Published in Microbial Ecology 71, issue 3, 725-734, 2016
which should be used for any reference to this work

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

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Abstract Most groups of higher organisms show a decrease in species richness toward high altitude, but the existence of such a pattern is debated for micro-eukaryotes. Existing data are scarce and mostly confounded with the diversity of habitats that also decreases with elevation. In order to disentangle these two factors, one approach is to consider only similar types of habitats occurring across an elevational gradient. We assessed the diversity and community structure of testate amoebae in two specific habitats: (1) natural *Calluna vulgaris* litter and (2) *Minuartia sedoides* cushions 7 years after their transplantation along a vertical transect from 1770 to 2430 m in the subalpine and alpine zones of the Swiss Alps. Analyses of co-variance and variance showed that testate amoeba species richness, equitability, and diversity declined with elevation and were significantly correlated to habitat type. In a redundancy analysis, the variation in the relative abundance of the testate amoeba taxa in *Calluna vulgaris* litter was equally explained by elevation and litter pH. This is the first study documenting a monotonic decrease of protist diversity in similar habitats across an elevational gradient.

Keywords Biodiversity patterns · Elevational gradient · Micro-eukaryotes · Pot experiment · Protists · Soil

Introduction

Examining the distribution of organisms along elevational gradients is a classical approach used for understanding the broad-scale distribution of species, the mechanisms that shape their biogeographical patterns and the influence of global change on protist communities. In most groups of macroorganisms, species diversity peaks at low latitudes and decreases toward higher latitudes (e.g., [1]), whereas along elevational gradients, a monotonic decrease in biodiversity toward higher altitude or a mid-elevational peak along the elevational gradient is generally observed [2–4]. Although microorganisms dominate biodiversity on earth and play essential roles in biogeochemical cycles and food web processes [5, 6], it still remains unclear whether patterns of diversity are similar for soil microorganisms and in particular for micro-eukaryotes.

With respect to micro-eukaryotes, diversity patterns along elevational gradients were mostly studied for diatoms (Stramenopiles) and testate amoebae (Amoebozoa, Rhizaria, and Stramenopiles [7]). Gremmen et al. [8] reported a negative relationship between moss-inhabiting diatom species diversity along a 550-m altitudinal gradient on the sub-Antarctic

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Electronic supplementary material The online version of this article (doi:10.1007/s00248-015-0687-3) contains supplementary material, which is available to authorized users.

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Kerguelen Island. With respect to testate amoebae, Todorov [9] reported less diverse communities in the subalpine zone than in the mountain forest, at lower elevation. In contrast, Mitchell et al. [10] found no significant correlations between testate amoeba diversity and communities in the moss *Hylocomium splendens* along an elevational gradient in the Alps. In a tropical mountain rain forest in Ecuador, Krashevska et al. [11–13] observed a higher species richness of testate amoebae in soils and epiphyte habitats at an intermediate elevation (2000 m above sea level (a.s.l.)) as compared to lower 1000 and 3000 m. In two vegetation types of the subarctic tundra, Tsyganov et al. [14] revealed a non-linear pattern of testate amoeba along a 400-m altitudinal range while Lamentowicz et al. [15] found a peak of diversity at 1400 m when he investigated testate amoeba from four *Sphagnum* peatlands along an altitudinal gradient in Switzerland (580–1880 m a.s.l.). More recently, Shen et al. [16] examined the eukaryotic microbial diversity along an elevational transect in China and found no correlation between the total eukaryotic community and elevation.

These contrasting patterns of diversity along elevational gradients may seem puzzling but might, to a large extent, be due to methodological biases related to (1) the variability of the investigated altitudinal ranges among transects, (2) the number of sites investigated along the transect, and (3) the variability of habitats investigated at the different altitudes (Table 1). This last point is of particular importance since the diversity of plant functional groups was shown to affect testate amoeba species diversity [17]. It is therefore impossible to conclude whether the observed diversity patterns are primarily explained by elevation (and, hence, climate) or habitat diversity changes. To disentangle these two effects, one solution is to consider only single habitats occurring across an altitudinal gradient. Mitchell et al. [10] followed this approach by assessing testate amoeba diversity from one very specific habitat (*Hylocomium splendens* mosses) along an altitudinal range. This study was limited to the forested ecozone, and the authors highlighted the need to perform a similar study across the treeline where the (micro) climatic conditions might be more contrasted.

Here, we (i) investigated testate amoeba diversity and community structure in two habitats along the same altitudinal gradient in the Alps and (ii) evaluate which environmental factors are correlated with testate amoeba diversity. We investigated a steep altitudinal transect crossing the forest and tree lines from 1770 to 2430 m a.s.l. (subalpine and alpine zones) in the Swiss Alps in two habitats: natural *Calluna vulgaris* (L.) Hull litter which is found across a broad altitudinal range and *Minuartia sedoides* (L.) Hiern cushion plants transplanted in the field.

We hypothesized that (1) testate amoeba density and diversity would decline with elevation, (2) testate amoeba density and diversity be higher in *Calluna* litter than *Minuartia* cushions owing to the more stable micro-environmental conditions (i.e., more stable moisture and temperature under than shade of the *Calluna* bushes), and (3) the proportion of shells with adaptations to drought would increase at higher elevation (i.e., “plagiostomic” and “cryptostomic” types with more hidden aperture than the “acrostomic” type). The two selected substrate types provide complementary data along an altitudinal gradient. The *Calluna* litter provides information on testate amoeba community from a natural environment while the *Minuartia* cushions planted in pots filled with identical soil allow us to rule out the potential impact of soil differences on protist communities along the altitudinal gradient.

Testate amoebae are an ideal group of micro-eukaryotes for assessing questions related to patterns of free-living terrestrial protistan diversity because they are diverse and abundant in soils and mosses, play an important role in microbial food webs, especially in acidic litter [18, 19], and respond to environmental factors such as the moisture content, pH, and temperature [20–22]. Furthermore, testate amoebae produce a shell which allows the identification of morphospecies more easily than in the vast majority of other protists [23].

Material and Methods

Study Site

The altitudinal transect is located in the Val d’Arpette (7° 04′ E; 46° 01′ N) at the eastern limit of the Mt Blanc Massif in the Swiss Alps. This mountainous area is characterized by a moderately continental climate. Mean annual temperature (1993–1999) at 1860 m a.s.l. is 4.38 °C with averages of 11.81 °C in summer (June–August) and −2.78 °C in winter (December–February) and mean annual precipitations (1994–1996) of 1575 mm. Precipitations (1996–1999) during the growing period (June–September) increase only slightly between 1800 and 2600 m a.s.l. The bedrock consists of granite. The sampled area ranges from 1720 to 2480 m a.s.l. along a steep, south to southeast oriented slope (mean 38°). The tree line is located at an altitude of approximately 2200 m a.s.l. *Calluna vulgaris* is common in the open, subalpine coniferous forest dominated by *Larix decidua*, *Picea abies*, *Pinus mugo*, and *Pinus cembra*, as well as in the intermingled, thermophilous heaths, followed by the mixed heath-swards above, dominated by *Arctostaphylos uva-ursi*, *Juniperus communis* subsp. *alpina*, *Vaccinium vitis-idaea*, *Vaccinium gaultherioides*, *Carex sempervirens*, *Nardus stricta*, and *Festuca scabriculmis* subsp. *luedii* (nomenclature according to [24]). Along the sampled area, Podzol and Ochric Podzol soil types were identified on the base of nine soil profiles [25, 26]. A previous study reported a decrease of vascular plants and bryophyte richness with increasing altitude along this transect [27].

Table 1 Non-exhaustive compilation of studies comparing testate amoebae diversity data at different altitudes

Reference	Study site	Ecosystem	Sample type	Samples collected along a transect(s) or from different slopes	Elevational range (m a.s.l.)	Number of elevational sites	Elevation between sampling sites (m)	Species diversity pattern[a]
Todorov [9]	Pirin Mountains, Bulgaria	Forests and meadows	Different moss and soil samples	No transects, different sites in the Pirin Mountains	400–2500	N.A.	Samples from various altitudes	Highest diversity at 900–1500 m a.s.l.
Mitchell [10]	Southeastern Alps of Italy	Forest	*Hylocomium splendens* moss	Three transects	1000–2200	7	ca. 200	No significant relationship
Krashevska et al. [12]	Southern Ecuador	Tropical mountain rain forest	Litter and soil samples	Three transects	1000–3000	3	ca. 1000	Peaks at intermediate level (ca. 2000 m a.s.l.)
Krashevska et al. [13]	Southern Ecuador	Tropical mountain rain forest	Epiphyte samples	Three transects	1000–3000	3	ca. 1000	Peaks at intermediate level (ca. 2000 m a.s.l.)
Lamentowicz et al. [15]	Jura and Swiss Alps	Peatland	*Sphagnum* sp. moss	Distinct regions	580–1880	4	ca. 400	Peak at intermediate level (ca. 1400 m a.s.l.)
Tsyganov et al. [14]	Northern Sweden	Meadow and willow in the subartic Tundra	Soil samples	One transect	500–900	5	ca. 100	U-shaped relationships with elevation
Present study	Swiss Alps	Forest and alpine meadow	*Calluna vulgaris* litter and *Minuartia sedoides* cushions	One transect	1780–2400	5 (3 for *Minuartia sedoides* samples)	ca. 150 (330 for *Minuartia sedoides* samples)	Linear decreased with altitude

[a] Conclusions based on living and empty shells, except in Tsyganov et al. [14] where conclusions are based on empty shells

Sampling of *Calluna vulgaris* Litter

Calluna litter samples were collected every ca. 150 m of elevation between 1770 and 2400 m a.s.l. on August 24, 2006. At each of the five different altitudes, three replicate litter samples were taken at least 25 m apart under dense natural *Calluna vulgaris* dwarf shrubs, at the same altitude and in macroscopically similar conditions. The value of pH was recorded from each *Calluna vulgaris* sample and ranged from 4.02 to 4.94 (average=4.58; SE=0.06).

Establishment of the Transplantation Experiment and Sampling of *Minuartia sedoides* Cushions

During the summer 1999, *Minuartia* plants were collected with roots in the alpine belt near the Orny glacier, above Champex-Lac (between 2500 and 2700 m a.s.l.), and planted in six distinct planter trays (50×29 cm), each with 15 pots 8 cm in diameter and 10 cm deep, for a total of 90 pots. The pots contained a draining mixture of siliceous sand and potting soil and were kept in the alpine garden nursery of Champex-Lac for about 3 months. At the end of October 1999, the plates were transported and installed at three different altitudes, i.e., 1770, 2100, and 2430 m a.s.l. Two plastic plates, each with 15 living *Minuartia* cushions, were positioned in rows on two leveled beds adding to a total of 30 plants per site along the altitudinal transect. Some testate amoeba taxa were probably present in *Minuartia* cushions before the transplantation experiment, but protist community differences among pots were certainly low because all *Minuartia* plants were collected from the same site and then kept in similar environmental conditions for about 3 months. In the field, the vegetation around the plates (i.e., 20 cm) was removed at the beginning of the transplantation experiment and any plant colonizing this bare surface was removed each year. In 2006, there were 18 *Minuartia* cushions left at 1770 m a.s.l., four at 2100 m a.s.l. and two at 2430 m.

Minuartia cushion samples were collected from two distinct pots at each altitude on August 24, 2006. In order to minimize the potential variability due to fine-scale spatial heterogeneity, three sub-samples consisting of about 8 cm^3 (2×2×2 cm) were pooled to make up each replicate sample. Measurements of pH values were not possible because of the limited amount of *Minuartia* cushion material available. However, it is unlikely that pH varied much along the altitudinal gradient as the substrate as well as the plants were identical.

Testate Amoeba Analyses

Testate amoebae extraction was done by washing the samples over filters of 700- and 200-μm-mesh size to remove coarse particles. In order to maximize the extraction of tests, the sieving was done with high water flow and then concentrated by centrifugation (5 min at 2500 rpm). Glutaraldehyde was added to fix the material (2 % final concentration). In each sample, 100 testate amoebae were identified to morphospecies level at ×400 magnification. The absolute concentration (density) of testate amoebae in our samples was expressed per gram of air-dried material. To evaluate the influence of the soil moisture content on testate amoeba communities, we assessed the position of the aperture of testate amoebae taxa as a functional trait. The increasing protection of the aperture in testate amoebae is believed to be an adaptation to decreasing soil moisture content [28]. As described in Fournier et al. [28], testate amoeba taxa were assigned to four different aperture types, ranging from completely exposed to completely cryptic apertures: axial aperture, acrostomic aperture, and plagiostomic and cryptostomic apertures. The aperture-type ratio was calculated as the proportion of specimens characterized by plagiostomic and cryptostomic apertures versus acrostomic apertures. Since we were interested in assessing the long-term effect of environmental factors on testate amoeba communities, we combined both living and empty tests in our analyses.

Statistical Analyses

In order to determine the relationship between testate amoeba species richness and equitability and habitat type (*Calluna* litter versus *Minuartia* cushions) along the altitudinal gradient, we used an analysis of covariance (ANCOVA) with altitude (i.e., m above sea level) as covariable. In addition, ANCOVA was used to assess the relationships between testate amoeba aperture type (i.e., the proportion of testate amoeba specimens characterized by plagiostomic and cryptostomic apertures) and habitat type along the altitudinal gradient. We used analysis of variance (ANOVA) to assess the relationships between altitude and testate amoeba diversity (Shannon-Wienner) and density for each habitat separately. For these last analyses, ANCOVA could not be used since significant interactions between altitude and the substrates were identified [29]. The density of testate amoebae was log-transformed to homogenize variances. ANCOVA and ANOVA tests were applied after corroboration of the assumptions of normality and variance homogeneity with the Wilk-Shapiro and Bartlett tests. Furthermore, we used redundancy analysis (RDA) to test the relationship between testate amoeba communities of the *Calluna* litter and their environment variables (i.e., altitude, pH, and the absence or presence of the forest). Data from *Minuartia sedoides* were not included in this analysis since pH was not measured. Seven taxa (*Centropyxis constricta*, *Cyclopyxis kahli*, *Difflugia pristis*, *Euglypha compressa*, *Euglypha tuberculata*, *Heleopera petriocola*, *Nebela militaris*) reported each from only one sample were excluded from this analysis in order to eliminate the unwanted effect of rare species (i.e., species with a relative abundance

≤1 %). A species matrix, corresponding to the abundance of testate amoeba species at each site, was then Hellinger transformed [30]. ANOVA permutation tests (999 permutations) were used to assess the significance of the variables, the individual axes, and the overall model. In addition, the forward selection procedure [31] was performed to identify environmental variables that significantly explained parts of the variation in testate amoeba species data. Finally, a second RDA was performed on the entire species dataset to evaluate whether or not the habitat type (i.e., *Minuartia* cushion and *Calluna* litter) has a significant impact on the composition of testate amoebae. Species matrix was transformed, and rare taxa were excluded as described above. We used altitude as numeric explanatory variable as well as the absence/presence of forest and the substrate type as categorical explanatory variables. ANOVA permutation tests (999 permutations) of each explanatory variable were used to assess the significance of the substrate type and the other variables. All analyses were performed in R version 3.0.1. [32], using the package “vegan” [33].

Results

Testate Amoeba Diversity and Species Composition in *Calluna vulgaris* and *Minuartia sedoides*

The analysis of 15 *Calluna* litter and 6 *Minuartia* cushion samples revealed a total of 31 morphospecies (15 arcellinids and 16 euglyphids) belonging to 16 genera. The three most abundant taxa in *Calluna* litter were *Corythion dubium*, *Assulina muscorum*, and *Trinema lineare*; together, they accounted for 59 % of the total count. In *Minuartia* cushions, *Phryganella acropodia*, *Centropyxis aerophila*, and *Euglypha rotunda* were the three most abundant species and represented 81 % of the total count (Table 2). RDA analysis revealed a significant impact of the substrate type on protist community composition ($F_{1,17}$=17, P<0.001). Species richness was significantly higher in *Calluna* litter than in *Minuartia* cushion samples (Fig. 1a). In *Calluna* litter samples, the species richness per sample varied from 12 to 21 taxa (average=15; SE=0.7) and from 6 to 11 taxa in *Minuartia* cushion samples (average=9; SE=0.7). Eighteen taxa were reported only in *Calluna* litter, 13 taxa were found both in *Minuartia sedoides* and in *Calluna vulgaris*, while no taxa were reported only in *Minuartia* cushion samples. The Shannon-Wiener equitability, Shannon-Wiener diversity, and the density of testate amoebae were significantly higher in *Calluna* litter samples than in *Minuartia* cushion samples (Fig. 1). The density of testate amoebae varied between 17,773 and 114,666 individuals per gram of dry weight in *Calluna* litter (average=53,167; SE=5292) and between 1427 and 13,671 in *Minuartia* cushion samples (average=7782; SE=2182). In *Calluna* litter, the species richness, Shannon-Wiener diversity, Shannon-Wiener equitability, and the density of testate amoebae increased with increasing pH values, but these correlations were not significant (Supplementary material 1). The proportion of testate amoebae characterized by plagiostomic and cryptostomic apertures was not significantly higher in *Minuartia* cushions than in *Calluna* litter.

Patterns of Testate Amoeba Diversity and Community Structure Along the Altitudinal Gradient

Overall, testate amoeba species richness, equitability, and diversity in *Calluna vulgaris* and *Minuartia sedoides* samples decreased significantly with increasing altitude (Fig. 1a–c). The density of testate amoebae decreased significantly with increasing altitude in *Minuartia* cushions but not in *Calluna* litter (Fig. 1d). Four species, *Centropyxis constricta*, *Euglypha compressa*, *E. denticulata*, and *Heleopera petriocola*, were found only at the lowest altitudinal site, i.e., at 1770 m in *Calluna* litter, while no taxa were reported exclusively from the highest altitudinal site at 2400 m. Four taxa, *Centropyxis aerophila*, *Euglypha rotunda*, *Plagiopyxis declivis*, and *Trinema lineare*, were present in all altitudes and both types of habitats while seven taxa, *Cyclopyxis kahli*, *D. pristis*, *Difflugia tenuis*, *E. compressa*, *E. denticulata*, *E. polylepis*, *Heleopera petriocola*, and *Nebela militaris*, were reported at one elevational site only.

The proportion of specimens characterized by plagiostomic and cryptostomic apertures, which offer a better protection from drying than the other types of apertures, decreased with elevation. This correlation was, however, not significant (Supplementary material 2). In *Calluna* litter, no correlation was found between pH and the aperture-type ratio of testate amoebae (Supplementary material 3).

Redundancy analysis (RDA) showed that altitude and litter pH were significantly correlated to compositional differences in testate amoeba data and explained 12.3 % of the variance (adjusted R^2) (P<0.05). Variance partitioning revealed that 5.5 % (adjusted R^2) of the variance was explained by the unique effect of altitude and 5.5 % of the variance by the unique effect of pH, and 1.3 % of the variance was jointly explained by both variables (P<0.05). The presence/absence of the forest did not significantly explain the composition of testate amoeba communities in *Calluna* litter.

The RDA analysis also revealed that the most abundant taxa, *Corythion dubium* and *Assulina muscorum*, were positively correlated to altitude while *Cyclopyxis eurystoma* or *Tracheleuglypha dentata* were negatively correlated (Fig. 2). Despite the fact that we focused on a single habitat and that the pH gradient was therefore short (i.e., 4.02–4.94), several species were either positively (e.g., *Trachelocorythion pulchellum*, *T. dentata*, *Cyclopyxis eurystoma*) or negatively

Table 2 Relative abundance of testate amoebae species in *Calluna vulgaris* litter and *Minuartia sedoides* cushions along an altitudinal gradient in the Swiss

	Average relative abundance in *Calluna vulgaris* litter (%) (SE)					Average relative abundance in *Minuartia sedoides* cushions (%) (SE)		
	1780 m	1940 m	2100 m	2260 m	2400 m	1780 m	2100 m	2400 m
Arcella arenaria Greff	0.3 (0.3)	1.3 (0.9)	1 (0.6)	0.3 (0.3)	1 (1)	1 (0)	0 (0)	0.5 (0.5)
Assulina muscorum Greff	9.7 (1.8)	19.3 (6.1)	19.3 (2.6)	19.7 (3.8)	14 (2.5)	0 (0)	1 (1)	0.5 (0.5)
Centropyxis aerophila Deflandre	7 (1)	3 (0.6)	1.7 (0.3)	5.7 (4.2)	2.7 (1.2)	9.5 (1.5)	55 (10)	45.5 (37.5)
Centropyxis constricta Ehrenberg[a]	1.7 (1.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Corythion dubium Taranek	19.3 (4.6)	33 (8.6)	31 (3.8)	29.7 (2.3)	29.3 (5.5)	8.5 (1.5)	0 (0)	0 (0)
Cyclopyxis eurystoma Deflandre[a]	3 (2)	2.6 (1.8)	1 (0.6)	0.3 (0.3)	0.3 (0.3)	0 (0)	0 (0)	0 (0)
Cyclopyxis kahli Deflandre	2 (2)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1)	2 (2)	1 (0)
Difflugia pristis Penard[a]	0 (0)	0 (0)	0.3 (0.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Difflugia tenuis (Penard) Chardez	1 (1)	0.6 (0.7)	0 (0)	0 (0)	0 (0)	0 (0)	0.5 (0.5)	0 (0)
Euglypha ciliata Ehrenberg	6.3 (2.2)	8.6 (1.5)	6 (0.6)	5.7 (1.8)	4.3 (1.5)	8 (7)	1.5 (1.5)	0 (0)
Euglypha compressa Carter[a]	0.3 (0.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Euglypha denticulata Brown[a]	2 (1.5)	0 (0)	0 (0)	0 (0)	1 (1)	0 (0)	0 (0)	0 (0)
Euglypha dolioliformis Bonnet	0 (0)	1.6 (0.9)	0.7 (0.7)	1.7 (0.9)	0.7 (0.3)	0 (0)	0 (0)	0.5 (0.5)
Euglypha laevis Perty[a]	1.7 (1.7)	0.3 (0.3)	0 (0)	0 (0)	0.3 (0.3)	0 (0)	0 (0)	0 (0)
Euglypha polylepis Bonnet	0 (0)	0 (0)	0 (0)	1.3 (1.3)	1 (0.6)	0.5 (0.5)	0 (0)	0 (0)
Euglypha rotunda Wailes	5.3 (1.8)	2.6 (1.5)	3.7 (2)	3.7 (1.5)	4.3 (0.9)	15.5 (0.5)	5 (2)	1 (1)
Euglypha tuberculata Dujardin	0 (0)	0 (0)	0 (0)	0 (0)	1 (1)	0 (0)	0 (0)	0 (0)
Heleopera petricola Leidy[a]	0.3 (0.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Nebela militaris Penard[a]	0 (0)	0 (0)	0 (0)	0.7 (0.7)	0 (0)	0 (0)	0 (0)	0 (0)
Nebela tincta Awerzinzew[a]	1 (1)	0 (0)	0 (0)	3.3 (3.3)	0 (0)	0 (0)	0 (0)	0 (0)
Padaungiella wailesii Lara & Todorov (Deflandre)[a]	1.3 (1.3)	0.3 (0.3)	0.7 (0.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Phryganella acropodia Hopkinson	6 (2.6)	6.3 (2.3)	4 (1.7)	5.3 (3)	0.3 (0.3)	43.5 (11.5)	25 (15)	44 (33)
Plagiopyxis callida Penard	1 (1)	0.3 (0.3)	0.3 (0.3)	1 (0.6)	1 (1)	3 (3)	0 (0)	0 (0)
Plagiopyxis declivis Penard	1.3 (0.7)	1 (0.6)	1.7 (0.7)	2.3 (0.7)	9.3 (8.3)	2 (2)	3.5 (3.5)	4 (1)
Pseudodifflugia gracilis var. *terricola* Bonnet & Thomas	0.3 (0.3)	0.6 (0.3)	1 (0.6)	1.7 (1.2)	0 (0)	0.5 (0.5)	1.5 (0.5)	2.5 (2.5)
Tracheleuglypha dentata Deflandre	1.7 (1.2)	0.3 (0.3)	0.3 (0.3)	0 (0)	0 (0)	0 (0)	0.5 (0.5)	0 (0)
Trachelocorythion pulchellum Bonnet	1.3 (0.7)	0 (0)	1.7 (0.3)	1 (1)	0.7 (0.7)	0 (0)	1.5 (1.5)	0 (0)
Trinema complanatum Penard[a]	4.7 (1.5)	4.3 (0.7)	6.3 (2.3)	2.7 (1.2)	6 (1.7)	0 (0)	0 (0)	0 (0)
Trinema enchelys (Ehrenberg) Leidy[a]	2.3 (0.9)	0.3 (0.3)	1.7 (0.9)	0.7 (0.7)	0.7 (0.7)	0 (0)	0 (0)	0 (0)
Trinema lineare Penard	15.3 (1.2)	9 (2.1)	13.3 (4.1)	12.7 (3.2)	19.3 (3.7)	2.5 (0.5)	3 (1)	0.5 (0.5)
Trinema penardi Thomas & Chardez	3.6 (1.2)	4 (1.2)	4.3 (1.3)	0.7 (0.3)	2.7 (2.9)	4.5 (1.5)	0 (0)	0 (0)

[a] Species observed only in *Calluna vulgaris* litter

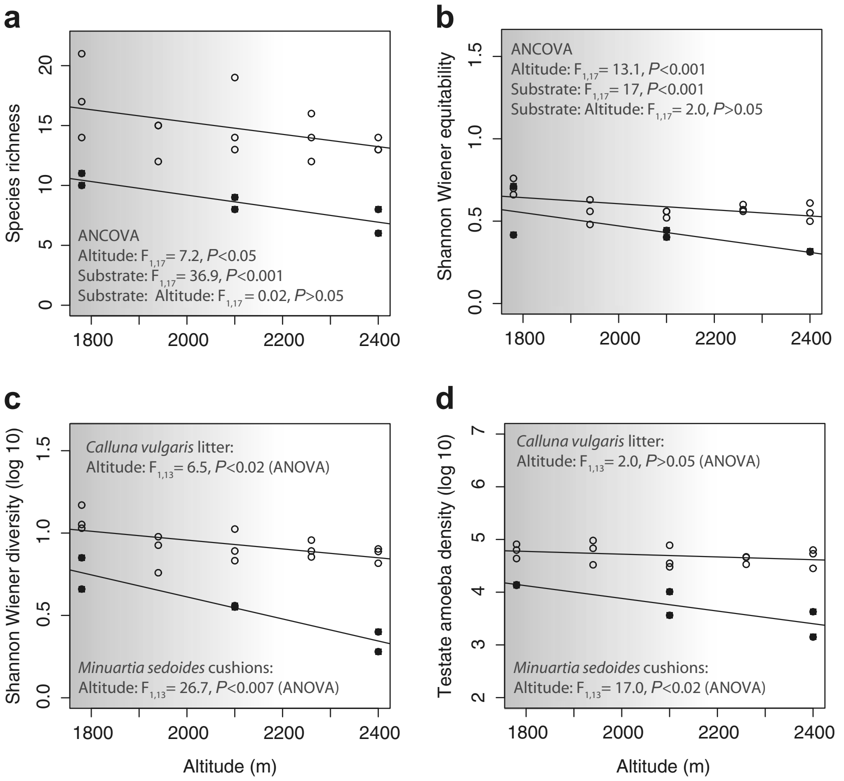

Fig. 1 Relationships between testate amoeba species richness (**a**), Shannon-Wiener equitability (log10-based) (**b**), Shannon-Wiener diversity index (**c**), and density (individuals/g) (**d**) and altitude. *Squares* and *open circles* represent, respectively, data recorded from *Calluna vulgaris* litter and *Minuartia sedoides* cushions. The *grey sector* represents the presence of the forest along the altitudinal gradient

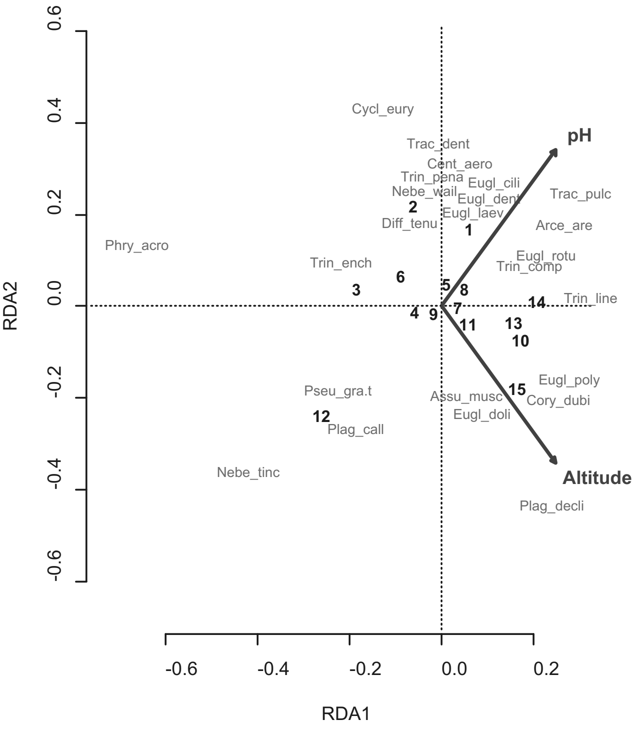

Fig. 2 Redundancy analysis (RDA) of the 15 testate amoebae samples analyzed in *Calluna vulgaris* litter (samples 1–3: alt=1770; samples 4–6: alt=1940; samples 7–9: alt=2100; samples 10–12: alt=2260; samples 13–15: alt=2400). Altitude and pH explain 12.3 % of the variation in the species data (adjusted R^2, P<0.05) (for full names of the taxa, see Table 2)

(e.g., *Nebela tincta*, *Plagiopyxis callida*, *Phryganella acropodia*) correlated to litter pH.

Discussion

In this study, we examined the diversity and community structure of testate amoebae along an altitudinal gradient. To minimize the variability due to soil heterogeneity and vegetation type, two specific substrates were investigated: (1) litter samples from *Calluna* plants and (2) *Minuartia* cushions, which naturally occur across this altitudinal range, from a pot experiment. To the best of our knowledge, this is the first study assessing soil protist diversity patterns in specific habitats across the treeline.

Diversity Patterns of Testate Amoebae Along the Altitudinal Gradient

Our results revealed that testate amoeba diversity from similar habitats decreased significantly with altitude in both investigated substrates. Our results contrast with the conclusions from Mitchell et al. [10] where testate amoebae were investigated in a very specific habitat (i.e., *Hylocomium splendens*) along the elevational gradient. The authors did not find any significant relationship between the altitude and testate amoeba diversity, although the altitudinal range of their study (1000–2200 m a.s.l.) partially overlaps the altitudinal range of the present study (1770–2430 m a.s.l.). Several possibilities might explain these different results. Firstly, Mitchell et al. [10] did not extend their study above the treeline, where the microclimatic conditions can be much more variable than below the treeline, under the forest canopy [34, 35]. Secondly, different types of substrates (i.e., *Calluna* litter, *Minuartia* cushions, and *Hylocomium splendens* mosses) have been investigated in these two studies and each of them might influence testate amoeba communities differently along the elevational gradient. For instance, some of these substrates might keep the water content more constant along the elevational gradient than others. The lack of pattern observed by Mitchell et al. [10] may indeed be explained by the fact that only the third segment of the mosses was sampled, excluding the upper segments where diversity was lower and the lower segment where diversity was higher but soil taxa appeared. *Hylocomium splendens* mosses produce new segments sequentially, but these do not necessarily correspond to annual growth (Bjartmar Sveinbjörnsson personal communication).

In contrast, our results tend to be in line with Lamentowicz et al. [15] who found in *Sphagnum* mosses a peak of testate amoeba diversity at 1406 m. However, this comparison has to be taken with caution since Lamentowicz and colleagues investigated only one site above 1406 m and the *Sphagnum* species investigated along the gradient were potentially different (i.e., slightly different substrate).

Testate Amoeba Composition in *Calluna vulgaris* Litter Versus *Minuartia sedoides* Cushions

The altitudinal pattern of testate amoeba diversity was similar in *Calluna* litter and *Minuartia* cushions. However, the composition of testate amoebae differed significantly between the two types of habitats. Particularly, the overall diversity of testate amoebae was much higher in *Calluna* litter than in *Minuartia* cushions. This difference might be due to a higher water stress in *Minuartia* cushions than in *Calluna* litter. Indeed, as documented by several studies, testate amoebae and soil protist, in general, are highly sensitive to soil moisture content [36, 37]. In *Calluna* litter, evaporation is likely reduced by the shade provided by the *Calluna* plants. Moreover, the relatively thick layer of *Calluna* litter helps to keep this habitat moist. By contrast, the *Minuartia* cushions were fully exposed to the sun and wind and especially so in the experimental setting where the vegetation had been removed around the black plastic plates.

Potential Factors Affecting Protist Diversity Along the Elevational Gradient

The investigated altitudinal transect, from 1770 to 2430 m a.s.l., corresponds to a steep climatic gradient. Annual average temperatures can be estimated to decrease by approximately 4 °C from the bottom to the top of the transect, assuming a linear relationship of −0.6 °C per 100 m [38]. Beyond temperature, solar radiation and air pressure are also correlated to elevation as well as factors such as precipitation, wind speed, seasonal patterns of soil moisture and air humidity, and soil composition but might have a more complex relationship with altitude [39–41].

In this study, we expected that elevation would explain an important percentage of the variation in the testate amoeba species composition dataset because (1) temperature [22, 37, 42] and soil moisture content [43] have been shown to influence testate amoeba communities, (2) our sampling design aimed to keep most of the other environmental variables, including pH, constant along the gradient, and (3) the sampling effort has been identical at each elevation removing potential sampling biases [44]. In agreement with this hypothesis, testate amoeba species composition in *Calluna* litter was significantly explained by elevation in the RDA. In addition, our data confirmed that testate amoeba species richness was lower in the drier, less stable substrate (i.e., *Minuartia sedoides*) than in the substrate with higher soil moisture (i.e., *Calluna vulgaris*) along the studied elevational gradient.

Litter pH, which also significantly explained testate amoeba community structure in the RDA, was not correlated to altitude, illustrating that other, relatively minor (as determined by our sampling approach), environmental gradients can influence testate amoeba community structure. Our results therefore agree with the observation of Shen and colleagues that soil micro-eukaryotic communities are correlated to pH [16]; in addition, our study revealed a correlation with elevation.

Clearly, additional factors than altitude and pH influence testate amoebae communities, and at least some of these variables are not strongly correlated to either of these factors. Numerous abiotic factors such as bioavailable nitrogen, calcium, and sulfate might influence testate amoeba communities [45, 46]. Besides these variables, several biotic variables such as the availability of food resources have been shown to drive testate amoeba community structure, as demonstrated by Krashevska et al. [11] along an elevational gradient of a tropical mountain rain forest.

Finally, although we focused this study on specific habitats to overcome the possible bias of changes in habitat diversity with elevation, this factor could still play a role in determining the regional species pool and thus affecting the diversity simply through mass effect [47]. In order to assess this effect, one approach would be to place sticky slides to record the input of testate amoebae at a different elevation as done recently by Wanner and colleagues in a quite different setting [48].

Perspectives

While the characterization of plant and animal communities along the slopes of mountains started about 200 years ago

[44], data on the elevational patterns of micro-eukaryotic diversity remains extremely limited. For the first time, our approach allowed us to disentangle the effect of elevation and habitat diversity on testate amoeba diversity and communities. Our results from the subalpine and alpine zones showed a monotonic decrease of testate amoeba diversity and changes in community patterns along an elevational gradient as well as an effect of habitat type and pH. Further research including observational studies along broader elevational ranges, the assessment of aerial dispersal of testate amoebae, and controlled field or laboratory manipulative experiments are needed to further explore the distribution patterns of testate amoebae and protists and micro-eukaryotes, in general, and the factors that control these patterns.

Acknowledgments This work was supported by the Swiss National Science Foundation (projects no. 205321-109709/1). T.J. Heger is currently supported by the Swiss National Science Foundation (project no. PA00P3_145374). We thank Alexandre Buttler, François Gillet, Jean-Michel Gobat, Claire Guenat, Julia Gustavsen, Michael De la Harpe, Marcie Kamerzin, Tanja Schwander, Aurélie Thébault, and Jean-David Teuscher for constructive discussion, laboratory assistance, help with statistical analyses, collection and transplantation of *Minuartia sedoides*, and/or comments on the manuscripts. We thank two referees for insightful comments that helped in improving several aspects of this paper.

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