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Does Climate Warming Stimulate or Inhibit Soil Protist Communities? A Test on Testate Amoebae in High-Arctic Tundra with Free-Air Temperature Increase

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Soil testate amoebae assemblages in a grassland area at Zackenberg (Northeast Greenland) were subjected to simulated climate-warming during the growing season using the Free-Air Temperature Increase technique. Samples were collected in upper (0 – 3 cm) and deeper (3 – 6 cm) soil horizons. Mean temperature elevations at 2.5 and 7.5 cm depth were 2.58 ± SD 1.11 and 2.13 ± SD 0.77 °C, respectively, and did not differ significantly. Soil moisture in the top 11 cm was not affected by the warming. During the manipulation, the densities of living amoebae and empty shells were higher in the experimental plots but only in the upper layer. Possibly, testate amoebae in the deeper layer were limited by other factors, suggesting that warming enhances the carrying capacity only in favourable conditions. Species richness, on the other hand, was only increased in the deeper horizon. Warming did not change the percentage of individuals belonging to small-sized species in any of the living assemblages, contrary to our expectation that those species would quickly increase their density. However, in the empty shell assemblages, the proportion of small-sized individuals in the experimental plots was higher in both layers, indicating a rapid, transient increase in small amoebae before the first sampling date. Changes in successional state of testate amoebae assemblages in response to future climate change might thus be ephemeral, whereas alterations in density and species richness might be more sustained.

Key words: ecosystem manipulation; global warming; Greenland; soils; temperature; testate amoebae.

Introduction

According to various scenarios of global warming, the Earth's surface temperature is expected to increase by 1.1 – 6.4 °C by the end of the 21st century (IPCC 2007). The increase is likely to be greater at higher latitudes, where current tempera-

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Abbreviations: DOY, day of the year; FATI, free air temperature increase.

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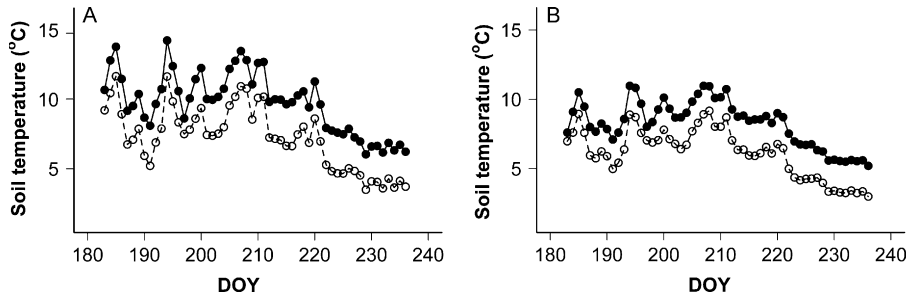

Figure 1. Mean diurnal soil temperature in control and experimental plots at 2.5 cm (**A**) and 7.5 cm depth (**B**) during the warming period. SE bars are not shown because they overlapped with the symbols. At 2.5 cm depth, SE's ranged from 0.03 to 0.29 and from 0.06 to 0.32 °C in the control and warming treatment, respectively; at 7.5 cm depth they ranged from 0.04 to 0.17 °C in both treatments. Solid symbols and lines represent warmed plots, open symbols and dotted lines control plots. DOY: day of the year.

tures are below 0 °C most of the year and growing season temperatures are only a few degrees higher (Rummukainen et al. 2003; Shaver et al. 2000). In these regions, even a small rise in average temperature will significantly extend the unfrozen period and increase degree-day accumulations (Billings 1987). The response of testate amoebae assemblages to global climate change at high latitudes is of particular interest because they represent a common and abundant group of free-living terrestrial protists in the Arctic (about 300 taxa; Beyens and Chardez 1995). They also play an important role in food webs as decomposers of organic matter, and as intermediates between bacterial and invertebrate communities (Gilbert et al. 1998).

Climate conditions, especially temperature and precipitation, appear to be essential for the distribution, and consequently the species composition, of testate amoebae. For example, in the southern hemisphere, a broad trend of decreasing species richness with increasing latitude and declining mean January temperatures was noted by Smith (1996) and by Smith and Wilkinson (1987). Yang et al. (2006) found that both the species and genus richness of the large fraction (> 64 μm) of planktonic testate amoebae correlate well with the mean temperature of the warmest month and with mean annual precipitation. These biogeographic trends hint at a potential for testate amoebae assemblages to respond significantly to changes in global climate. An in vitro experiment on the testate amoeba *Arcella vulgaris* Ehrenberg has shown that warming indeed promotes reproduction (up to 20 °C; Laybourn and Whymant 1980). Testate amoebae also react to other environmental factors such as moisture and pH. These responses are quite well known and are widely used in paleoclimate reconstructions and biomonitoring (Beynes and Meisterfeld 2001; Charman 2001; Heal 1962, 1964; Lousier 1974; Mitchell et al. 2008; Tolonen 1986).

So far, we are aware of only a single study on testate amoebae responses to climate manipulation in the field. Beyens et al. (2009) studied the effect of a short-term (two-week) heatwave on the structure of soil testate amoebae in dry heath tundra in Qeqertarsuaq (Disko Island, West Greenland), and found transient shifts in species populations during the exposure and ultimately an increase in species richness weeks after the heatwave had ended. The question thus arises whether continuous warming will induce lasting changes in diversity and community structure? We examined this using the same climate manipulation technique (infrared irradiation), investigating both upper and deeper soil layers and both the living testate amoebae assemblage and the empty shell assemblage (thanatocoenosis). Our study was carried out in the plots of the Marchand et al. (2004a, 2004b) studies at Zackenberg (Northeast Greenland), which simultaneously investigated the responses of the plant community.

Results

Warming Effects on Soil Parameters

During the manipulation period, the soil was significantly warmer at 2.5 than at 7.5 cm depth, both in the control plots and the experimental plots (Fig. 1A and B, significant layer effect, repeated measures ANOVA, $F_{1,286} = 205.36$, $P < 0.001$). Mean control temperatures were 7.23 ± SD 3.03 in the upper layer and 6.16 ± SD 2.07 °C in the deeper layer. The experimental warming significantly increased these temperatures (Fig. 1A and B, significant treatment

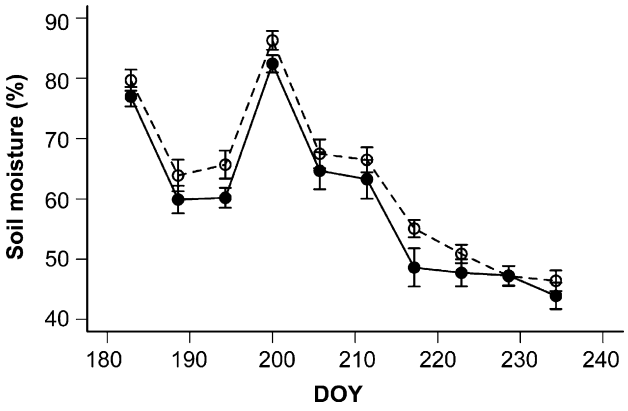

Figure 2. Volumetric soil moisture (mean ± SE) over the upper 11 cm horizon in the control and experimental plots during the warming period. Solid symbols and lines represent warmed plots, open symbols and dotted lines control plots. DOY: day of the year.

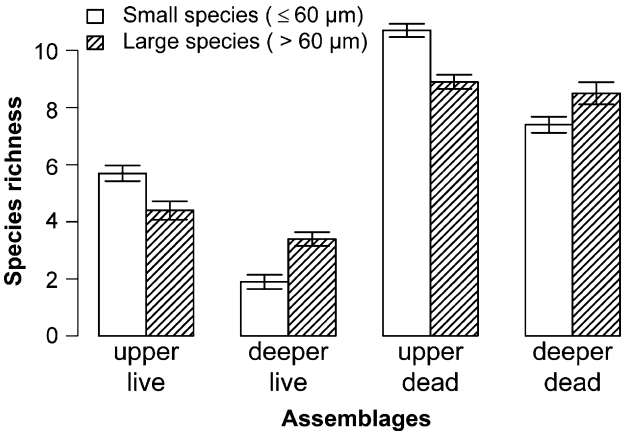

Figure 3. Species richness (mean ± SE) by shell size of the testate amoebae assemblages.

effect, repeated measures ANOVA, $F_{1,286} = 264.17$, $P < 0.001$), by 2.58 ± SD 1.11 °C at 2.5 cm depth and by 2.13 ± SD 0.77 °C at 7.5 cm depth. As the treatment × layer interaction was not significant (repeated measures ANOVA, $F_{1,286} = 3.36$, $P > 0.05$), soil warming was similar at both depths. Vegetation temperature was elevated by on average 2.48 ± SD 0.52 °C above an ambient mean of 8.37 °C in the control plots. During the growing season, mean thawing depth augmented from 29.8 ± SE 1.0 to 65.9 ± SE 0.6 cm in the control plots and from 30.5 ± SE 1.4 to 72.7 ± SE 0.4 cm in the warmed plots (significant treatment effect, repeated measures ANOVA, $F_{1,22} = 22.35$, $P < 0.05$), while mean soil moisture was lowered from 76.8 to 46.4% (control) and from 75.1 to 44% (treatment). Soil moisture was consistently lower in the warmed plots, though not significantly (Fig. 2, repeated measures ANOVA, $F_{1,22} = 3.76$, $P > 0.05$).

Testate Amoebae Assemblages before the Manipulation

A total of 47 testate amoebae taxa belonging to 15 genera were identified in 60 samples (all data combined). An alphabetical list of the taxa and their abbreviations used in this study is given in Appendix 1. Living individuals were observed for 35 species, empty shells for all taxa. Species that were only represented by empty shells were generally not abundant (*Cyclopyxis kahli, Cyphoderia* sp1*, Difflugia bryophila, Difflugia lucida, Difflugiella oviformis* v. *fuscum, Difflugiella pusila, Difflugiella* sp2*, Hyalosphenia papilio, Hyalosphenia* sp2*, Nebela lageniformis, Nebela marginata, Trinema lineare* v. *truncatum*).

Before the onset of the warming, the community indices were not significantly different between control and experimental plots in any of the soil layers (ANOVA, $P > 0.05$ for all community indices), whereas they were different between the two sampling depths (Table 1). Both the living assemblage and the thanatocoenosis in the upper layer were characterized by greater population density and species richness, less equitable species densities (lower evenness) and predominance of small-sized testate amoebae (≤60 µm), relative to the deeper layer (Table 1, Mann-Whitney U-test, $P < 0.05$ for all the variables). The distribution of species richness by shell size groups in the assemblages is shown in Figure 3. The diversity index was also significantly greater in the upper layer but only for the living assemblage (Table 1, Mann-Whitney U-test, $P < 0.05$), whereas it did not differ between the upper and deeper thanatocoenosis.

The upper assemblage of living amoebae was dominated by *Trinema lineare* (29.8 ± SE 3.9%), with *Trinema complanatum* (8.9 ± SE 1.7%), *Centropyxis aerophila* (8.4 ± SE 1.6%), *Euglypha laevis* (6.1 ± SE 1.6%), and *Corython dubium* (6.9 ± SE 1.9%) as subdominants. In the deeper layer, on the other hand, the most abundant species were *Centropyxis aerophila* v. *sphagnicola* (25.0 ± SE 6.5%), *Centropyxis aerophila* (15.0 ± SE 2.3%), *Difflugia globulus* (15.1 ± SE 5.0%), *Centropyxis sylvatica* (7.6 ± SE 3.6%), and *Trinema lineare* (6.4 ± SE 1.8%). The species composition of the thanatocoenosis generally followed that of the living testate amoebae assemblages, with some exceptions. Firstly, in the deeper thanatocoenosis, the relative abundance of *Trinema lineare* was much

Table 1. Community characteristics of the testate amoebae assemblages. Means ± SE before (two sampling dates) and after (three sampling dates) the start of the warming.

	Control		Warmed	
	Before	After	Before	After
Living assemblage				
Upper layer (0–3 cm)				
Density, x 1000 individuals cm^{-3}	5.6 ± 1.7	6.1 ± 0.5	7.3 ± 1.7	9.7 ± 1.3
Species richness	10.2 ± 0.9	11.4 ± 1.0	9.2 ± 1.1	9.6 ± 0.5
Shannon-Wiener index	2.1 ± 0.1	2.1 ± 0.1	1.9 ± 0.1	1.9 ± 0.1
Evenness	0.8 ± 0.0	0.7 ± 0.0	0.8 ± 0.0	0.7 ± 0.0
% of small individuals (≤60 μm)	72.3 ± 5.0	63.3 ± 3.6	68.8 ± 6.6	71.5 ± 3.8
Deeper layer (3–6 cm)				
Density, x 1000 individuals cm^{-3}	2.3 ± 0.6	1.8 ± 0.5	2.8 ± 0.6	1.9 ± 0.2
Species richness	5.3 ± 0.1	4.1 ± 0.3	6.3 ± 0.6	5.9 ± 0.6
Shannon-Wiener index	1.6 ± 0.1	1.3 ± 0.1	1.6 ± 0.1	1.6 ± 0.1
Evenness	0.9 ± 0.0	0.9 ± 0.0	0.9 ± 0.0	0.9 ± 0.0
% of small individuals (≤60 μm)	26.3 ± 10.6	36.3 ± 8.1	31.3 ± 5.8	43.4 ± 7.6
Thanatocoenosis				
Upper layer (0–3 cm)				
Density, x 1000 individuals cm^{-3}	50.6 ± 13.6	37.7 ± 2.7	75.5 ± 19.0	63.1 ± 7.2
Species richness	19.0 ± 0.8	20.4 ± 0.8	19.3 ± 0.7	19.4 ± 0.6
Shannon-Wiener index	2.3 ± 0.1	2.4 ± 0.1	2.4 ± 0.1	2.5 ± 0.1
Evenness	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0
% of small individuals (≤60 μm)	77.4 ± 1.6	69.3 ± 1.8	73.0 ± 2.3	75.0 ± 0.5
Deeper layer (3–6 cm)				
Density, x 1000 individuals cm^{-3}	31.1 ± 8.5	29.5 ± 4.3	31.1 ± 4.6	33.6 ± 4.5
Species richness	14.8 ± 1.7	15.0 ± 0.7	15.7 ± 1.1	17.7 ± 0.7
Shannon-Wiener index	2.3 ± 0.1	2.3 ± 0.0	2.3 ± 0.1	2.4 ± 0.0
Evenness	0.7 ± 0.1	0.7 ± 0.0	0.7 ± 0.0	0.6 ± 0.0
% of small individuals (≤60 μm)	61.4 ± 2.1	43.0 ± 1.6	54.8 ± 2.5	52.3 ± 3.1

greater (18.6 ± SE 1.9%) than in the corresponding living assemblage. Secondly, *Assulina muscorum* was a subdominant species of the thanatocoenosis in both layers (5.8 ± SE 0.9% and 7.4 ± SE 1.2% in the upper and deeper layer, respectively).

Warming Effects on Community Indices

During the treatment, the total density of living testate amoebae in the upper layer became significantly greater in the warmed than in the control plots (Table 1, Fig. 4A, ANOVA, $F_{1,16} = 4.54$, $P < 0.05$). In the deeper layer, on the other hand, the warming caused no significant changes in density. The same pattern was observed in the thanatocoenosis (Table 1, Fig. 4B, ANOVA, $F_{1,16} = 11.88$, $P < 0.01$).

The increase in density in the upper layer in response to the warming was not accompanied by changes in species richness. Surprisingly, in the deeper layer where density was not affected, richness did become higher upon exposure, both in the living assemblage and the thanatocoenosis (Table 1, Fig. 4C and D, ANOVA, $F_{1,16} = 5.81$ and $F_{1,16} = 8.04$, $P < 0.05$, respectively). A similar pattern was detected in the the Shannon-Wiener diversity index, though it was only significant for the living assemblage (Table 1, Fig. 4E and F, ANOVA, $F_{1,16} = 7.08$, $P < 0.05$). The difference in species richness between the control and experimental plots was primarily due to species which were encountered occasionally with few specimens (*Arcella hemisphaerica*, *Euglypha ciliata*, *Euglypha compressa* v. *glabra*, *Euglypha rotunda* v. *dorsalis*, *Difflugiella pusilla, Trinema lineare* v. *truncatum*). At the same time, species evenness was not affected by the warming (Table 1, Fig. 4G and H, ANOVA, $P > 0.05$ for all the assemblages).

Warming did not change the percentage of individuals belonging to small-sized species (≤60 μm) in any of the living testate amoebae assemblages (Table 1, Fig. 4I). However, in the thanatocoenosis

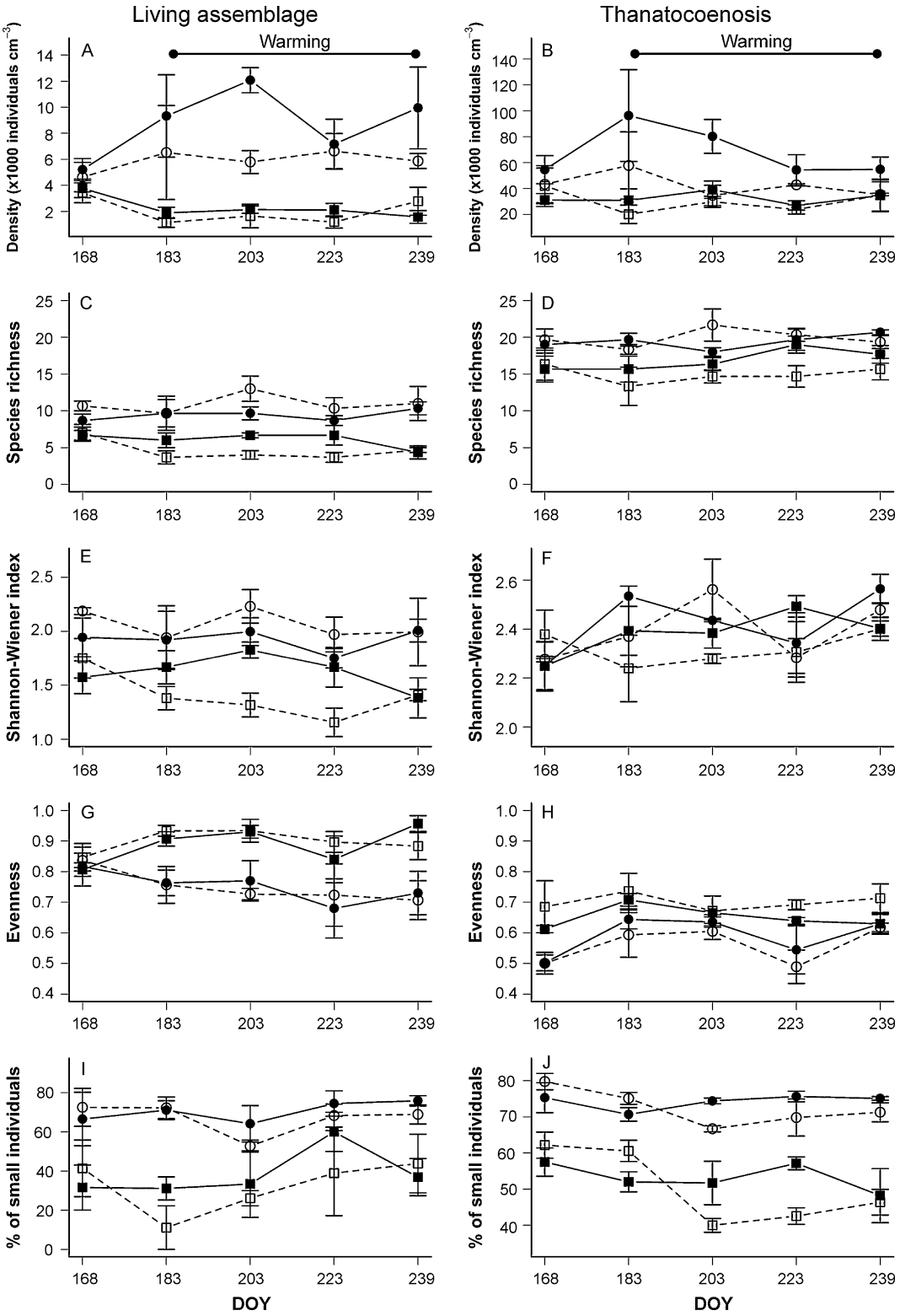

Figure 4. Testate amoebae community characteristics (**A**, **B** – density, **C**, **D** – species richness, **E**, **F** – Shannon-Wiener index, **G**, **H** – evenness, **I**, **J** –percentage of individuals belonging to small-sized ($\leq$60 μm) species) in the upper (circles) and deeper (squares) layer during the experiment (mean $\pm$ SE). Solid symbols and lines represent warmed plots, open symbols and dotted lines control plots. DOY: day of the year.

of both layers, the proportion of small-sized individuals was significantly greater in the experimental plots in comparison to the control plots (Table 1, Fig. 4J, ANOVA, $F_{1,16} = 9.37$ and $F_{1,16} = 6.05$, $P < 0.05$, for the upper and deeper layer, respectively).

Warming Effects on Species Composition

Warming significantly affected the community structure of the upper living assemblage and the deeper thanatocoenosis (global permutation test, $P < 0.05$ for both assemblages). In these two assemblages, warming accounted for respectively 14.4 and 14.8% of the variation in species composition, which is a considerable part of the variance given the typical noisy nature of testate amoebae data. The co-variables, associated with spatial and temporal variation, explained respectively 19.5 and 29.8% of the total variation in those assemblages.

The resulting triplots are presented in Figure 5. The first two axes of the diagram of the upper living assemblage (Fig. 5A) represent 73.7% of the variation explained by the environmental variables (38.3% on axis 1 and 35.4% on axis 2). In the upper layer, the changes in relative abundance caused by the warming balanced each other, both in the small and the large-sized species. This explains why the earlier analysis of variance did not reveal any significant differences in the proportion of individuals belonging to small-sized species between the control and experimental plots. Within the small species, the increase of *Difflugia globulus* (DOY 203, 223, 239), *Euglypha laevis* (DOY 203, 223, 239), *Trinema lineare* (DOY 203, 223), and *Assulina muscorum* (DOY 203, 223) compensated for the decrease of *Trinema enchelys* (DOY 203 and 239) and *Trinema companatum* (DOY 203, 239). Within the large species, the increase of *Euglypha tuberculata* (DOY 203, 223) and *Euglypha strigosa* (DOY 203, 223) compensated for the decrease of *Centropyxis aerophila v. sphagnicola* (DOY 203, 223, 239), *Centropyxis aerophila* (DOY 203, 223) and *Centropyxis platysoma* (DOY 203, 223).

On the ordination diagram of the deeper thanatocoenosis (Fig. 5B), the first two axes represent 85.6% of the variation explained by the environmental variables (51.5% on axis 1 and 34.1% on axis 2). Here, the changes in relative abundance of small and large-sized testate amoebae induced by the warming were not as balanced as in the living assemblage. In particular, the increase of the large-sized species *Difflugia penardi* (DOY 239), which originally occurred at a minor density, did not outweigh the decrease of the dominant species *Centropyxis aerophila* (DOY 203, 223, 239) and *Centropyxis aerophila v. sphagnicola* (DOY 203, 223, 239). Moreover, the increase in the small-sized species *Trinema lineare* (DOY 203, 223) and *Trinema enchelys* (DOY 223) was not compensated for at all. Combined, these changes in species composition associated with the warming increased the proportion of small-sized species in both the upper and deeper thanatocoenosis.

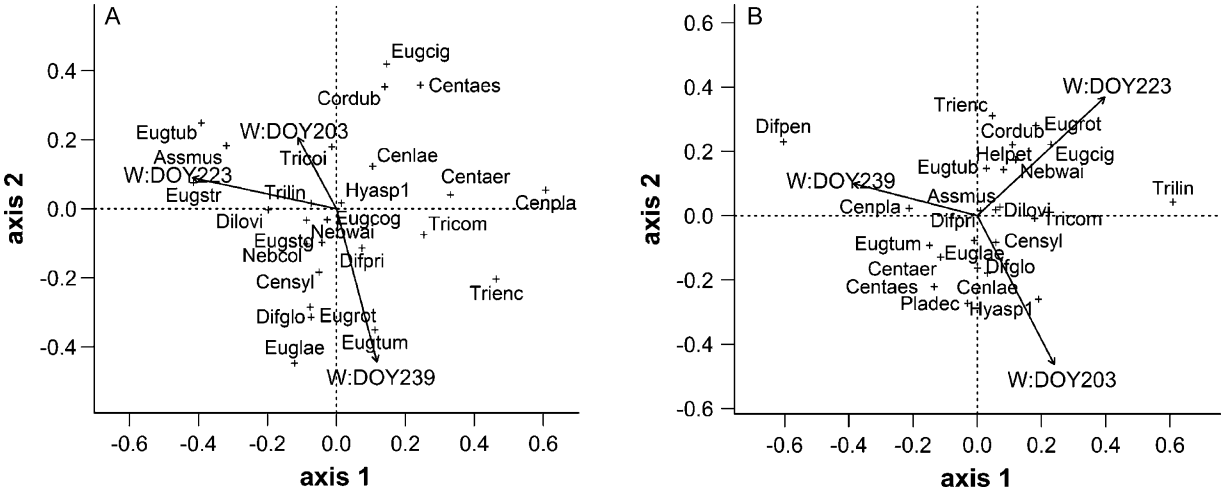

Figure 5. Redundancy analysis ordination diagram showing relationships between the taxa and the experimental warming (**A** – living testate amoebae assemblage in the upper layer, **B** – thanatocoenosis in the deeper layer). W × DOY 203, W × DOY 223, W × DOY 239 – interaction between warming and sampling day. See Appendix 1 for species abbreviations.

Discussion

In the present work, we investigated effects of warming during the growing season on testate amoebae assemblages in a high arctic soil. The experimental treatment increased soil temperature and active layer depth, but did not significantly alter the volumetric water content of the upper 11-cm soil horizon. The latter can probably be ascribed to the "wet" local soil moisture regime and/or the moderate exposure intensity, as infrared irradiation of dry heath tundra at greater intensity in Qeqertarsuaq (Disko Island, West Greenland) did lower the soil water content (Beyens et al. 2009). Possibly, originally dry soil habitats undergo more significant changes in moisture regime than humid ones. Other factors that might affect soil biota and could be altered by global warming, such as growing season length and number of freeze-thaw cycles, were not included in the present experiment as we did not irradiate during winter, basically because it is unknown whether the effect of the FATI system on snow is realistic. The observed differences between the testate amoebae assemblages in the control and experimental plots can therefore be considered as direct effects of the imposed temperature increase and its possible indirect consequences through changes in biotic factors such as food supply, competition, etc.

The testate amoebae assemblage sampled here is highly similar to that in a previous study on soils in the Zackenberg area (Trappeniers et al. 2002). Moreover, the observed predominant taxa *Trinema lineare*, *Centropyxis aerophila, Centropyxis aerophila* v. *sphagnicola* and *Difflugia globulus* have been reported as the most common and abundant testate amoebae species in the Arctic (Balik 1994; Beyens et al. 1990). Our findings may therefore have bearing beyond the limits of the studied location.

Our data show that the testate amoebae assemblage at the experimental site was horizontally and vertically heterogeneous. The horizontal heterogeneity was less pronounced and mostly became apparent in a deviation from mean testate amoebae density in one experimental plot on sampling day 183, as illustrated by the large error bars at that time. This did not lead to significant differences between the control and experimental plots before the warming started, but visually suggests an increase in testate amoebae density in the experimental plots prior to the warming (Fig. 4A and B). Such a deviation is not surprising provided that testate amoebae have a complex spatial distribution pattern even within biotopes that look uniform from a macroscopic point of view (Mitchell et al. 2000). The distribution reflects to some extent the heterogeneity of soil resources and of the physical and chemical characteristics of the environment (Ettema et al. 1998). However, the size of testate amoebae aggregations usually does not exceed 10 – 20 cm (Mitchell et al. 2000), hence the observed density deviation is unlikely to have affected our results as subsequent samples were taken in other parts of the experimental plot. This conclusion is in agreement with the first sampling time not showing such a difference, and with subsequent sampling also showing fluctuations over time. These fluctuations, however, did not preclude the statistics from detecting a significant effect of the warming. The vertical variation of the testate amoebae assemblage along the soil profile was more pronounced. Both the living assemblage and the thanatocoenosis in the upper layer were characterized by greater population density and species richness, less equitable species densities (lower evenness) and predominance of small-sized testate amoebae ($\leq$60 μm), relative to the deeper layer. Earlier studies showed that vertical distribution of testate amoebae in soils is highly variable and primarily depends on soil bulk density, quality of organic litter and hydrological regime (Bonnet 1964; Coûteaux 1972; Korganova 1975; Lousier 1975; Schönborn 1962; Vincke et al. 2006). In the current study, the aforementioned differences between the layers likely reflect the presence of more variable and abundant organic resources and a more favourable temperature regime in the upper horizon.

A main finding of our experiment was the positive effect of the warming on the density of the living assemblage and thanatocoenosis in the upper layer. In contrast, in their experimental simulation of a heatwave, Beyens et al. (2009) did not detect any changes in density of live testate amoebae in that layer, probably because the treatment was shorter, or because it was more intense and reduced soil moisture to levels limiting population growth (mean soil moisture was always below 45%, the lowest limit in the present manipulation). In the current experiment the soil water content was measured in the 0 – 11 cm horizon, hence desiccation of the 0 – 3 cm and 3 – 6 cm horizons in the warmer environment can in principle not be excluded. However, given the crucial importance of water for protists, this would have limited growth instead of stimulating it. Moreover, significant reductions in soil moisture in the 0 – 3 cm or 3 – 6 cm layers would have altered the total volumetric water content in the 11-cm soil layer (which did not occur), unless opposite

changes in the underlying layer of 6 – 11 cm would have compensated for this. The latter is unlikely, because rising temperatures modify soil moisture content not only through increased evapotranspiration, drying the top soil, but also by means of ameliorated drainage, due to melting of the permafrost, which further desiccates deeper soil layers (Oechel and Billings 1992). Unexpectedly, in our experiment, the thecamoebae density in the deeper layer did not respond to the warming, in spite of the similar temperature increase there. This can be explained by the lower temperature in that layer or by other limitations such as spatial constrictions or food supply. Combined, our data support the hypothesis that warming enhances the overall carrying capacity of testate amoebae in favourable conditions.

In spite of the unaffected population density in the deeper layer, species richness in that horizon was increased in both the living assemblage and the thanatocoenosis. This was mostly caused by the appearance of species represented by only few specimens. Some of these species (e.g. *Euglypha ciliata*, *Euglypha compressa* v. *glabra*) were characteristic of the upper layer, suggesting that changes caused by the warming in the upper layer might affect the deeper assemblage. Possible mechanisms for such an effect are active locomotion and passive downwards transfer of the testate amoebae. Although the mobility of testate amoebae is limited to the order of 1 – 3 cm per day, depending on moisture and porosity of substrates (Adl 2007; Stout and Heal 1967), this seems sufficient to explain the observed patterns. Passive vertical movements also seem feasible, but remain speculative due to lack of knowledge. Besides the apparent colonisation by species from the top layer, other species (e.g. *Arcella hemisphaerica* and *Difflugiella pusilla*) that appeared in the deeper layer during the warming were new to both layers. The appearance of these new species indicates that interspecific competition at this originally species-poorer and less populated depth was lower. In the upper layer, on the other hand, competition may have been more intense given the greater initial density, which was further enhanced by the warming. This would explain the observed lack of changes in species richness in that layer. In the heatwave experiment of Beyens et al. (2009), species richness did increase in the upper layer, but only weeks after the heatwave had ended. Apparently, the original testate amoebae assemblage was not able to respond to the disturbance quickly enough because of soil desiccation, which did however allow new species to settle after some time.

In terms of species response, the effect of elevated temperature was mixed. Positive as well as negative correlations with the treatment were observed both in small and large-sized species. As a result, the proportion of individuals belonging to small-sized species in the living assemblage did not change in any of the layers. Smaller protists are characterized by higher reproductive rates than larger ones (Baldock et al. 1980; Finlay 1977). Hence, one would expect a rapid increase in the proportion of small-sized testate amoebae in response to the more favourable conditions achieved in the experiment. The reason why this was not observed might be the relatively long time intervals of our sampling design, which was aimed at detecting more sustained changes. Testate amoebae are known for their fast generation times (Hedley and Ogden 1973, 1974; Hedley et al. 1974; Schönborn 1975, 1982), so any transient increase in the density of small species before DOY 223, within three weeks of warming, may have remained undetected. This would explain why the proportion of individuals belonging to small-sized species increased significantly in the thanatocoenosis. We conclude that the successional state of testate amoebae assemblages appears to be fairly robust, meaning that a balance between small and large-sized species might be quickly re-established.

Overall, our data demonstrate the ability of testate amoebae to increase their density in response to warming, provided that other factors are not limiting. We also showed that changes in density in one soil horizon may affect species richness in other layers. In terms of successional state, testate amoebae assemblages exhibited a moderate resistance. We therefore suggest that changes in the carrying capacity of the biotope in a warmer climate would be more sustained than alterations in the successional state.

Methods

Research site: The study was conducted in a grassland area surrounding the Zackenberg research station in Northeast Greenland (74°28'N, 24°34'W, 25 m a.s.l.). At this wet High Arctic tundra site, the growing season lasts approximately two months; the mean annual air temperature is -10.4 °C with annual precipitation of 215 mm (means for 1961 – 90, Danish Meteorological Institute). The site has a well developed Podzol soil and is in the zone of continuous permafrost with an active layer between 20 and 80 cm below ground level (Meltofte and Thing 1997).

Experimental design: At the end of the 1998 growing season, six tundra plots (40 × 50 cm) were selected with similar plant species composition, measured with a 500-point pinframe

(Marchand et al. 2004a). The plots were dominated by *Salix arctica* Pall. and *Arctagrostis latifolia* Griseb., with *Carex bigelowii* Torr. ex Schwein, *Polygonum viviparum* L., and *Dryas* spp. as subdominants. From 2 July to 26 August 1999, three of these plots were continuously warmed by a regulated flux of infrared radiation (0.8 – 3 μm), in order to obtain a 2.5 °C increase in surface temperature of the vegetation. To this end, individual irradiation units were placed on the north side of each plot. This Free Air Temperature Increase (FATI) method was designed to homogeneously warm limited areas of short vegetation in realistic field conditions, i.e. in the absence of enclosure (for more details see Nijs et al. 1996, 2000). The other three plots served as controls and had 'dummy' FATI-units without lamps to ensure they received similar solar radiation. Over the period of warming, the surface temperature of the vegetation was monitored by non-contact semiconductor sensors ('infracouple', type OS39-MVC-6, Omega engineering, Stamford, CT, USA). Soil temperatures at the 2.5 and 7.5 cm depths were measured with NTC-thermistors (EC95, Thermometrics, New Jersey, US). Data loggers (16 kb, 12-bit, eight-channel; DL2E, DeltaT, Cambrige UK) recorded the aforementioned parameters once every 30 min from 2 July 1999 1200 h until 29 August 1730 h Local Day Time (LDT). Soil volumetric water content was measured with time domain reflectometry (TRIME-FM, IMKO Micromodultechnik, Ettligen, Germany) over the upper 11-cm soil horizon (one reading per quadrant per plot) ten times during the season. Simultaneously, thawing depth was sampled in the centre of each plot with a fibreglass rod of 5 mm diameter.

Soil sampling: The centre of the plots was used to study plant reactions, which are reported in Marchand et al. (2004a, 2004b). For the current experiment on soil biota, soil samples were collected close to the borders of the plots, twice before the warming started [on 17 June (day of the year, DOY 168) and 2 July (the day when the warming was turned on, DOY 183)] and three times during the warming [on 22 July (DOY 203), 11 August (DOY 223) and 27 August (DOY 239)]. Two cylindrical soil cores (diameter 20 mm) were collected per sampling date per plot with a cork drill at 0–60 mm depth under the vegetation. Each sample was divided into two parts, the upper 0–30 mm and the deeper 30–60 mm. The two replicates per plot for each depth were pooled and analysed as one sample. Samples were stored in plastic vials, and fixed with 3% neutralized formaldehyde.

Slide preparation and counting: In the laboratory, the soil samples were thoroughly and carefully mixed in distilled water with 10 tablets of *Lycopodium* spores (Batch 483216, Lund University, Sweden) as an exotic marker which allows quantitative rhizopod analysis (Stockmarr 1971). The samples were washed through a sieve (300 μm mesh) to remove coarse detritus and subsequently concentrated by low speed centrifugation (5 min at 2000 rpm). Rose Bengal was added to distinguish empty tests from organisms which were alive at the moment of fixation. Testate amoebae were identified using an Olympus BX-50 microscope at × 400 magnification. Counting was continued until a minimum of 200 tests per sample were identified. Encysted testate amoebae were counted as living organisms. Identification was mainly based on Decloître (1962, 1981), Deflandre (1928, 1929, 1936), Grospietsch (1964, 1965), Mazei and Tsyganov (2006), Ogden (1983), Ogden and Hedley (1980).

Numerical analyses: Four community indices were used to describe the changes in the testate amoebae assemblages during the experiment. These were organism density, species richness, the Shannon-Wiener diversity index (Shannon and Weaver, 1949), and Pielou's evenness (Pielou 1966). Successional state was estimated using the percentage of individuals belonging to small-sized (≤60 μm) species in the assemblages. The species were assigned to the size class according to the mean value of the largest dimension based on morphometrical studies (Bobrov and Mazei 2004; Foissner and Korganova 2000; Lüftenegger et al. 1988; Lüftenegger and Foissner 1991) or, if data were not available, based on the size mentioned in their original descriptions (Appendix 1). All indices were calculated both for the living testate amoebae assemblage and for the empty shell assemblage (thanatocoenosis).

Univariate analysis of variance (ANOVA) was used to detect the difference between community indices in control and experimental plots, both before the warming when the equipment was set up after snow melt (DOY 168 and 183) and during the warming (DOY 203, 223, 239). All variables were examined for normality and heterogeneity of variance and log transformed if necessary. Differences between community characteristics in upper and deeper soil layers were assessed using the Mann-Whitney two-sample rank-sum test, because the data did not meet the assumptions of the parametric tests even after transformation. Environmental data, gathered on the same plots during the experiment, were analyzed with repeated measures ANOVA with date of measurement as within-subject factor and treatment as between-subject factor. Soil depth was included in the model as within-subject factor in order to estimate the temperature increase in the upper and deeper soil layers. Greenhouse-Geisser adjusted P-values were used to control for Type I error when the sphericity assumption was violated (Von Ende 2001). Statistical tests were considered significant at $P < 0.05$.

Partial redundancy analysis (partial RDA; Ter Braak 1988; van den Wollenberg 1977) based on the covariance matrix was applied in order to estimate the warming effect on the species composition of the testate amoebae assemblages. The choice of the linear model was based on an examination of gradient lengths following Detrended Correspondence Analysis (Hill and Gauch 1980). As the data constitute repeated observations that include the baseline (before treatment) measurements, the interaction between treatment and time (W × DOY 203, W × DOY 223, W × DOY 239) is of greatest interest and corresponds to the effect of the experimental manipulation (Lepš and Šmilauer 2003). In order to partial out spatial and seasonal variation in the testate amoebae assemblages, plot identifiers and sampling time were included in the analysis as covariates. Before conducting the RDA analysis, the data set was screened to remove unrepresentative taxa (with relative abundance below 2% in at least one sample). Species data were log $(x + 1)$ transformed in order to downweight dominant taxa. Statistical significance of the relationships between the explanatory variables and species data were assessed using a permutation test restricted by type of treatment (number of permutations 999).

All calculations were performed using the statistical program R version 2.9.1 (R Development Core Team 2009). In particular, partial RDA was performed with the package Vegan (Oksanen et al. 2009).

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Appendix 1. List of observed taxa, size groups, and abbreviations used in the figures

Species name	Abbreviation	Size (μm)
Arcella arenaria Greef	Arcare	>60
Arcella arenaria v. *sphagnicola* Deflandre	Arcars	>60
Arcella hemisphaerica Perty	Archem	≤60
Assulina muscorum Greef	Assmus	≤60
Centropyxis aerophila Deflandre	Centaer	>60
Centropyxis aerophila v. *sphagnicola* Deflandre	Centaes	>60
Centropyxias laevigata Penard	Cenlae	>60
Centropyxis platystoma (Penard) Deflandre	Cenpla	>60
Centropyxis sylvatica (Deflandre) Bonnet & Thomas	Censyl	>60
Corython dubium Taránek	Cordub	≤60
Cryptodifflugia compressa Penard	Crycom	≤60
Cyclopyxis kahli Deflandre	Cyckah	>60
Cyphoderia sp1	Cyphsp1	≤60
Difflugia bryophila (Penard) Jung	Difbry	>60
Difflugia globulus (Ehrenberg) Cash & Hopkinson	Difglo	≤60
Difflugia lucida Penard	Difluc	>60
Difflugia penardi Hopkinson	Difpen	>60
Difflugia pristis Penard	Difpri	≤60
Difflugiella oviformis (Penard) Bonnet & Thomas	Dilovi	≤60
Difflugiella oviformis v. *fuscum* (Penard) Bonnet & Thomas	Dilovf	≤60
Difflugiella pusila Playfair	Dilpus	≤60
Difflugiella sp2	Dilsp1	≤60
Euglypha ciliata (Ehrenberg) Leidy	Eugcil	>60
Euglypha ciliata v. *glabra* Wailes	Eugcig	>60
Euglypha compressa Carter	Eugcom	>60
Euglypha compressa v. *glabra* Wailes	Eugcog	>60
Euglypha laevis (Ehrenberg) Perty	Euglae	≤60
Euglypha rotunda Wailes	Eugrot	≤60
Euglypha rotunda v. *dorsalis* Wailes	Eugrod	≤60
Euglypha strigosa (Ehrenberg) Leidy	Eugstr	>60
Euglypha strigosa v. *glabra* Wailes	Eugstg	>60
Euglypha tuberculata Dujardin	Eugtub	>60
Euglypha tuberculata v. *minor* Taránek	Eugtum	≤60
Heleopera petricola Leidy	Helpet	>60
Hyalosphenia papilio Leidy	Hyapap	>60
Hyalosphenia sp1	Hyasp1	>60
Hyalosphenia sp2	Hyasp2	>60
Nebela collaris (Ehrenberg) Leidy	Nebcol	>60
Nebela lageniformis Penard	Neblag	>60
Nebela marginata Penard	Nebmar	>60
Nebela wailesi Deflandre	Nebwai	>60
Plagiopyxis declivis Thomas	Pladec	>60
Trinema complanatum Penard	Tricom	≤60
Trinema complanatum v. *inflata* Decloître	Tricoi	≤60
Trinema enchelys (Ehrenberg) Leidy	Trienc	≤60
Trinema lineare Penard	Trilin	≤60
Trinema lineare v. *truncatum* Chardez	Trilit	≤60

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