How does litter quality affect the community of soil protists (testate amoebae) of tropical montane rainforests?

Valentyna Krashevska, Mark Maraun & Stefan Scheu

J.F. Blumenbach Institute of Zoology and Anthropology, Animal Ecology, Georg August University Göttingen, Göttingen, Germany

Correspondence: Valentyna Krashevska, J.F. Blumenbach Institute of Zoology and Anthropology, Animal Ecology, Georg August University Göttingen, Berliner Str. 28, 37073 Göttingen, Germany. Tel.: +49 0 551 395557; fax: +49 0 551 395448; e-mail: valentyna.krashevska@biologie.uni-goettingen.de

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Abstract

Litter quality and diversity are major factors structuring decomposer communities. However, little is known on the relationship between litter quality and the community structure of soil protists in tropical forests. We analyzed the diversity, density, and community structure of a major group of soil protists of tropical montane rainforests, that is, testate amoebae. Litterbags containing pure and mixed litter of two abundant tree species at the study sites (Graffenrieda emarginata and Purdiaea nutans) differing in nitrogen concentrations were exposed in the field for 12 months. The density and diversity of testate amoebae were higher in the nitrogen-rich Graffenrieda litter suggesting that nitrogen functions as an important driving factor for soil protist communities. No additive effects of litter mixing were found, rather density of testate amoebae was reduced in litter mixtures as compared to litterbags with Graffenrieda litter only. However, adding of high-quality litter to low-quality litter markedly improved habitat quality, as evaluated by the increase in diversity and density of testate amoebae. The results suggest that local factors, such as litter quality, function as major forces shaping the structure and density of decomposer microfauna that likely feed back to decomposition processes.

Introduction

Density and species composition of decomposers depend on abiotic factors, such as micro- and macroclimatic conditions, but also on biotic factors, such as plant litter quality and diversity (Swift et al., 1979; Couèteaux et al., 1995; Cadish & Giller, 1997; Loranger et al., 2002; Hättenschwiler et al., 2005; Wardle et al., 2006). Litter quality varies markedly among litter species, and therefore, effects of individual litter species are likely to be modified by the presence of other species, with such mixing effects ranging from negative to positive (Wardle et al., 1997; Gartner & Cardon, 2004; Hättenschwiler & Gasser, 2005). However, most studies investigating litter mixing effects focused on litter mass loss, that is, litter decomposition, ignoring the organisms responsible for the decomposition process. Very little is known on the role of litter identity and litter mixing as structuring forces for microbial and microfauna communities (Wardle et al., 2006; Hossain et al., 2010), and this applies in particular to tropical ecosystems. Both the response of microorganisms and microfauna likely are linked, as microfauna, such as protists and nematodes, represent major predators of microorganisms.

We analyzed the diversity, density, and community structure of testate amoebae, a major protist microfauna of tropical forest ecosystems (Krashevska et al., 2007), as affected by litter identity and litter mixing using litterbags with two litter types strongly differing in quality as indicated by different nitrogen concentrations (Illig et al., 2008). As nitrogen was shown to function as a limiting factor for testate amoebae in the studied rainforests (Krashevska et al., 2010), we hypothesized that the density and diversity of testate amoebae in nitrogen-rich litter of Graffenrieda emarginata exceed that in the nitrogen-poor litter of Purdiaea nutans. Further, increases in litter diversity and thereby increases in the number of niches are likely to result in increased diversity of testate amoebae; therefore, we expected the diversity of testate amoebae to be at a maximum in litter mixtures (Hättenschwiler et al., 2005).
Materials and methods

Experimental design

The study was performed in southern Ecuador in the area of the Reserva Biologica San Francisco at 1850 m a.s.l. (S 3°58′38″, W 79°04′66″). Leaf litter of two dominating tree species at the study sites, that is, G. emarginata Triana (Melastomataceae) and P. nutans Planch (Cyrillaceae) (Homeier et al., 2002), was sampled and dried at 65 °C for 72 h. Litterbags (20 × 20 cm; 1-mm mesh size) with 10 g dry litter from G. emarginata, P. nutans and a mixture of both with 5 g of each litter type were exposed in the field in February 2004 and retrieved after 1 year in February 2005; more details on the experiment and study area are given in Illig et al. (2008). Each treatment was replicated four times.

Extraction and analysis of testate amoebae, chemical, and microbial parameters

After transfer of the litterbags into the laboratory, testate amoebae were extracted by washing samples over a filter of 500-μm mesh and then back-sieving the filtrate over 20-μm mesh. Microscopic slides were prepared, and tests of amoebae were identified and counted at 200× and 400× magnification with an upright Leitz Ortholux II and a Nikon Inverted Microscope DIAPHOT-TMD. The number of tests was expressed per gram of air dry litter material. Determination of species was based on morphological characters (morphospecies); for details see Krashevska et al. (2007).

The remaining material was used for investigation of environmental factors that likely affect testate amoebae and function as indicator of habitat quality. Litter material was milled, dried at 65 °C for 72 h, and analyzed for total C and N concentrations using an elemental analyzer (Carlo Erba, Milan, Italy). Water content was determined gravimetrically. Microbial biomass (Cmic) was measured using the substrate-induced respiration method (Anderson & Domsch, 1978; Scheu, 1992). Mass loss was calculated as differences among the initial dry mass and the litter dry mass remaining after 12 months and expressed as percentages of initial.

Statistical analysis

Differences in species number and density of testate amoebae between the litter treatments were analyzed by ANOVA (type III sum of squares) using SAS 9.13 (SAS Institute Inc., Cary). Further, Shannon–Wiener diversity and evenness were analyzed to test for differences among three litter treatments.

Relationships between testate amoebae communities and environmental factors were analyzed using redundancy analysis (RDA) as implemented in CANOCO (Ter Braak, 1988–1992). RDA allows to relate dependent variables (species data) to a set of independent variables (environmental factors) by direct ordination. Environmental variables included N concentration, Cmic, mass loss, and water content. All data were ln(x + 1) transformed. Only species present in more than two of the four replicates were included in RDA. Monte–Carlo tests (999 permutations) were performed to evaluate the significance of environmental variables, overall significance, and individual axes (Ter Braak, 1996). Treatments (G. emarginata, P. nutans, and Mixture) were included as passive variables.

Results

Diversity and density of testate amoebae

In total, 106 taxa of testate amoebae were indentified with the total number of species being low in P. nutans litter (41) and similar in the litter mixture (68) and G. emarginata litter (70) (Supporting Information, Table S1). Average species richness followed the same pattern with 18 ± 6, 33 ± 12, and 35 ± 9 species in P. nutans litter, litter mixture, and G. emarginata litter, respectively (F2,9 = 3.88, P = 0.05). In contrast, Shannon–Wiener diversity among G. emarginata litter (2.31 ± 0.38), P. nutans litter (2.47 ± 0.40), and litter mixture (2.59 ± 0.52) did not differ significantly; however, the Shannon–Wiener evenness increased significantly in the order G. emarginata litter ≤ litter mixture < P. nutans litter with 0.65 ± 0.09, 0.74 ± 0.06, and 0.86 ± 0.08, respectively (F2,9 = 6.2, P = 0.01).

In G. emarginata litter, four species were dominant (contributing > 5% to total abundance) and accounted for 74% of the total abundance: Trinema lineare Penard, 1890 (35%), T. lineare minuscula Chardez, 1968 (17%), Euglypha laevis (Ehrenberg, 1832) Perty, 1849 (12%), and Euglypha cristata Leidy, 1879 (10%). In P. nutans litter, six species were dominant and accounted for 58% of the total abundance: Cryptodiﬂugia oviformis fusca Bonnet, Thomas, 1955 (20%), T. lineare (10%), E. laevis (9%), Cyclopyxis eurystroma parvula Bonnet, Thomas, 1960 (7%), Hyalosphenia subflava Cash, 1909 (6%), and Archeolla sp. (5%). In the litter mixture, four species were dominant and accounted for 63% of total abundance: Cr. oviformis fusca (24%), T. lineare minuscula (20%), T. lineare (10%), E. laevis (8%).

Similar to species richness, the density of testate amoebae significantly increased in the order P. nutans litter < litter mixture < G. emarginata litter with 513 ± 186,
2163 ± 579, and 3123 ± 762 ind. g⁻¹ litter dry weight, respectively ($F_{2,9} = 22.25, P = 0.0003$).

**Response of the testate amoebae community to the litter treatments**

In the forward selection procedure of RDA, only one of the four quantitative variables (see Materials and methods) was significant ($P < 0.05$; Fig. 1). Together, these variables explained 49% of the variation in species data, with the trace being significant ($0.489; F = 1.67, P = 0.016$). N concentration accounted for 30% of total variation in species data ($F = 4.20, P = 0.001$). Axes 1 was significant and explained 31% of the data ($F = 3.15, P = 0.001$), and axis 2 explained only 7%.

Axis 1 of the RDA correlated positively with N concentration, water content, and mass loss, whereas axis 2 correlated negatively with Cmic (Fig. 1). Further, N concentration and water content clustered in the vicinity of *G. emarginata* litter. Cmic was associated with litter mixture. A large number of testate amoebae were associated with high litter N concentration and clustered between *G. emarginata* litter and litter mixture. The ordination reflects that the three litter treatments supported different communities of testate amoebae. Along axis 1 testate amoebae in *G. emarginata* litter separated from those in the litter mixture and *P. nutans* litter, reflecting, for example, that *G. emarginata*, *Trinema*, and *Euglypha* species were more abundant, whereas in the litter mixture, *Centropyxis*, *Nebela*, and *Cyclopyxis* species reached high abundance and/or diversity. Further, some taxa were found only in one of the three treatments, for example, *Sphenoderia fissa* Penard, 1890 in *G. emarginata* litter, whereas *Nebela militaris* Penard, 1890 in the litter mixture. In *P. nutans* litter, only three species reached high density compared to the other treatments, that is, *Archerella flavum* Archer, 1877, *Euglypha strigosa* Ehrenberg, 1871, and *H. subflava*.

**Discussion**

The mobility of testate amoebae is limited (Lousier, 1982); nevertheless, a high number of species colonized the litterbags and reached high abundance, although the density and diversity were lower than in the litter layer of the forest (Krashesvka *et al.*, 2007). As hypothesized, their density and diversity in the nitrogen-rich litter of *G. emarginata* exceeded that in the nitrogen-poor litter of *P. nutans*. Further, most variation in testate amoebae community structure was explained by litter nitrogen.

![Fig. 1. RDA ordination of testate amoebae with environmental variables represented by arrows [N, litter nitrogen concentration; Cmic, microbial biomass; H₂O, litter water content; mass loss, litter mass loss after 12 months]. The types of litter in the litterbags, that is, *Graffenrieda emarginata*, *Purdiaea nutans*, and the mixture of both, were included as passive variables. For authorities of species, see Table S1.](image-url)
concentration. This supports earlier conclusions (Krashevska et al., 2010) that testate amoebae at the studied montane rainforest are limited by N and that litter with high N concentration improves the availability of N to testate amoebae. Further, high litter N concentration also correlated with litter mass loss and microbial biomass supporting earlier assumptions that plant litter quality represents an important driver of decomposer communities, in particular in tropical regions with little seasonal variations in climate and plant litter input (Swift et al., 1979; Aerts, 1997; Loranger et al., 2002). Therefore, in tropical ecosystems, the role of litter quality as structuring force for decomposer communities may indeed be as important or even surpass the importance of abiotic factors such as microclimate as hypothesized before (Aerts, 1997; Loranger et al., 2002).

In forest ecosystems, plant litter material typically is mixed, thereby increasing the number of local niches, and this applies in particular to tropical forests such as the montane rainforest studied which is exceptionally rich in tree species (Homeier et al., 2002). Because of this increase in local niches, we assumed that the density and diversity of testate amoebae are at a maximum in litter mixtures. In contrast to this assumption, mixing of litter of G. emarginata with that of P. nutans did not result in increased diversity and density of testate amoebae, rather the diversity was similar to that in G. emarginata litter, and the density was intermediate between G. emarginata and P. nutans litter. This suggests that favorable conditions in litter of G. emarginata were detrimentally affected by admixture of litter of P. nutans. Similar results have been reported for microarthropods by Illig et al. (2008). However, in G. emarginata litter, typical abundant species such as Trinema and Euglypha predominated, whereas in the litter mixture, species of Centropyxis, Nebola, and Cyclopyxis were also abundant. Presumably, mixed litter with higher microbial biomass improved the availability of food resources or of test-building material for these taxa.

In conclusion, this study suggests that litter quality is one of the most important factors structuring the community composition of testate amoebae and, therefore, microfauna food webs in tropical montane rainforests. Further, the results suggest that adding of high-quality litter to low-quality litter markedly improves habitat quality for testate amoebae with their density and diversity reaching similar levels as in the presence of high-quality litter only. Therefore, habitat quality for testate amoeba in this ecosystem is reflected predominantly by litter nitrogen concentration. However, further investigations are needed to understand the mechanisms how litter nitrogen improves habitat quality for testate amoebae and the role of litter diversity for the diversity of protists in tropical rainforests. These investigations are currently under way at our study site.

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References


Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Testate amoebae species in litterbags with litter of G. emarginata, P. nutans, and the mixture of both exposed in the field for 12 months given as individuals per gram of air dry litter.

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