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# Response of Testate Amoebae (Protozoa) to N and P Fertilization in an Arctic Wet Sedge Tundra

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## Abstract

There are few data on the effect of long-term manipulations on soil protozoa, and almost nothing is known about soil protozoa in Alaska. I studied the response of testate amoebae to nitrogen and phosphorus addition in an Arctic fen, at Toolik Lake Long-Term Experimental Research (LTER) Station, Alaska. Testate amoebae were extracted from *Sphagnum* mosses in control and fertilized plots. Of the 35 testate amoebae taxa recorded, 7 are first observations for the Arctic (excluding Russia) and 14 for Alaska. The total density and biomass of testate amoebae were significantly reduced, by 77% and 84%, in the fertilized plots. The structure of testate amoebae communities was also modified in those plots, although for most taxa the changes were not significant. Four taxa (*Amphitrema flavum*, *Assulina muscorum*, *Placocista spinosa* ssp. *hyalina*, and *Hyalosphenia papilio*) accounted for over half of the population in the control plots but only for 11% in the N and P plots. The densities of *A. muscorum* and *Diffflugia oviformis* were significantly lower in the N and P-treated plots. The relative abundance and contribution to biomass of *Centropyxis aerophila*, *Phryganella acropodia*, and *Tracheleuglypha dentata* increased in the fertilized plots, while that of *D. oviformis* decreased. These effects suggest that testate amoebae respond to nutrient manipulations in the Arctic.

## Introduction

The use of microorganisms, and testate amoebae in particular, as bioindicators is often promoted because they have short generation times, high densities, and diversity, and they are likely to respond to changes more rapidly than other components of the ecosystem. The response of soil protozoa communities to environmental gradients has been established in a broad range of ecosystems, from mineral and organic soils to aquatic systems. These relationships have also been used to infer past environmental conditions based on testate amoebae tests (shells) preserved in lake and peat sediments (Charman, 2001).

Soil protozoa, and protists in general, have also been used in a number of studies to assess the biotic effect of a range of environmental perturbation on different ecosystems. Heavy metals (Kandeler et al., 1992) and air pollution (Balik, 1991) had negative effects on testate amoebae abundance and diversity. Several species of soil algae were able to grow in highly acidic soil polluted by heavy metals, but diatoms were absent from the most polluted sites (Shubert et al., 2001). The addition of organically enriched magnesite fertilizers reduced the abundance but not the diversity of soil ciliates (Aesch and Foissner, 1993). The abundance or biomass of testate amoebae was increased by solar ultraviolet-B radiation (Searles et al., 1999), elevated CO<sub>2</sub> (Lussenhop et al., 1998), the addition of nutrients to mineral soils as pig slurry (Griffiths et al., 1998), L-lysine (Hodge et al., 1999), and nitrogen (Maraun et al., 2001). However, elevated atmospheric CO<sub>2</sub> also had contrasting effects on soil protozoa in mineral soils. The density of flagellates tended to increase, whereas the density of amoebae significantly declined (Treonis and Lussenhop, 1997). In a *Sphagnum* peatland, N addition increased the relative importance of cyanobacteria, euglenophyceae, diatoms, and ciliates and decreased the relative importance of heterotrophic bacteria, other microalgae, and testate amoebae (Gilbert et al., 1998b). In the same site, the supply of nutrients (PKCa and NPKCa) resulted in increases of the relative biomasses of heterotrophic bacteria, diatoms, and ciliates and a decrease in the relative proportion of testate

amoeba and other micro-algae (Gilbert et al., 1998a). Soil acidification due to atmospheric pollution did not affect protozoan biomass (Couteaux et al., 1998).

Taken together, these studies suggest that protozoa, or protists in general, have potential as biomonitors in most cases. But this approach is currently limited by (1) taxonomic uncertainties (Griffiths et al., 2001), (2) an understanding of the importance of phenotypic plasticity in response to environmental conditions (Wanner, 1999), (3) the debate over global versus local distribution of protozoa (Wilkinson, 1994; Finlay et al., 2001; Wilkinson, 2001), and (4) the scarcity of experimental studies that include protists. Most of the existing studies using protists as bioindicators focused either on existing gradients of environmental perturbations such as long-term pollution in highly industrialized regions, or on short-term experiments, often under artificial conditions. Long-term manipulative experiments in natural ecosystems are comparatively rare in ecology, and even more so in the Arctic. Furthermore, the soil biota is usually less intensively studied than the aboveground component of ecosystems.

There are almost no data on the effect of long-term controlled manipulations on soil protozoa, and almost nothing is known about testate amoebae in Alaska. The objectives of this study were therefore (1) to assess how an experimental manipulation of N and P might affect the abundance and diversity of testate amoebae in the Arctic, and (2) to initiate a list of testate amoebae from Alaska. In view of the marked effect of N and P on the vegetation and carbon dynamics (Shaver et al., 1998) a clear response of testate amoebae was hypothesized.

## Material and Methods

### STUDY SITE AND FERTILIZATION EXPERIMENT

Toolik field station, the Arctic Long Term Experimental Research (LTER) site, is located in the northern foothills of the Brooks Range, Alaska (68°38'N, 149°43'W, elevation 760 m a.s.l.). This area has continuous permafrost and no trees. The dominant vegetation type is

tussock tundra vegetation of sedges and grasses mixed with dwarf birch (*Betula nana*, *B. glandulosa*) and low willows (*Salix* spp.). The climate at the site is typical of arctic regions, with a mean annual air temperature of about  $-10^{\circ}\text{C}$  and low precipitation (45% of the 20–40 cm of precipitation falls as snow). During the summer the daily average air temperature is  $7\text{--}12^{\circ}\text{C}$  with the sun continuously above the horizon from mid-May to late July. The snow-free season lasts from late May to mid-September, with below-freezing temperatures possible at any time.

Several long-term fertilization experiments are being carried out at the Toolik LTER site. This study focuses on a nitrogen and phosphorus enrichment experiment that was initiated in 1989 in two wet sedge tundra sites (wet, nutrient poor, fen community) 2 km apart. One site was located near the main inlet to Toolik Lake, and the other was located near the outlet of the lake. The sites were almost flat, with up to 5 cm of standing water and occasional moss hummocks. The vegetation at the experimental sites was dominated by sedges, *Eriophorum angustifolium*, *Carex rotundata*, and *C. cordorrhiza*, with scattered hummocks of bryophytes, including *Sphagnum*. Nitrogen was applied every year as granular ammonium nitrate at a rate of  $10\text{ gN m}^{-2}\text{ yr}^{-1}$ . Phosphorus was applied the first year as granular superphosphate at a rate of  $10\text{ gP m}^{-2}\text{ yr}^{-1}$  and in subsequent years at a rate of  $5\text{ gP m}^{-2}\text{ yr}^{-1}$  (Shaver et al., 1998).

#### SAMPLING AND ANALYSIS

Five to 10 *Sphagnum* samples (top 5 cm) were taken on 28 July 2000 in 3 control and 3 experimental plots. For each treatment 2 plots were located at the outlet site and 1 at the inlet site. In one of the fertilized plots of the outlet site duplicate samples were taken, and the average of these 2 samples was used in the data analyses for that plot. The moss samples were first dried at  $65^{\circ}\text{C}$  and then weighed. Testate amoebae were extracted from the mosses using a sieving and back-sieving method that retained all particles with a size between 10  $\mu\text{m}$  and 300  $\mu\text{m}$  (Hendon and Charman, 1997). Exotic *Lycopodium* spores tablets were added to estimate the density of testate amoebae (Stockmarr, 1971). Wet mounts were analyzed under the microscope at 200 $\times$  and 400 $\times$  magnifications. Testate amoebae were identified and counted with a goal of reaching 150 individuals. One rotifer species, *Habrotrichia angusticollis*, which is frequently found in *Sphagnum*, was also observed and counted. The results for this species are presented, but it was not included in the numerical analyses of testate amoebae communities. Biovolumes of testate amoebae and the rotifer *Habrotrichia angusticollis* were estimated by assuming geometrical shapes using the following formulae where L is the length (in most cases the longest dimension from the aperture to the tip of the shell), w is the width (the longest dimension perpendicular to L), h is the height (perpendicular to both L and w), r is the radius, and d is the diameter in the cases where w would be equal to h:

For ovoid shells (flattened bottle-shaped; most species):

$$v = 2/3 * L * w * h \quad (1)$$

For hemispheric shells, e.g., *Phryganella acropodia*:

$$v = 0.5 * 4/3 * \text{Pi} * r^3 \quad (2)$$

For saucer-shaped shells, e.g., *Arcella*:  $v = 1/2 * \text{Pi} * r^2 * h$  (3)

For cylindrical-ovoid (bottle-shaped) shells, e.g., *D. bacillifera*:

$$v = 4/5 * L * d^2 \quad (4)$$

Biovolumes were converted to carbon using the following conversion factors: testate amoebae,  $1\mu\text{m}^3 = 1.1 \times 10^{-7}\text{ }\mu\text{gC}$  (Weisse et al., 1990); rotifers  $1\mu\text{m}^3 = 1.25 \times 10^{-7}\text{ }\mu\text{gC}$  (Gilbert et al., 1998a). The effect of the treatment on testate amoebae densities, diversity, biomass carbon, relative abundance (% of total testate amoebae count), and relative contribution to biomass (% of total testate amoebae esti-

mated biomass) were assessed using t-tests. The data was transformed to homogenize variances using the logarithm [ $x' = \ln(x + 1)$ ], or the square root when needed. If neither of these transformations homogenized the variance, the *P*-values for nonhomogeneous variances of the t-test on untransformed data were used.

## Results

#### ABUNDANCE, DIVERSITY, AND BIOMASS

A total of 35 testate amoebae taxa were recorded in the 7 samples. The species richness of individual samples varied between 13 and 19 species (Table 1). The density of testate amoebae species varied between 0 and 34,654 individuals per g dry weight of *Sphagnum*, and the estimated biomass of species varied between 0 and 399  $\mu\text{gC g}^{-1}$ . Total testate amoebae densities varied among the samples from 5723 to 103,403 individuals  $\text{g}^{-1}$ . Total estimated biomass varied from 45 to 1345  $\mu\text{gC g}^{-1}$ . The density of the rotifer *Habrotrichia angusticollis* varied between 0 and 2728 individuals  $\text{g}^{-1}$ , and its biomass varied between 0 and 103.7  $\mu\text{gC g}^{-1}$ .

#### DIVERSITY, DENSITY, AND COMMUNITY STRUCTURE IN THE CONTROL AND FERTILIZED PLOTS

After 12 yr of N and P addition, the total density of testate amoebae was 77% lower in the fertilized plots (74,530 individuals  $\text{g}^{-1}$  in the control plots and 16,838 individuals  $\text{g}^{-1}$  in the N and P plots;  $P = 0.052$ ). The biomass carbon was 84% lower in the fertilized plots (988  $\mu\text{gC g}^{-1}$  in the control plots and 159  $\mu\text{gC g}^{-1}$  in the N and P plots;  $P = 0.036$ ). However, no significant effect was observed for the diversity, as measured with the Shannon's entropy test (data not presented), or the species richness (Table 1).

The density and biomass C of almost all testate amoebae taxa differed between the control and fertilized plots, but in most cases these trends were not significant owing to high variability (Table 1). Although the percentage treatment effect was very important for many taxa, some of which were absent from either the control or the fertilized plots, it was statistically significant only for 2 species, *Assulina muscorum* and *Diffugia oviformis*. The density of both species was lower in the N and P-fertilized plots ( $-85\%$ ,  $P = 0.024$ , and  $-99\%$ ,  $P = 0.008$ ).

The structure of testate amoebae communities, measured as the species' relative frequencies and relative contribution to the total testate amoebae biomass C, was also different between the control and fertilized plots, but as for density and biomass, in most cases these trends were not significant owing to high variability (Table 2). In the control plots, 4 taxa (*Amphitrema flavum*, *Assulina muscorum*, *Placocista spinosa hyalina*, and *Hyalosphenia papilio*) accounted for over half (51%) of the population. These same 4 taxa accounted for only 11.1% of the population in the N and P-fertilized plots. Two species (*Centropyxis aerophila* and *Phryganella acropodia*) accounted for nearly half (47%) of the population in the fertilized plots but only 18% of the population in the control plots. However, the percentage treatment effect on relative frequencies was different than the effect on densities. As the total density was significantly reduced in the fertilized plots, the relative frequency of several species that did not show any response in terms of density was clearly affected, and in some cases this effect was significant. The relative proportion of *Arcella discoides*, *Centropyxis aerophila*, *Phryganella acropodia*, and *Tracheleuglypha dentata* more than doubled, and their relative contribution to the total testate amoebae biomass C increased by 6- to 14-fold. This effect was significant or marginally significant for the last 3 species. Among the many taxa that decreased in relative frequency and relative contribution to the total testate amoebae biomass C in the fertilized

TABLE 1

Density and biomass carbon of testate amoebae species in Sphagnum samples from control and N- and P-fertilized wet sedge tundra plots in Toolik LTER, Alaska

Species	First observations for:		Density [ind/gd.w.]						Biomass Carbon ( $\mu\text{C/gd.w.}$ )					
			Control		N & P		% effect	P-value <sup>a</sup>	Control		N & P		% effect	P-value <sup>a</sup>
	Alaska	the Arctic #	Mean	SE	Mean	SE			Mean	SE	Mean	SE		
<i>Amphitrema flavum</i>	1	1	13608	10673	77	32	-99	n.s.	22	18	0.13	0.05	-99	n.s.
<i>Amphitrema wrightianum</i>	1	1	0	0	12	12	100	n.s.	0.00	0.00	0.05	0.05	100	n.s.
<i>Arcella discoides</i>			865	740	862	433	-0.4	n.s.	9.1	7.8	9.0	4.5	-0.4	n.s.
<i>Assulina muscorum</i>			9337	1634	1402	806	-85	0.024	16	2.8	2.4	1.4	-85	0.024
<i>Assulina seminulum</i>			1118	1118	0	0	-100	n.s.	8.6	8.6	0.00	0.00	-100	n.s.
<i>Centropyxis aculeata</i>			86	86	0	0	-100	n.s.	0.88	0.88	0.00	0.00	-100	n.s.
<i>Centropyxis aerophila</i>			5857	3251	4839	1847	-17	n.s.	56	31	46	18	-17	n.s.
<i>Centropyxis aerophila</i> <i>v. sphagnicola</i>	1		0	0	155	104	100	n.s.	0.00	0.00	1.1	0.8	100	n.s.
<i>Centropyxis platystoma</i>			520	520	56	56	-89	n.s.	3.0	3.0	0.32	0.32	-89	n.s.
<i>Corythion dubium</i>			171	171	235	235	37	n.s.	0.50	0.50	0.69	0.69	37	n.s.
<i>Cyclopyxis arcelloides</i>			0	0	327	327	100	n.s.	0.00	0.00	3.6	3.6	100	n.s.
<i>Cyclopyxis kahli</i>	1		130	130	19	19	-86	n.s.	1.7	1.7	0.25	0.25	-86	n.s.
<i>Diffflugia bacillifera</i>			0	0	70	70	100	n.s.	0.00	0.00	4.2	4.2	100	n.s.
<i>Diffflugia globulosa</i>	1		0	0	19	19	100	n.s.	0.00	0.00	0.8	0.8	100	n.s.
<i>Diffflugia oviformis</i>	1	1	919	184	12	12	-99	0.008	5.8	1.2	0.07	0.07	-99	0.008
<i>Euglypha ciliata</i>	1		1914	1014	683	305	-64	n.s.	8.8	4.7	3.2	1.4	-64	n.s.
<i>Euglypha rotunda</i>			0	0	117	84	100	n.s.	0.00	0.00	0.2	0.1	100	n.s.
<i>Heleopera petricola</i>			7653	3859	30	16	-100	n.s.	248	125	1.0	0.5	-100	n.s.
<i>Heleopera rosea</i>	1		5851	4099	1244	1090	-79	n.s.	152	107	32	28	-79	n.s.
<i>Hyalosphenia elegans</i>			3111	2918	0	0	-100	n.s.	14.8	13.9	0.00	0.00	-100	n.s.
<i>Hyalosphenia papilio</i>			9111	7268	115	58	-99	n.s.	125	100	1.6	0.8	-99	n.s.
<i>Nebela collaris</i>			877	341	254	227	-71	n.s.	21.5	8.4	6.2	5.6	-71	n.s.
<i>Nebela dentistoma</i>			5888	5633	117	117	-98	n.s.	121	116	2.4	2.4	-98	n.s.
<i>Nebela griseola</i>	1	1	86	86	0	0	-100	n.s.	0.67	0.67	0.00	0.00	-100	n.s.
<i>Nebela militaris</i>			86	86	59	59	-31	n.s.	0.3	0.3	0.20	0.20	-31	n.s.
<i>Nebela tincta</i>			3893	1776	1598	1514	-59	n.s.	41	19	17	16	-59	n.s.
<i>Nebela tincta v. major</i>	1	1	0	0	117	84	100	n.s.	0.00	0.00	3.4	2.4	100	n.s.
<i>Nebela tubulosa</i>			909	909	94	94	-90	n.s.	125	125	13	13	-90	n.s.
<i>Nebela wailesii</i>			0	0	47	47	100	n.s.	0.00	0.00	0.4	0.4	100	n.s.
<i>Phryganella acropodia</i>			1031	680	3043	1806	195	n.s.	2.0	1.3	6.0	3.6	195	n.s.
<i>Phryganella paradoxa</i>	1		0	0	23	23	100	n.s.	0.00	0.00	0.02	0.02	100	n.s.
<i>Quadrullella symmetrica</i>	1	1	130	130	19	19	-86	n.s.	0.9	0.9	0.13	0.13	-86	n.s.
<i>Tracheleuglypha dentata</i>	1		691	459	916	125	32	n.s.	2.0	1.3	2.7	0.4	32	n.s.
<i>Trinema enchelys</i>			689	507	279	125	-59	n.s.	0.44	0.32	0.18	0.08	-59	n.s.
<i>Trinema lineare</i>			257	257	141	141	-45	n.s.	0.64	0.64	0.35	0.35	-45	n.s.
Total for testate amoebae			74530	16215	16838	8040	-77	0.052	988	199	159	92	-84	0.036
Total number of first observations #	13	6												
Testate amoebae species richness			16.3	1.8	16.0	1.5	-2.0	n.s.						
Habrotrochoa angusticollis MURRAY*			1096	832	59	59	-95	n.s.	42	32	2.2	2.2	-95	n.s.

<sup>a</sup> t-tests of raw data or data transformed using  $x' = \ln(x + 1)$  or the square root homogenize variances; n.s.:  $P > 0.1$ . Man-Whitney tests  $P$ -values were equal to 0.0495 for all tests where the t-test  $P$ -value was  $< 0.1$ .

\* Rotifera; Bdelloidea.

# "Arctic" is here only the regions covered in Beyens and Chardez 1995, which exclude the Russian arctic.

plots, the treatment effect was significant only for one, *Diffflugia oviformis* (-97%,  $P = 0.015$ ).

## Discussion

In this study I aimed at assessing the long-term (12 yr) effects of N and P addition on the testate amoebae of an arctic fen. To my knowledge this is only the second published data set on testate amoebae from the Alaskan Arctic (Beyens and Chardez, 1995) and the first report of the response of testate amoebae to experimental environmental manipulation in the Arctic.

A total of 35 taxa were recorded in the control and fertilized wet sedge tundra plots of Toolik Lake LTER Station. To my knowledge, 7 of these had previously never been recorded in the Arctic (between 27°E and 168°W), and 7 more (14 in total) had not been recorded in Alaska before this date (Beyens and Chardez, 1995; Van Kerckvoorde et al., 2000) (Table 1). These first observations for the arctic are: *Amphitrema flavum*, *Amphitrema wrightianum*, *Diffflugia oviformis*, *Nebela griseola*, *Nebela tincta* var. *major*, *Placocista spinosa* ssp. *hyalina*, and *Quadrullella symmetrica*. In addition, the following 7 taxa are first observations for Alaska: *Centropyxis aerophila* var. *sphagnicola*, *Cyclopyxis kahli*, *Diffflugia globulosa*, *Euglypha ciliata*,

TABLE 2

Relative abundance and relative contribution to the total testate amoebae biomass C of testate amoebae species in Sphagnum samples from control and N- and P-fertilized wet sedge tundra plots in Toolik LTER, Alaska

Species	Relative abundance (% of total testate amoebae count)						Relative contribution to biomass (% of total testate amoebae biomass C)					
	Control		N & P		% effect	P-value <sup>a</sup>	Control		N & P		% effect	P-value <sup>a</sup>
	Mean	SE	Mean	SE			Mean	SE	Mean	SE		
<i>Amphitrema flavum</i> ARCHER	15.5	9.8	0.7	0.3	-95	n.s.	2.5	1.8	0.2	0.1	-94	n.s.
<i>Amphitrema wrightianum</i> ARCHER	0.0	0.0	0.0	0.0	100	n.s.	0.0	0.0	0.0	0.0	100	n.s.
<i>Arcella discoides</i> EHRENBERG	1.3	1.0	5.5	3.5	340	n.s.	0.7	0.6	7.4	5.3	893	n.s.
<i>Assulina muscorum</i> GREEFF	13.1	2.1	8.8	3.0	-33	n.s.	1.7	0.0	1.8	0.7	8	n.s.
<i>Assulina seminulum</i> PENARD	1.1	1.1	0.0	0.0	-100	n.s.	0.9	0.9	0.0	0.0	-100	n.s.
<i>Centropyxis aculeata</i> STEIN	0.2	0.2	0.0	0.0	-100	n.s.	0.1	0.1	0.0	0.0	-100	n.s.
<i>Centropyxis aerophila</i> DEFLANDRE	9.0	4.5	32.3	4.2	260	0.019	5.3	2.2	37.5	7.4	601	0.037
<i>Centropyxis aerophila v. sphagnicola</i> DEFLANDRE	0.0	0.0	1.0	0.6	100	n.s.	0.0	0.0	4.0	4.0	100	n.s.
<i>Centropyxis platystoma</i> DEFLANDRE	0.7	0.7	1.0	1.0	38	n.s.	0.0	0.0	0.9	0.5	100	n.s.
<i>Corythion dubium</i> TARANEK	0.4	0.4	0.7	0.7	100	n.s.	0.2	0.2	0.7	0.7	224	n.s.
<i>Cyclopyxis arcelloides</i> PENARD	0.0	0.0	2.7	2.7	100	n.s.	0.1	0.1	0.2	0.2	164	n.s.
<i>Cyclopyxis kahli</i> DEFLANDRE	0.2	0.2	0.3	0.3	83	n.s.	0.1	0.1	0.6	0.6	332	n.s.
<i>Diffugia bacillifera</i> PENARD	0.0	0.0	0.2	0.2	100	n.s.	0.0	0.0	1.2	1.2	100	n.s.
<i>Diffugia globulosa</i> DUJARDIN	0.0	0.0	0.3	0.3	100	n.s.	0.0	0.0	1.8	1.8	100	n.s.
<i>Diffugia oviformis</i> CASH	1.4	0.5	0.0	0.0	-97	0.043	0.6	0.2	0.0	0.0	-97	0.015
<i>Euglypha ciliata</i> EHRENBERG	2.9	1.4	5.3	2.5	85	n.s.	0.8	0.3	3.2	1.7	279	n.s.
<i>Euglypha rotunda</i> WAILES	0.0	0.0	0.8	0.7	100	n.s.	0.0	0.0	0.2	0.2	100	n.s.
<i>Heleopera petricola</i> LEIDY	12.1	7.6	0.4	0.3	-97	n.s.	32.2	17.6	1.5	1.3	-95	n.s.
<i>Heleopera rosea</i> PENARD	8.0	5.6	4.9	2.9	-39	n.s.	12.7	7.2	13.3	6.6	4	n.s.
<i>Hyalosphenia elegans</i> LEIDY	3.1	2.8	0.0	0.0	-100	n.s.	1.5	1.5	0.0	0.0	-100	n.s.
<i>Hyalosphenia papilio</i> LEIDY	10.3	6.6	1.2	0.9	-89	n.s.	13.8	10.1	2.0	1.6	-86	n.s.
<i>Nebela collaris</i> LEIDY	1.3	0.5	1.1	0.6	-16	n.s.	2.1	0.4	2.7	1.5	32	n.s.
<i>Nebela dentistoma</i> PENARD	8.2	7.7	0.4	0.4	-96	n.s.	9.3	8.5	0.7	0.7	-92	n.s.
<i>Nebela griseola</i> (PENARD) JUNG	0.2	0.2	0.0	0.0	-100	n.s.	0.1	0.1	0.0	0.0	-100	n.s.
<i>Nebela militaris</i> PENARD	0.2	0.2	0.2	0.2	0	n.s.	0.0	0.0	0.1	0.1	32	n.s.
<i>Nebela tinctoria</i> AWERINZEW	5.8	3.0	5.7	4.3	-1	n.s.	5.1	2.4	6.1	4.3	19	n.s.
<i>Nebela tinctoria v. major</i> DEFLANDRE	0.0	0.0	0.8	0.7	100	n.s.	0.0	0.0	3.2	2.9	100	n.s.
<i>Nebela tubulosa</i> PENARD	1.2	1.2	0.3	0.3	-77	n.s.	9.3	9.3	3.8	3.8	-59	n.s.
<i>Nebela wailesii</i> DEFLANDRE	0.0	0.0	0.4	0.4	100	n.s.	0.0	0.0	0.4	0.4	100	n.s.
<i>Phryganella acropodia</i> HOPKINSON	2.0	1.5	15.0	4.1	656	0.073	0.3	0.2	3.4	0.9	1180	0.015
<i>Phryganella paradoxa</i> PENARD	0.0	0.0	0.1	0.1	100	n.s.	0.0	0.0	0.0	0.0	100	n.s.
<i>Quadrullella symmetrica</i> WALLICH (SHULZ)	0.2	0.2	0.3	0.3	83	n.s.	0.1	0.1	0.3	0.3	332	n.s.
<i>Tracheleuglypha dentata</i> DEFLANDRE	1.1	0.6	7.7	2.4	616	0.057	0.2	0.1	2.8	1.0	1396	0.060
<i>Trinema enchelys</i> LEIDY	0.7	0.5	1.9	0.5	165	n.s.	0.0	0.0	0.1	0.0	238	n.s.
<i>Trinema lineare</i> PENARD	0.5	0.5	0.4	0.4	-20	n.s.	0.1	0.1	0.1	0.1	6	n.s.

<sup>a</sup> t-tests of raw data or data transformed using  $x' = \ln(x + 1)$  or the square root to homogenize variances; n.s.:  $P > 0.1$  Mann-Whitney tests  $P$ -values were equal to 0.0495 for all tests where the t-test  $P$ -value was  $< 0.1$ .

*Heleopera rosea*, *Phryganella paradoxa*, and *Tracheleuglypha dentata*. These findings indicate only that very few studies of testate amoebae have been done in Alaska and only a few more in other parts of the Arctic. In addition, some of the taxa listed here, especially *A. flavum* and *A. wrightianum*, are found almost exclusively on *Sphagnum* mosses, which have not been extensively sampled in the published arctic studies. Clearly, many more data are needed before any conclusions can be drawn regarding possible broad-scale distribution patterns of testate amoebae in Alaska or the Arctic.

The experimental addition of N and P to wet sedge tundra had profound effects on the vegetation and ecosystem functioning after 6 yr (Shaver et al., 1998). The total plant biomass increased nearly 3-fold. This effect was due mostly to the increase in vascular plants, although a significant increase in moss biomass was observed in the inlet site. The vegetation composition was also affected, with an increase of *Carex cordorrhiza*, a decrease of *Carex rotundata*, and a disappearance of *Trichophorum caespitosum*. The mass of N and P in the living plant biomass strongly increased with N and P addition. The gross ecosystem production, net ecosystem production, and ecosystem respiration all increased with N and P addition.

The testate amoebae results suggest an apparent response to the experimental addition of N and P, which was significant for 2 species and for the total testate amoebae density and biomass. The strong significant reduction in total testate amoebae density (-77%) and biomass C (-84%) may be due to a deterioration of living condition or availability of prey species for testate amoebae. Alternatively, it may partly be an artifact related to the treatment effect on the bryophytes communities. I aimed at collecting *Sphagnum* mosses only, but in 2 of the fertilized plots the samples contained either a mix of *Sphagnum* and other mosses or only non-*Sphagnum* mosses. The bulk density of *Sphagnum* mosses is much lower and the water-holding capacity is higher than that of other mosses. Thus, differences in bulk densities may at least in part explain the effect on total density and biomass.

The experimental setup did not allow for more replicate samples. Therefore, the data set was limited, and the clear trends observed for many taxa did not emerge as statistically significant. For example, density of *Phryganella acropodia* nearly tripled with N and P addition, and several species (*Assulina seminulum*, *Centropyxis aculeata*, *Hyalosphenia elegans*, and *Nebela griseola*) were absent from the fertilized plots. Other taxa showed a clear reduced density in the fertilized plots.

Furthermore, if a conservative significance threshold were applied, such as a Bonferroni correction (0.05 per number of taxa), none of the taxa's responses would be significant. This remark does not apply to the tests on the oval abundance and biomass, as these can be considered as single tests. Conversely, several taxa were present only in the fertilized plots. With small data sets such as this one, the likelihood of a type 2 statistical error is high. Therefore, the statistical significance of the results presented here (or lack thereof) should not be over-emphasized.

From a bioindication standpoint, the response of several species is in agreement with their known ecology. Two examples may be highlighted, although neither was statistically significant. (1) *Phryganella acropodia* is not characteristic for *Sphagnum* but is also found in forest litter and bryophytes other than *Sphagnum*. The higher density of this species in the fertilized plots suggests that conditions in *Sphagnum* fens are not optimal for this species and that the perturbation caused by the fertilization made the environment more favorable for this species. (2) By contrast, *A. flavum* is often cited as a good indicator species for bog pools (wet depressions in ombrotrophic *Sphagnum* peatlands). The dramatic decrease of this species in the fertilized plots could be due to physical or chemical changes in the microhabitat colonized by amoebae, or by changes in food sources. Given the sensitivity of testate amoebae to microenvironmental conditions at a very fine scale (Mitchell et al., 2000a) and the existence of vertical trophic gradients within the surface of *Sphagnum* peatlands (Mitchell et al., 2000b), a *Sphagnum* hummock in a relatively nutrient-rich peatland (the site was a minerotrophic fen as opposed to an ombrotrophic bog) may represent an environment similar to a bog pool.

At this site the vegetation also changed as a result of the treatment, with a clear reduction in *Sphagnum* cover. Therefore, an indirect effect of N and P fertilization on testate amoebae through shifts in bryophyte community cannot be excluded and is indeed most likely given the sensitivity of these organisms to microenvironmental gradients. In this study the effect of N and P on the ecosystem was clear before looking at testate amoebae, and their hypothesized response was therefore highly likely. But the identity of the species and the magnitude of their response were unknown. The clear effect of N and P on total testate amoebae density and biomass, the significant effect on 2 species for density and C biomass and 4 species for relative frequency and relative contribution to total testate amoebae biomass, and the clear but non-significant response of most other species suggest that testate amoebae are strongly responsive to nutrient manipulations in the Arctic.

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