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The Distribution of Soil Testate Amoebae under Winter Snow Cover at the Plot-scale Level in Arctic Tundra (Qeqertarsuaq/Disko Island, West Greenland)

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Summary. Understanding the spatial distribution of soil protozoa under the snow cover is important for estimation of ecosystem responses to climate change and interpretation of results of field experiments. This work explores spatial patterns of soil testate amoebae under the snow cover at the plot scale (the range of metres) in arctic tundra (Qeqertarsuaq/Disko Island, West Greenland). To explain spatial patterns in abundance, species diversity and assemblage composition of testate amoebae, we measured microtopography, snow depth and substrate density. The results indicate that the abundance of active testate amoebae under the snow cover was quite low. The empty shell assemblage was characterised by the presence of linear spatial trends in the species composition across the site, whereas no patterns were detected within the plot. The distribution of the abundance and the species diversity were unstructured. The linear trends in the species composition corresponded to the site microtopography and were controlled by the topography-related soil moisture. Snow depth also affected the linear trends presumably by controlling soil temperatures. Overall, the results suggest that population processes do not generate spatial patterns in protozoan assemblages at the plot scale so that protozoan distribution can be considered random at macroscopically homogeneous plots.

Key words: Arctic tundra, PCNM, plot-scale, snow depth, soil testate amoebae, winter ecology.

Abbreviations: PCNM – Principal Coordinates of Neighbour Matrices.

INTRODUCTION

Soil protozoa represent a common and diverse component of soil ecosystems (Foissner 1987). They play an important role in soil decomposer food webs as secondary and higher-level consumers. Grazing on bacteria,

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they increase bacterial turnover rates and thereby the release of the nutrients immobilised in bacterial tissue (Bardgett and Griffiths 1997, Coûteaux and Darbyshire 1998). These feeding activities result in a greater availability of nutrients for plant uptake. Besides, protozoa represent good model organisms for studying ecological processes in experimental setups thanks to their small size, short generation times and high sensitivity to environmental conditions. The combination of the ecological importance and the possibility of testing hypothesis in experiments makes soil protozoa potentially

interesting objects for studying ecosystem responses to climate change (Andrushchyshyn *et al.* 2009). However, for a better understanding of ecological processes and a proper interpretation of results of field experiments, the knowledge on effects of winter climate on soil protozoa is necessary. This is particularly important for arctic ecosystems which are subjected to winter climate conditions most of the year and which are expected to experience considerable shifts in winter climate due to global change (IPCC 2007).

The most important climate parameters which affect soil biota in winter are thickness and timing of snow cover (Marchand 1996). Being an efficient insulator, winter snow cover protects the ground against low winter air temperatures and regulates soil temperatures in late autumn, winter and early spring, rates of soil cooling and warming, and frequency of freeze-thaw cycles (Coulson *et al.* 1995). Normally, soil protozoa produce cysts resistant to freezing in order to withstand unfavourable winter conditions. However, many groups of soil biota (insects, fungi, bacteria, etc.) have been shown to remain active in the subnivean environment under a continuous snow cover throughout the winter (Bleak 1970, Aitchison 1983, Merriam 1984). Rogerson and Berger (1981) found that protozoan populations were active in garden soil in Canada for short periods on many occasions throughout the winter. Thus, snow cover may affect soil protozoa by influencing encystment, excystment, cyst survival and activity of soil protozoa in winter.

Snow distribution and timing is controlled by surface topography, wind, and vegetation (Walker *et al.* 1993). Considering the above mentioned effects of snow cover on soil biota, the variation in snow depth can be partly responsible for spatial patterns in soil protozoan communities at the plot scale (range of metres). Soil biota is characterised by spatially predictable patterns at various scales (Ettema and Wardle 2002). The variation in soil biota at the plot-scale level is structured by plant growth, activity of burrowing animals and grazing (Ettema and Wardle 2002, Ritz *et al.* 2004, Fortin and Dale 2005). Besides, the spatial aggregations at the plot scale can be also influenced by intrinsic population processes, such as dispersal, reproduction and competition (Ettema and Wardle 2002). The complexity of spatial patterns should be taken into account when using soil protozoa in field manipulation experiments (Mitchell *et al.* 2000) as spatial aggregations can cause difficulties in interpretation of experimental results by violating the assumption of data independence in standard parametric statistics. Thus, studies on the environmental variables which control spatial patterns of soil protozoa at the plot scale are essential for a better understanding of soil ecosystems processes and interpretation of field manipulation experiments.

One of the most interesting groups of soil protozoa for ecological studies is testate amoebae. Testate amoebae are shelled protozoa which constitute a considerable part of soil biota in terms of biomass and biodiversity (Schönborn 1992, Finlay *et al.* 2000, Schröter 2003, Esteban *et al.* 2006). They prey on a wide range of organisms, including bacteria, protozoa, microalgae, fungi and micrometazoa, and may also consume dead organic matter (Gilbert *et al.* 2000, Wilkinson and Mitchell 2010, Jassey *et al.* 2012). Testate amoebae survive unfavourable winter conditions by producing cysts (Ogden and Hedley 1980). Previous studies on testate amoebae have shown that they can be active during the winter and have well defined spatial structures in soils. Smith and Headland (1983) reported that testate amoebae can maintain some reproductive activity under the snow cover on a subantarctic island. Moreover, Lousier and Parkinson (1984) found that some species of testate amoebae could maintain higher than normal densities, had seasonal peaks of abundance and biomass, or higher than normal rates of reproduction and turnover in aspen woodland soil under 60–90-cm-thick snow cover during the winter. Several studies provide evidences for well defined fine-scale (up to one metre) spatial patterns of testate amoeba assemblages along a soil transect across a visible environmental gradient (Balik 1996) and in a macroscopically homogeneous *Sphagnum* carpet (Mitchell *et al.* 2000, Jassey *et al.* 2011).

However, the spatial distribution of soil protozoa at the plot scale in arctic tundra and the environmental variables which influence it remain basically unknown. The aims of the present study were twofold: (1) to investigate the winter state of a testate amoeba assemblage in soil; (2) to explore spatial patterns of the testate amoeba assemblage at the plot scale and to identify the environmental predictors for the patterns. For that purpose, we studied the distribution of soil testate amoebae in an 8 × 15 m plot located in Qeqertarsuaq/Disko Island (West Greenland) in December 2006. We focused on the abundance, species diversity and composition of the testate amoeba assemblage and hypothesised: (1) that testate amoebae maintain activity under the snow cover during the winter (so we expected to find some active individuals); (2) snow cover regulates spatial patterns of soil testate amoeba assemblages at the plot scale.

MATERIAL AND METHODS

Study site

The study was performed in the *Vaccinium*/*Empetrum* heath tundra area near the Arctic Station (N69°15′, W53°30′, 30 m a.s.l.) on Qeqertarsuaq/Disko Island on the west coast of Greenland. The island is located in the transitional zone between the low and high Arctic and has an arctic maritime climate (Aleksandrova 1980). According to the meteorological measurements of the station (1991–2004; Hansen *et al.* 2006) the mean air temperatures of the warmest (July) and the coldest (February–March) months are 7.1 and –16.0°C, respectively. The mean annual soil temperature at the depth of 5 cm is –1.9°C. Mean annual precipitation amounts to 436 mm of which 42% falls as snow. The soil is frozen from early October or late September till late May or early June (1991–1994; Humlum 1998). Below-freezing conditions typically prevail in the entire active soil layer from late December till early July. Most of the years, a complete snow cover appears in October and approaches its maximum thickness in November–December.

Sampling strategy and environmental variables

Soil testate amoeba assemblages and environmental variables were studied in a square plot with the dimensions of 8 × 15 m on December 16–17, 2006. Soil samples for testate amoeba analysis were collected in staggered order at regular sampling intervals of 1.5 m (Fig. 1). Before sampling, snow depth was measured at the same spots using a measuring rod. At each point, a soil sample was extracted using a cylindrical soil drill (2 cm in diameter) by pushing into the soil till the depth of 3 cm. The sample strategy resulted in a total of 57 soil samples. All samples were stored in screw top plastic vials and fixed with 3% neutralised formaldehyde. A Total Station was used to determine spatial position (*x* and *y* coordinates in metres) and microtopography (*z*, surface elevations or depressions in metres relative to the position of the Total Station) at each sampling point.

Testate amoeba analysis

The soil samples were prepared for counting of testate amoebae following a slightly modified version of the water based procedure (Hendon and Charman 1997). First, the soil samples were oven-dried at 40°C for 48 h to normalise the water content. Then, the dry samples were carefully sieved over a 2 mm sieve to disaggregate soil particles and remove large-size material, such as gravel and plant roots, which normally are not suitable substrata for testate amoebae but which can cause variation in testate amoeba abundance affecting sample weight. The sieving residue was discarded and the fine fraction was weighted for determination of the substrate density. A subsample of the fine fraction (1–2 grams) was mixed with arbitrary amount of deionised water and five tables of *Lycopodium* spores (Batch No. 177745, Lund University, Sweden) as exotic marker spores for quantitative analysis (Stockmarr 1971). After 12 hours of soaking, the samples were thoroughly shaken for 5 min. to extract testate amoebae from soil particles. The suspension was sequentially passed through sieves with mesh openings of 300 and 10 μm, and the fraction between the two sieves was retained. We used a finer sieve mesh for back sieving instead of the 15 μm
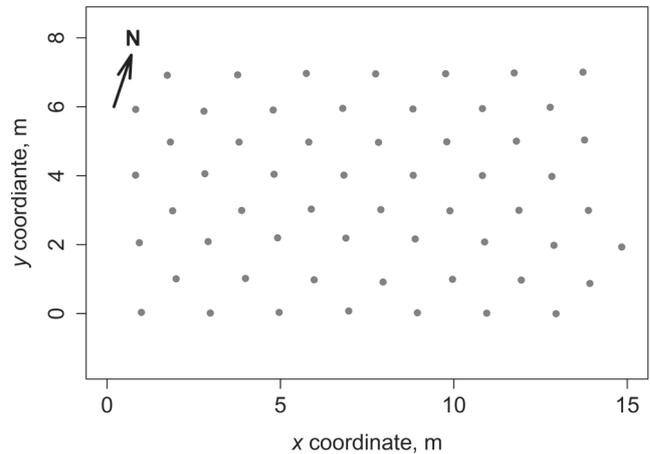
Fig. 1. The map of soil sampling (57 samples) for the study on distribution of soil testate amoebae within an 8 × 15 m plot located in an arctic tundra on Qeqertarsuaq/Disko Island (West Greenland).

suggested by the original technique in order to reduce the possible loss of small species (Payne 2009). Rose Bengal was added to the samples in order to distinguish empty shells from amoebae which were alive at the moment of sampling. Testate amoebae were identified and counted in a drop of the concentrate mixed with glycerol at magnification × 400 using a light microscope (Olympus BX-50). Live individuals and empty shells were counted separately. However, a minimum total of 150 shells in each sample could be achieved for empty shells only.

Statistical analyses

The numerical calculations and statistical analyses were performed in the R language environment (R Development Core Team 2011). Spatial patterns of testate amoebae were estimated using both univariate characteristics and species composition of the testate amoeba assemblage. The following univariate characteristics of the testate amoeba assemblage were calculated: (1) total concentrations ($\times$ 10^3 shells g^{-1} of soil dry weight), (2) species richness (species number per sample), (3) Shannon-Wiener's diversity index, (4) Pielou's evenness index. Patterns in assemblage composition of testate amoebae were analysed using the species abundance data. In order to eliminate unwarranted effects of rare taxa on the results of the multivariate analysis we removed species which were encountered in one sample only (*Arcella discoides*, *Arcella megastoma*) before the analysis. After that, species data were Hellinger transformed (function 'decostand' in the package 'vegan'; Oksanen *et al.* 2011) in order to preserve Hellinger distance in further multivariate analysis. The Hellinger transformation helps to avoid problems arising from Euclidian distance, where the distance between two sites sharing no species in common can be smaller than between two sites sharing common species (Legendre and Gallagher 2001). Among the univariate variables, substrate density, total concentrations and species richness were log-transformed [$x' = \log_e(x)$] prior to further analyses as it improved their normality.

The analysis of spatial structures in both univariate and multivariate data was performed by the Principal Coordinates of Neighbour Matrices (PCNM) approach. This method was proposed by Borcard and Legendre (2002) for modelling spatial patterns across the whole range of scales perceptible with a given data set. The general idea and application of this approach to the analysis of ecological data sets were fully described by Borcard *et al.* (2004, 2011). Briefly, at the first step, a set of orthogonal (linearly independent) spatial variables is calculated based on a truncated matrix of Euclidian distances among sampling locations using Principal Coordinate Analysis. Truncating the matrix serves to retain only the explicit spatial information on the closest neighbouring samples (threshold = 1.43 m; samples greater than this distance from each other are set to the threshold value multiplied by a factor of 4 as suggested by Borcard and Legendre 2002). The generated variables reflect spatial structure at all scales encompassed by the data matrix and can be further used as explanatory variables in multiple regression or RDA. The PCNM variables for the present study were computed using 'pcnm' function in the 'vegan' package (Oksanen *et al.* 2011). Principal coordinate decomposition of the distance matrix produced 39 positive PCNM axes, which were used as explanatory variables in further analyses.

The response data were checked for the presence of linear trends prior to the application of PCNM analysis. Linear trends indicate the presence of spatial structures at broader scales than the sampling extent, in other words a gradient across the entire studied area (Borcard *et al.* 2004). Linear spatial trends can be simply modelled by regression of the response data on the x–y coordinates of the sample locations. Although PCNM analysis is capable of recovering linear trends, it is recommended to remove them before the analysis so that all PCNM variables are used for modelling finer spatial structures which are normally more difficult to recover (Borcard *et al.* 2004). We tested the presence of linear trends in our response data by using linear multiple regression for the univariate assemblage characteristics and redundancy analysis (RDA) for the species abundance data. If the test indicated the presence of significant linear trends the data were detrended by retaining only the residuals for further PCNM analysis. Effects of significant linear trends were analysed then separately as described further.

In order to determine which of the 39 PCNM variables had significant influence on the spatial patterns of the univariate characteristics and the species composition, a forward selection procedure was performed. Before the forward selection, the significance of the full model including all the 39 PCNM variables was tested by using a linear multiple regression for the univariate assemblage characteristics and redundancy analysis (RDA) for the species abundance data. If the results were significant the forward selection procedure was performed using the function 'step' (the package 'stats'; R Development Core Team 2011) for the univariate assemblage characteristics and 'ordiR2step' (the package 'vegan'; Oksanen *et al.* 2011) for the species abundance data. The function 'step' achieves automatic stepwise model building using Akaike's information criterion (AIC, Sakamoto *et al.* 1986). The function 'ordiR2step' performs an enhanced forward selection procedure based on the double stopping criterion suggested by Blanchet *et al.* (2008). The selection stopped if either the usual significance level P or the global R^2_{adj} were exceeded. The same forward selection procedures were also performed for the selection of the x–y location coordinates and the environmental variables. Significant spatial patterns in the species composition were visualised using the results of redundancy analyses. Site scores from significant canonical axis were plotted against their location coordinates using 's.value' function in the package 'ade4' (Dray and Dufour 2007). The same function was used to produce distribution maps of the environmental variables and the univariate characteristics of the assemblage. The testate amoeba species which had the highest correlations with the canonical axes were indentified in order to assess the patterns in the assemblage composition. Statistical significance of the relationships between explanatory variables and the assemblage composition data was estimated by 999 Monte Carlo permutations under the reduced model. Statistical tests were considered significant at $P < 0.05$; the tendencies towards significance ($P < 0.1$) were also reported.

RESULTS

General observations

The descriptive statistics of environmental variables measured at the studied site (microtopography, snow depth and substrate density) and the maps of their spatial patterns are presented in the Table 1 and Fig. 2, respectively. The plot was characterised by overall southward sloping microtopography with some finerscale depressions and elevations (Fig. 2A). Generally, topographic depressions were associated with a thicker snow cover, whereas the snow cover at elevations was thinner (Fig. 2B). However, the negative correlation between microtopography and snow cover depth was weak and only marginally significant (Pearson's correlation: $r = –0.23$; $t = –1.74$; $P = 0.09$).

The soil testate amoeba assemblage in the winter was characterised by predominance of empty shells, whereas living individuals were scarce. The proportion of living individuals at the site was 5.5% of the total counts. Living individuals were mostly encountered as encysted forms, i.e. shells containing stained and enclosed cytoplasm (4.8% of the total counts). Besides, a small proportion of coloured cells (0.7% of the to-

Table 1. Descriptive statistics of the environmental variables at the 8 × 15 m plot (57 samples) in arctic tundra in Qeqertarsuaq/Disko Island (West Greenland) in the winter.

	Mean	SE	Median	Min	Max
Microtopography, m	0.10	0.03	0.07	–0.31	0.75
Snow depth, m	0.25	0.01	0.24	0.07	0.39
Substrate density, g cm^{-3}	0.14	0.01	0.13	0.08	0.54
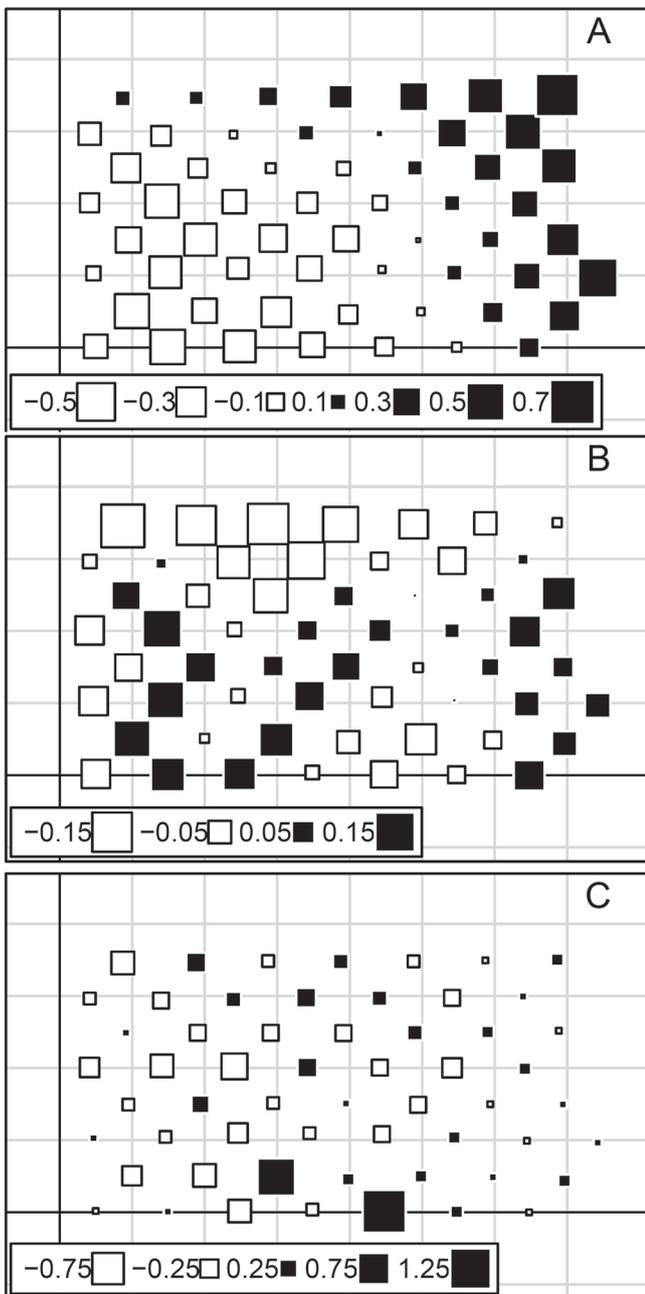
Fig. 2. Maps of the spatial distribution of the explanatory environmental variables (**A** – microtopography; **B** – snow depth; **C** – log$_e$(substrate density)) plotted against their spatial coordinates at 57 sampling locations within an 8 × 15 m plot in arctic tundra in Qeqertarsuaq/Disko Island (West Greenland). All data are centred on 0, so square sizes are proportional to the deviations from the mean values at the plot. Open symbols are used for negative values and the filled symbols are used for positive values. Spatial patterns are visualised as aggregations of similar size and colour.

tal counts) had no enclosure and could represent active forms, although no direct evidence for their activity were detected. Overall, the counts of living individuals were not sufficient for reliable estimation of the assemblage composition and spatial distribution so only the empty shell counts were used in the further analyses.

In total, 53 testate amoeba taxa belonging to 14 genera were indentified in the samples (Appendix, Table A.1). All the species were encountered as empty shells whereas living individuals were found for 42 taxa only. The empty shell assemblage was dominated by *Difflugia globulus* (12.6% of the total counts of empty shells), *Centropyxis aerophila* (8.2%), *Euglypha tuberculata minor* (8.0%), *Centropyxis aerophila sphagnicola* (7.7%), *Centropyxis sylvatica* (5.9%), *Nebela (Argynnia) dentistoma* (5.2%), *Centropyxis minuta* (5.0%), *Trinema enchelys* (3.5%). These taxa were also characterised by high occurrence (in more than 70% of the samples) and thus constituted the core of the testate amoeba assemblage at the experimental site. Seven species (*Arcella catinus*, *Heleopera petricola*, *Bullinularia indica*, *Difflugia linearis*, *Arcella discoides*, *Arcella megastoma*) were present in less than 10% of the samples. The descriptive statistics of the univariate characteristics of the testate amoeba assemblage is presented in the Table 2.

Spatial patterns of the univariate assemblage characteristics

The maps of the spatial distribution of the univariate characteristics (total concentrations, species number, Shannon-Wiener's diversity index and Pielou's evenness index) of the testate amoeba assemblage at the site are shown in the Fig. 3. Multiple regression of the univariate characteristics on *x–y* coordinates showed a marginally significant linear trend in the species num-

Table 2. Descriptive statistics of the univariate characteristics of the testate amoeba assemblage at the 8 × 15 m plot (57 samples) in arctic tundra in Qeqertarsuaq/Disko Island (West Greenland) during the winter.

	Mean	SE	Median	Min	Max
Abundance, × 10³ shells g⁻¹ soil dry weight	39.1	5.2	28.9	4.2	248.9
Species number, taxa sample⁻¹	27.2	0.4	27.0	20.0	37.0
Shannon's diversity	2.83	0.02	2.87	2.15	3.26
Pielou's evenness	0.86	0.01	0.86	0.68	0.94
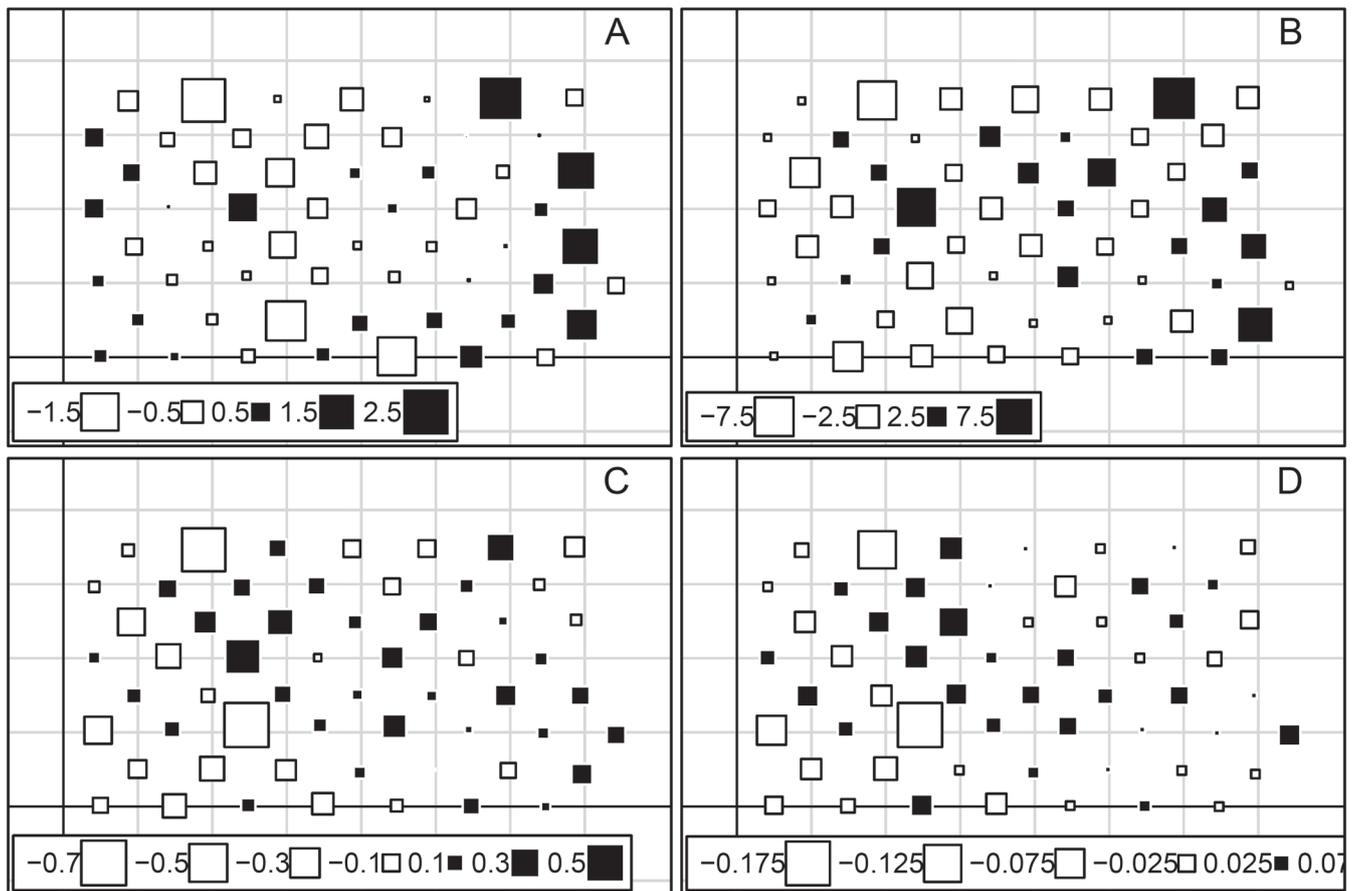
Fig. 3. Maps of the univariate characteristics of the soil testate amoeba assemblage (**A** – log$_e$(total concentration, × 10³ empty shells g⁻¹ of dry soil weight); **B** – species number (taxa sample⁻¹); **C** – Shannon-Wiener's diversity index; **D** – Pielou's evenness index) plotted against their spatial coordinates at 57 sampling locations within an 8 × 15 m plot in an arctic tundra in Qeqertarsuaq/Disko Island (West Greenland). All data are centred on 0, so square sizes are proportional to the deviations from the mean values at the plot. Open symbols are used for negative values and the filled symbols are used for positive values. Spatial patterns are visualised as aggregations of similar size and colour.

ber ($P < 0.1$) whereas no linear trend were detected for the other variable ($P > 0.05$ for all variables). Consequently, the undetrended data were used for testing effects of the PCNM variables. The test of the full model (with all PCNM variables included) did not reveal significant patterns in any of the univariate characteristics of the testate amoeba assemblage ($P > 0.05$) either. Thus, the univariate characteristics of the testate amoeba assemblage at the site were characterised by a random spatial distribution.

Spatial patterns in the assemblage composition

The redundancy analysis of the species abundance matrix indicated the presence of a significant linear trend in the assemblage composition of testate amoebae

at the site (pseudo-$F_{2,54} = 1.96$, $P < 0.001$). The species data were detrended and the full model which included all the PCNM variables was tested. The test was not significant (pseudo-$F_{39,17} = 0.94$, $P = 0.741$) indicating that there were no spatial structures in the assemblage composition within the range of spatial scales covered by the experimental design. As there were no spatial patterns within the plots, we modelled the general linear trend only. The forward selection procedure of *x*–*y* coordinates showed that both the variables should be retained in the parsimonious model meaning that there were significant linear trends in the assemblage composition along both *x* and *y* transects. The model explained 3.3% (R^2_{adj}) of the total variation in the assemblage composition of testate amoebae at the site. Both

canonical axes were significant (RDA1: pseudo-$F_{1,54}$ = 2.30, P = 0.003; RDA2: $F_{1,54}$ = 1.62; P = 0.025). The first canonical axis accounted for 1.9% (R^2_{adj}) of the total variation whereas the second axis explained 1.4% (R^2_{adj}) of the variance.

The spatial linear trends in the assemblage composition are illustrated in the Fig. 4. In terms of species responses, the most pronounced spatial linear trend (RDA1, Fig. 4A) was associated with decreasing proportions of hydrophilous species (*Arcella vulgaris*, *Difflugia pristis*, *Difflugia parva*) and increasing proportions of species with wide ecological preferences (*Trinema enchelys*, *Trinema complanatum*) (Table 3). The variation in the assemblage composition along the second linear trend (Fig. 4B) was related to the species which do not seem to have common ecological preferences (Table 3). In order to explain the detected patterns we regressed the site scores on the available environmental variables. Forward selection demonstrated that both microtopography and snow depth significantly
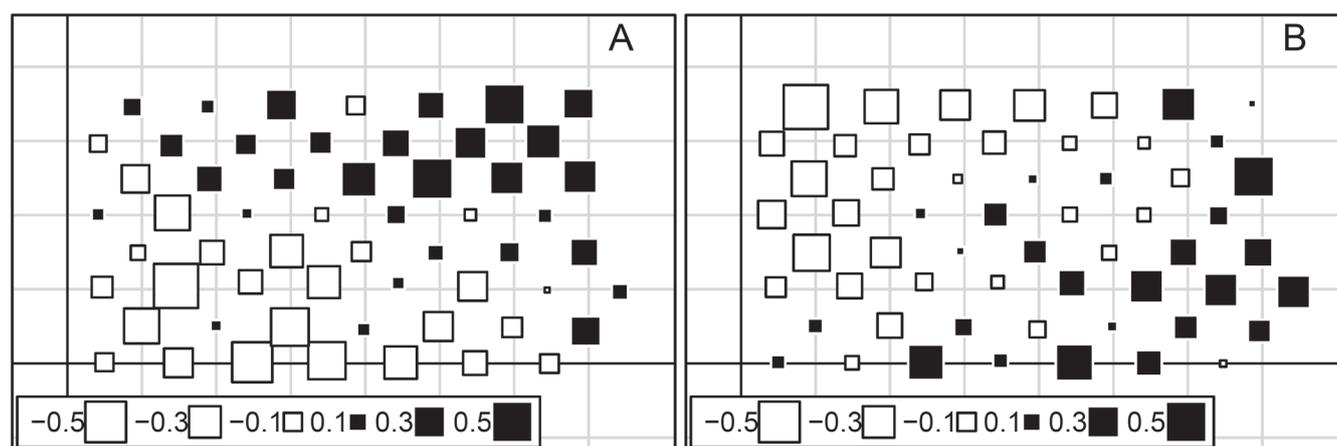
Fig. 4. Maps of the site scores extracted from the linear trend (**A** – the 1st canonical axis (RDA1); **B** – the 2nd canonical axis (RDA2)) plotted against their spatial coordinates at 57 sampling locations within an 8 × 15 m plot in an arctic tundra in Qeqertarsuaq/Disko Island (West Greenland). Square sizes are proportional to the absolute values of the site scores, the open symbols are used for negative values and the filled symbols are used for positive values. The squares of similar size and colour represent aggregations of testate amoebae with the similar species composition. Species which contributed the most to the changes in the assemblage composition across the linear spatial trends are presented in the Table 4.

Table 3. Species scores which demonstrated the strongest negative and positive correlations between testate amoeba species and the linear spatial trends modelled by the 1st (RDA1) and the 2nd (RDA2) axes of the redundancy analysis. The species can be related to the corresponding site scores in the Fig. 4.

Axes	Negative correlation	r	Positive correlation	r
RDA1	*Arcella vulgaris*	–0.46	*Trinema enchelys*	0.47
	Centropyxis aerophila sphagnicola	–0.40	*Trinema complanatum*	0.39
	Centropyxis cassis	–0.39	*Euglypha strigosa*	0.38
	Difflugia pristis	–0.36	*Centropyxis elongata*	0.28
	Difflugia parva	–0.23	*Centropyxis constricta*	0.25
RDA2	*Centropyxis sylvatica*	–0.30	*Nebela collaris*	0.40
	Nebela (Argynnia) virtae	–0.26	*Trinema lineare*	0.37
	Euglypha cristata decora	–0.25	*Arcella arenaria compressa*	0.37
	Trinema penardi	–0.21	*Euglypha tuberculata*	0.29
	Tracheleuglypha dentata	–0.21	*Euglypha ciliata glabra*	0.28

Table 4. The results of the multiple regressions showing the environmental variables which explained the spatial patterns in the assemblage composition of testate amoebae at an 8 × 15 m plot (the site scores along the first two canonical axes: RDA1 and RDA2; see Fig. 4). Estimate refers to the estimated partial regression slope. $^{+}P < 0.1$, $*P < 0.05$, $**P < 0.01$; $***P < 0.001$.

Parameter	Estimate	Standard Error	t-value	P
RDA1				
Intercept	−0.20	0.17	−1.15	
Microtopography	0.65	0.09	7.06	***
Snow depth	−0.66	0.30	−2.23	*
$\log_e$(Substrate density)	−0.15	0.08	−1.92	+
RDA2				
Intercept	−0.01	0.18	−0.03	
Microtopography	0.33	0.10	3.42	**
Snow depth	1.29	0.31	4.18	***
$\log_e$(Substrate density)	0.17	0.08	2.19	*

explained the variation in the assemblage composition along the canonical axes, whereas substrate density significantly contribute to the explanation of the variation along the second axis only (Table 4). The variation in the assemblage composition along the first axis was positively related to microtopography and negatively to the snow cover depth (Table 4, Fig. 4A, Fig. 2). The model explained 53.3% (R^2_{adj}) of the variation along the first canonical axis ($F_{3,53} = 22.26$, $P < 0.001$). The second canonical axis was positively related to microtopography, snow depth and substrate density (Table 4, Fig. 4B, Fig. 2) which explained together 32.1% (R^2_{adj}) of the variation along the axis ($F_{3,53} = 9.84$, $P < 0.001$).

DISCUSSION

General observations

The results of the present study indicate that concentrations of active testate amoebae in soils under the snow cover was quite low so that their contribution to the winter ecological processes is rather minor. These results contradict to the observations of Lousier and Parkinson (1984) who showed that some testate amoeba species had an increase in abundance and reproduction in aspen woodland soil in western Canada throughout the winter period. The differences can be explained by the fact that our study site was located further to the north in more severe climate conditions so that the snow cover often established upon frozen ground which can considerably limit the activity of soil protozoa during the winter. Hence, in the arctic climate, winter climate conditions seem to control soil testate amoeba assemblages mostly by affecting cyst formation and/or cyst survival rather than by controlling activity of testate amoebae under the snow cover.

The spatial distribution of the testate amoeba assemblages was analysed basing on the counts of empty shells only. Empty shell assemblages are a product of activity of the corresponding living assemblages and decomposition processes (Lousier and Parkinson 1981). In addition, it can be partly affected by passive translocations of shells with water flows and by other means. However, the effects of decomposition and the passive transfer of empty shells do not normally prevent empty shell assemblages from being used as an indicator of environmental conditions (Charman 2001; Mitchell *et al.* 2008). Overall, empty shells integrate activity of the corresponding living assemblage over long time periods and reflect more general environmental trends without confounding short-term variations.

The soil testate amoeba assemblage at the site was characterised by high taxonomic diversity with relatively high proportions of scanty taxa. The assemblage was mostly dominated by typical soil-dwelling and ubiquitous taxa (*Difflugia globulus*, *Centropyxis aerophila*, *Euglypha tuberculata minor*, *Centropyxis aerophila sphagnicola*, *Centropyxis sylvatica*, *Nebela (Argynnia) dentistoma*, *Centropyxis minuta*, *Trinema enchelys*). The other species in the assemblage were also either soil-dwelling or ubiquitous. The species composition corresponded well to the description of the testate amoeba assemblage studied in the same area in the summer of 2003 (Beyens *et al.* 2009). The dominant species have been reported to be the typical component of soil ecosystems in the Arctic region (Beyens *et al.* 1990, Balik 1994, Trappeniers *et al.* 2002, Carlson *et al.* 2010, Tsyganov *et al.* 2011). Overall, the assemblage composition was typical for testate amoeba in arctic tundra soils suggesting that our findings may have bearing beyond the limits of the studied location.

Spatial patterns of univariate assemblage characteristics

Our results do not indicate any spatial structures in the univariate characteristics of the testate amoeba assemblage in soil, i.e. there were neither linear trends

across the studied site nor patterns within the plot. Interestingly, the univariate characteristics were spatially unstructured whereas linear trends in the assemblage composition were detected. This is in accordance with the results of Mitchell *et al.* (2000) who found fine-scale spatial patterns in assemblage composition of testate amoebae in a macroscopically homogeneous *Sphagnum* carpet (40 × 60 cm) whereas the biomass, species number and total abundance of testate amoebae were generally unstructured except for the negative autocorrelations of total abundance for the distances between 15 and 20 cm. Following Mitchell *et al.* (2000) we conclude that spatial variation of testate amoebae is better pronounced in assemblage composition than in univariate assemblage characteristics. Overall, distribution of the univariate characteristics of soil testate amoeba assemblages is spatially unstructured at the plot-scale level.

Spatial patterns of assemblage composition

The assemblage composition of soil testate amoebae was characterised by the presence of linear spatial trends across the studied site whereas there were no spatial patterns within the plot. The detected spatial patterns of testate amoebae corresponded well to the microtopography at the site which had an overall sloping surface. Microtopography has been already demonstrated to be the main factor controlling the spatial patterns in the assemblage composition of testate amoebae at the fine-scale level (up to 1 m) (Mitchell *et al.* 2000) and at the range of tens of meters (Jassey *et al.* 2011). Altogether these data hint at the essential role of topography in regulation of spatial patterns of assemblage composition of testate amoeba at various spatial scales. Besides, the absence of spatial patterns within the plot is in line with the results of Griffiths (2002) who found that assemblage composition of protozoa was spatially unstructured within a 12 × 12 m soil plot of pasture with homogeneous vegetation and microtopography. These data suggest that in the absence of spatially structured environmental factors (such as topography, vegetation, animal droppings, etc.) population processes (reproduction, distribution, mortality, etc.) do not generate any spatial structures in assemblage composition of testate amoebae at the plot-scale level. So that spatial distribution of assemblage composition of testate amoebae at this scale can be considered as random within macroscopically homogeneous areas.

Topography is a complex ecological variable determining a number of other environmental characteristics such as soil moisture, disturbance by water movements, micro-climatic conditions (northern slopes are cooler and wetter), concentration of nutrients, snow depth, etc. Our results show that the most pronounced topography-related patterns in the assemblage composition of testate amoebae were associated with the distribution of species with different hydrological preferences. Hydrophilous species predominantly occurred in surface depressions whereas species with broad hydrological preferences inhabited topographical elevations. This hints at the essential role of topography-related soil moisture in regulation of spatial patterns of testate amoeba assemblages. The mechanism which could explain the observed patterns is water accumulation in surface depressions that makes them generally wetter than elevated parts.

Besides, the spatial patterns in the assemblage composition of testate amoebae at the studied site were also affected by snow depth and to a lesser degree by substrate density. Although microtopography can determine variation in snow depth by accumulating snow in depressions, the weak relationship between microtopography and snow depth at the site can be ascribed to the great influence of the other factors which influence the variation in the snow depth, for instance wind speed and direction (Walker *et al.* 1993). However, wind-dependent snow depth seems to generate spatial patterns in soil protozoa at wider scales than the plot-scale level. The relationships between the snow depth and the assemblage composition can be explained by the fact that a thicker snow cover in winter supports higher soil temperatures (Jones *et al.* 2001) and thus favour cyst survival of thermophilic species. These mechanisms can be important for maintenance of sustainable populations of thermophilic species in testate amoeba assemblages during the summer as well. However, some detected spatial patterns in the species distribution of testate amoebae could not be explained based on the known ecology of testate amoebae. Therefore, further studies on the effects of the other topography-related variables on testate amoebae can contribute considerably to our understanding of spatial patterns of soil protozoa.

We conclude that winter activity of soil testate amoebae under snow cover is rather limited in the arctic climate. The winter climate conditions rather influence soil testate amoebae by affecting cyst formation and/or cyst survival. The spatial distribution of soil testate amoebae was better pronounced in the species composition than in the univariate assemblage characteristics which were spatially unstructured. The assemblage composi-

tion was characterised by linear trends across the studied site whereas no spatial patterns were detected within the plot. These patterns corresponded well to the site microtopography, supporting the hypothesis about the essential role of microtopography in structuring of the distribution of soil protozoa at various spatial scales. The patterns were mainly controlled by topography-related soil moisture which favours development of hydrophilous species in surface depressions. Besides, snow depth also contributed to the regulation of spatial patterns in the assemblage composition of soil testate amoebae presumably by controlling soil temperature in the winter. However, wind-dependent snow depth seems to generate spatial patterns of soil protozoa at wider scales than the plot-scale level. Overall, the unstructured distribution of testate amoebae within the studied plot suggests that population processes do not generate spatial patterns in testate amoeba assemblages at the plot-scale level so that their distribution can be considered random at macroscopically homogeneous plots.

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Appendix

Table A.1. Summary table (relative abundance, % of the total counts; occurrence, % of the total number of samples; concentrations, × 10³ empty shells g⁻¹ of dry soil weight; and relative abundance, % of the sample counts) for the testate amoeba taxa encountered in 57 soil samples in the 15 × 8 m plot in Qeqertarsuaq/Disko Island (West Greenland) in December 2006. Species are sorted in decreasing order of their relative abundance to the total counts. Occurrence was calculated as the percentage of the number of samples in which the taxon was encountered. Species marked with an asterisk are excluded from the multivariate analysis.

Species names	Relative abundance,	Occurrence,	Concentrations, × 10³ g⁻¹ dry weight			Relative abundance, %		
	%	%	Mean ± SD	Max	CV, %	Mean ± SD	Max	CV, %
Difflugia globulus	12.6	100	4.92 ± 7.68	40	156	10.92 ± 6.14	34	56
Centropyxis aerophila	8.2	100	3.19 ± 2.49	16	78	9.33 ± 3.92	17	42
Euglypha tuberculata minor	8.0	100	3.11 ± 3.27	20	105	8.09 ± 3.2	22	40
Centropyxis aerophila sphagnicola	7.7	98.2	3.02 ± 2.97	14	98	8.29 ± 5.06	23	61
Centropyxis sylvatica	5.9	70.2	2.29 ± 3.73	24	163	6.24 ± 6.93	25	111
Nebela (Argynnia) dentistoma	5.2	98.2	2.04 ± 1.89	8.8	93	5.66 ± 3.72	16	66
Centropyxis minuta	5.0	89.5	1.95 ± 2.17	9.2	111	4.93 ± 4.02	18	82
Trinema enchelys	3.5	100	1.39 ± 2.12	12	153	2.97 ± 1.88	7.3	63
Nebela penardiana	3.4	94.7	1.32 ± 1.14	4.6	86	3.83 ± 2.62	13	68
Centropyxis elongata	3.2	94.7	1.27 ± 2.28	12	180	2.59 ± 2.03	8.4	78
Trinema complanatum	3.2	84.2	1.26 ± 2.43	13	194	2.33 ± 2.01	7.4	86
Centropyxis orbicularis	2.8	73.7	1.10 ± 1.65	10	150	2.99 ± 3.45	14	115
Euglypha laevis	2.5	91.2	0.97 ± 1.09	5.7	113	2.70 ± 2.50	13	93
Trinema lineare	2.5	89.5	0.98 ± 2.07	12	212	1.84 ± 1.77	7.9	96
Centropyxis cassis	2.4	84.2	0.93 ± 1.11	6.9	119	2.79 ± 2.64	14	95
Nebela wailesi	2.3	86.0	0.89 ± 0.78	3.5	88	2.62 ± 1.9	6.3	72
Nebela tubulosa	1.9	70.2	0.74 ± 1.05	5.1	142	2.29 ± 3.09	13	135
Nebela collaris	1.5	70.2	0.58 ± 0.96	5.1	166	1.43 ± 1.62	7.7	113
Difflugia pristis	1.4	57.9	0.55 ± 1.27	8	231	1.05 ± 1.43	7.9	136
Plagiopyxis declivis	1.4	47.4	0.54 ± 1.20	6.6	221	1.23 ± 2.11	7.9	171
Tracheleuglypha dentata	1.3	49.1	0.50 ± 1.46	10	294	1.63 ± 5.11	36	313
Corythion dubium	1.2	75.4	0.49 ± 0.85	5.1	175	1.12 ± 1.1	4.7	99
Cyclopyxis kahli	1.1	38.6	0.43 ± 1.01	5.3	232	1.52 ± 3.4	19	224
Euglypha rotunda	1.1	50.9	0.44 ± 1.06	5.6	241	0.73 ± 1.02	3.9	140
Heleopera rosea	1.1	77.2	0.45 ± 0.46	2.3	103	1.72 ± 2.19	12	127
Heleopera sylvatica	1.0	71.9	0.39 ± 0.57	3.5	144	1.00 ± 0.87	3.2	87
Euglypha ciliata glabra	0.9	36.8	0.33 ± 1.06	7.4	318	0.56 ± 1.05	4.7	188
Trinema penardi	0.9	57.9	0.33 ± 0.61	3.5	182	0.84 ± 1.15	4.9	136
Assulina muscorum	0.8	59.6	0.33 ± 0.72	4.4	219	0.84 ± 1.18	5.8	141
Nebela lageniformis	0.8	52.6	0.31 ± 0.77	5.1	247	0.61 ± 1.09	5.1	180
Euglypha strigosa glabra	0.7	59.6	0.26 ± 0.41	2.5	159	0.68 ± 0.79	2.9	116
Arcella vulgaris	0.6	45.6	0.24 ± 0.38	1.5	162	0.86 ± 1.38	5.5	161
Difflugia tenuis	0.6	47.4	0.22 ± 0.3	1.3	139	0.55 ± 0.74	2.3	134
Difflugia parva	0.5	40.4	0.2 ± 0.39	2.5	197	0.65 ± 1.14	5.9	174
Euglypha strigosa	0.4	38.6	0.16 ± 0.51	3.8	328	0.31 ± 0.49	1.7	158
Euglypha tuberculata	0.4	36.8	0.16 ± 0.55	4	352	0.26 ± 0.49	2.8	189

Arcella arenaria compressa	0.3	31.6	0.1 ± 0.24	1.3	226	0.23 ± 0.57	4	252
Euglypha ciliata	0.3	19.3	0.12 ± 0.47	3.2	385	0.15 ± 0.36	1.6	241
Arcella arenaria	0.2	14	0.06 ± 0.22	1.3	361	0.14 ± 0.50	3.4	357
Centropyxis constricta	0.2	12.3	0.09 ± 0.32	1.6	340	0.40 ± 1.41	7.3	348
Euglypha cristata decora	0.2	21.1	0.06 ± 0.15	0.6	247	0.13 ± 0.31	1.8	243
Nebela (Argynnia) virtae	0.2	15.8	0.07 ± 0.19	0.8	270	0.19 ± 0.52	2.9	272
Arcella catinus	0.1	7.0	0.06 ± 0.28	1.9	488	0.1 ± 0.46	3.3	479
Difflugia globulosa	0.1	10.5	0.03 ± 0.12	0.8	384	0.12 ± 0.54	3.9	443
Difflugia lucida	0.1	14.0	0.05 ± 0.17	1	328	0.11 ± 0.33	1.7	294
Euglypha cristata	0.1	10.5	0.04 ± 0.14	0.8	375	0.1 ± 0.36	2.2	365
Heleopera petricola	0.1	5.3	0.05 ± 0.29	1.9	540	0.03 ± 0.15	0.8	435
Pontigulasia incisa	0.1	10.5	0.04 ± 0.15	0.8	361	0.11 ± 0.38	1.9	345
Arcella discoides*	0.0	1.8	0.02 ± 0.13	1	755	0.07 ± 0.55	4.2	755
Arcella megastoma*	0.0	1.8	0.01 ± 0.09	0.7	755	0.05 ± 0.35	2.6	755
Bullinularia indica	0.0	3.5	0.01 ± 0.05	0.3	582	0.02 ± 0.13	0.9	550
Difflugia linearis	0.0	3.5	0.01 ± 0.03	0.2	542	0.03 ± 0.15	1	558
Nebela (Porosia) bigibbosa	0.0	3.5	0.01 ± 0.05	0.4	566	0.03 ± 0.14	1	557