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FULL PAPER

Seasonal occurrence and distribution of myxomycetes on different types of leaf litter in a warm temperate forest of western Japan

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Abstract The seasonal occurrence and distribution of myxomycetes on different types of newly defoliated leaf litter were examined in a secondary forest in a warm temperate region of western Japan. The two types of leaf litter (deciduous trees, *Prunus verecunda* and *Quercus variabilis*, and evergreen trees, *Q. glauca* and *Cinnamomum camphora*) were incubated in trays on the forest floor. A total of 45 myxomycete species were recorded from 3021 collected samples that occurred at the July peak during the warmest and humidest season from April to November. The occurrence of species was significantly related to the changes in mean temperature and minimum temperature on both leaf types under humid conditions. Myxomycete assemblages were divided into three seasonal phases. Most of the species occurred in June–September, while a few species demonstrated characteristic distributions; i.e., *Didymium melanospermum* appeared in April–May and *Diderma umbilicatum* appeared in October–November. The respective leaf types supported the reproduction of myxomycetes with high species richness and diversity, with 34 species and $H' = 2.59$ on deciduous trees and similarly 30 species and $H' = 2.49$ on evergreen trees. Several species, however, exhibited a preference for either the deciduous tree or evergreen tree leaves. Thus, a mixed forest that defoliates during different two seasons yields a greater species diversity of myxomycete assemblage.

Keywords Deciduous tree leaf · Evergreen tree leaf · Foliicolous myxomycetes · Preference for leaf type · Seasonal distribution

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Introduction

Forests are the main biotopes for myxomycetes, and they provide myxomycetes with a wide variety of potential habitats. Myxomycete fruiting bodies are often found in great profusion on decaying wood or bark, fallen leaves, other herbaceous matter, or soil in forests (Gray and Alexopoulos 1968). The nutrition of myxomycetes depends on the ingestion of prokaryotes as well as eukaryotic organisms and nutriments by reproductive swarm cells; myxamoebae and plasmodia, including fungal spores, fruit bodies and mycelium (Madelin 1984). The layer of leaf litter on the forest floor is particularly beneficial for myxomycetes, where the myxamoebae and plasmodia feed and migrate to drier surfaces to sporulate (Ing 1994). Some myxomycetes are known to associate with litter (Gray and Alexopoulos 1968), and Ing (1994) defined foliicolous myxomycetes as those that appear on fallen leaves. Most systematic accounts mention litter on the forest floor (which mostly consists of dead leaves) as a habitat for myxomycetes, whereas our knowledge of their ecology in forest litter remains in its infancy. Only a few studies (Härkönen 1981; Stephenson 1989; Stephenson et al. 1999, 2008) have examined the assemblages of species found in this microhabitat. Therefore, in the present study, we really focused on the myxomycetes occurring in leaf-litter masses, which were experimentally incubated in trays on the

forest floor, with the exception of soil-feeding myxomycete plasmodia.

Härkönen (1981) reported that *Alnus* litter harbored the greatest number of myxomycete specimens, whereas *Betula* leaves appeared to be the most unfavorable substrate in a moist-chamber culture. Also, Stephenson (1989) provided comparative differences of foliicolous myxomycetes on deciduous leaf and coniferous leaf litter. These results may indicate an association of specific myxomycete species with the leaves of certain tree types. In warm temperate regions of Japan, deciduous litter fall occurs in autumn, whereas that of evergreens occurs in late spring. We should expect that both the defoliation season and tree species would affect the seasonal occurrence of myxomycetes. As the annual cycle of new leaf-litter substrate and decomposition progresses, the leaf litter ultimately provides a highly varied and nourishing habitat for myxomycetes.

Foliicolous myxomycetes have short-lived fruiting bodies, forming microscopic sporocarps of around 1 mm or less in height on leaf litters, which renders these organisms much less amenable for systematic ecological study. Although several studies on dead wood have revealed that the myxomycete distribution is associated with seasonal changes and/or wood types in forests (e.g., Takahashi and Hada 2008a; Takahashi et al. 2009), relatively little is known about the ecological features of foliicolous myxomycetes. The objective of the present study was to characterize the changes in the seasonal occurrence of foliicolous myxomycetes, as well as to identify the species associated with leaf litter types that defoliate during different seasons (i.e., deciduous trees and evergreen trees) by monitoring throughout the entire fruiting season.

Materials and methods

Study site

Coppiced woodlands in western Japan, which were historically used for timber resources, have remained unused since the 1960s. The study site was located in the coppiced woodland in the Okayama University of Science Natural Botanical Garden in Okayama, Yokoi (34°42.8′N, 133°55.0′E, 70 m above sea level). The forest mainly consists of deciduous trees [*Quercus variabilis* Blume and *Q. serrata* Murray in the crown mixed with *Prunus verecunda* (Koidz) Koehne] and evergreen trees [*Quercus glauca* Thunb. and *Ilex chinensis* Sims as well as *Cinnamomum camphora* (L.) Siebold]. The forest crown reaches over 20 m in height. A portion of the study forest also harbors a 45-year-old *Pinus densiflora* Siebold & Zucc. The bush layer under the crown is dominated by *Eurya japonica* Thunb. interspersed with young *Q. glauca*. Leaf fall occurs primarily in late spring and autumn, supplying a new, rich litter layer. The soil layer can reach over 10 cm in depth and is covered by both litter and humus layers.

The fallen leaves of two tree types—deciduous trees (*P. verecunda* and *Q. variabilis*) and evergreen trees (*Q. glauca* and *C. camphora*)—were collected from the forest floor under their respective tree species in areas where these species dominated. Deciduous tree leaves were collected in December–January, and evergreen tree leaves were collected at the end of April, during their respective defoliation seasons. All twigs over 5 mm in diameter were removed from the litter masses. The leaf-litter masses of each individual tree species were placed into trays (60 L in volume, 96 × 69 × 20 cm) at the end of January for deciduous trees and end of April for the evergreens. Three or more trays for each tree species were incubated on the forest floor along the northern slope where the land was flatter (Fig. 1a). Each tray had four small holes bored into the bottom to allow adequate humidity from rainfall for the litter masses and to drain excess water. A plastic sheet was placed under each tray to separate it from the soil or humus on the forest floor. The trays were also covered with mesh to exclude additional fallen leaves during the study period.

At a neighboring study site, annual precipitation was 1047.5 mm, and mean annual temperature was 14.4°C (2006–2009, at a Japan Meteorological Agency observation point, 34°45.4′N, 133°51.3′E, 239 m above sea level). Hourly temperature and humidity measurements on the forest floor (10 cm above forest floor) of the study site were recorded for three years from February to November using data loggers. Mean values of temperature and humidity data were averaged using two-week periods over the three years to illustrate seasonal changes in weather conditions (Fig. 2). Mean air temperature and minimum temperature of the forest floor gradually ascended from April to the beginning of August, and peaked at 26.5 and 22.6°C, respectively, before starting to decrease until November. Mean temperatures exceeded 15°C from mid-May until the end of October. The minimum temperature exceeded 8°C in mid-May and then fell to 8°C at the end of October. Humidity increased in April due to spring rains, peaked at 88% during the rainy season late in July, and became dry from August until the beginning of September. Subsequently, the humidity returned due to rains at the start of autumn. Forest floor moisture levels remained at 70% or higher from May to November. The correlations of mean temperature, minimum temperature, and humidity as climatic factors with myxomycete assemblages were examined.

Measurements of leaf litter decomposition

To measure the decomposition of the leaf litter, we used the litter bag method (Crossley and Hoglund 1962). Bags

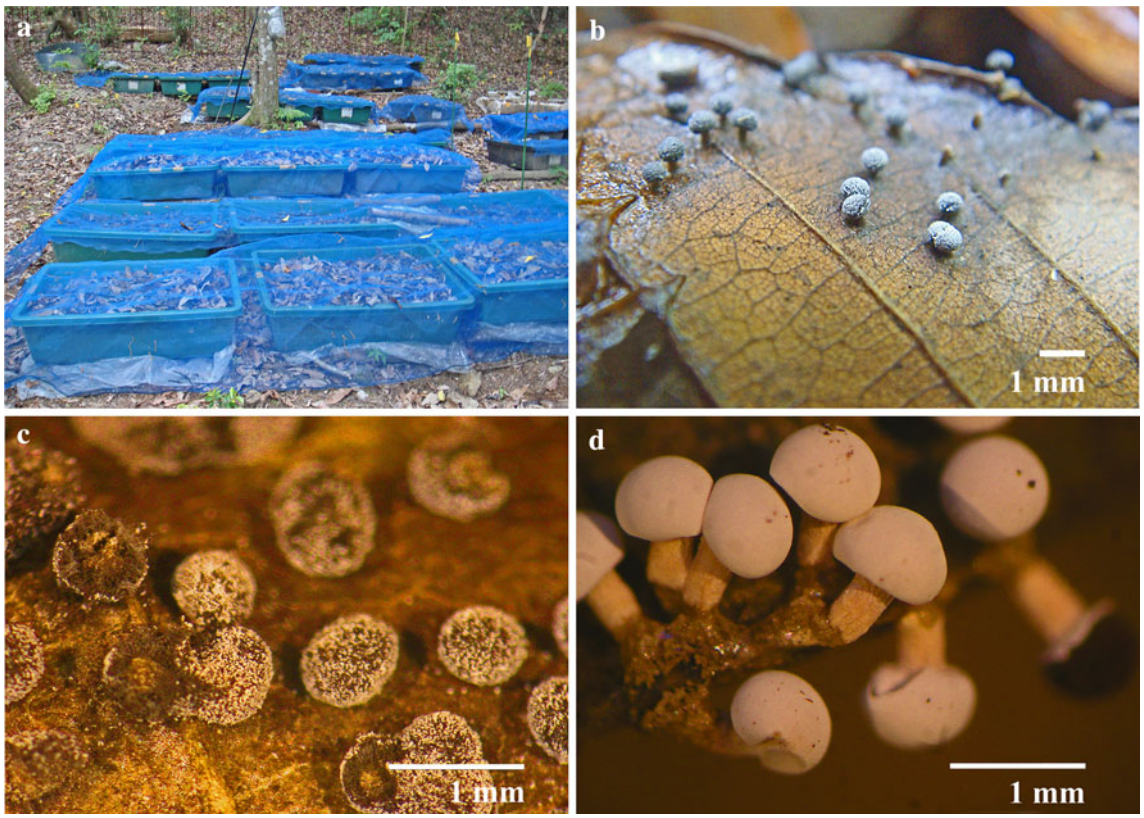

Fig. 1a–d Trays of leaf-litter masses at the forest study site and fruiting bodies of myxomycetes. **a** Nylon mesh-covered trays containing leaf litter. **b** Myxomycete fruiting bodies on a leaf. **c** Fruiting bodies of *Didymium melanospermum*. **d** Fruiting bodies of *Diderma umbilicatum*. *Bars* 1 mm

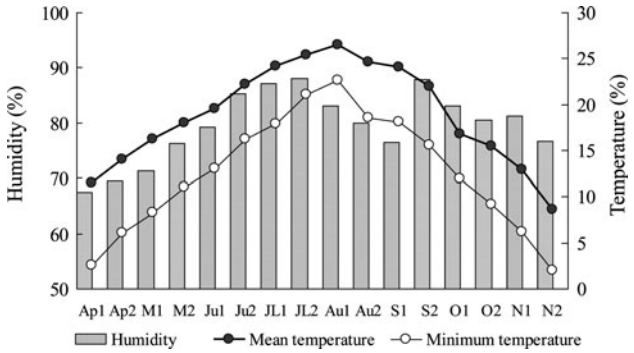

Fig. 2 Three-year (2007–2009) averages of the variations in humidity and mean temperature on the forest floor of the study site (each *bar* refers to the average for a two-week period)

(18 × 25 cm) made of 2 mm mesh nylon were filled with 10 g of dry leaves of each leaf species and simultaneously buried under the leaf-litter masses in trays. Bags were placed in every tray of deciduous litter mass at the beginning of February and in every tray of evergreen litter mass at the end of April in 2009. The remaining mass of leaf litter was measured on the 15 November 2009, and the averages for each of the four species were obtained for the five bags after drying at 60°C. The remaining masses differed among tree species (49.9% in *P. verecunda*, 50.3% in *Q. variabilis*, 53.8% in *C. camphora*, and 59.4% in *Q. glauca*). Defoliation of deciduous *P. verecunda* trees occurred in early autumn, such that decomposition was more advanced than in *Q. variabilis* or in the evergreen species.

Myxomycete occurrence

The occurrence of myxomycete fruiting bodies in the leaf litter masses was monitored with both the naked eye and loupes at approximately two-week intervals from March to January for three years. The fruiting that occurred was removed from the tray, allowed to dry, and then glued inside a small paper box suitable for long-term storage. The myxomycete occurrences were quantified based on the number of leaves and/or twigs to which three or more sporocarps adhered (considered to be one sample). If myxomycete sporocarps occurred on a leaf fragment that was ≤ 1 cm^2 in area or on twig that was ≤ 1 cm in length, the samples were excluded from analyses. One leaf contained one species of myxomycete (Fig. 1b), and there were few or no samples with mixed species on a leaf. The collected specimens were identified via microscopic

examination, and the nomenclature followed that of Yamamoto (1998, 2006), referring to Lado (2001).

Species richness and characteristic species of myxomycetes were compared between the deciduous tree leaves and the evergreen tree leaves and across surveyed months and seasons. Relative abundance of myxomycetes was calculated as the ratio of the number of samples in every two-week period to the total number of samples on the deciduous leaves or the evergreens throughout the season. Species diversity of myxomycete assemblage was calculated using the Shannon–Wiener index (H'; Shannon and Weaver 1963) and the evenness component (J'; Pielou 1966), as described in previous studies on myxomycetes (Stephenson 1989, Takahashi 2010). Species diversity (H'), defined as

$$H' = -\sum_{i=1}^{s} Pi \ln Pi,$$

where Pi is the proportion of the total number of samples represented by species i, was calculated for the different survey codes. The species evenness component (J') was calculated using the formula $J' = H'/\ln S$, where S represents the number of species present in the assemblage.

Test for seasonal distribution of myxomycete assemblages

The study season was divided into 16 time periods each lasting two weeks from April to November, as described in the survey codes in Table 2. Among the three years of study data, there were eleven myxomycete assemblages on deciduous leaves and ten assemblages on evergreen leaves throughout the fruiting season. As a whole, the assemblages on both types of tree leaves were periodically combined into thirteen assemblages (Table 2).

To compare the seasonal distributions of myxomycete assemblages, we analyzed them using nonmetric multidimensional scaling (NMDS; Kenkel and Orlóci 1986), following cluster analysis (Ward's method) depending on the scores of the first two NMDS axes. According to the blade with bootstrap proportions of ≥70% (Hillis and Bull 1993), the thirteen assemblages were classified into three groups. For the ordination method that was used to reveal the relationship among the myxomycete assemblages (e.g., Takahashi 2010), the number of samples of a particular species was computed using the computer application PAST (http://folk.uio.no/ohammer/past/).

Seasonal distribution of species

The seasonal distributions of the 27 dominant myxomycete species (more than ten samples of each were recorded) were quantified using a succession index. The succession index (SI) and the successional deviation (D) for a given species were calculated using the following formulae (Hasegawa 1997):

$$\mathrm{SI} = \left(\sum_{i=1}^{16} i \times ni/N\right), \quad D = \sqrt{\left(\sum_{i=1}^{16} ni(i - \mathrm{SI})^2\right)/N},$$

where i is the number of two-week periods from the start of the survey in April, ni is the number of samples of a particular species, and N is the total number of samples of a particular species throughout the season. Therefore, SI indicates the stage with the greatest abundance of a species, and indicates the preferred fruiting season for a given species.

Test for preference for leaf type

Data from samples of each species on the four tree species were combined into two tree types. The occurrence of myxomycete species on the two leaf types (deciduous or evergreen) was analyzed using Fisher's exact probability test of independence (e.g., Sokal and Rohlf 1973). When the frequency of species on any tree type was significantly higher ($p < 0.01$) than 0 according to Fisher's exact probability test, the species was considered to frequently occur in association with a particular tree type, with the exception of those species with <10 total observations. Analysis of variance was achieved using the Excel Statistics (version 5.0) software package (Esumi Co. Ltd., 2001).

Results

Myxomycetes on leaves

Throughout the study, 3021 samples yielded 45 myxomycete species (with varieties treated as species), indicating that the different leaf litters in this forest of western Japan harbored a rich diversity of myxomycete species, with the leaf litters providing between 19 and 26 myxomycete species (Table 1). Twenty-two myxomycete species from 82 mean samples per tray occurred on *P. verecunda* leaf masses, 24 species from 63 mean samples occurred on *Q. variabilis* leaf masses, 26 species from 31 mean samples occurred on *Q. glauca* leaf masses, and 19 species from 51 mean samples occurred on *C. camphora* leaves. Thus, the species richness of myxomycetes did not appear to differ among the four tree species. However, the mean number of samples per tray was higher among deciduous than among evergreen leaves. *P. verecunda* leaf masses harbored the highest abundance of myxomycetes. There were 34 species of myxomycetes and a species diversity of $H' = 2.59$,

Table 1 Mean number of samples per tray year^{-1} and species diversity on the leaf litters of different tree species

Leaf litter	Trays	Mean samples per tray year^{-1}	Species diversity		
			Number of species	H'	J'
Deciduous tree leaves					
Prunus verecunda	7	82	22	2.06	0.67
Quercus variabilis	27	63	24	2.25	0.71
Evergreen tree leaves					
Quercus glauca	19	31	26	2.26	0.69
Cinnamomum camphora	3	51	19	2.34	0.79
Total	56	54	45	2.76	0.72

$J' = 0.73$ on deciduous tree leaves, while evergreen tree leaves had a similar species richness of 30 species and $H' = 2.49$, $J' = 0.73$, meaning that species diversity was $H' = 2.76$, $J' = 0.72$ in total.

Twenty-seven species were dominant (these occurred frequently, with ten samples or more; Table 2). The most abundant and common species, which occurred with 200 samples (6.6% of all samples) or more, were *Physarum melleum*, followed by *Didymium iridis*, *Physarum cinereum*, and *Physarum roseum*. Species of Physarales, including the genera *Craterium*, *Diachea*, *Diderma*, *Didymium*, and *Physarum*, represented a total of 39 species, which equaled 87% of all species on the leaf litters.

The species composition of myxomycetes varied significantly between the assemblages of the deciduous trees and the evergreens (Table 2). Eighteen species appeared on both tree types, whereas several species clearly showed a preference for a particular tree type according to Fisher's exact probability test. The characteristic species on the deciduous tree leaves included *Didymium melanospermum* (Fig. 1c), *D. minus*, *D. megalosporum*, *Craterium minutum*, *C. leucocephalum* var *scyphoides*, *Physarum roseum*, and *Diachea subsessilis*, and evergreens included *Diachea lecopodia*, *Physarum cinereum*, *Diderma saundersii*, *Didymium squamrosum*, *P. oblatum*, *Craterium leucocephalum* var. *cylindricum*, *Diderma effusum*, *D. rugosum*, *D. umbilicatum* (Fig. 1d) and *D. globosum.*

Seasonal pattern of myxomycete assemblages

Spore production began in late April, when only one myxomycete species occurred on the deciduous tree leaves (Fig. 3). The fruiting season lasted from April to November, and the relative abundance of myxomycete samples peaked in early June on deciduous tree leaves and in late June on evergreen tree leaves. Myxomycetes subsequently reappeared in early September and then fruiting decreased in late October. Myxomycete occurrence exhibited different seasonal patterns for the deciduous tree leaves and the evergreens—the start and end of the period of occurrence was earlier on the deciduous tree leaves than on the evergreens. However, the species richness of deciduous tree leaves demonstrated a similar pattern to that of the evergreens, and this peaked in July for both leaf types, when 27 species were recorded in total.

Thirteen myxomycete assemblages on both leaf litters from monthly field studies (Table 2) were statistically clarified into groups using cluster analysis that calculated the first two NMDS scores of the assemblages (Fig. 4). The assemblages were divided into three groups, which indicated the three fruiting seasons: April–May (early phase), June–September (middle phase), and October–November (late phase).

Seasonal occurrence of myxomycete species

Twenty-seven species that were recorded with ten or more samples were arranged in order of increasing succession index (Fig. 5). Thus the successional position (SI) and deviation (D) were ranked in temporal order for two-week steps. The early phase primarily included two species, the middle phase twenty-three species, and the late phase two species. *Didymium melanospermum*, for which SI was 2.2, chiefly appeared on *P. verecunda* leaves in April, following *D. minus*, for which SI was 4.8. The middle phase indicated SI values ranging from $5.0 \leq \text{SI} \leq 13.0$, and exhibited associations with e.g., *Didymium iridis* (SI = 5.5) and *D. megalosporum* (SI = 5.6), *Physarum melleum* (SI = 6.2), *Craterium minutum* (SI = 6.6), *P. cinereum* (SI = 7.3) and *Diderma effusum* (SI = 9.7). Several species occurred for particularly long periods of time, e.g., *D. saundersii*, *P. cinereum*, and *D. effusum*, indicating broad deviations, $D = 2.3$, $D = 2.3$, and $D = 2.2$, respectively. *D. rugosum* (SI = 12.5) and *P. roseum* (SI = 12.9) overlapped in occurrence with the late phase. In the late phase, indicating SI values ranging from $13.0 \leq \text{SI} \leq 16.0$, *Diderma spumarioides* (SI = 13.0) occurred on

Table 2 Myxomycete species and number of samples on the leaf litters during each month, and differences between the deciduous tree leaves and the evergreen tree leaves

Myxomycete species	Spring				Summer								Autumn				Total	Leaf type	
	Ap1	Ap2	M1	M2	J1	J2	Jul1	Jul2	Au1	Au2	S1	S2	O1	O2	N1	N2		D	E
Species recorded with ten or more samples																			
Didymium melanospermum (Pers.) T. Macbr.		148	6	15	1												170	170**	
Didymium minus (Lister) Morgan				114	42	12	26		1								195	189**	6
Didymium iridis (Ditmar) Fr.					220	15	9	28	4		3						279	198	81
Didymium megalosporum Berk. & M.A. Curtis				4	115	75	18	1			4						217	215**	2
Diachea leucopoda (Bull.) Rostaf.					16	3	13	2									34		34
Physarum melleum (Berk. & Broome) Massee					265	162	214	85	9		2		3				740	539	201
Craterium minutum (Leers) Fr.					4	71	68	12									155	150**	5
Didymium nigripes (Link) Fr.					1	17	10	7	1		1						37	31	6
Craterium leucocephalum var. *scyphoides* (Cooke & Balf. f. ex Massee) G. Lister						13	94	21									128	128**	
Physarum cinereum (Batsch) Pers.				11	26	86	12	18	24		22	10	5				214	130	84
Diderma saundersii (Berk. & Broome ex Massee) Lado					31	1	9	17	5		13		1				77	47	30
Didymium squamulosum (Alb. & Schwein.) Fr.					7	2	3	16	3		1						32		32
Physarum oblatum T. Macbr							22	1	3								26	8	18
Craterium leucocephalum var. *cylindricum* (Massee) G. Lister						6	12	13			2						33	17	16
Physarum serpula Morgan							1	23	2		1						27	19	8
Diderma effusum (Schwein.) Morgan						3	58	13	9		68	21	19				191	67	124
Diderma rugosum (Rex) T. Macbr.							1				1		13				15		15
Physarum roseum Berk. & Broome							1	4				1	195	3			204	202**	2
Diderma umbilicatum Pers.													6	12			18		18
Diderma spumarioides (Fr.) Fr.													10				10	10	

Table 2 continued

Myxomycete species	Spring				Summer								Autumn				Total	Leaf type	
	Ap1	Ap2	M1	M2	J1	J2	Jul1	Jul2	Au1	Au2	S1	S2	O1	O2	N1	N2		D	E
Diachea subsessilis Peck				4	5	5	11										25	25**	
Diderma globosum Pers.				4	2		4	5	1		1						17	4	13
Didymium serpula Fr.				7	47	1		5									60	48	12
Physarum bivalve Pers.					3		5	3									11	6	5
Didymium leoninum Berk. & Broome						8	1	9									18	18	
Comatricha pulchella (C. Bab.) Rostaf.						1	3	1	13				1				19	19	
Hemitrichia serpula (Scop.) Rostaf.							1	1	2		3	1	2				10	8	2
Species recorded with nine or less samples																			
Physarum mutabile (Rostaf.) G. Lister								1			7						8		8
Diderma cingulatum Nann.-Bremek.											6	1					7	7	
Physarum myricanum Y. Yamam.									6								6		6
Didymium flexuosum Yamash.					1				4								5		5
Craterium aureum (Schumach.) Rostaf.												4	1				5	5	
Physarum hongkongense Chao H. Chung					4												4		4
Trichia favoginea var. *persimilis* (P. Karst.) Y. Yamam.											1						1	1	
Physarum nutans Pers.							2	1									3	1	2
Physarum leucopus Link					2	1											3	1	2
Physarum florigerum (Meyl.) Y. Yamam.								3									3	3	
Physarum bogoriense Racib.						2	1										3		3
Craterium concinnum Rex								1	2								3		3
Diderma hemisphaericum (Bull.) Hornem.					1			1									2	2	
Arcyria globosa Schwein.								2									2	2	
Lamproderma scintillans (Berk. & Broome) Morgan					1												1	1	
Craterium leucocephalum (Pers.) Ditmar							1										1	1	

Table 2 continued

Myxomycete species	Spring				Summer								Autumn				Total	Leaf type	
	Ap1	Ap2	M1	M2	J1	J2	Jul1	Jul2	Au1	Au2	S1	S2	O1	O2	N1	N2		D	E
Craterium dictyosporum (Rostaf.) H. Neubert, Nowotny & K. Baumann							1										1		1
Arcyria cinerea (Bull.) Pers.						1											1	1	
Total no. samples		148	6	159	794	485	601	294	89	0	136	38	256	3	12	0	3021	2273	748

Abbreviations of survey codes show *Ap* April, *M* May, *J* June, *Jul* July, *Au* August, *S* September, *O* October, *N* November and *1* first two weeks in a month, *2* last two weeks in a month, *D* deciduous, *E* evergreen

** Significant difference between the deciduous tree leaves and the evergreen tree leaves by Fisher's exact probability test of independence ($p < 0.01$)

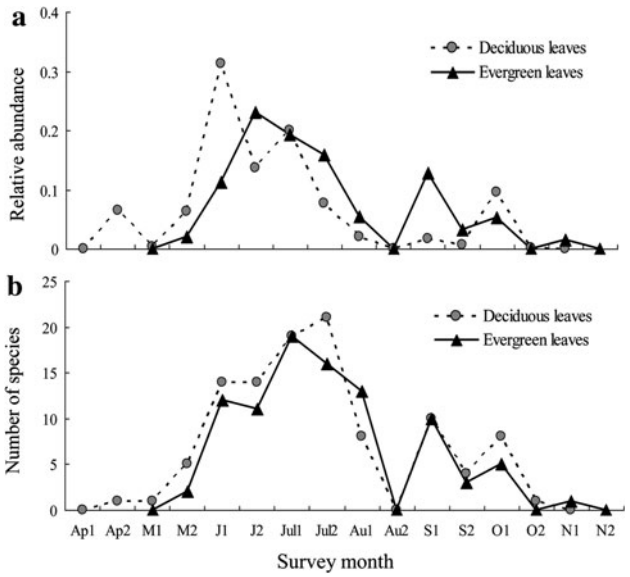

Fig. 3 Occurrence patterns of myxomycetes on the deciduous and the evergreen leaf-litter masses during the entire fruiting season. The *x*-axis indicates survey codes, *Ap1* represents the first two weeks of April, and *Ap2* represents the last two weeks of April; subsequent months follow this system

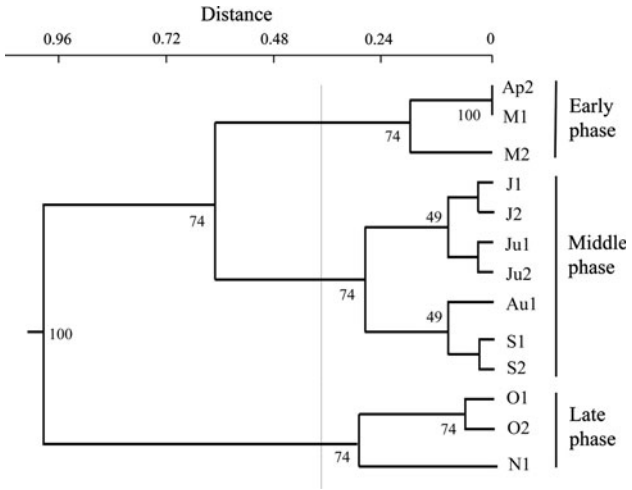

Fig. 4 Dendrograms of myxomycete communities from a monthly field survey, obtained using cluster analysis based on the first two NMDS scores. Thirteen seasonal communities were statistically divided into three groups: early phase (April–May), middle phase (June–September), and late phase (October–November) according to blade with bootstrap proportions of ≥70%. Abbreviations indicate the survey codes, as given in Table 2

P. verecunda leaves and *D. umbilicatum* (SI = 14.3) occurred on *C. camphora* leaves with narrow deviations. Thus, species showed clear seasonal changes across the fruiting season, and deviations of the early and later phases did not overlap. The species composition of the myxomycete assemblage clearly shifted from April–May to June–September and again in October–November.

Discussion

Elliot and Brazier (1933) grew myxomycetes in traps with wood and leaves in a garden in Warwickshire, England. This approach confirmed the general view that as long as moisture and temperature conditions are appropriate and the food supply is adequate, myxomycetes can colonize and complete their life cycles within a wide range of both natural and artificial environments. Similarly, our method of using litter-mass trays provided a unique medium within which to measure ecological characteristics of myxomycetes occurring on forest leaf litter. The leaf-litter trays created nurseries for myxomycete growth on the forest floor and assisted in the quantification of myxomycete occurrence. Using the present method, leaf litters were found to harbor abundant myxomycete specimens, and 45 species that inhabited the surface litter layer (excluding soil) of the forest were observed. Myxomycete occurrence

was estimated mainly by counting the number of leaves on which several fruiting bodies were attached, allowing for statistical analysis of the temporal changes associated with leaf-litter type.

Our study is the first to document ecological characteristics of myxomycetes associated with seasonal changes on newly defoliated leaf litters. Foliicolous myxomycetes occurred on different types of leaf litter under the influence of climatic changes. Most of the myxomycetes fructified sporangia during the period of stable humidity levels of over 70%, minimum air temperatures of over 8°C, and average temperatures of over 15°C, except during the dry period in August. Seasonal changes in species richness and species diversity (H') had a significantly high correlation to mean temperature, minimum temperature, and humidity on deciduous leaves and was also correlated to mean temperature and minimum temperature on evergreen leaves (Table 3). From these results, we deduced that the fructification of most myxomycetes is primarily affected by seasonal changes in temperature under humid conditions. The temporal pattern of species distribution that assembled in warm and humid seasons differed from that of wood-inhabiting myxomycetes in the forest of the same temperate region (Takahashi and Hada 2008a).

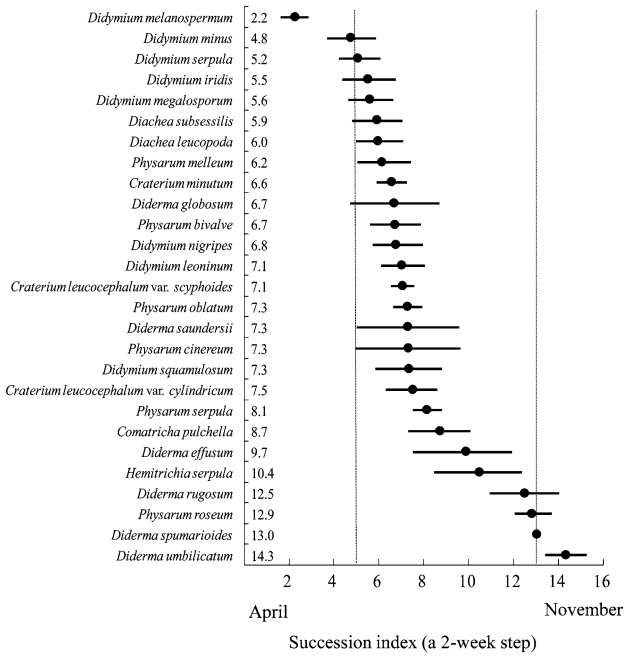

Fig. 5 Seasonal distribution of 27 myxomycetes in order of succession index from April to November. The *number* along the *x*-axis represents the number of two-week periods from the start of the survey in April. The fruiting season was divided into three phases by *dotted lines*: early phase (April–May), middle phase (June- September), and late phase (October–November), according to Fig. 4

In April, the beginning of the fruiting season, *Didymium melanospermum* chiefly occurred on the leaves of *P. verecunda*, which defoliated from early autumn and decomposed faster. Another species, *Diderma umbilicatum*, occurred in November on the leaves of *C. camphara*, which defoliated from the end of April and decomposed. Therefore, certain species clearly appeared to occur in association with leaf-litter type and decomposition. To assess the effects of litter quality on the decomposition rate, Takeda et al. (1987) revealed that the decomposition of leaf litter was related to the litter quality of several tree species. In the present study, however, it is unclear if myxomycete species were explicitly affected by the decay of leaves of different tree species. Foliicolous myxomycete occurrence may adapt to annual leaf defoliation time and the progression of leaf decomposition.

Associations between certain myxomycete species and various types of leaf litter have been reported based on previous observations performed in Finland (Härkönen 1981). Stephenson (1989), using the moist chamber culture technique, investigated foliicolous myxomycetes in five forest areas in the Mountain Lake area of southwestern Virginia. The study indicated that several species showed distribution trends for deciduous litter or coniferous litter, and that *Arcyria cinera* was an exceedingly common species among all of the 34 species studied. Further pH seemed to be an important factor in determining distribution patterns of particular species of myxomycetes. In the present study from a warm temperate region, *Physarum melleum* was the most abundant and common species, and the different types of leaf litter that defoliated in different seasons yielded different myxomycete assemblages (i.e., several

Table 3 Correlation coefficients between species richness (S) and species diversity (H') of myxomycete assemblages on the deciduous leaf litter and the evergreen leaf litter, and climatic conditions

	Deciduous leaf litter		Evergreen leaf litter	
Climatic conditions	S	H'	S	H'
Mean temperature	0.707**	0.881**	0.897**	0.823**
Minimum temperature	0.704**	0.853**	0.877**	0.817**
Humidity	0.644**	0.668**	0.481	0.130

Significance level; ** $p < 0.01$

dominant species preferred the deciduous or the evergreen leaves of broad-leaved trees). Cavender and Raper (1968) demonstrated that the dominant cellular slime mold species in the soil were related to the dominant trees in the forest canopy. This suggests that particular myxomycete species associated with specific tree leaves share certain ecological requirements; however, the nature of these requirements remains unknown. Takahashi and Hada (2008b) demonstrated in a summer survey that a diverse canopy consisting of deciduous and evergreen trees affected the species diversity of foliicolous myxomycetes. Consequently, the results of the present study support the idea that a mixed forest can yield great species diversity of myxomycete assemblages.

Feest and Madelin (1985, 1988) reported that changes in the populations of amoebae, ciliates, myxomycetes, and dictyostelids in the soil were sometimes synchronized with changes in bacterial populations, which exhibited 9- to 35-fold seasonal variations in abundance at four woodland sites. Kayang (2001) studied a subtropical monsoon area in India, and reported that seasonal changes in temperature and humidity were related to patterns of fungal and bacterial communities on newly fallen leaves of the alder *Alnus nepalensis* D. Don. Viable bacterial propagules of alder leaf litter exhibited seasonal variations and two peaks in July and September, which corresponded to heavy rainfall and warm temperatures. This pattern is similar to that seen for the myxomycete occurrence in the present study. Food supply (i.e., nourishment from the seasonal decomposition of leaves and bacteria) may be a potential factor regulating changes in myxomycete species composition. Since we provided suitable climactic conditions for litter decomposition and/or the occurrence of myxomycetes in the trays, differences in litter quality are likely a regulatory factor for myxomycete occurrence. Knowledge of the ecological requirements of most bacterial and fungal populations in the wild is lacking; thus, we can only assume a link between myxomycetes and the leaf-litter feeding environment. Rayner and Boddy (1988) stated that myxomycetes may play a role in nutrient cycling in woodlands. Myxomycetes may represent the biotic characteristics of forest leaf litter, particularly in light of the high sporocarp production and species richness in leaf-litter masses. Therefore, further study of myxomycete occurrence on leaf litters may elucidate the ecological role of myxomycetes in forests.

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