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Myxomycete diversity and ecology in the Baotianman National Nature Reserve, a subtropical mountain forest in central China

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A B S T R A C T

Diversity of myxomycetes has been extensively studied in temperate and tropical areas. However, there is still limited information about these organisms for the subtropics. The current study investigated the community of myxomycetes, characterizing the influence of elevation, season and forest type on myxomycete diversity in a subtropical mountain forest of China. Five study sites were established along an elevational gradient that extended from 1212 to 1626 m. Collection of field specimens and substrates for moist chambers was carried out at monthly interval from April to October in 2016. A total of 71 species were identified, with *Arcyria cinerea*, *Hemitrichia minor*, *Perichaena depressa*, *Diderma effusum* and *Perichaena corticalis* recorded as abundant. The diversity-based estimators were mainly affected by the sampling month and forest type, rather than by elevation. Different ecological patterns were observed among the microhabitats. Bark-inhabiting communities were closely linked with forest type, while communities of litter-inhabiting myxomycetes changed seasonally. Canonical Correspondence Analyses showed that the bark-inhabiting species were closely related to bark features, while the litter-inhabiting species were mainly determined by climate factors.

1. Introduction

The myxomycetes (plasmodial slime molds or Myxogastria) are a group of fruiting amoebae. Their life cycle includes myxamoebae, swarm cells, multinucleate plasmodia, microcysts, sclerotia and spore-bearing fruit bodies (sporocarps) (Martin and Alexopoulos, 1969). The myxamoebae and plasmodia feed on bacteria, yeasts and other microorganisms associated with soil, plant litter and other decomposing plant debris and exert a major control on the populations of these organisms (Martin and Alexopoulos, 1969; Madelin, 1984; Feest and Stephenson, 2014). This group comprises approximately 1000 morphospecies (Lado, 2005–2017) and forms a firmly established clade in Amoebozoa (Fiore-Donno et al., 2010; Cavalier-Smith, 2013; Kang et al., 2017).

Myxomycetes are known from every major type of terrestrial ecosystem, including tropical, temperate and boreal forest, forest tundra, desert and alpine snowbank (Liu et al., 2015). Like other eukaryotic microorganisms, most of the known myxomycetes have been considered to be cosmopolitan, but some appear to be moderately endemic (Stephenson et al., 2008). Most researches on myxomycete diversity have focused on temperate and tropical forests, describing different ecological patterns of these organisms (Stephenson, 1988; Lado et al., 2003; Stephenson et al., 2004a,b; Schnittler et al., 2016). However, few have been carried out in subtropical forests (Ukkola et al., 2001; Härkönen et al., 2004; Liu et al., 2013).

Environmental factors such as temperature, moisture, elevation, vegetation and pH have a considerable influence on both the trophic and reproductive stages of the myxomycete life cycle (Stephenson, 1989; Ndiritu et al., 2009). As a result, the distribution of myxomycetes is not random and shows spatial and seasonal variability in nature. Elevation is an important factor shaping the gradients of myxomycete diversity. Although previous studies (Stephenson et al., 1999, 2004a; Schnittler and Stephenson, 2000; Rojas and Stephenson, 2008) carried out within elevational ranges have usually indicated a negative association between myxomycete diversity and elevation, Rojas et al. (2016) found no differences related to elevation when both elevational resolution and floristic differences were considered. A similar pattern was observed in a mid-mountain forest in Philippines (Dagamac et al., 2012). These results may indicate that myxomycetes can survive to some extent

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in a wide range of elevations.

There are dramatic seasonal differences in myxomycete communities in different ecosystems. Species richness and diversity in temperate forests tend to reach the highest levels in summer (Stephenson, 1988; Takahashi and Hada, 2012). In Neotropical forests of Costa Rica and southwestern Amazon basin, diversity-based estimators were predominantly higher during the dry period when field-laboratory methodology was applied to acquire myxomycete specimens (Rojas and Stephenson, 2007, 2012). However, it has also been reported that species richness was higher in the warm-wet season than in the cool-dry season in tropical forests in Thailand (Tran et al., 2006; Ko Ko et al., 2011). The paradoxical patterns may be attributed to the sampling methods used and local climate conditions.

To gain a more comprehensive understanding of the ecological patterns of myxomycetes, investigation from a wider region is needed. We chose the Baotianman National Nature Reserve in central China, a mountainous subtropical forest, as a sampling ground. The primary objectives were to: (i) characterize the community of commonly occurring species by assessing their abundances of fructifications; (ii) obtain data on the distribution of myxomycetes along elevational gradient, vegetation and seasons; and (iii) assess the relationship between myxomycetes and environmental factors such as the elevation, forest type, meteorological factor and bark feature.

2. Materials and methods

2.1. Study area

The Baotianman National Natural Reserve (33°20′-33°36′N, 111°47′-112°04′E), Henan Province, China, is the easternmost part of the Qinling Range (Fig. 1), with an average elevation of 1400 m a.s.l. The Reserve is located in a transitional zone from north subtropic to south warm temperate. The mean annual air temperature is 15.1 °C and the mean monthly air temperature ranges from 3.6 °C in January to 25.5 °C in August. The mean annual precipitation is 900 mm, of which 60% falls in July to August (Luan et al., 2011). Based on temperature and precipitation, four seasons in a year are recognized: winter (December to February), spring (March to May), summer (June to August), autumn (September to November). Vegetation in the reserve mainly consists of deciduous broadleaf forest and coniferous forest (Song, 1994).

In April of 2016, five study sites (ca. 0.3 ha) situated along a transect were set up, which represent an elevational gradient and cover the main vegetation types in the reserve (Fig. 1). Information on geographic location, elevation, and dominant vegetation is shown in Table 1. Qiulin Fall (abbr. QF; 33°29′59″N, 111°55′38″E, 1212 m a.s.l.), located on a north-facing slope in a valley, is the first study site. The vegetation type is mixed broadleaf-conifer forest, with *Pinus tabuliformis*, *Cyclobalanopsis glauca*, *Diospyros lotus* and *Quercus aliena* var. *acutiserrata* as common tree species. Understory plants include shrubs of *Lespedeza bicolor*, *Abelia engleriana* along with ferns of *Pentarhizidium intermedium*. The second study site, named Botanic Garden (abbr. BG), is located in a flat area at the foot of the mountain slope (33°29′79″N, 111°55′51″E; 1302 m a.s.l.). The vegetation is typical of coniferous forest dominated by *Pinus armandii*, *Metasequoia glyptostroboides* and *P. tabuliformis*. Associate understory plants consist of *Rubus phoenicolasius* and *Celastrus orbiculatus*. Ice Fall (abbr. IF; 33°30′48″N, 111°56′0″E; 1412 m a.s.l.) represents the evergreen broadleaf forest in the reserve. The canopy is dominated by *C. glauca* and *Quercus acrodonta*. The understory is dominated by *Campylotropis macrocarpa*. At higher elevation, vegetation appears to be uniform in its composition, dominated mainly by *Quercus variabilis*, *Q. aliena* var. *acutiserrata*. Two representative study sites of deciduous broadleaf forest type were selected: Baozi Ridge (abbr. BR; 33°30′43″N, 111°56′4″E; 1508 m a.s.l.) and Jingxin Pavilion (abbr. JP; 33°30′73″N, 111°56′14″E; 1626 m a.s.l.). The shrub layer of the two study sites is sparse and includes *L. bicolor* and *Lonicera japonica*.

2.2. Field work

All the meteorological parameters were collected from a nearby weather station 2.5 km away from the study area. Myxomycete specimens were derived from field collections (FC) and moist chambers (MC) prepared with two types of substrates (barks and ground litters). At each site, 5 plots (numbered A to E, 10 m × 10 m) were established at the corners and in the middle. Seven survey trips (including examination of fruiting specimens and substrate collection) were made to the 25 plots at monthly intervals from the end of April to the end of October in 2016. Fruiting specimens of myxomycetes in each plot were exhaustively searched for during all the field trips. Sporocarps with a piece of the substrate, were placed in small plastic box, labeled, brought back to the laboratory and dried at room temperature. For the substrate collection, three trees in each plot were randomly selected. The outer bark at breast height and the ground litter under the selected trees were sampled. A total of 1050 substrate samples were obtained (5 sites × 5 plots per site × 2 types of substrates × 3 trees per plot × 7 sampling month). The substrate samples were placed in separate plastic bags and brought back to the laboratory for moist chamber cultures.

2.3. Moist chamber cultures

Moist chamber cultures were prepared following the protocol provided by Gilbert and Martin (1933). Briefly, the substrate was placed on top of filter paper previously placed in a Petri dish. Sterile distilled water adjusted to a pH of 7 was added to the Petri dish. The pH was measured using a flat plate pH meter (Fisher Accumet Model PHS-3C, JingKe Company, Shanghai, China) within the first 2 d after the substrates were soaked in the water. The water retention of bark was measured following the method described by Snell and Keller (2003). All cultures were incubated at room temperature (23–25 °C) under diffused light for approximately one month and observed every 2 or 3 d for the presence of plasmodia or sporocarps. Mature sporocarps were harvested, air-dried and mounted in small plastic boxes.

2.4. Taxonomy

All the specimens were identified to morphospecies using standard monographs for the group (Martin and Alexopoulos, 1969; Li et al., 2008a,b). Nomenclature followed Lado (2005–2017). Specimens were deposited in the Center of Microbial Cultures at Nanjing Normal University (MCCNNU).

2.5. Data analysis

For ecological analyses, both the relative abundance and absolute abundance of myxomycete records were quantified following Novozhilov et al. (2000). The abundance dataset was subdivided into four assemblages based on method of data collecting and substrate, including MC-bark, FC-bark, MC-litter and FC-litter. Since there were only 15 records in the FC-bark assemblage, the data was counted in the myxomycete checklist of the reserve but abandoned in the statistical analyses. All the statistical analyses were conducted with the R software (http://cran.r-project.org; R Development Core Team, 2014). To estimate the completeness of the sampling effort for each assemblage, Chao1 estimators were