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The Effects of Release from Cold Stress on the Community Composition of Terrestrial Gymnamoebae: A Laboratory-based Ecological Study Simulating Transition from Winter to Spring

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Summary. Changes in community composition of terrestrial gymnamoebae were analyzed in laboratory microcosms established with thoroughly mixed soil from a northeastern U. S. A. site taken during the onset of winter 2002-2003, when the soil temperature was 5°C. The temperature of one of the microcosms, maintained in constant temperature chambers, was increased from 5°C to 25°C at 5 intervals of two weeks each for a period of 10 weeks. Another microcosm was maintained for the 10 weeks at 5°C to serve as a control for the lapse of time and to assess the effects of prolonged treatment at 5°C simulating winter conditions. Small subsamples (0.01 g) of the microcosm soil were taken using a modified 1 cm^3 syringe with the tip cut off to make a cylindrical corer. Based on a culture enrichment technique, the morphospecies richness, heterogeneity of distribution within the samples (morphospecies uniqueness) and community patchiness were determined by microscopic observation of the morphospecies that grew out in the cultures of each 0.01 g sample. The data showed that with increasing temperatures up to 20°C, the richness and patchiness of morphospecies increased while the uniqueness did not change within statistical significance. Two-weeks treatment at 25°C simulating summer conditions, produced a marked decline in richness of morphospecies and a substantial increase in the percent that were encysted suggesting less favorable conditions for growth, perhaps resulting from thermal stress and depletion of nutrients and other resources. Overall, the evidence from this study indicated that release from cold stress during the transition from winter to spring produced increased richness of morphospecies and greater patchiness of the gymnamoeba communities. This "bloom," however, was followed by a period of less productivity and retrenchment with increasing numbers of encysted gymnamoebae as the temperature was increased above 20°C.

Key words: amoebae, biotic complexity, community structure, microcosm, seasonal temperature effects.

INTRODUCTION

Research on the ecology of terrestrial gymnamoebae is relatively limited compared to other protozoa such as testate amoebae, ciliates and flagellates (e.g., Heal 1964; Bamforth 1971; Lousier and Parkinson 1984; Foissner 1987, 1991, 1994; Darbyshire *et al.* 1989). More research appears to have been done on the role of gymnamoebae in the ecology of agricultural soils (e.g., Clarholm 1989, Griffiths 1994, Zwart *et al.* 1994, Anderson and Griffin 2001) and as bioindicators and regulators of soil pathogens (Old 1986, Foissner 1994). Recently, there have been increasing investigations of fundamental aspects of terrestrial gymnamoeba abundance, spatial distribution, and ecology (e.g., Sawyer 1989; Anderson and Bohlen 1998; Bischoff and Anderson 1998; Anderson 2000, 2002; Bass and Bischoff 2001; Bischoff

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2002). However, little is known about major interactions of gymnamoebae with physical characteristics of the environment, especially terrestrial environments. Terrestrial gymnamoebae as is also the case for other protists exhibit a wide range of adaptive morphologies and sizes (e.g., Page 1988, Rogerson and Patterson 2000, Corliss 2002) including forms that are broad in outline and fan-shaped with sizes in the micron range, a variety of forms with peripheral extended pseudopodia in a size range of microns to hundreds of microns, limax (worm-shaped) species with sizes up to hundreds of microns, and reticulate plasmodial forms of very large sizes in the millimeter size range. These markedly different life forms and sizes suggest that terrestrial gymnamoeba species have highly varied niches and ecological roles that may vary across small spatial dimensions in the millimeter range. Nonetheless, only a few studies have focused on the distribution of terrestrial gymnamoebae in micro-size dimensions of soil particles (e.g., Anderson 2002, 2003). Furthermore, there is clear evidence that seasonal and climatic factors influence the abundance and diversity of protozoa, although much of the work has been done in aquatic environments (e.g., Baldock and Sleigh 1988, Schmidt-Halewicz 1994, Mathes and Arndt 1995) including studies focused on gymnamoebae (Anderson and Rogerson 1995). Annual variations in abundances of terrestrial testate amoebae have been studied rather carefully (e.g., Lousier and Parkinson 1984), but there has been less emphasis on time series studies of seasonal effects on terrestrial gymnamoebae (e.g., Anderson 2002). Moreover, there have been limited analyses of how differences in temperature associated with seasonal changes affect the composition of terrestrial gymnamoeba communities.

This study examined how the composition of terrestrial gymnamoeba communities, grown in laboratory microcosms, varied during release from cold stress simulating a transition from winter to spring. The change in community composition was analyzed using a biotic complexity model previously applied to a variety of terrestrial environments (Anderson 2003). This model is used to analyze changes in gymnamoeba abundances within small (0.01 g) soil samples in relation to three parameters: (1) the morphospecies richness (number of morphospecies in each small soil sample), (2) the heterogeneous spatial distribution (uniqueness) of individual morphospecies among the small soil samples, and (3) patchiness in the distribution of morphospecies across the soil samples. The purpose of this study was to demonstrate the application of the biocomplexity model to ecological studies of amoeboid protists and to examine a hypothesis about the relationship of the variables in the biocomplexity model to the effects of temperature changes during release from cold-stress simulating winter conditions. The study was not intended to describe the ecology of an entire natural site, but rather to use microcosms to test a theory-based hypothesis

Based on prior findings that gymnamoeba communities are distributed in a patchy pattern in microbiocoenoces (Anderson 2000) and theory related to community biocomplexity (Anderson 2002), the following hypothesis was tested.

With increasingly favorable temperatures for growth, the richness of gymnamoebae morphospecies will increase leading to a proliferation of gymnamoebae in the most favorable niches, while less favorable niches are less populated, thus giving rise to increased patchiness of morphospecies across small spatial domains. Concomitantly, there will be a decline in the number of uniquely occurring individual morphospecies within the small-scale spatial domains. The latter is predicted due to the proliferation and migration of morphospecies throughout larger regions of the soil medium as the temperature became more favorable, thus reducing the uneven distribution of a particular morphospecies throughout the soil environment.

MATERIALS AND METHODS

Sample site and preparation of laboratory microcosms

Soil samples were obtained from a location covered by leaf litter beneath a sparse stand of deciduous trees on the Lamont-Doherty Earth Observatory campus in January, 2003 when soil temperature (*ca* 5°C) was representative of early winter conditions in coastal northeastern U. S. A. Three core samples were taken approximately 10 cm apart from beneath the leaf litter using a LaMotte model EP soil corer to a depth of approximately 5 cm. The soil samples were combined yielding approximately a 500 g mass that was thoroughly mixed to ensure greater initial homogeneity. Prior experience with microcosm studies of this kind (e.g., Anderson 2002) has shown that soil communities are very heterogeneous even within small dimensions of centimeters. Thus, to establish uniform initial conditions within the microcosms, it is important to thoroughly mix the soil samples before placing them in the Pyrex microcosm dishes. A portion of the soil (*ca* 200 g) was placed in each of two Pyrex culture dishes (9 cm diameter and 5 cm deep) that had been sterilized with 70 % (w/v) aqueous ethanol solution. Each of the soil-containing culture dishes served as a microcosm for the laboratory research. The dishes were covered with a sheet of parafilm ™ and a Pyrex glass cover to control for loss of moisture. One dish served as a control and

was placed at 5°C for the duration of the experiment. Since this dish contained the same thoroughly mixed soil sample as the temperature-treated dish, but was not subjected to increasing temperatures during the course of the experiment, the enumeration of gymnamoebae from this dish served as a baseline measure of the density of gymnamoebae resulting from continuous treatment at 5°C during the duration of the experiment. The other dish, designated the temperature-treatment dish, was placed initially at 5°C, but every subsequent two weeks it was transferred to warmer temperatures (10°C, 15°C, 20°C, and 25°C) simulating cold release during transition from winter into spring and early summer. The duration of the experiment was 10 weeks. At each two-week interval, beginning with the 10°C temperature treatment, soil samples were taken for analysis as described below. The time-control dish kept at 5°C was sampled at the end of the experiment (10 weeks) when the final sample was taken. This served as the 5°C sample and simulated the effects of extended treatment of winter cold stress.

Analysis of soil samples

At each two-week sampling point, small cores of soil were extracted from the microcosms using a 1 ml syringe that had been modified by cutting off the tip to produce an open cylindrical corer (Anderson 2002). The plastic syringe cylinder was inserted into the soil while the plunger was withdrawn to create a slight vacuum during penetration into the soil, thus enhancing uptake of the soil into the corer. Four replicate cores were taken at each biweekly sample to assess the total number of viable morphospecies using a culture-enrichment technique (Anderson 2002) as follows. To obtain small *ca* 0.01 g subsamples of the soil core, each of the cores was gently extruded by use of the syringe plunger. As each increment was extruded, a small disc of the soil from the protruding tip (*ca* 2 mm length) was cut from the core using an ethanol-sterilized, thin razor blade. The disc was immediately cut into 4 pieces (each piece was *ca* 0.01 g as described previously in Anderson 2002). Each piece was placed in a culture well of a 24-well plastic sterile culture dish (Falcon 35-3047 multi-well tissue culture dish) where each well contained 2 ml of 0.45 µm pore-size Millipore-filtered pond water. A small cube of agar (*ca* 2 mm^3) containing malt and yeast extract (Anderson 2002) was added to each well to promote growth of bacteria used as food by the gymnamoebae. After two weeks of culture at 25°C to permit outgrowth of viable morphospecies in each well containing the soil core sample, the culture dish was examined with a Nikon Diaphot inverted compound microscope using phase contrast optics and a phase contrast 40x objective. The purpose of maintaining the enrichment cultures at a uniform temperature of 25°C is to ensure a standard procedure for enumerating the categories of gymnamoebae that grow out from each of the 0.01 g samples. The number of different morphospecies that grow out is tallied, not the total number of gymnamoebae found in the well, hence the use of a constant temperature ensures a consistent measure of the total suite of morphospecies that grow out from each 0.01 g sample, but it does not bias the affect of the temperature treatment on the soil cultures in the microcosms. That is, this analysis (e.g., Anderson 2002) can only detect the number of morphospecies already present in the small soil sample and is intended to provide an optimum temperature and enrichment culture condition to reliably detect as many as possible of the existing morphospecies within each subsample of soil. Each well was thoroughly scanned and each morphospecies identified. Since it is difficult to uniformly identify all of the observed gymnamoebae to species level based on taxonomic characteristics, morphospecies categories were used. Morphospecies are identified by noting the morphology, size and locomotion using methods previously published (e.g., Anderson and Rogerson 1995; Anderson 2000, 2002). A list of morphospecies occurring in each well was tabulated. The total number of individuals tallied during the 10 week experiment was *ca* 6,600 as observed in the 480 wells all-totaled for the 20 dishes (four replicate dishes per treatment) used for the assays in the five temperature treatments. Each kind of morphospecies occurring in each of the wells of the culture dish was tabulated in order to compute the indices as explained below. The list of morphospecies observed in each well of the culture dish represented morphospecies that were present in the small 0.01 g sample of soil and grew out from it. Based on this tabulation, the following indices are computed following the procedure of Anderson (2002). Mean values were computed for the four replicated samples taken for each temperature treatment.

Indices of gymnamoeba community composition and complexity

Morphospecies richness (I_R). The number of individual morphospecies enumerated per well was summed across all 24 wells and divided by the number of wells in the dish and multiplied by 100 to better scale the index. This index of richness (I_R) represents the mean number of morphospecies identified per small sample of soil from the core.

(1) $I_R = F_C / N \times 100$ where F_C is the total count of morphospecies and N is the number of wells.

Morphospecies spatial uniqueness (I_D). When the morphospecies identified in each well are fully tabulated, the data are examined to determine if there are any morphospecies that occurred in only one of the 24 wells in the culture dish. The number of such uniquely occurring species (F_U) is divided by the total number of morphospecies tabulated in the wells (F_C). This index of spatial uniqueness represents a form of spatial diversity across the soil samples and is symbolized as I_D.

(2) $I_D = F_U / F_C \times 100$ where F_U is the number of morphospecies that occurred in only one of the 24 wells in the culture dish.

Patchiness of morphospecies (I_P). The patchiness of the morphospecies identified from the small soil samples was assessed using an index applied to metazoan populations (e.g. Taylor 1984). The variance (V_F) in the morphospecies counts across the 24 wells is divided by the mean number of morphospecies per well (M_F).

(3) $I_P = (V_F / M_F) \times 100$ where V_F is the variance in the counts of morphospecies among the 24 wells and M_F is the mean count per well.

These three indices were used to characterize the community composition of the gymnamoebae in the soil samples. The three indices are tabulated for each treatment. Additionally, the values of the three indices are plotted as a three-dimensional graph where each index serves as one of the three axes of the Euclidean plot. Thus, the data for each sample is reduced to a plotted point in the field of the three-dimensional graph. Mean values of the four replicate samples for each of the three axes were used to plot the coordinates on the graph. The Euclidean distance of each point from the origin is used as an overall index of complexity (C) of the organization of the gymnamoeba communities. A standard Shannon-Wiener index of diversity (H) also was computed using the formula: $H = - \Sigma\, p_i \log_2 p_i$.

Where, p_i = the proportion of times a given morphospecies occurred compared to the total counts.

Estimation of encysted stages

In addition to the assay of the total number of morphospecies in a soil sample, the proportion of encysted forms also was determined. A replicate sample of the soil in each temperature treatment was taken with the syringe core sampler. However, each small segment of soil was added initially to a dry culture dish well without water. The soil particles were rapidly air dried for 15 min under gentle flowing air at ambient temperature before adding the 2 ml of micropore-filtered pond water to each well. The drying step has been shown to kill trophic stages but preserve the viability of encysted terrestrial gymnamoebae (Anderson 2000). The count of morphospecies that grew out from the dried samples after two weeks of culture divided by the counts obtained with the non-dried samples, expressed as a percent, provides evidence of the number of encysted morphospecies in the soil sample. This technique has been used successfully previously (Anderson 2000). It yields a strong correlation between the number of encysted forms and the extent of dryness of the soil sample.

Soil chemical and physical characteristics

The organic content, percent moisture, and pH of the soil in the microcosms was determined at the end of the 10 week experiment for the temperature-treated microcosm and the time-control microcosm kept at 5°C using methods previously published (Anderson 2000, 2002). There were no major differences between the two microcosm samples (moisture content = *ca* 43 to 44 %, organic content = 20 %, and pH = 6.0) indicating that these physico-chemical variables were not altered by the temperature increases compared to the constant temperature control. Organic content was determined by combustion. An air-dried portion of the soil was weighed and combusted for at least 16 h at a temperature of 375°C. The difference in weight between the air-dried and combusted sample was used to calculate the percentage of organic matter. Percent moisture was obtained by weighing a freshly collected portion of moist soil, oven drying the sample overnight at 109°C and re-weighing the fully dried sample. The difference in weight was used to determine moisture content expressed as a percent. To measure pH, 5 g of the soil from the microcosm was suspended in 50 ml of tap water and the pH of the slurry was determined using an Accumet model 15 pH meter (Fisher Scientific Co.).

Statistical analyses

The purpose of this study was to evaluate the hypothesis stated in the introduction using the microcosms as the sampling universe. A series of 4 samples each were taken independently from the dishes using a small coring device. It has been shown previously that samples of soil taken at centimeter distances apart are highly different in community composition; therefore, each core sample was considered sufficiently independent of the other samples to apply inferential statistical analyses. A linear regression model was used with each of the four complexity variables (I_R, I_p, I_D and C, the overall coefficient of complexity) as a dependent variable and the treatment temperatures as the independent variable for the regression analysis. A StatView 512+ computer program (Abacus Concepts, Calabasas, CA) was used.

RESULTS

The results of the measurements of the three indices of gymnamoeba community composition (I_R, I_p and I_D), and the overall index (C) are presented as mean values for each of the four replicate samples in Table 1. The percent of gymnamoebae that were encysted in each treatment condition and the Shannon-Wiener index of diversity (H) also are included in Table 1. There was a statistically significant increase in species richness ($R = 0.8$, $p < 0.01$, $df = 14$), patchiness ($R = 0.6$, $p < 0.05$, $df = 14$), and the overall complexity coefficient (C) ($R = 0.9$, $p << 0.01$, $df = 14$) during the release from cold as the temperature was increased from 5°C to 20°C simulating a transition from winter into spring. The mean index for richness (I_R) increased from 255 to 397, the mean index for patchiness (I_p) increased from 41 to 61, and the complexity coefficient (C) increased from 259 to 402. Concurrently, the mean index of uniqueness (I_D) decreased from 19 to14, but this small decline was not statistically significant ($R = - 0.3$, $p = 0.2$, $df = 14$). After two additional weeks at 25°C, which was more typical of summer temperatures, the morphospecies richness declined rather markedly from 397 to 138, but the patchiness and uniqueness variables changed less markedly. The decline in richness of morphospecies at 25°C was accompanied by a major increase in encysted forms (*ca* 50%) relative to 20°C (31%). The two-week sustained treatment at 25°C appears to have produced less favorable growing conditions for the gymnamoebae. This may be due to thermal stress during the two-week incubation at 25°C, or perhaps due to depletion of nutrients and a decline in prey during the last two weeks of the entire10-week experiment.

The data are plotted as a graph (Fig. 1) following the standard procedures used in the complexity model (Anderson 2002). However, only a two-dimensional graph is presented here since the third variable (I_D), expressing the degree of morphospecies uniqueness, was not significantly related to change in temperature. The Euclidean distance from the origin to each plotted point is used as a measure of overall complexity (C) as reported in Table 1. The clear trend of an increase in richness and patchiness is apparent in the pattern of plotted points.

Table 1. Temperature treatments and gymnamoeba community composition variables[a].

Temperature (°C)	Complexity (C)	I_R	I_P	I_D	R_C/R_T (%)[b]	H[c]
5	259 ± 34	255 ± 35	41 ± 5	19 ± 4	61	3.9
10	315 ± 17	278 ± 70	52 ± 13	25 ± 8	56	4.3
15	325 ± 27	304 ± 55	56 ± 8	20 ± 4	44	4.0
20	402 ± 23	397 ± 24	61 ± 18	14 ± 7	31	4.4
25	153 ± 24	138 ± 20	58 ± 24	24 ± 9	52	3.2

[a] The ± values are standard errors of the mean for the four samples. [b] Percent of encysted forms derived from the ratio of encysted forms (R_C) to total forms (R_T) observed to grow out in culture from the air dried sample and the undried sample, respectively, as explained in the Materials and Methods section. [c] Shannon-Wiener diversity coefficient.

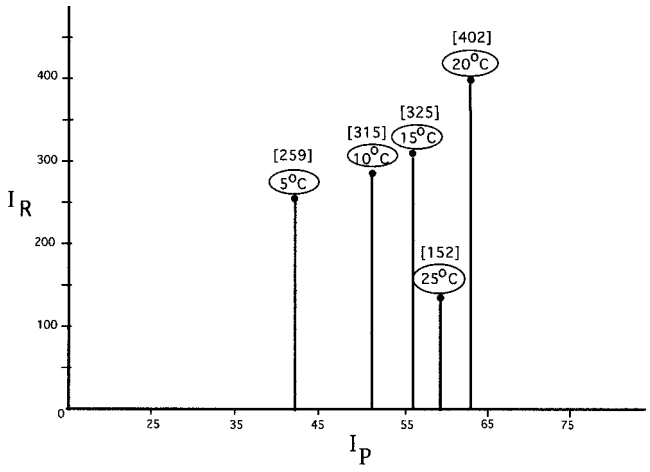

Fig. 1. Plot of the indices for richness (I_R) and patchiness (I_p), for each of the temperature treatments. The numbers in brackets are the complexity coefficient values (C). As temperature increased from 5°C to 10°C there was a statistically significant increase in richness and patchiness indicating a marked change in community composition. At 25°C, a major decrease in richness occurred, probably representing stress. There was a marked increase in encysted forms at 25°C. Data are based on a total of 6,600 observed individuals.

Overall, the combined data from Table 1 and Fig. 1 indicate that release from cold stress during the transition from winter temperatures (5°C) toward spring temperatures (20°C) is accompanied by increased richness and patchiness of the gymnamoeba community as predicted in the hypothesis. However, the predicted decline in the index for uniqueness was not statistically supported, though there is a trend in the predicted direction. The decline in percent of encysted individuals (*ca* 60% to 30%) during this sequence of temperature treatments at constant moisture indicated that growth conditions improved markedly. These results suggest that there was a gradual proliferation of gymnamoeba species during improved growing conditions resulting in greater species richness within the soil samples, but patchiness also increased due to enhanced growth of gymnamoebae within those portions of the soil aggregates that particularly support population growth. That is, with increasingly favorable temperatures, those small soil aggregates that were most suitable to sustaining gymnamoeba populations gave rise to substantial increases in population richness, while other small aggregates with less favorable growth conditions were relatively sparsely populated. Hence the net result was increased patchiness within the soil communities.

Based on light microscopic morphology, 96 different morphospecies were identified and some could be assigned with confidence to the following genera: *Acanthamoeba*, *Mayorella*/ *Korotnevella*, *Vannella*, *Platyamoeba*, *Arachnula*, *Glaeseria*, *Saccamoeba*, *Hartmannella*, *Thecamoeba*, *Leptomyxa*, and *Cochliopodium*. As is increasingly clear from analyses of gymnamoebae in various environments, there are many examples of morphospecies that are taxonomically undefined based on current species descriptions. This is especially true for the smallest forms that are less than 10 µm. Undoubtedly, many of these unusual morphospecies observed in this experiment represent new taxa, but cannot be fully described without substantially more light microscopic and fine structural analyses. During the course of this experiment, there were no marked changes in the occurrence of categories of morphospecies in the samples, all tended to remain at about the same level or increased substantially in num-

bers. That is, there is no evidence that increasing temperature favored the abrupt appearance or disappearance of any of the morphospecies identified in this study.

DISCUSSION

The ecology of soils is highly complex, due in part to the large variations in particle size in the soil, varying diameters of soil pores, and the fine-scale variations in nutrients, moisture, and gases within the pore spaces of many soils (Tate 1995). Previous research on abundance of gymnamoebae in soils of varying porosity and organic content has shown that the number and diversity of terrestrial gymnamoebae can vary substantially across very small spatial scales within soil samples at a northeastern U. S. A. site (Anderson 2002). This evidence indicates that microniches for gymnamoebae can vary across small spatial dimensions of soil on the order of millimeters and within small volumes in the range of 0.01 g. Terrestrial microbial communities in temperate climates incur large extremes in growth conditions, especially major variations in temperature during the annual seasonal cycle. Soil temperatures in winter under litter and snow cover hover near or below freezing while in summer in the same location temperatures reach 25°C or higher depending on solar radiation. In addition to extremes of temperature, terrestrial environments incur large variations in moisture content. In some cases, the surface soil may dry out completely under drought conditions while extended precipitation can produce heavy water saturation of the soil. It is widely established that most terrestrial gymnamoebae produce cysts or other desiccation-resistant stages that permit survival of terrestrial species during periods of starvation and soil water stress (Fenchel 1987, Cowling 1994).

While considerable evidence has been gathered on the desiccation tolerance of gymnamoebae, there is less research on the ecological significance of temperature stress. The research reported here specifically addresses the question of how the composition of gymnamoeba communities is affected by release from cold stress in a laboratory simulation of the changes that occur during temperature transitions from winter to spring and into summer. A controlled experiment using microcosm cultures was used to ensure that only temperature and associated temperature effects were the key variables. The soil sample was thoroughly mixed prior to the experiment to establish a uniform baseline for the experimental work. A laboratory study was done, since sampling from the natural environment introduces so many uncontrolled variables that it is not feasible to isolate temperature effects from co-occurring confounding variables. Moreover, plant cover and associated variations in rhizosphere, soil texture and organic content in the natural environment varies appreciably even within a small quadrat making it almost impossible to ensure that small core samples taken over a period of several months during a seasonal cycle come from portions of the soil medium that are sufficiently similar to control for non-temperature-related variables. Also, major changes in soil moisture in the natural environment may mask any effects of temperature. The composition of the gymnamoebae in the microcosm soil samples was determined by a culture enrichment technique (Anderson 2002). Laboratory culture enrichment techniques for enumerating protists have limitations. Only those morphospecies that grow out in laboratory culture can be analyzed by these techniques and there is always the possibility that some taxa that are not amenable to laboratory cultivation are missed. However, as noted in the Results section, 96 different morphospecies were detected by use of the culture enrichment technique including forms with unique characteristics that appear to be previously undescribed taxa. Moreover, all-totaled over 6,000 observations were tallied during the course of this experiment suggesting that this technique which has been used in various research settings provides a reasonable estimate of the major occurring gymnamoebae (e.g., Anderson and Rogerson 1995; Darbyshire *et al.* 1996; Bischoff and Anderson 1998; Anderson 2000, 2002; Anderson *et al.* 2001; Bass and Bischoff 2001).

The results of this study provide some insights into changes in communities of soil gymnamoebae when moisture is held constant and as cold stress is released over a range of temperatures simulating natural environmental changes from winter to spring. Morphospecies richness (the number of different kinds of gymnamoebae occurring in each 0.01 g of soil sample) increased markedly after the temperature reaches 10°C probably due to excystment and proliferation of species that are dormant at lower temperatures. This is consistent with prior research (Anderson 1996, Fig. 6) indicating that numerical abundances of gymnamoebae in aquatic environments also increase substantially after the temperature reaches 10°C.

In the study reported here, the statistically significant increase in morphospecies richness combined with an

increase in patchiness among the 0.01 g samples of soil support the predictions made in the hypothesis presented in the Introduction. Further research is needed to determine if there is a significant change in the uniqueness of morphospecies since no significant trend was found with the limited set of samples used in this study. This hypothesis is based on a theory that soil communities are compartmentalized into small biocoenoses existing in microscale soil aggregates as small as *ca* 0.01 g (Anderson 2002). These small domains vary substantially in the number and kind of microbiota that they can support. Optical examination of such small soil aggregates indicates that they are heterogeneous in composition and vary in mineral particle content, particle size and amount of bulk organic matter. Thus, some of these microdomains can potentially support larger populations of microbiota due to favorable nutrient composition, available surface area for attachment, and enhanced water-holding capacity compared to other microdomains. The number of encysted morphospecies available to support population growth undoubtedly varies across these small domains providing variations in potential for species richness among the small-scale niches available in the soil. When cold temperature stress decreases, giving rise to more favorable growth conditions, gymnamoebae excyst and proliferate. The most favorable niches support more luxuriant growth than less favorable ones, thus increasing the variance in the distribution of morphospecies richness across the microdomains leading to increased patchiness. This theory may account for the concomitant increase in gymnamoeba richness (I_R) and patchiness (I_p) as observed in this study.

It is interesting to note that the values of the Shannon-Wiener index (H), typically used to assess community diversity, increased during the increment in temperature from 5 to 10°C (Table 2), but remained fairly constant in the 10 to 20°C range and in this study at least, did not reflect major changes in the composition of the gymnamoeba communities. The biotic complexity measure used here, however, did reflect subtle changes that occur during release from cold stress above 10°C (Table 1).

Overall, this microcosm-based research indicated that during release from cold stress at constant moisture, the percent of active gymnamoebae versus encysted forms, morphospecies richness, and community patchiness increased as the temperature increased from 5°C to 20°C simulating a transition from winter to spring. However, since this work was based on only one soil sample, further research is needed to determine the generality and reproducibility of these findings.

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