Assessing the ecological impact of whole watershed acidification on the myxomycetes associated with forest floor leaf litter

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Abstract

The ecological impact of whole watershed acidification on the myxomycetes (plasmodial slime molds or myxogastrids) associated with forest floor leaf litter was investigated on the Fernow Experimental Forest in West Virginia. Since 1989, ammonium sulfate fertilizer has been applied to a small forested watershed (WS3) on the Fernow. Samples of litter collected from this watershed and another adjacent watershed (WS7), which served as a control, were used to prepare a series of 100 moist chamber cultures for each of the two watersheds. Cultures prepared with samples from WS3 yielded more positive (some evidence of plasmodia or fruiting bodies) cultures (78%) than WS7 (73%), but the percentage of cultures in which fruiting bodies appeared was higher for WS7 (63%) than for WS3 (52%). This was also reflected in the total number of records of fruiting bodies, with 66 for WS3 and 92 for WS7. Sixteen species of myxomycetes were recorded from the two watersheds, but *Arcyria cinerea* was the overwhelming dominant, appearing in almost 40% of all moist chamber cultures. Four species (*Perichaena chrysosperma*, *Physarum bivalve*, *Ph. lateritium*, and *Ph. viride*) were common in one watershed and absent or represented by only one or two records in the other watershed. Although some differences were apparent for the assemblages of myxomycetes present in the two watersheds, these were all relatively minor. However, these minor differences do at least suggest that whole watershed acidification possibly has had some minimal effect on the myxomycetes associated with forest floor leaf litter, possibly as a result of lowering the pH of the actual substrates upon which these organisms occur.

Key words – ecology – litter microhabitat – slime molds – substrate pH

Introduction

The myxomycetes (plasmodial slime molds or myxogastrids) are eukaryotic microorganisms usually present and sometimes abundant in terrestrial ecosystems, where they are associated with the microhabitats represented by various types of plant detritus. The myxomycete life cycle encompasses two very different trophic stages, one consisting of uninucleate amoebae, with or without flagella (the term "amoeboflagellate" is used to refer to both types), and the other consisting of a distinctive multinucleate structure, the plasmodium (Martin et al. 1983). Under favorable conditions, the plasmodium gives rise to one or more fruiting bodies containing spores. The fruiting bodies produced by myxomycetes are somewhat suggestive of those produced by higher fungi, although they are considerably smaller (usually no more than 1-2 mm tall). The spores of myxomycetes are thought to be

largely wind-dispersed and complete the life cycle by germinating to produce the uninucleate amoeboflagellate cells. These feed and divide by binary fission to build up large populations in the various microhabitats in which these organisms occur. Ultimately, this stage in the life cycle gives rise to the plasmodium. Bacteria apparently represent the main food resource for both trophic stages, but plasmodia are also known to feed upon yeasts, algae (including cyanobacteria), and fungal spores and hyphae (Stephenson & Stempen 1994).

The association of myxomycetes with the microhabitat represented by forest floor leaf litter is well known (Gray & Alexopoulos 1958) and has been the focus of a number of studies (e.g., Härkönen 1981, Stephenson 1989, Stephenson & Stempen 1994, Stephenson et al. 1999, Tran et al. 2008, Ko Ko et al. 2009, Rollins & Stephenson 2012). Indeed, certain species appear to be restricted largely to the litter microhabitat, since they are rarely recorded from other microhabitats. However, many of the species of myxomycetes associated with litter tend to be rather inconspicuous or sporadic in their occurrence and thus not always easy to detect in the field. Moreover, fruiting bodies of most species are relatively ephemeral and do not persist in nature for very long. The moist chamber culture technique as it applies to myxomycetes (Stephenson & Stempen 1994) provides a convenient and often very productive method of supplementing field collections, especially when studying such microhabitats as forest floor leaf litter.

In 1989, a watershed acidification experiment began on the Fernow Experimental Forest (39.03°N, 79.67°W) in north-central West Virginia (Adams et al. 2006, 2007). Ammonium sulfate fertilizer (35.5 kg N ha^{-1} yr^{-1} and 40.5 kg S ha^{-1} yr^{-1}) was applied to a forested watershed (WS3) at a rate that approximately doubled the estimated bulk deposition inputs of N and S to this part of West Virginia. Aerial application of fertilizer has taken place in spring, summer and fall of each year since 1989 (Fig. 1). At the time of the first application, the forest on WS3 had been cut about 20 years earlier and thus would have been considered early second-growth. The forest in another nearby watershed (WS7) was about the same age. Both forests are dominated by broadleaf trees, with tulip-poplar (*Liriodendron tulipifera* L.), black cherry (*Prunus serotina* Ehrh.), red maple (*Acer rubrum* L.) and black birch (*Betula lenta* L.) among the major species present. These forests are typical of those found throughout the Central Appalachians (Muzika et al. 1999).

Materials & Methods

In June of 2014, bulk samples of forest floor leaf litter were collected from a series of 20 microsites located along two transects in WS3 and a similar series of 20 microsites located along two transects in WS7 (Fig. 2). Each bulk sample was placed in a paper bag, returned to the laboratory and then used to prepare moist chamber cultures. Five moist chamber cultures were prepared from each of the original bulk samples of litter, for a total of 100 cultures from WS3 and another 100 from WS7. The moist chamber cultures were prepared in the manner described by Stephenson & Stempen (1994) and consisted of plastic disposable Petri dishes (100 mm diameter) lined with filter paper. Enough sample material was placed in each dish to cover the bottom, and then this material was moistened with distilled water. After a period of approximately 24 hours, the pH of each culture was determined with a portable pH meter, after which excess water in the Petri dish was poured off. Moist chamber cultures were placed out of direct sunlight and maintained at room temperature for a period of several months. Cultures were checked for the presence of myxomycetes (either plasmodia or fruiting bodies) at regular intervals over this entire period.

When fruiting bodies of myxomycetes appeared in a culture, a portion of the substrate upon which they occurred was removed, glued to a small paper tray, and the latter mounted in a pasteboard box for permanent storage. All occurrences of the same species in a single moist chamber culture were considered to represent one record. Identifications were made with the use of standard monographs such as Martin & Alexopoulos (1969). Nomenclature used herein essentially follows Lado (2005-2016) except for *Stemonitis nigrescens*, where the treatment used is that of Martin & Alexopoulos (1969). Voucher specimens of all species are deposited in the herbarium of the University of Arkansas (UARK).
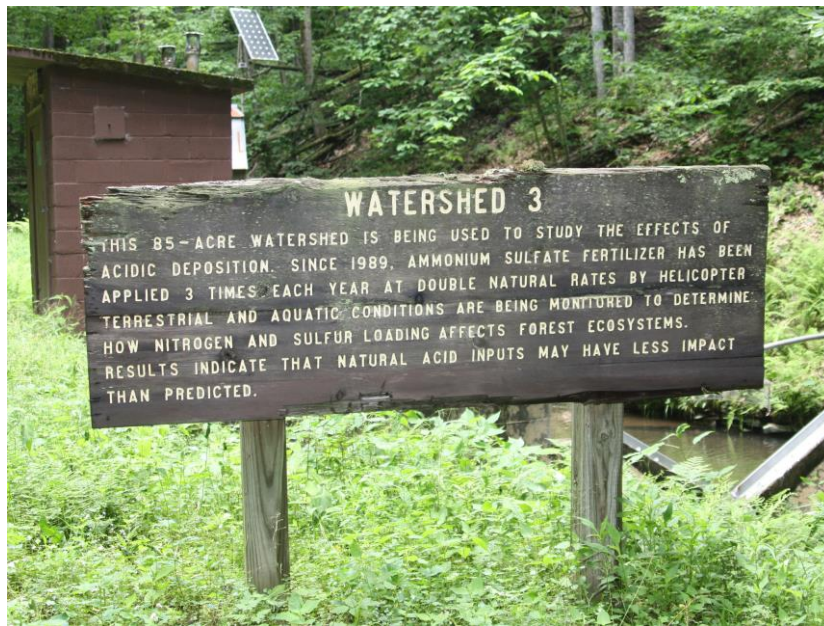
Fig. 1 – Watershed 3 on the Fernow Experimental Forest in West Virginia.

Fig. 2 – Forest floor leaf litter layer in Watershed 3.

Results

Sixteen species were recorded from the moist chamber cultures prepared with samples of forest floor leaf litter from WS3 and WS7 (Table 1). The 16 species belong to four different taxonomic orders (Liceales, Physarales, Stemonitales, and Trichiales), with the Physarales represented by the most species (8) and the Trichiales represented by the most records (99 or 63% of all records). Samples from WS3 yielded more positive (some evidence of plasmodia or fruiting bodies) cultures (78%) than WS7 (73%), but the percentage of cultures in which fruiting bodies appeared was higher for WS7 (63%) than WS3 (52%). This was also reflected in the total number of records of fruiting bodies, with 66 for WS3 and 92 for WS7. Thirteen species of myxomycetes were recorded from WS3 and 10 from WS7. However, *Arcyria cinerea* was the overwhelming dominant in both watersheds, appearing almost 40% of all moist chamber cultures (51% in WS7 and 27% in WS3). Four species (*Perichaena chrysosperma*, *Physarum bivalve*, *Ph. lateritium*, and *Ph. viride*) were common in one watershed and absent or represented by only one or two records in the other watershed.

Diversity indices (Shannon's formula [Shannon & Weaver 1963]) calculated for the assemblages of species in the two watersheds were 1.87 for WS3 and 1.43 for WS7. A coefficient of community index (Mueller-Dombois & Ellenberg 1974) used to compare two assemblages was (0.61). This index is based solely on the presence or absence of species in the particular communities (or assemblages) being considered.

Values of pH recorded for moist chamber cultures prepared with samples of litter from WS3 ranged from 4.5 to 6.6 (mean = pH 5.4), whereas the corresponding values for WS7 were 5.0 to 6.9 (mean = pH 6.1).

Discussion

Most of the myxomycetes recorded in the present study are species typically associated with litter (Martin & Alexopoulos 1969), although several examples (e.g., *Arcyria denudata* and *Hemitrichia serpula*) are generally considered to be lignicolous (i.e., associated with decomposing wood). The relative dominance of *Arcyria cinerea* was not surprising, since this species is one of most common and widespread of all myxomycetes (Stephenson & Stempen 1994). As already noted, the number of species of myxomycetes recorded from WS3 (13) was higher than the number (10) recorded

Table 1 Myxomycetes recorded from moist chamber cultures prepared with samples of forest floor leaf litter from the two watersheds. Data are numbers of records.

Taxon	WS3	WS7
Arcyria cinerea (Bull.) Pers.	27	51
Physarum lateritium (Berk. & Ravenel) Morgan	12	1
Physarum bivalve Pers.	10	0
Diderma effusum (Schwein.) Morgan	5	2
Trichia botrytis (J.F. Gmel.) Pers.	3	2
Cribraria microcarpa (Schrad.) Pers.	2	0
Didymium iridis (Ditmar) Fr.	1	1
Physarum viride (Bull.) Pers.	2	17
Stemonitis nigrescens Rex	2	2
Diderma saundersii (Berk. & Broome ex Morgan) E. Sheld.	1	0
Stemonitis cf. *flavogenita* E. Jahn	1	0
Didymium clavus (Alb. & Schwein.) Rabenh.	1	0
Didymium ovoideum Nann.-Bremek.	1	0
Perichaena chrysosperma (Curr.) Lister	0	10
Hemitrichia serpula (Scop.) Rostaf. ex Lister	0	4
Arcyria denudata (L.) Wettst.	0	2
Total	66	92

from WS7. However, the total number of fruitings was higher for WS7 (99) than WS3 (66). Perhaps more importantly, plasmodia that did not produce fruiting bodies were more common in WS3. It is well known that the pH of the substrates upon which myxomycetes develop represents an environmental factor that affects the distribution of these organisms (Stephenson & Stempen 1994). The mean pH of cultures prepared with samples of litter from WS3 (5.4) was lower than the corresponding value for samples from WS7 (6.1). Consequently, the relatively more acidic conditions for WS3 could have been a limiting factor with respect to the ultimate number of positive cultures, although the assemblage of species of myxomycetes present in WS3 was characterized by higher values of diversity and species richness.

As a general observation, the plasmodia and fruiting bodies of myxomycetes were slower to develop in moist chamber cultures prepared with samples of litter from WS3. For example, approximately 60 days after the cultures had been started, only 26% of those prepared with litter from WS3 displayed any evidence of myxomycetes, whereas plasmodia or fruiting bodies of myxomycetes were already present in 50% of the cultures prepared with litter from WS7. Adams & Angradi (1996) examined the decomposition rates and nutrient dynamics of leaf litter collected from the same two watersheds of the Fernow Experimental Forest. At the time their study was carried out, fertilizer had

been applied to WS3 for three years. They reported that freshly fallen leaves from three different species of trees collected from WS3, placed in litter bags and left in place for a period of two years decomposed more slowly than those of the same three species of trees collected from WS7. The differences were statistically significant, which suggested that the artificial acidification was having some effect. However, the rates of nutrient loss from the leaves did not show the same pattern, with no real differences apparent. The authors concluded that the acidification treatment had only minimally affected the quality of the litter on WS3. As such, it is possible that myxomycetes developed more slowly in cultures prepared with litter from WS3 because the samples of litter decomposed at a somewhat slower rate. Presumably, this would have resulted in lower numbers of the microorganisms (particularly bacteria) which represent the primary food resource for myxomycetes and thus been reflected in a somewhat restricted development of their early trophic stages. However, there is no way to confirm that this was indeed the case, and certain other as yet unknown factors may have been involved. For example, values of pH recorded for the two sets of cultures were appreciably different (5.4 for WS3 and 6.1 for WS7), and the distribution of particular species of myxomycetes is known to be influenced by the pH of the substrates upon which they occur (Stephenson & Stempen 1994).

The percentages of positive cultures (78% for WS3 and 73% for WS7) were only slightly different. Both values are comparable to those reported by Stephenson (1989) for a study of the assemblages of myxomycetes associated with forest floor leaf litter in the upland forests of southwestern Virginia. Moreover, the number of species he recorded for a particular type of forest ranged from 11 to 19, which is similar to the total (16) recorded in the present study. The coefficient of community index for the assemblages of species recorded from the two watersheds was 0.61, which is relatively high. As such, there appeared to be no major different in the taxonomic composition of the assemblage characteristic of each of the two watersheds.

In summary, although a number of differences were apparent in the assemblages of myxomycetes recorded from the two watersheds, these were all relatively minor. However, these minor differences do at least suggest that whole watershed acidification possibly has had some minimal effect on the myxomycetes associated with forest floor leaf litter, possibly as a result of lowering the pH of the actual substrates upon which these organisms occur.

Acknowledgments

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