; H: humus content; L: light; R: soil reaction (pH); T: temperature

Table 2

Table 2. Relations between (a) richness and elevation, and (b) richness and average annual air temperature. Relationships were modelled with both linear (L) and quadratic (Q) models. The better model was selected using the Akaike's information criterion.
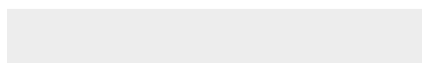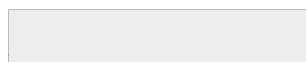
	Relations	Taxon	Linear model		Quadratic model		Better Model
			R^2	P	R^2	P	
(a)	Richness vs. elevation	Soil protist group	-0.699	0.002	0.699	0.353	L
		Bacillariophyta	-0.886	0.000	0.896	0.222	L
		Cercomonadida	-0.853	0.000	0.847	0.425	L
		Ciliophora	-0.579	0.006	0.785	0.021	Q
		Euglyphida	-0.776	0.000	0.842	0.077	L
		Kinetoplastida	-0.593	0.006	0.877	0.003	Q
(b)	Richness vs. average annual air 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

Table 3

Table 3. Phylobetadiversity in soil protists. Variation in phylogenetic composition among sites (PhyloSor) and its underlying phenomena, namely phylogenetic turnover (PhyloSor$_{Turn}$) and phylogenetic nestedness (PhyloSor$_{PD}$), were estimated for each soil protist taxa using betapart [43]. All taxa exhibited higher than expected phylobetadiversity, primarily represented by phylogenetic turnover among sites.

Taxon	PhyloSor		PhyloSor$_{Turn}$		PhyloSor$_{PD}$	
	Observed	Expected	Observed	Expected	Observed	Expected
Soil protist group	0.60	0.45	0.56	0.44	0.04	0.00
Bacillariophyta	0.57	0.32	0.50	0.41	0.07	0.00
Cercomonadida	0.64	0.51	0.60	0.48	0.04	0.00
Ciliophora	0.66	0.55	0.59	0.51	0.07	0.00
Euglyphida	0.61	0.47	0.55	0.42	0.06	0.00
Kinetoplastida	0.61	0.51	0.51	0.44	0.09	0.00

ESM1

ESM2

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