Myxogastrid distribution within the leaf litter microhabitat

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The moist chamber culture technique was used to examine the distribution of myxogastrids at four different levels (or strata) of the forest floor leaf litter microhabitat. Each stratum was characterized by a distinct assemblage of species; moreover, both richness and abundance varied throughout. Many species appeared to exhibit a preference for a particular stratum, whereas others were widespread. Overall, the species richness recorded in the study was relatively high considering the total area sampled. These data suggest that for any study of the forest floor litter microhabitat, an effort should be made to examine all strata so as not to miss any of the species present.

Key words – ecology – forest floor – myxomycete – sampling strategy – slime mold – soil

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Introduction

Myxogastrids (also known as myxomycetes or plasmodial slime molds) are bacterivorous fruiting amoebae belonging to the Amoebozoa supergroup. These organisms have been documented to occur in association with decaying plant material across all major terrestrial biomes (Rollins & Stephenson 2011). Although scientists have been aware of this group since the 17th century, relatively little is known with respect to their biogeography and ecology (Stephenson et al. 2008a). The majority of studies for this group have been carried out in temperate regions of the northern hemisphere; however, more recent studies have focused on other regions such as the Neotropics (e.g., Schnittler & Stephenson 2000), the old world tropics (e.g., Tran et al. 2006), and high latitudes (e.g., Stephenson et al. 2000). Collectively, studies have suggested that the type of microhabitat (*sensu* Stephenson 1989) and the associated parameters of temperature, moisture, and pH are among the major factors determining the distribution of myxogastrids in nature. Myxogastrids are characterized by four major (and various minor) ecological groups. These are (a) lignicolous species, (b) corticolous species, (c) litter-inhabiting species, and (d) coprophilous species. The lignicolous group is the best known, whereas the litter-inhabiting group has received relatively little study and is still poorly understood. The objective of this study was to develop a better understanding of the patterns of occurrence for myxogastrids throughout the leaf litter microhabitat.

The leaf litter microhabitat represents a rather complex and heterogeneous mixture of decaying plant detritus (Stephenson 2011) that includes items such as leaves, fruits, flowers, seeds, bark fragments, and twigs that are intimately associated with (and ultimately contribute to the formation of) the soil. Given the heterogeneity of this microhabitat, it could conceivably be quite productive for myxogastrids. As such, it is surprising that so

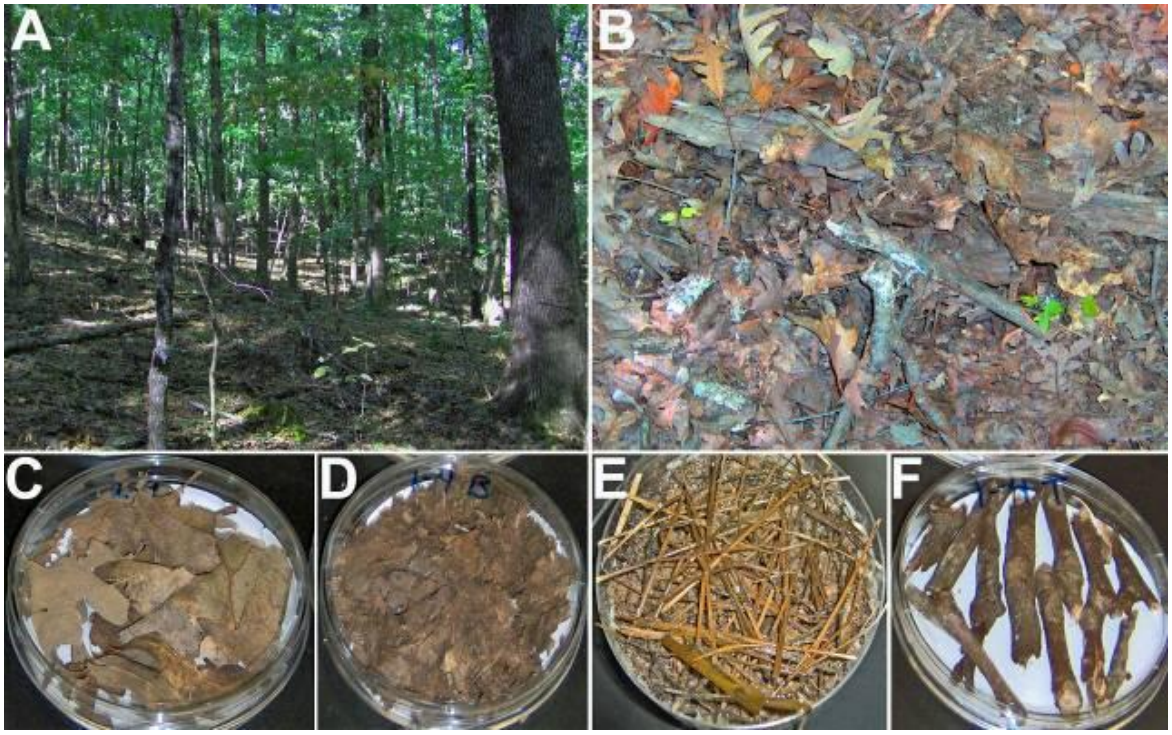

Fig. 1 The general aspect of the study area **A** dominated by species of oak and hickory **B** the general appearance of the leaf litter microhabitat, **C** moist chamber prepared with leaf litter from the O-1 stratum, **D** moist chamber prepared with leaf litter from the O-2 stratum, **E** modified moist chamber prepared with soil of the A-1 stratum overlaid with autoclaved straw, and **F** moist chamber prepared from the twig stratum.

little attention has been paid to the microhabitat. More recently, certain components of the leaf litter microhabitat, such as fruits (e.g., Novozhilov et al. 2001) and twigs (Stephenson et al. 2008b), have been given some attention. Furthermore, it is generally accepted, although very little quantitative data exist, that myxogastrids are normal and sometimes quite abundant components of the soil microbiota and likely have a substantial role in nutrient cycling and the detrital food chain (Stephenson et al. 2011). Other than a single paper by Stephenson & Landolt (1996), little information exists with respect to the distributional patterns of myxogastrids within the leaf litter microhabitat. The purpose of this paper is to contribute additional information for this understudied microhabitat.

Methods

A square 6.25 m^2 permanent fixed area plot was established during October 2005 in an oak-hickory (*Quercus-Carya*) dominated deciduous forest (Fig. 1A) in the Ozark National Forest of Arkansas, USA (N 35.77516 W -94.24221; elevation 475 m). The plot was divided into 25 0.25 m^2 subplots, and four samples, each from a different stratum of the leaf litter microhabitat (Fig. 1B), were collected and used to prepare moist chamber cultures, for a total of 100 cultures. The strata recognized and sampled essentially followed the soil horizons as defined by Brady (1974). The O-1 stratum samples (Fig. 1C) consisted of the upper layer of decaying leaves that still could be easily identified to the genus or species level. The O-2 stratum samples (Fig. 1D) consisted of leaves that were highly decomposed and could not be assigned readily to a particular taxon. A-1 stratum samples (Fig. 1E) consisted of an admixture of humus and the upper mineral soil (i.e., the soil-litter interface), while the fourth stratum consisted of twigs (Fig. 1F) interspersed throughout the above described strata.

The O-1, O-2, and twig strata were investigated using the traditional moist chamber technique as it applies to myxogastrids (Stephenson & Stempen 1994). In short, this involved placing the dried sample material into plastic Petri dishes (100 × 15 mm) lined with filter paper. The sample material

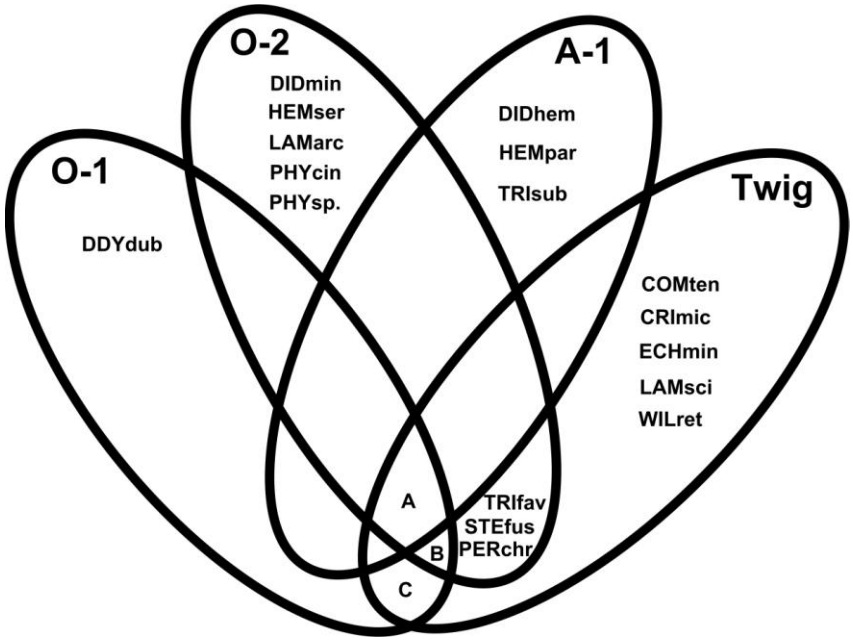

Fig. 2 The distribution of species among the different strata of the leaf litter microhabitat. Note: A = DIDeff and METves, B = ARCcin, and C = DDYmin. The species abbreviations used in this figure are the same as those listed in Table 1.

was then soaked with water for approximately 24 hours, at which time the pH was determined and the excess water decanted. The cultures were then examined for the appearance of fruiting bodies with the use of a compound microscope for a period of three months.

Samples obtained from the A-1 stratum were examined using a modified moist chamber technique. All rocks of appreciable size were removed and the soil mixture was placed into Petri dishes lined with filter paper. The soil was then saturated with water and allowed to soak for approximately 24 hours, at which time the pH was determined. Autoclaved straw was then placed over the soil, providing a substrate (and moisture gradient) to encourage myxogastrids to migrate from the soil and produce fruiting bodies. Five moist chambers were prepared as controls by placing autoclaved straw inside Petri dishes lined with filter paper and keeping the contents of the dishes moist.

Collections, which consisted of one or more fruiting bodies that presumably developed from a single plasmodium (Stephenson 1989), were glued to a paper tray and placed into a small box and stored in the herbarium at the University of Arkansas (UARK). Identifications were made using the morphological species concepts as reflected in the nomenclatural treatment of Lado (2001). Species diversity indices and subsequent analyses were carried out in a manner similar to that described by Rollins & Stephenson (2010).

Results

Ninety-six collections of myxogastrids were obtained, representing 21 species distributed among 14 genera (Table 1). The greatest number of species and the highest diversity (as assessed by both the Shannon and Simpson diversity indices) were recovered from the twig stratum, whereas the O-1 and A-1 strata, with five species each, yielded the fewest numbers of species and the lowest calculated diversity values. All of the cultures prepared from the O-1 stratum were positive for the occurrence of myxogastrids, while those prepared from O-2 stratum produced the lowest percentage of positive cultures. The five control moist chambers were all negative for the occurrence of myxogastrids.

Distinct myxogastrid assemblages were associated with each stratum as evidenced by low calculated values for both percent similarity and the Sørensen coefficient of community (Table 2). Pairwise values for the A-1 and twig strata were the most dissimilar. Five species (*Comatricha tenerrima*, *Cribraria microcarpa*, *Echinostelium minutum*, *Lampro-*

Table 1 The relative abundances of the recovered species per stratum and associated summary statistics.

Species	Abbreviation	Stratum O-1	O-2	A-1	Twig	Total
Arcyria cinerea	ARCcin	2.9	10.7	0.0	15.0	
Comatricha tenerrima	COMten	0.0	0.0	0.0	5.0	
Cribraria microcarpa	CRImic	0.0	0.0	0.0	5.0	
Diderma cf. *hemisphaericum*	DIDhem	0.0	0.0	14.3	0.0	
Diderma cf. *miniatum*	DIDmin	0.0	3.6	0.0	0.0	
Diderma effusum	DIDeff	38.2	10.7	64.3	5.0	
Didymium cf. *dubium*	DDYdub	5.9	0.0	0.0	0.0	
Didymium minus	DDYmin	50.0	0.0	0.0	10.0	
Echinostelium minutum	ECHmin	0.0	0.0	0.0	5.0	
Hemitrichia pardina	HEMpar	0.0	0.0	7.1	0.0	
Hemitrichia serpula	HEMser	0.0	7.1	0.0	0.0	
Lamproderma arcyrionema	LAMarc	0.0	7.1	0.0	0.0	
Lamproderma scintillans	LAMsci	0.0	0.0	0.0	5.0	
Metatrichia vesparia	METves	2.9	25.0	7.1	5.0	
Perichaena chrysosperma	PERchr	0.0	7.1	0.0	5.0	
Physarum cinereum	PHYcin	0.0	14.3	0.0	0.0	
Physarum sp.	PHYsp	0.0	3.6	0.0	0.0	
Stemonitis fusca	STEfus	0.0	3.6	0.0	25.0	
Trichia favoginea	TRIfav	0.0	7.1	0.0	10.0	
Trichia subfusca	TRIsub	0.0	0.0	7.1	0.0	
Willkommlangea reticulata	WILret	0.0	0.0	0.0	5.0	
% Positive Cultures.		100	80	88	96	91
Number of Collections		34	28	14	20	96
Species Richness		5	11	5	12	21
Shannon Diversity		1.09	2.21	1.13	2.29	2.40
Simpson Diversity		0.62	0.90	0.59	0.92	0.89
Mean pH		5.4	6.2	5.6	5.9	5.9

derma scintillans, and *Wilkommlangea reticulata*) were recovered only from the twig stratum, and *Diderma* cf. *hemisphaericum*, *Hemitrichia pardina*, and *Trichia subfusca* were collected only from the A-1 stratum (Fig. 2). Only two species (*Diderma effusum* and *Metatrichia vesparia*) were recovered from all sampled strata.

Members of the Physarales dominated (i.e., were characterized by the greatest relative abundance) the O-1 and A-1 strata, where they accounted for 94% and 78% of the total number of collections, respectively. The O-2 horizon was dominated by the Trichiales, where members of that group accounted for 57% of the total collections. The Stemonitales and Trichiales were codominant in the twig stratum, where each accounted for 35% of the total collections.

Discussion

The values for species richness (21 species distributed among 14 genera) and diversity (Shannon diversity 2.4) obtained in the current study (100 moist chambers collected from a single 6.25 m^2 plot) appear to be quite high when compared to other studies that have examined the leaf litter microhabitat. For example, Stephenson (1989) prepared 507 moist chambers collected from five 0.1 hectare plots (each from an ecologically distinctive type of forest community) and obtained 34 species representing 16 genera, with Shannon diversity values ranging from 0.4 to 0.9. A study carried out in Puerto Rico utilized 500 moist chambers prepared with samples collected across five forest types and yielded 24 species in 12 genera (Stephenson et al. 1999). In addition, a study in Alaska (Novozhilov et al. 2007) recovered 16 species in 11 genera, with a Shannon diversity value of 2.16, from 156 moist chambers prepared with samples collected from four study sites (two burned and two control). Ko Ko et al. (2009), in what was apparently the first molecular-based study to examine the litter microhabitat, recovered only two species from small disks taken from decaying leaves collected from the

Table 2 Calculations of percent similarity (upper right) and Sørensen coefficient of community (lower left) for the fixed area plot.

	O-1	O-2	A-1	Twig
O-1		16.6	41.2	20.9
O-2	0.38		17.9	36.4
A-1	0.40	0.25		10.0
Twig	0.47	0.52	0.24	

forest floor (presumably from the O-1 stratum) in northern Thailand.

Stephenson & Landolt (1996) recorded the occurrence of myxogastrids appearing in serial dilutions of soil/humus samples plated out on hay infusion agar and reported these organisms from all three of the strata (O-1, O-2, and A-1) sampled. Although they frequently obtained vigorous plasmodia in their plates, very few were ever induced to produce fruiting bodies and therefore were never identified to species. However, they believed that many of these plasmodia represented *Didymium ovoideum* or other members of the Physarales. It is commonly thought that members of the Physarales account for the majority of the species associated with the leaf litter and soil microhabitats. This was the case for the O-1 and A-1 strata (in the current study); however, it is interesting to note that the Trichiales were the dominant group (in terms of total number of collections) recovered from the O-2 stratum. However, the dominance of the Trichiales was attributed largely to the relative abundance of just two species (*Metatrichia vesparia* and *Trichia favoginea*), and this might have been the result of an unusually favorable microsite for the species involved. As such, this pattern may not have held up if samples had been collected over a larger study area. As was the case for the study reported by Stephenson & Landolt (1996), the results of the current study indicated that myxogastrids represent organisms commonly associated with all strata of the leaf litter microhabitat, but the data reported herein suggest that the previous study likely underestimated their richness and diversity.

The current study, and that of Stephenson & Landolt (1996), both appear to indicate that notable differences exist with respect to the number of collections/plasmodia among the sampled strata. Furthermore, the current study found marked differences for the assemblages of species occurring among the strata. It is conceivable that these patterns are a result of interspecific competitive interactions; however, they may also be a reflection of the available food resource (e.g., bacteria) or microenvironmental conditions (e.g., carbon dioxide concentrations) associated with the various strata. The complete explanation is likely a combination of these and other currently unrecognized factors. These clearly represent very worthwhile questions that should be addressed by further observational studies and manipulative experimentation. Moreover, any study that examines the leaf litter microhabitat should make a concerted effort to sample the different strata in order to miss as few species as possible. Using the current study as an example, if an investigator collected leaves only from the uppermost layer of the forest floor he/she would miss approximately 76% of the recovered species.

The patterns that Stephenson & Landolt (1996) observed with respect to the strata they sampled varied between two different sampling sites (each of which represented a different type of forest community). Unfortunately, this phenomenon cannot be addressed by the current study, but it does represent an interesting possible extension of the research described herein. For example, what patterns would be observed if the current study were expanded to include several forest types within a particular area (e.g., the Great Smoky Mountains National Park) or comparisons were made among high latitude, temperate, and tropical forest communities? Another interesting comparison would be between forests of the Old World tropics (e.g., northern Thailand, where a nicely defined vertical stratification within the leaf litter microhabitat has been observed by the author) and the New World tropics (e.g., Belize, where the author has not observed a well defined vertical stratification to occur within the leaf litter layer).

Finally, it is interesting to note that several investigators have reported the (sometimes very abundant) occurrence of plasmodia in soils plated on agar media but experience very limited success in inducing these to produce fruiting bodies. The modified moist chamber technique employed for the A-1 strata (in the current study) resulted in a 56% success rate with respect to obtaining fruiting bodies from soil. It may be that placing autoclaved straw (or cornmeal or rolled oats) over the soil may create a gradient of moisture (from the soil to the substrate) that is conducive to inducing myxogastrids to exit the soil and produce fruiting bodies.

In summary, the leaf litter microhabitat represents a heterogeneous and understudied microhabitat that can be very productive (at least in temperate forests) for myxogastrids. Even when species richness is relatively low for a given stratum, the total number of collections can be appreciable. A well defined stratification of myxogastrid assemblages was evident and may represent the effects of competition and/or other microenvironmental influences. The highly decomposed A-1 stratum and twigs were found to be exceptionally diverse for myxogastrids in the current study. The relatively high diversity of the twig stratum is probably not surprising, since Stephenson et al. (2008b) reported more than 40 species associated with twigs in a series of studies carried out at a number of different localities around the world. These data clearly illustrate that an effort should be made to examine all strata of the leaf litter microhabitat in order to avoid missing some of species present.

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