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Testate Amoebae (Protista) Communities in *Hylocomium splendens* (Hedw.) B.S.G. (Bryophyta): Relationships with Altitude, and Moss Elemental Chemistry

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We studied the testate amoebae in the moss *Hylocomium splendens* along an altitudinal gradient from 1000 to 2200 m asl. in the south-eastern Alps of Italy in relation to micro- and macro-nutrient content of moss plants. Three mountainous areas were chosen, two of them characterised by calcareous bedrock, the third by siliceous bedrock. A total of 25 testate amoebae taxa were recorded, with a mean species richness of 9.3 per sampling plot. In a canonical correspondence analysis, 63.1% of the variation in the amoebae data was explained by moss tissue chemistry, namely by C, P, Ca, Mg, Al, Fe, and Na content and a binary site variable. We interpreted this result as an indirect effect of moss chemistry on testate amoebae through an influence on prey organisms. Although two species responded to altitude, there was no overall significant relationship between testate amoebae diversity or community structure and altitude, presumably because our sampling protocol aimed at minimizing the variability due to vegetation types and soil heterogeneity. This suggests that previous evidence of altitudinal or latitudinal effects on testate amoebae diversity may at least in part be due to a sampling bias, namely differences in soil type or moss species sampled.

Introduction

This study addresses two fundamental questions regarding the distribution of free-living protists in terrestrial ecosystems: 1) Do they respond to altitudinal or latitudinal gradients and 2) do they respond to the chemistry of their living environment? To answer these questions, we focus on a common group of free-living protists, the testate amoebae.

Testate amoebae are a group of shelled protozoa that have been shown to be good indicators of hydrology and water chemistry in lakes, rivers, and peatlands (Beyens et al. 1990; Charman and Warner 1997; Mitchell et al. 1999; Schönborn et al. 1965; Tolonen et al. 1992; Trappeniers et al. 1999; Warner

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and Chmielewski 1992). They also respond to soil development and soil chemistry in mineral soils (Beyens et al. 1991; Bonnet 1961; Bonnet 1978; Trappeniers et al. 2002).

With respect more specifically to the moss-dwelling testate amoebae, Bonnet (1973) found that the limiting factor for testate amoebae is water availability and the species found in mosses of dry habitats are characterized by a relatively small size and shell morphology adapted to a thin water film. Bonnet further distinguished the mosses growing on trees, which are drier and usually colonized by a ubiquitous and cosmopolitan fauna (e.g. *Assulina muscorum* and *Corythion dubium*) and the mosses growing on soils, which, in addition are also colonized by soil-specific taxa (e.g. *Plagiopyxis* sp.). In the latter case the soil type influences the community structure (Bonnet 1973). However, to our knowledge the existing studies on testate amoebae living in mosses mostly focused on biogeography and the structure of communities in different habitats, but the relationship between testate amoebae and the elemental chemistry of mosses other than *Sphagnum* has not yet been studied.

Bryophytes lack a true vascular system and are dependent mostly on precipitation, condensation and capillarity for their supply of water and mineral nutrients. Given this constraint, it is not surprising that they have evolved a high capacity to retain minerals, especially cations, from aqueous solutions through cation exchange, a mechanism that is especially well documented in the case of *Sphagnum* mosses (Clymo 1963; Gstoettner and Fisher 1997). These characteristics have led to the extensive use of terrestrial and aquatic bryophytes as biomonitors of anthropogenic pollution (for a recent review see Onianwa (2001)). Among bryophytes, *Hylocomium splendens* has been the focus of much interest because of its growth pattern where segments are produced in a sequence of tiers, which are often believed to represent annual growth markers and thus to allow an estimate of heavy metal deposition rates (Zechmeister 1998).

To protistologists and microbial ecologists, mosses are especially interesting because they represent a habitat for microbial communities, which may also respond to elemental chemistry related to natural causes or to pollution. However, although the response of different microbial groups to pollutants has be studied quite extensively in aquatic systems and soils (Admiraal et al. 1999; Balik 1991; Foissner 1987; Foissner 1999; Johnson et al. 1998; Kandeler et al. 1992; Madoni 2000; Madoni and Bassanini 1999; Mitchell 2004; Shubert et al. 2001), very little is known on the extent to which micro-organisms living in bryophytes may be affected by their elemental chemistry. The elemental chemistry of mosses is determined by their ability to retain particles brought by wet and dry atmospheric deposition as well as the characteristics of the substrate on which they grow if capillarity allows for significant upward movement of solutes. Pollutants may affect microorganisms and their predators both directly and indirectly through their effect on the mosses (Sims and Lacey 2000). It is unclear if microorganisms are more or less sensitive to pollution than the bryophytes themselves. But it is likely that their response will be different, just as different taxonomic groups have generally been shown to respond differently to environmental gradients (Bragazza and Gerdol 2002; Kabirov et al. 2000; Mitchell et al. 2000b). Therefore including more than one species and more than one trophic level in biomonitoring programs could improve such tools. But before we can even suggest what taxonomic groups to use, we need to know more about the responses of different taxonomic groups to the physical and chemical characteristics of their natural living environment.

The aims of this study were 1) to explore the patterns of testate amoebae community composition along three altitudinal gradients located in contrasting climatic and geological settings but in a single moss species common to all sites, and 2) to determine if the testate amoebae community structure was correlated to elemental moss tissue chemistry.

Plant communities and soil types are generally strongly correlated to altitudinal gradients (Odum 1971). Hence it is not surprising that soil living communities also respond strongly to this gradient if they are analysed in the different soil types found along an elevation gradient (Loranger et al. 2001; Todorov 1998). To our knowledge only one study exists on the relationship between testate amoebae and altitude (Todorov 1998). Todorov (1998) observed a reduction in the diversity of soil testate amoebae in the subalpine zone (2000–2500 m asl.) as compared to lower elevations (400–2000 m asl.), but his samples were not taken in the same soil types along the gradient. More work has been done on the patterns of diversity in relation to latitude. For example a decrease in Nebelid species richness was observed towards high southern latitudes (Smith 1992; Smith 1996; Smith and Wilkinson 1987; Wilkinson 1994). These observations, combined with the similarities of patterns along latitudinal and altitudinal gradient (Odum 1971), led us to hypothesise (H1) that the species richness and diversity as measured by various indices should decrease with altitude.

Figure 1. General location of the study sites.

Our approach was different from the previous studies on altitudinal or latitudinal gradients and elemental chemistry on an important point: We specifically sampled a single moss species, *Hylocomium splendens,* growing in similar situations and extracted testate amoebae from a specific part (the third tier from the top) of these mosses. This approach aimed to minimize the amount of variation due to obvious broad environmental gradients such as soil and vegetation types in order to focus on a set of specific variables: moss elemental chemistry, related either to the bedrock or to atmospheric deposition, and variables other than the vegetation and soil type, such as mean annual temperature, precipitation, or the length of the growing season, that are directly correlated with the altitude and may influence the living conditions of testate amoebae. Our approach however did not allow us to explore the influences of fluctuations in microclimatic conditions that may influence microorganisms more than the changes in climate associated with the general altitudinal gradient; instead we aimed to minimize this heterogeneity in our sampling protocol.

With respect to our second goal, we hypothesised (H2) that the testate amoebae communities would be more similar between the two calcareous regions than between either of the calcareous sampling areas and the siliceous sampling area because the nature of the bedrock was likely to influence the moss elemental chemistry. We also hypothesised (H3) that the correlation between testate amoebae and elemental chemistry would be species-specific. Finally, we hypothesised (H4) that testate amoeba communities would be correlated with measured macro- and micro-nutrients. The rationale for this last hypothesis was that nutrient might control directly the productivity of the mosses and the abundance or type of autotrophic prey organisms and, through these, influence heterotrophic prey organisms indirectly.

Results

Chemical Variables

The summary elemental chemistry data of *Hylocomium splendens* mosses from the three studied sites is given in Table 1. Concentrations of C, N, and P did not differ significantly between transects located on calcareous (Marmarole and Civetta) and siliceous (Lagorai) bedrocks. Fe concentrations were significantly higher in the Marmarole mosses compared to Civetta and Lagorai mosses ($P < 0.01$), whereas Al was significantly higher in the Marmarole mosses compared to Lagorai mosses ($P < 0.05$). Na

Table 1. Elemental chemistry of *Hylocomium splendens* mosses from three altitudinal transects in Northern Italy.

	Lagorai		Civetta		Marmarole	
	Average	SE	Average	SE	Average	SE
C [%]	41.9	0.4	41.4	0.2	42.4	0.4
N [%]	1.29	0.19	1.11	0.07	1.29	0.07
P [ppm]	891	176	667	41	673	60
Fe [ppm]	521	61	541	69	770	61
Al [ppm]	596	67	667	72	889	108
Na [ppm]	115	38	58	8	89	9
Ca [ppm]	2646	366	4006	251	6027	510
K [ppm]	3505	306	2864	327	3172	279
Mg [ppm]	832	80	1131	157	1352	215

was significantly higher in the Lagorai mosses ($P < 0.01$), whereas Ca and Mg showed significantly higher concentrations in the Marmarole and Civetta mosses ($P < 0.05$). K did not differ significantly among mosses collected in the three study areas (Table 1).

Testate Amoebae Diversity, Abundance, and Community Composition: General Results

A total of 25 testate amoeba taxa were recorded in the 21 samples analysed (Table 2). The average species richness of the samples was 9.3 (SE 0.54, min = 5, max =16). The considerable variation in diversity among the samples was mostly due to the presence or absence of rare species. This is illustrated by two characteristics of the data. First, the total species richness of a sample was positively correlated to the number of species recorded as a single individual (out of a total of 150 or more) in that sample (linear correlation: $r^2 = 0.65$, $p < 0.0001$). Second, of the 25 species recorded, seven accounted for 97.1% of the total count on average (min = 88; max = 100%), and the average relative density of three of these species, *Corythion dubium*, *Euglypha ciliata* and *Assulina muscorum* together represented 73.8% of the community (min = 48.3; max = 97.6%). These same three species were the only ones to be present in all samples. By contrast, eight species were recorded each as a single individual in our samples. The estimated total testate amoebae density of samples ranged for most samples between $1.24 * 10^4$ and $4.38 * 10^4$ ind. g moss d.w.$^{-1}$ (average $2.95 * 10^4 \pm$ SE $0.30 * 10^4$) but one extreme sample had a higher value at $7.57 * 10^4$ ind. g moss d.w.$^{-1}$. Shannon-Wiener equitability was weakly positively correlated to total density when the extreme sample mentioned above was excluded ($r^2 = 0.21$, $P = 0.043$) but species richness and Shannon-Wiener diversity were not.

General Patterns of Diversity and Density Along the Three Altitudinal Transects

Total testate amoebae density was higher in the Civetta and Lagorai samples than in the Marmarole samples, but this difference was not significant (Table 3). The species richness and Shannon-Wiener

Table 2. Overall frequency, density and relative density of 25 testate amoebae species in *Hylocomium splendens* along three altitudinal transects in the Alps of Northern Italy (N total = 21).

Taxon	Frequency	Average density [ind g^{-1}]; SE		Average relative density [%]; SE	
Amphitrema flavum Archer	1	9	9	0.03	0.03
Arcella sp.	7	225	133	0.65	0.34
Argynnia dentistoma (Penard)	1	8	8	0.03	0.03
Assulina muscorum Greeff	21	4299	608	14.41	1.30
Assulina seminulum (Ehrenberg)	2	47	33	0.14	0.10
Centropyxis aerophila Deflandre	18	1302	350	4.61	1.23
Centropyxis platystoma Deflandre	1	8	8	0.03	0.03
Corythion dubium Taranek	21	12666	1448	44.23	3.20
Difflugia sp.	3	56	45	0.24	0.19
Euglypha ciliata Ehrenberg	21	4826	812	15.11	1.55
Euglypha compressa (Carter)	1	7	7	0.03	0.03
Euglypha laevis (Ehrenberg)	20	1379	240	4.78	0.80
Euglypha strigosa (Ehrenberg)	1	11	11	0.03	0.03
Heleopera petricola Leidy	1	11	11	0.03	0.03
Heleopera rosea Penard	5	34	15	0.14	0.06
Heleopera sylvatica Penard	3	36	20	0.11	0.06
Nebela tincta (Leidy)	17	2531	708	8.35	2.04
Nebela tincta v. *major* Deflandre	3	36	21	0.14	0.08
Nebela wailesii Deflandre	9	106	38	0.47	0.16
Phryganella acropodia (Hertwig & Lesser)	20	1684	255	5.64	0.74
Porosia bigibbosa (Penard)	4	42	25	0.17	0.09
Tracheleuglypha dentata (Penard)	1	8	8	0.03	0.03
Trigonopyxis arcula (Leidy)	2	17	12	0.06	0.04
Trinema enchelys (Ehrenberg)	11	133	42	0.50	0.14
Trinema lineare Penard	1	4	4	0.03	0.03

Table 3. Summary data for testate amoebae density, species richness and diversity indices in *Hylocomium splendens* along three altitudinal transects in Northern Italy. Differing letters among transects indicate significant differences (one way ANOVA, Turkey-Kramer test).

	Lagorai		Civetta		Marmarole	
	Average	SE	Average	SE	Average	SE
Total density [1000 ind/g d.w.]	31.3	8.0 a	33.7	2.3 a	23.5	3.5 a
Species richness	7.7	0.8 a	8.9	0.5 ab	11.3	1.0 b
Shannon-Wiener diversity (ln)	1.29	0.14 a	1.66	0.03 ab	1.72	0.11 b
Shannon-Wiener eveness	0.63	0.05 a	0.77	0.02 b	0.71	0.03 ab

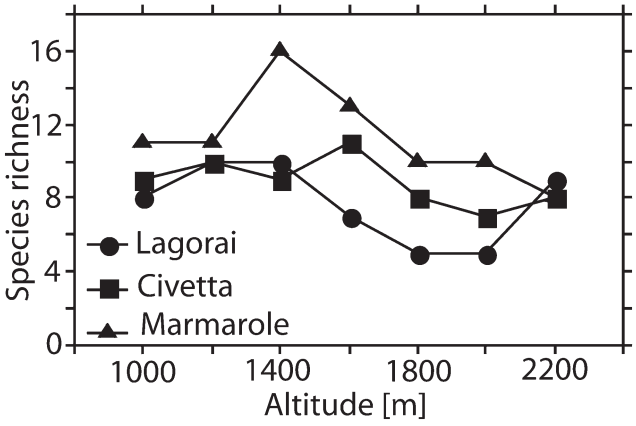

Figure 2. Relationship between altitude and testate amoebae species richness in *Hylocomium splendens* along three altitudinal transects in Northern Italy.

diversity differed significantly (one way ANOVA respectively P = 0.015, 0.021) and the Shannon-Wiener eveness differed marginally significantly (P = 0.054) among the three transects. Species richness and Shannon-Wiener diversity were significantly higher in Marmarole than Lagorai while Shannon-Wiener equitability was significantly higher in Civetta than Lagorai (Turkey-Kramer test; Table 3). Although the diversity of all sites decreased or was stable between 1400 m and 2000 m, there was no overall significant correlation between altitude and either species richness (Fig. 2), total density, diversity, or eveness (the last three not illustrated). Despite this overall lack of relationship, two species were significantly correlated to altitude: *Nebela tincta* increased from 0–535 ind. g^{-1} at 1000 m to 2297–13518 ind. g^{-1} at 2200 m (linear correlation, P = 0.019), while *Euglypha laevis* decreased from 1070–4242 ind. g^{-1} at 1000 m to 0–605 ind. g^{-1} at 2200 m (P = 0.013). Several species showed differential frequency of occurrence or relative density among the three transects (Table 4) and these patterns were responsible for differences in species richness (Fig. 2). Some of these are detailed further.

Correlation with Variables: Multivariate Approach

In the forward selection procedure of the CCA, seven quantitative variables (C, P, Al, Fe, Ca, Mg, and Na) and one binary site variable (Marmorale) were significant when tested as a first variable of the model ($P < 0.05$). Some of these variables were strongly correlated (e.g. Al and Fe) and therefore when one was included in the model the other was no longer significant. We chose to include in the model all variables that were significant when tested as a first variable. Then the remaining variables were tested and one more significant variable (P) was included in the model. Altitude was not significant in this analysis.

The resulting selected variables explained 63.1% of the variation in the species data in the CCA model. Axes 1 and 2 were significant and respectively explained 25.9 and 15.7% of the total variance and are illustrated in Figure 3. Axis 1 is best explained by Ca, Mg, Fe and Al, while axis 2 is most strongly correlated to C and to a lesser extend positively to Al and Fe and negatively to P and Na (Fig. 3A). The position of samples in the ordination (Fig. 3B) reveals that the Marmorale samples are clearly separated from the Lagorai and Civetta samples but the latter two groups cannot be separated. The species biplot (Fig. 3C) revealed that the less frequent to rare species (n = 2–11) were responsible for the structure of the data while the most frequent ones (n = 17–21) were poorly correlated with the environmental variables. Three species *Assulina seminulum, Amphitrema flavum* and *Eulgypha strigosa*, which were present only in Marmarole samples, are clearly separated from the others. Of these only *A. seminulum*, which was found in the

Table 4. Frequency, density and relative density of 25 testate amoebae species in *Hylocomium splendens* along three altitudinal transects in the Alps of Northern Italy.

	Frequency			Average density [ind g^{-1}]; SE						Average relative density [%]; SE					
	Lagorai	Civetta	Marmarole	Lagorai		Civetta		Marmarole		Lagorai		Civetta		Marmarole	
Amphitrema flavum	0	0	1	0	0	0	0	26	26	0.00	0.00	0.00	0.00	0.08	0.08
Arcella sp.	2	4	1	106	88	538	380	31	31	0.34	0.25	1.44	0.94	0.17	0.17
Argynnia dentistoma	0	0	1	0	0	0	0	23	23	0.00	0.00	0.00	0.00	0.09	0.09
Assulina muscorum	7	7	7	4072	1481	6004	637	2821	494	11.62	1.75	18.04	1.68	13.58	2.70
Assulina seminulum	0	0	2	0	0	0	0	141	92	0.00	0.00	0.00	0.00	0.42	0.28
Centropyxis aerophila	4	7	7	528	351	1854	842	1524	480	1.36	0.95	4.96	2.04	7.52	2.63
Centropyxis platystoma	0	0	1	0	0	0	0	23	23	0.00	0.00	0.00	0.00	0.09	0.09
Corythion dubium	7	7	7	15863	3449	12540	1396	9594	1962	55.42	6.25	36.86	2.93	40.41	4.75
Difflugia sp.	0	0	3	0	0	0	0	169	132	0.00	0.00	0.00	0.00	0.73	0.55
Euglypha ciliata	7	7	7	6017	2121	5903	712	2557	541	16.73	2.99	18.39	2.84	10.21	0.95
Euglypha compressa	1	0	0	22	22	0	0	0	0	0.09	0.09	0.00	0.00	0.00	0.00
Euglypha laevis	7	7	6	1423	376	1973	525	740	177	4.45	0.92	6.18	2.03	3.70	0.97
Euglypha strigosa	0	0	1	0	0	0	0	32	32	0.00	0.00	0.00	0.00	0.09	0.09
Heleopera petricola	0	1	0	0	0	34	34	0	0	0.00	0.00	0.09	0.09	0.00	0.00
Heleopera rosea	0	1	4	0	0	31	31	72	28	0.00	0.00	0.08	0.08	0.34	0.12
Heleopera sylvatica	1	1	1	30	30	31	31	47	47	0.08	0.08	0.08	0.08	0.16	0.16
Nebela tincta	5	5	7	1723	783	2407	1015	3462	1764	5.87	3.02	7.08	2.85	12.10	4.53
Nebela tincta v. *major*	0	0	3	0	0	0	0	107	57	0.00	0.00	0.00	0.00	0.43	0.22
Nebela wailesii	1	2	6	18	18	56	37	244	87	0.09	0.09	0.18	0.11	1.13	0.35
Phryganella acropodia	6	7	7	1309	588	2010	444	1732	258	3.31	0.76	5.69	0.92	7.93	1.52
Porosia bigibbosa	1	0	3	22	22	0	0	103	68	0.09	0.09	0.00	0.00	0.42	0.24
Tracheleuglypha dentata	0	0	1	0	0	0	0	23	23	0.00	0.00	0.00	0.00	0.09	0.09
Trigonopyxis arcula	1	0	1	30	30	0	0	23	23	0.08	0.08	0.00	0.00	0.09	0.09
Trinema enchelys	4	6	1	89	41	299	91	11	11	0.45	0.19	0.95	0.31	0.08	0.08
Trinema lineare	0	0	1	0	0	0	0	11	11	0.00	0.00	0.00	0.00	0.08	0.08

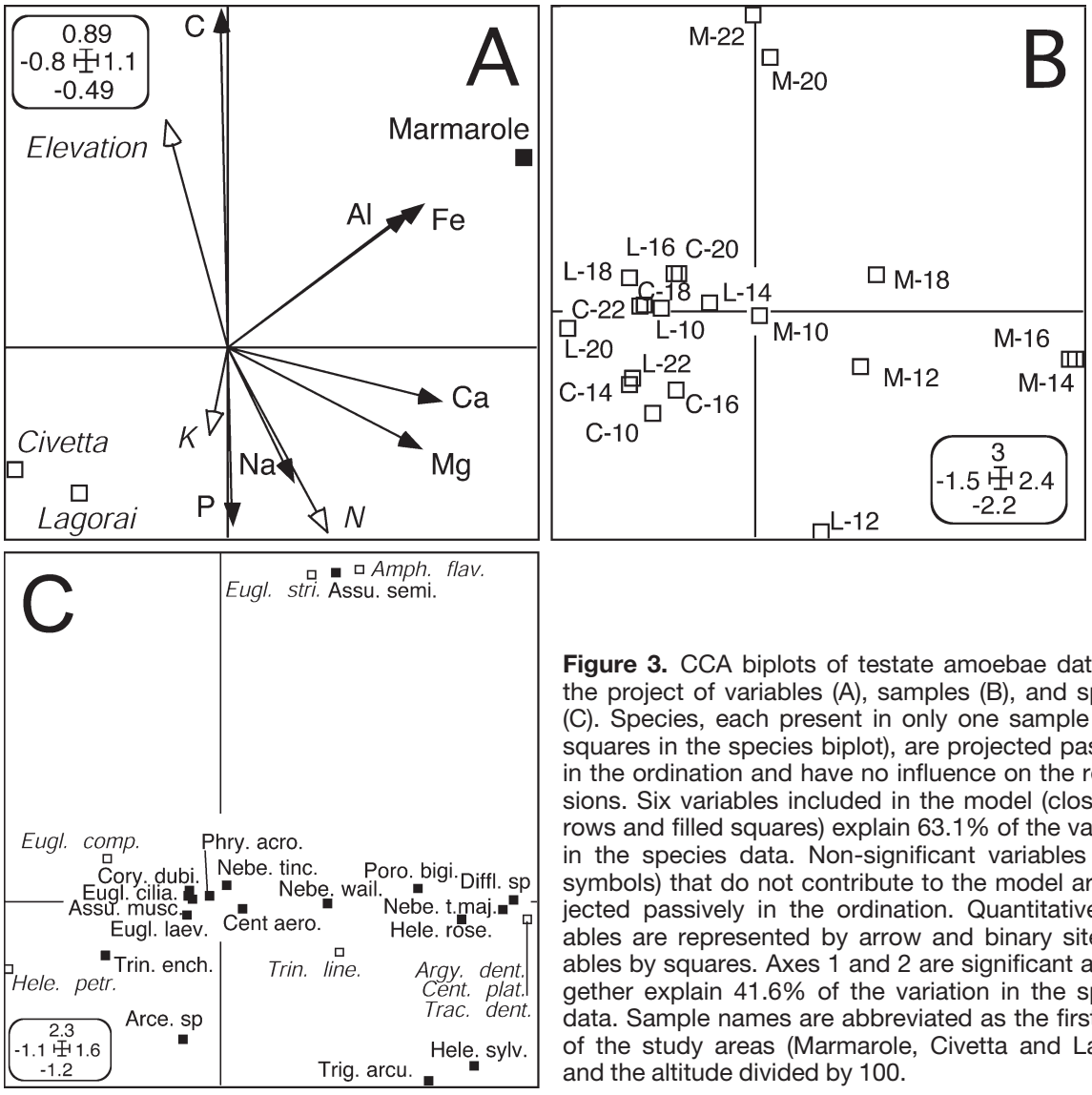

Figure 3. CCA biplots of testate amoebae data with the project of variables (A), samples (B), and species (C). Species, each present in only one sample (open squares in the species biplot), are projected passively in the ordination and have no influence on the regressions. Six variables included in the model (closed arrows and filled squares) explain 63.1% of the variation in the species data. Non-significant variables (open symbols) that do not contribute to the model are projected passively in the ordination. Quantitative variables are represented by arrow and binary site variables by squares. Axes 1 and 2 are significant and together explain 41.6% of the variation in the species data. Sample names are abbreviated as the first letter of the study areas (Marmarole, Civetta and Lagorai) and the altitude divided by 100.

two highest elevation (2000 and 2200 m) of the Marmarole site, contributed actively to the analysis, the other two being projected passively because they occurred in only one sample each (respectively Marm 2000 m and 2200 m). Six active, but overall relatively rare (n = 2–5) species are positively correlated with axis 1: *Difflugia sp., Nebela tincta major, Helopera sylvatica, Heleopera rosea, Trigonopyxis arcula,* and *Porosia bigibbosa* and were found exclusively or in higher abundance in the Marmarole samples (Table 4, Fig. 3C). Seven species, *Assulina muscorum, Centropyxis aerophila, Corythion dubium, Euglypha ciliata, Euglypha laevis, Nebela tincta,* and *Phryganella acropodia*, were found in most or all samples (n = 17–21) (Table 4) and were poorly correlated with either of the first two axes (Fig. 3C). Two other relatively rare species (n = 7 and 11), *Arcella sp*. and *Trinema enchelys* reached their highest abundance in the Civetta and to a lesser extent Lagorai samples (Table 4) and were negatively correlated to both axes (Fig. 3C).

Variance Partitioning

The summary results of the three CCAs used for the variance partitioning are given in Table 5 and Figure 4. Of the total variation in the testate amoebae data, the only significant site variables, Marmarole, explained 21.4% (fractions a + b in Fig. 4), while the chemical variables that were significant in the for-

Table 5. Summary results of the CCA used to partition the variance of the testate amoebae data.

	Groups of variables tested		
	Sites	Chemical variables	Sites + chemical variables
Variables included in the model	Marmarole	C, Ca, Mg, Na	Marmarole, C, Ca, Mg, Na
Total variance	0.626	0.626	0.626
Sum of canonical eigenvalues	0.134	0.321	0.358
Fraction explained [%] [a]	21.4	51.3	57.2
Overall P value	0.001	0.001	0.001
Axis 1 P value	0.001	0.001	0.001
Axis 2 P value	n.a.	0.019	0.004
Axis 3 P value	n.a.	0.014	0.018
Axis 4 P value	n.a.	0.427	0.677

[a] Fraction explained = 100*sum of canonical eigenvalues/total variance.

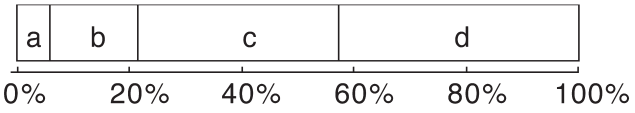

Figure 4. Partitioning of the variation in the testate amoebae community data from three altitudinal transects in Northern Italy. The total variation (100%) is divided into a fraction explained only by a single binary site variable (fraction a: 5.9%), a fraction explained by both the site variable and four significant chemical variables (fraction b: 15.5%), a fraction explained only by the four significant chemical variables (fraction c: 35.8%), and finally an unexplained fraction (d: 42.8%).

ward selection procedure explained 51.3% (fractions b + c in Fig. 4). When the binary site variable Marmarole and the significant chemical variables were used together, the fraction of variance explained was 57.2% (fractions a + b +c in Fig. 4). This latter figure is less than the CCA presented in Figure 3 in which all the variables that were significant when tested as first variable were included. Thus of the 21.4% of variance explained by the Marmarole site. 15.5% was also explained by the chemical variables and 5.9% was not. Symmetrically, of the 51.3% of the variance explained by the chemical variables, 15.5% was also explained (shared explanation of variance) by the Marmarole binary site variable. Finally 42.8% of the variation in the testate amoebae species data could neither be explained by the site nor by the measured chemical variables. Altitude was not included in the variance partitioning because it was never significant.

Discussion

Moss Elemental Chemistry

The mosses sampled in the two transects located in calcareous regions (Marmarole and Civetta) logically contained higher concentrations of Ca and Mg than the mosses from the siliceous region (Lagorai). However the mosses from Marmarole had higher Fe and Al contents than those of Civetta. The reason for this difference was not clear and may be due either to the characteristics of the bedrock or to atmospheric deposition. Interestingly no significant differences were found for the macronutrients C, N, P and K among the three transects (Table 1).

Testate Amoebae Overall Species Richness

In this study we observed 25 testate amoebae species in a single moss species. By comparison, in a study on testate amoebae living in mosses, Bonnet (1973) recorded a total of 49 species. However this represented 13 different moss species growing on a range of substrates (different soils and trees). When only a single moss species was considered, the total diversity was of 23 species in 15 samples. With respect to these 15 samples, Bonnet (1973) did not detect any pattern in the data that could be explained by the known preferences of the species for specific soil types (calcareous versus acidic) and he therefore concluded that the characteristics of the soil did not significantly affect the population of testate amoebae colonizing the mosses growing on the moss surface. The only noticeable structure in his data set was due to the general climate of habitat in which the mosses were taken: Samples taken under

trees in a moist climate were separated from those taken under isolated trees or more xerophylous forests. This observation supports our sampling strategy, which precisely aimed to avoid such a bias.

Nguyen et al. (2004) studied the testate amoebae communities colonizing the moss *Tortula ruralis* growing on wall, stones and other hard and dry substrates and recorded nine species in 30 samples. This low diversity may be attributed to the extremely dry conditions of the habitat sampled and to the fact that only the upper, photosynthetic part of the mosses was sampled, possibly leaving out some soil or litter species. Several other studies provide data on the species richness of testate amoebae in mosses, with values ranging from 9 to 53 taxa, but these always include several moss species (Beyens and Chardez 1994; Beyens et al. 1990; Beyens et al. 1986a; Todorov and Golemansky 1996; Todorov 1999; Van Kerckvoorde et al. 2000).

The overall species richness we recorded in 21 samples of *Hylocomium splendens* is high by comparison to these studies. However, it must be noted that eight of the 25 species recorded were very rare, being found each in a single sample. Furthermore, some of these species, such as *Amphitrema flavum*, are usually believed to be strictly restricted to *Sphagnum* peatlands. Their presence in the samples we analyzed could be an indication of their long-distance colonization potential, a subject that has not been studied very extensively yet.

Relationship to Altitude

Todorov (1998) observed a significantly lower diversity of testate amoebae at the subalpine zone than at lower elevations. Smith and Wilkinson (1987) observed a correlation between climate and the diversity of the genus *Nebela* in South America and were able to separate species in groups corresponding to climatic thresholds. Beyens and co-worker made a similar observation in the Arctic (Beyens et al. 1986b). Some small ubiquitous species such as *Corythion dubium* or *Assulina muscorum* show a high tolerance for dry and acidic conditions (Warner et al. 1992), but are also able to survive in a broad range of conditions and, perhaps for this reason are the most cosmopolitan of all species and among the few to have been regularly reported from Arctic and Antarctic regions (Beyens et al. 1994; Beyens et al. 1992; Searles et al. 2001; Van Kerckvoorde et al. 2000).

We expected (hypothesis 1) to find a negative correlation between altitude and the diversity and community structure of testate amoebae but our results did not support this hypothesis despite the significant correlations for two species. However, all the above-mentioned studies differ from our in one important point: They did not sample specifically a single habitat but sampled instead characteristic habitats of the geographical or altitudinal zones of interest. We therefore speculate that one likely reason why we did not find an overall significant relationship to altitude is our sampling protocol that specifically aimed to avoid any variability that would be due to the soil type or vegetation. The above-mentioned studies were mainly focused on the biogeography or local distribution patterns of testate amoebae. Because of this, they neither specifically aimed to avoid any sampling bias due to soil type or vegetation, nor did they include a numerical approach to address this question. Therefore the observed patterns of reduced diversity with increasing latitude or altitude may, at least in part, be due to an overall reduction in habitat diversity. This question could be answered either by using a protocol similar to the one used here, or by using the habitat diversity sampled as a covariable in the analysis of the relationship between testate amoebae species richness and altitude or latitude.

One limitation of our protocol however is that because *Hylocomium splendens* is rare or absent in the alpine zone our study could not be extended beyond the subalpine zone where microclimatic conditions are more variable and temperature extremes more marked than at lower elevations. Another limitation is that we did not address the question of microclimate, which arguably has more influence on testate amoebae than then general difference in climate associated with the altitudinal gradients, although our sampling protocol, in which we pooled mosses from two to three 50 × 50 cm areas without any tree cover, aimed to minimize the influence of fine-scale spatial variability that could be associated to microclimatic conditions. Further work on this subject should aim to overcome these limitations.

Testate Amoebae Diversity and Community Structure in the Three Sites

The climatic and geologic characteristics of the three transects led us to expect (hypothesis 2) more similarity between the testate amoebae from the two calcareous regions with a sub-oceanic climate, Marmarole and Civetta, than between either of them and Lagorai, the siliceous transect with a sub-continental climate. This was in part the case: The Lagorai samples had lower species richness and Shannon-Wiener diversity than Marmarole and had lower Shannon-Wiener equitability than Civetta (Table 3). In addition, in the CCA, the binary site variable “Mar-

marole" was significant. While these results confirm that the testate amoebae communities from Lagorai were different from those of Marmorale, and had a lower equitability than those of Civetta, the testate amoebae communities from the two calcareous areas were also clearly different. This is well illustrated by the clear separation between the Marmarole samples and the Civetta and Lagorai samples in the CCA ordination and the fact that the binary variable "Marmarole" was the only site variable to be significant (Fig. 3). These results point toward the existence of some difference between the two calcareous transects.

Relationship Between Testate Amoebae and Moss Elemental Chemistry

In agreement with our third hypothesis, the correlation of testate amoebae with elemental chemistry was species specific. The seven variables included in the CCA model (C, P, Al, Fe, Ca, and Mg) significantly explained a high fraction of the total variation in the species data (63.1%). Due to covariance among some of these variables (e.g. Fe and Al, Ca and Mg; Fig. 3), the number of variables that would have been included in the model following a forward selection procedure and the percentage of the total variation explained by these variables would be lower. For example if both quantitative and site variables were included in the forward selection, the included variables, in the order of selection would be: 1) the binary variable Mamorale, 2) C, and 3) Na. Together these would explain 46% of the variation in the testate amoebae data. If only quantitative variables were included, the sequence would be 1) Ca, 2) C, 3) Na, and 4) Mg, and the fraction of variance explained would be 51.3%. Four of the seven significant variables in the CCA (Al, Fe, Ca, and Mg) were significantly more concentrated either in mosses from Marmorale (Al and Fe) or from both Marmorale and Civetta (Ca and Mg). However, as two of the significant variables, C and P, did not differ among the sites, in these cases the response of testate amoebae to moss elemental chemistry cannot be related to the bedrock or the general climate of the sites.

Variance Partitioning

The variance partitioning (Table 5, Fig. 4) clearly demonstrated four main things:

First, a large fraction of the testate amoebae data was related to differences in the elemental chemistry of the mosses. This is discussed further.

Second, about one fifth of the variation could be explained by a single binary site variable. Contrary to our hypothesis, the area that stood out as having different testate amoebae communities was not Lagorai, the siliceous area, but Marmarole, one of the two calcareous areas. Taken alone this could suggest that testate amoebae are not responding to the nature of the bedrock but to other factors such as micro-climate (uncorrelated linearly to the altitudinal gradient) or atmospheric deposition.

Third, about 75% of the variation that could be explained by that single binary site variable was also explained by the elemental chemistry of the mosses. This suggests that the difference in testate amoebae communities between Marmarole and the other two sites is mostly due to differences in elemental chemistry. If, in turn, this elemental chemistry reflects the nature of the bedrock, then the bedrock of the two calcareous areas is significantly different. Unfortunately this data was not available to us. Alternatively, some other factor, such as climate or atmospheric deposition may differentiate the two calcareous areas.

Fourth, only about 1/3 of the variation that was explained by the chemical variables could also be explained by the binary site variable. This result suggests that the measured chemical species vary substantially within each area, but the testate amoebae communities respond well to these differences. The within-area variability of the moss elemental chemistry can possibly be attributed to regional heterogeneities of the bedrock, soil cover, vegetation, or slope and aspect, all of which could influence the mosses.

Are Testate Amoebae Communities Controlled by Nutrient Availability?

Although the structure of testate amoebae communities has been correlated to water or elemental chemistry in several studies, a true understanding of the causes for the observed patterns is still mostly lacking. Similar to our results, a correlation between testate amoebae and some macro- and micro-nutrients (Mg, Ca, Al+Fe, and P) was found in drained and restored *Sphagnum* peatlands in Finland, but it was not clear if all of these variables were significant (Jauhiainen 2002). In *Sphagnum* peatlands, testate amoebae communities were related to Ca^{2+}, NO_3^-, SO_4^{2-} (Mitchell et al. 2000b), and to a combination of physical variables such as moisture and chemical variables such as pH, N, Ca^{2+}, C:N, DOC, and C (Tolonen et al. 1994).

Our results partly support our fourth hypothesis: A significant correlation was found between testate amoebae community structure and macro- and micro-nutrients concentration in the moss tissues.

However, no correlation was found with two important, and often limiting nutrients: N and K. In the existing studies in which testate amoebae communities were studied in relation to their chemical environment many of the significant explanatory variables were also nutrients (Jauhiainen 2002; Mitchell et al. 2000b; Tolonen et al. 1994). Therefore we tentatively interpret these correlations as an indication that the availability of nutrients influences testate amoebae indirectly through an effect on prey organisms (mainly bacterial, fungi, or protists). However, the present knowledge on the functioning of microbial food webs in mosses does not allow us to interpret these results further and a detailed analysis of the microbial food webs was beyond the scope of this study. The results from this and previous studies nevertheless provide observational data that will be useful for hypothesis generation. When these are tested using manipulative experiments we will gain a better understanding of the causal relationships behind the observed correlations.

Methods

Study sites and sampling: The study was performed in three mountainous areas located in the South-Eastern Alps of Italy, i.e. the Civetta group (Cordevole Valley), the Marmarole group (Ansiei Valley), and the Lagorai group (Val di Fiemme Valley) (Fig. 1). The bedrock consists of limestone and dolomite for the Civetta and Marmarole groups, whereas the Lagorai group is mostly formed by quartz porphyry. The Civetta and the Marmarole group are characterised by a moderately suboceanic climate with a total annual precipitation between 1000 and 1500 mm, whereas the Lagorai group is characterised by a moderately continental climate with an annual precipitation <1000 mm culminating during the summer months. The mean annual temperature at 1000 m a.s.l. over the southeastern Alps can be estimated to be ca. +7 °C with an altitudinal gradient of ca. –0.5 °C/100 m (Fliri 1975).

Moss sampling was performed during the summer of 2002 along altitudinal transects starting at an elevation of 1000 m a.s.l. and ending at 2200 m a.s.l. Sampling was performed every *ca* 200 m of elevation yielding a total number of seven moss samplings at each study area. The tree line is located at an altitude of approximately 2050 m in the study area. The position of the tree line does not seem to be influenced by human activities. Therefore the uppermost sample represents the sub-alpine zone.

An important source of variation in soil micro-organism data is the spatial heterogeneity at the fine scale (mm to dm). Variations at this scale may exist even in macroscopically homogeneous surfaces (Mitchell et al. 2000a). To minimize this potential source of variability, at each elevation two to three sampling plots were subjectively chosen outside tree cover (to remove the variability due to interception and leachates from the trees) in microhabitats characterised by a dense and healthy cover of *Hylocomium splendens*. At each plot, all moss plants were collected using plastic gloves over a surface of ca 50 × 50 cm. All moss plants collected at each elevation were then bulked and, after return to laboratory, air-dried for several days.

The annual growth segments of each moss plant were detached and separated. Preliminary analyses revealed that the third segment contained a higher diversity of testate amoebae than the top two segments but still contained many species characteristic for dry micro-environment rather then species such as *Plagiopyxis* sp. usually found in the moister soil litter. This choice represented a compromise between the conflicting aims of having a maximum diversity while minimizing the direct influences of the soil habitat and thus allowing us to assume that the differences in testate amoebae community composition could be related primarily to the moss environment, including the moss elemental chemistry as influenced by atmospheric deposition. The third segment was also the lowest photosynthetic (i.e. green) segment. Finally, segments lower than the third were not always available for all the elevations being frequently highly decomposed.

Chemical analyses: Before performing chemical analyses, the moss segments were ground in a titanium mill through a 0.2 mm screen to ensure homogeneity of the sample. Sub-samples of powdered material were oven-dried for 72 h at 40 °C to convert air-dry weight as well as element concentrations to standard oven-dry conditions.

Carbon (C) and nitrogen (N) concentrations were determined using an elemental analyser (EA 1110, Carlo Erba). Mineral elements, i.e., calcium (Ca), magnesium (Mg), sodium (Na), potassium (K), aluminium (Al), and iron (Fe) were analysed by atomic absorption spectrophotometry after digestion of the moss powder with nitric acid in a microwave oven. Total phosphorus (P) was determined colorimetrically by the molybdenum blue method on the same solution used for mineral cation analyses (Allen 1989). Standard reference material (NIST Citrus leaves 1572, National Bureau of Standards) was analysed along with moss samples to ensure accuracy within 5% of known total element concentration. The chemical data for Ca, Mg, Al, Fe, K, and Na at elevation of 1200 m in the Civetta group were not

reliable, possibly due to an unsuspected local pollution of the sampling site. Therefore these values were excluded from the data set and this sample was not included in the numerical analyses in which the chemical data was used.

Testate amoebae analyses: Testate amoebae extraction was done by washing sub-samples of the ground moss samples over filters of 700 µm, 300 µm, and finally 150 µm mesh size, and then back-sieving the filtrate over a 20µm mesh filter. The sieving over 700, 300 and 150 µm was done with high water flow to maximize the extraction of tests. While some testate amoebae species are larger than 150 µm in their smallest dimension, such species are usually found in very wet habitats. Indeed an analysis of the fraction between 150 and 300 µm did not reveal any testate amoebae. The back sieving was done with a very low flow of water to maximize the retention of small particles. Most testate amoebae are larger than 20 µm but the microscopic examination of the final samples revealed a large amount of material smaller than 20 µm suggesting that our cautious back sieving was efficient in retaining a significant proportion of smaller particles through clogging of the filter. Nevertheless is it possible that some very small species such as *Cryptodifflugia oviformis* or very small *Difflugia* species have been overlooked. To estimate the absolute concentrations of testate amoebae in our samples we added a known number of exotic makers (*Lycopodium* spores, Dansk Droge Import A/S, Ishøj, Denmark) that were counted along with the testate amoebae shells (Stockmarr 1971). Testate amoebae were identified and counted to a minimum total of 150 shells per sample (mean: 164.4, min 151, max 188).

Numerical analyses: Student t-test and one-way ANOVAs were performed on element concentrations in moss samples, testate amoebae species richness and diversity indices (Shannon - Wiener's diversity and eveness), to test for differences among the three study areas. Simple correlations were computed between altitude and the same set of data.

The testate amoebae data was subjected to a canonical correspondence analysis (CCA) using the software CANOCO (Ter Braak 1988; Ter Braak 1988–1992). Species data was log-transformed [using $x' = \ln(x+1)$] and eight species that occurred each in a single sample were projected passively in the ordination space. The significance of chemical variables (quantitative) and dummy binary variables representing the three study areas was assesses using the forward selection procedure and Monte-Carlo permutation tests (999 permutations). Finally an overall significance test and tests on individual axes were performed (Monte-Carlo, 999 permutations). Non-significant variables were projected passively in the ordination as in an indirect gradient analysis.

To quantify the relative importance of sites versus chemical variables in controlling testate amoebae community structure, we performed a variance partitioning (Borcard et al. 1992). The aim of this analysis was to determine the fraction of variation explained by a) only the sites, b) both sites and chemical variables, c) only the chemical variables, and d) neither of the two. To do this, three CCAs need to be done. In the first only the site variables were included in the model. In the second only the chemical variables were included and in the third the site and chemical variables. In each case, the variables were tested one by one in the forward selection procedure using Monte-Carlo permutation tests (999 permutations). The separate CCAs on site or chemical variables determined the variables that would be used in the combined analysis.

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