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Myxomycetes on the bark of living *Metasequoia glyptostroboides* trees and their distribution along a rural–urban gradient

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ABSTRACT

Myxomycete distribution along urban–rural gradients remains to be studied in detail. The ancient plant *Metasequoia glyptostroboides* has been mainly planted in urban parks and green areas in Japan, and it provides new habitats for myxomycetes on its growing tree bark. Here, we examined myxomycetes on bark along urbanization gradients, estimated by land-use coverage types. Survey sites were selected at 20 locations in western Japan, where the bark was sampled from 10 trees at each site. The bark samples were cultured in 10 Petri dishes per tree using the moist chamber technique. Myxomycete fruiting colonies occurred in 71% of cultures, and 44 species were identified across surveys. *Diderma chondrioderma* occurred at all sites, with the next most abundant species being *Licea variabilis* and *Perichaena vermicularis*. Twenty-two myxomycete communities ordinated using non-metric multidimensional scaling showed a significant negative correlation with building coverage and bark pH, increasing along the first axis. Relative abundances of *Physarum crateriforme* and *Licea biforis* positively correlated with increasing building coverage. Overall, urbanization causes alternation of the myxomycete community structure without diversity loss, and intermediate urbanization diversified species diversity on *M. glyptostroboides* tree bark.

Keywords: building cover, corticolous myxomycetes, land use types, moist chamber culture, urbanization

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1. Introduction

Myxomycetes are fungus-like amoeboid protists that primarily inhabit plant detritus and humus in terrestrial ecosystems (Stephenson, 2011), preferring habitats such as dead wood, litter, soil, and living tree bark, based on differences in the substrates and microenvironments (Novozhilov et al., 2022). Their fruited sporangia mostly disperse spores by wind (Kamono et al., 2009) and reproduce on various substrates. Some species known as bark-dwelling myxomycetes (corticolous myxomycetes) extend their habitat to the bark surface of living trees, where they complete their life cycle (Keller & Brooks, 1973). Although these species mostly possess minute fruiting bodies with an ephemeral existence and are rarely observed in the field, they can be reliably detected using the moist chamber culture technique (Michell, 1977).

Trees of the species *Metasequoia glyptostroboides* Hu et W. C. Cheng were found as fossils from the Cretaceous by Dr. Shigeru Miki (Tsukagoshi et al., 2011) and later discovered as an extant plant in China (Hu, 1946; Hu & Cheng, 1948); the species is therefore regarded as a "living fossil." Even though the species became extinct in the Japanese archipelago during the 4th Cenozoic period (Momohara, 2011), present specimens in Japan derive from 100 seedlings that were a present from Dr. R. W. Chaney from the USA (Tsukagoshi et al., 2011). The species was then propagated to schools, companies, and the public through cuttings planted throughout the country and was used as a garden and roadside tree in green areas in conurbations, local cities, rural areas, and in afforestation to examine its usefulness for timber production (Tsukagoshi, 2016). The bark surface provides a habitat for many myxomycetes that grow on bark surface (Takahashi, 2014).

Urbanization is presumed to cause various environmental changes (Grimm et al., 2008; Güneralp & Seto, 2013) along with habitat loss and fragmentation, resulting in impacts on biodiversity and ecological processes (Liu et al., 2016). Urbanization has increased globally and is rapidly spreading. The urban population in three conurbations in Japan is expected to reach 56.7% of the country's total population by 2050 (Ministry of Internal Affairs and Communications, www.soumu.go.jp). Understanding the effects of urbanization on biodiversity is thus of vital importance. Although the effects of urbanization on species richness have been studied and discussed for large visible plants, animals, birds, and arthropods (Faeth et al., 2011; McKinney, 2008), the importance of saproxylic microbes and the effects of urbanization on microbial diversity remain to be studied in detail (Korhonen et al., 2022). As a great variety of microbes, such as bacteria, fungi, and viruses, including the protozoan myxomycetes, inhabit human living spaces,

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understanding the microbiome is important for maintaining the ecology of the city environment.

Urban ecology has been an understudied topic in myxomycete research (Rincón-Marín et al., 2021). Myxomycetes constitute useful indicator organisms for assessing the microbial ecology in urban centers and are affected by the degree of urbanization. Forest disturbance-associated human activities result in the loss of habitat and available substrates for myxomycetes, causing differences in fruiting body abundance (Dagamac et al., 2015; Macabago et al., 2017; Rojas & Doss, 2014; Rojas & Stephenson, 2013). However, evident differences in myxomycete communities between urban centers and outer zones are unlikely (Rojas et al., 2022). In Sydney, Australia, species diversity and myxomycete composition based on several substrates between inner-city and semi-urban parks did not display any impact from urbanization (Hosokawa et al., 2019). By contrast, an investigation in San Jose, Costa Rica, suggested that three different urbanization grades were positively correlated with the number of species found on ground litter, and that air currents may also affect the distribution of myxomycetes in urban conditions (Rincón-Marín et al., 2021). These studies used surveys based on varied substrate types or ground litter; however, the ecological complexity and urbanization-related trends of myxomycetes remain ambiguous and little studied.

As the balance of microbial ecosystems in the context of urban biodiversity conservation is an urgent issue worldwide (Dudka & Romanenko, 2006), maintaining various microbiomes in the urban environment is necessary for human health and safety. The bark of *M. glyptostroboides* trees planted in urban parks or forest areas is suitable for monitoring influences of landscape changes from rural to urban environments on myxomycete distribution. The present study investigated myxomycete diversity on the bark of *M. glyptostroboides* trees using a systematic sampling approach across urbanization grades from forest to urban centers. Understanding the myxomycete diversity on tree bark will deepen our understanding of the impacts of urbanization on biodiversity and ecology.

2. Materials and methods

2.1. Study sites

The present survey was selectively conducted at 20 sites in temperate western Japan (Fig. 1) where *M. glyptostroboides* trees are growing and form attractive and beautiful landscapes through their deciduous coniferous shapes (Fig. 2A). The geographical locations of the sites are shown in Table 1, ranging from a latitude of 33.06771 N° at Ureshino to 35.501461 N° at Tottori, and from a longitude of 129.992575 E° at Ureshino to 135.92546 E° at Kusatu. Altitude ranged from 3 m above mean sea level at Tsurumi Ryokuchi Park to 494 m at Hirata farm in Miyosi, over a distance of approximately 600 km (Table 1). Using population and population density as indicators of human activity, the largest city in the area was Osaka, with a population of 2.691 million people (12,236 people/km^2, 2015), followed by Hiroshima City, with 1.994 million people (1,312 people/km^2, 2015) and Kyoto City, with 1.475 million people (1,779 people/km^2, 2015). The smallest cities were Takashima City, Shiga Prefecture, with 46,284 people (66 people/km^2, 2023), and Ureshino City, Saga Prefecture, with 25,129 people (199

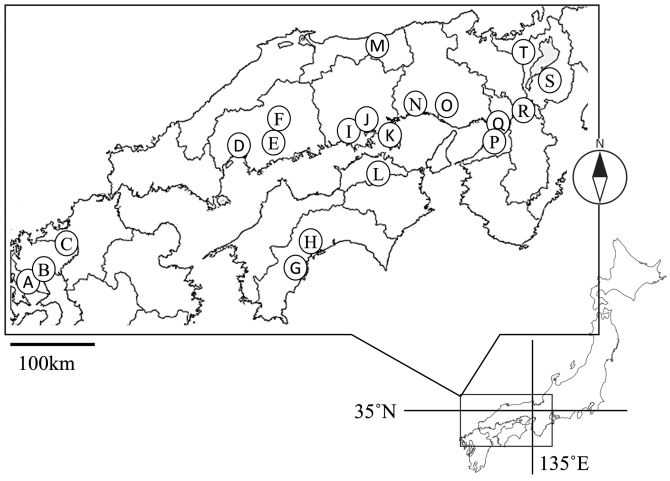

Fig. 1 – Location of the 20 survey sites in western Japan.

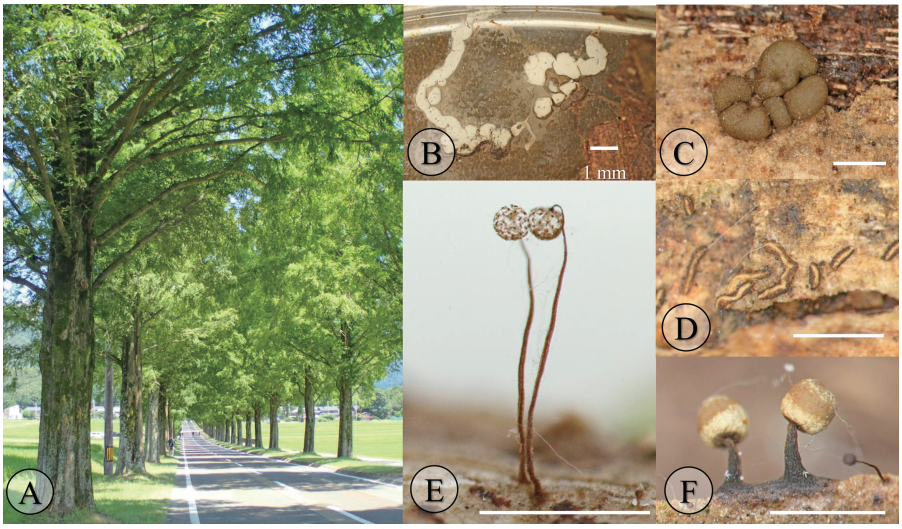

Fig. 2 – Landscape of *Metasequoia glyptostroboides* trees planted in a row, and images of myxomycete fruiting bodies appearing in moist chamber cultures. A: Makino Highland in Takashima City, Siga Pref., B: *Diderma chondrioderma*, with plasmodia crawling out of the bark and fruited sporangia, C: Fruiting bodies of *Licea variabilis*, D: Fruiting bodies of *Licea biforis*, E: Fruiting bodies of *Cribraria microcarpa*, F: Fruiting bodies of *Physarum crateriforme*. Scale bars indicate 1mm.

Table 1. Information of 20 survey sites in western Japan: Geographical location, the composition of landscape types, and averages of tree traits of ten trees sampled at a survey time.

			Location				Landscape types (%)					Tree traits	
Prefecture	City/Town	Site	Code	Longitude (E°)	Latitude (N°)	Altitude (m)	Buildings	Green area	Agriculture	Forest	Water	DBH (cm)	Bark pH
Saga	Ureshino	22nd Century Asian Forest	A	129.99275	33.06771	357	0	0	24	76	0	45	5.3
	Saga	Don-Don-Don no Mori	B	130.29275	33.26035	4	94	1	0	0	0	59	6.5
Fukuoka	Sawara-Ku	Shiihara Metasekoia no Mori	C	130.34612	33.45166	503	0	0	0	100	0	71	5.1
Hiroshima	Hiroshima	Higashi-Senda park	D1	132.45728	34.38141	1	83	7	0	0	3	58	6.8
			D2									55	7.0
	Saijo	Kagamiyama park	E	132.72742	34.40723	230	12	2	19	58	3	45	6.2
	Miyosi	Hirata Farm	F	132.91486	34.68455	494	3	0	19	78	0	62	6.2
Kochi	Shimanto	Farm Training Center	G	133.19240	33.22446	240	8	0	56	32	0	72	6.3
	Ochi	Yokogurayama	H	133.24400	33.53505	56	10	2	23	53	12	41	5.3
Okayama	Kurashiki	Rose Park	I	133.83377	34.64662	3	48	1	43	0	6	56	5.9
	Okayama	Multipurpose Grounds	J	133.92025	34.67935	4	73	23	2	0	0	63	6.4
		Asahi High School	K	133.94366	34.66008	6	59	7	0	25	7	69	7.4
Kagawa	Miki	Taikonomori Park	L	134.14775	34.23914	79	10	4	29	45	7	32	5.5
Tottori	Tottori	Fusesougou athletic Park	M	134.18620	35.50146	8	30	14	39	16	0	43	5.9
Hyogo	Himeji	Shosha	N	134.66034	34.86726	28	57	2	28	8	7	42	6.1
	Kobe	Kobe Municipal Arboretum	O	135.17788	34.74077	440	9	4	0	85	0	60	6.4
Osaka	Osaka	Nagai Park	P	135.52250	34.61455	7	78	22	0	0	0	55	6.7
		Tsurumi-ryokuti Park	Q	135.57761	34.71221	3	67	33	0	0	0	43	6.8
Kyoto	Kyoto	Ukyou-Ku Siinmizozaki Town	R	135.72855	34.99480	23	96	4	0	0	0	38	5.9
Shiga	Kusatsu	Lakeside Green Park	S	135.92546	35.05649	86	6	7	40	0	47	41	6.2
	Takashima	Makino Highland	T1	136.03656	35.47501	129	4	0	55	36	3	80	5.3
			T2									86	6.4

people/km^2, 2023).

Spatial environmental condition at each survey site was determined using aerial photographs following a previous study (Ukawa & Katoh, 2007), generally based on differences in land cover as classified using the classification scheme of the Geographical Survey Institute (http://maps.gsi.go.jp/, accessed on August 1, 2019). Urbanization was estimated from the composition of various landscape types (i.e., the extent of specific land-use types) surrounding the survey sites, following previous studies (Takahashi & Yano, 2021; Takahashi, 2021). A 1-km^2 grid comprising 100 cells of 1 ha was overlaid on each survey site, with the site itself at the center of the grid. Each cell was visually examined and classified into one of the following six broad land-use types: building areas, including industrial buildings and residences; green areas, such as parks and schools; agricultural areas; forests; water and other (wasteland, such as roads and vacant areas). If a land-use type occupied more than 50% of a cell, the cell was classified as that type. These classifications as indicators of spatial environmental conditions were similar to those of previous studies (Takahashi & Yano, 2021; Ukawa & Katoh, 2007). The matrix space of the 1-km^2 grid surrounding the survey site was estimated as the percentage of the first five land-use types among the 100 cells (Table 1).

Climate data for the surveyed region of western Japan was obtained from the Japanese Meteorological Agency (1991–2020) (http://www.data.jma.go.jp/obd/stats/etrn/index.php; accessed August 1, 2022). The coldest site was at Miyoshi City, Hiroshima Prefecture (159 m alt.), with an annual mean temperature of 13.5 °C and an annual precipitation of 1,931.3 mm. The warmest site was at Osaka city (23 m alt.), with an annual mean temperature of 17.1 °C and an annual precipitation of 1,338.3 mm, even though the most southern site was at Ureshino, Saga Prefecture, with an annual mean temperature of 15.3 °C and an annual precipitation of 2,323.7 mm.

2.2. Bark sampling

The outer bark of *M. glyptostroboides* is reddish-brown, arranged in a sheet, and easy to peel off. Bark sampling was performed from May to Nov 2020–2021. A previous study indicated that the species richness of corticolous myxomycetes at a site was approximately saturated within a 10-tree sampling effort (Takahashi et al., 2018); accordingly, 10 trees were sampled for each site. An additional second sample of 10 trees was taken in Higashisenda Park, located in the urban center of Hiroshima City, and in the Makino Highland, located in the rural agricultural area of Takashima City, resulting in a total of 220 trees. Sampled trees were selected based on a trunk diameter at breast height (DBH) greater than 20 cm. Outer bark fragments were manually stripped around the trunk surface at 0.5–2.0 m above ground level, excluding sections with epiphytes. Approximately 800 cm^2 of bark (approximately 50 g dry weight) was sampled from each tree, stored in a paper bag (23 × 35 cm), and labeled. The samples were then stored in a dry environment at room temperature (approximately 19–24 °C) for several weeks before myxomycete culture.

2.3. Myxomycete culture

The moist chamber (MC) culture method was used for myxomycete culturing, following a previous study (Stephenson, 1989). Each bark sample was cut into small pieces (approximately 2–6 cm in length), and approximately 50 cm^2 (4 g) of the bark was randomly selected and placed on its outer surface in a Petri dish (diameter: 9 cm) layered with filter paper (diameter: 7 cm), constituting the MC. Ten MCs were prepared for each tree, resulting in 100 MCs per site and 2200 MCs in total. The bark pieces in the Petri dishes were then soaked in 25 mL of distilled water (pH 6.9) and incubated at 23 °C without illumination. After 3 d, excess water was drained, and a lid was placed for 3 wk. The culture dishes were then opened halfway to allow the chambers to dry out slowly, encouraging the fruiting sporangia of myxomycetes to develop. Myxomycete fruiting bodies

were examined under a dissecting stereomicroscope at 4–5 wk after the beginning of culturing.

The bark pH of individual trees was examined using the excess water drained after 3 d of soaking. Bark pH was measured using a compact pH meter (Horiba, Kyoto, Japan), and the median pH for each tree was calculated. Site-level bark pH was calculated as the average of these values and was compared among the 22 site sampling instances.

Fruiting bodies of myxomycetes from all 2200 MCs were identified based on microscopic observations of sporangia, as described by Yamamoto (1998). Taxonomic assessment followed the most recent classification (http://eumycetozoa.com/data/genera.php). The incidence of a given species was recorded as the proportion of positive cultures in which myxomycete fruiting bodies occurred, while abundance was taken as the number of cultures containing the species. For each species, a bark piece containing the fruiting bodies was glued to the bottom of a paper collection box and the voucher specimens were deposited in the herbarium of the Wakayama Prefectural Museum of Natural History.

2.4. Data analyses

The abundance of each species was cross-tabulated per tree and determined for each site sampling instance. Those cumulative data were arranged into 22 communities. The relative abundance (%) of each species was calculated as follows: an abundance of a given species/total abundance in each community unit of grade ×100. The number of species recorded at each site (observed species richness, *Sobs*) was estimated using the presumed species richness (estimated species richness, *Sest*), which was calculated using the Chao1 method (Chao, 1984) based on *Sobs* and the abundance of a given species in each community of the site. Survey effort was evaluated by comparing *Sobs* and *Sest*, assuming *Sest* would be representative of a complete survey (*Sobs*/*Sest* × 100). The species diversity of myxomycete communities was obtained using the Shannon-Wiener index (*H'*) (Shannon & Weaver, 1963) and the equitability index (*J'*) (Pielou, 1966), as described in previous studies (Stephenson, 1989). These indices were also estimated for each community within each site using PAST software (Hammer et al., 2001; retrieved from http://folk.uio.no/ohammer/past/).

Ordination of the 22 myxomycete communities in the survey was carried out using non-metric multidimensional scaling (NMDS) based on the Bray-Curtis similarity, which is a useful method for comparing community structures (Takahashi et al., 2018) and performed using PAST software. The relationship between NMDS scores and environmental factors was analyzed using the Pearson product-moment correlation coefficient. The correlation between the measured characteristics was analyzed by a correlation coefficient (*r*) determined using Excel Statistics 2012 (SSRI Co., Ltd., Tokyo, Japan). The statistical significance threshold was set at $p < 0.01$.

The coverage percentage of built-up landscape surrounding a site was classified into six urbanization grades: grade + (<1%), grade I (≥1%), grade II (≥10%), grade III (≥25%), grade IV (≥50%), and grade V (≥75%), similar to the estimation method for official vegetation surveys (Ministry of the Environment: https://www.biodic.go.jp/moni1000/manual/vegetation_200803.pdf). The 22 communities were then aggregated into these six urbanization groups (Table 3). Species were arranged according to the ordination of species abundance and occurrence across grades (Table 4).

3. Results

3.1. Environmental variables

Relationships between environmental variables and 22 myxomycete communities (i.e., site-level communities) were examined. The 220 sampled trees had a bark pH ranging from 5.1 to 7.4, with an average of 6.2 (Table 1). Relationships among environmental variables such as geographical location, coverage of landscape types, and tree traits were tested by correlation analysis. The building cover increased as the forest ($r = -0.769$, $p < 0.01$) and agriculture covers decreased ($r = -0.607$, $p < 0.01$); further, an increase in building cover was related to an increase in bark pH ($r = 0.582$, $p < 0.01$), but bark pH negatively correlated with forest coverage ($r = -0.513$, $p < 0.05$).

Altitude increase was negatively correlated with the mean number of species per tree ($r = -0.561$, $p < 0.01$) and number of colonies ($r = -0.590$, $p < 0.01$) (Table 2). Building cover was positively correlated with number of colonies ($r = 0.520$, $p < 0.05$). Forest cover negatively correlated with the mean number of species per tree ($r = -0.557$, $p < 0.01$) and the number of colonies ($r = -0.583$, $p < 0.01$). The other environmental factors, such as latitude, longitude, green area, agriculture, water cover, DBH, and bark pH did not clearly correlate with myxomycete variety (Table 2).

3.2. Myxomycete communities

Myxomycete fruiting bodies developed positively in 46–93% of each survey instance (Table 3), resulting in a positive culture rate of 71% (1,557/2,200 dishes). The mean number of species per tree was 2.3–6.5 per survey, with 4.3 species on average across all surveys. Species richness was 8–17 (mean 12.5) across surveys. Species diversity (*H'*) ranged from 1.59 to 2.43 (mean 1.99), with an equitability range of 0.70–0.87 (mean 0.72). Survey completeness ranged from 46% to 100% (mean 86%), indicating that survey efforts were

Table 2. Correlation coefficients between the diversity of 22 myxomycete communities surveyed and environmental variables. Significance, **p < 0.01, *p < 0.05.

	Species richness	Mean species number per tree	Number of colonies	Species diversity	Equitability
Altitude	0.035	-0.561 **	-0.590 **	0.044	0.042
Latitude	0.145	0.378	0.352	0.170	0.118
Longitude	0.058	0.291	0.286	0.102	0.099
Building cover	-0.272	0.269	0.520 *	-0.254	-0.151
Green area cover	-0.168	0.202	0.453 *	-0.177	-0.128
Agricultural cover	0.265	0.241	-0.198	0.397	0.438 *
Forest cover	0.189	-0.557 **	-0.583 **	0.081	-0.084
Water cover	-0.027	0.200	0.091	0.013	0.059
DBH	0.336	-0.018	-0.040	0.367	0.263
Bark pH	-0.331	0.082	0.452 *	-0.391	-0.376

Table 3. Positive culture (%), number of species, species diversity, and completeness at surveys according to six building coverage grades.

Building grades	Site (Code)	Positive culture (%)	Mean number of species	Species richness	Shannon (*H′*)	Equitability (*J′*)	Completeness (%)
Grade V (≥75%)							
	Kyoto (R)	81	5.7	10	*1.94*	*0.84*	100
	Saga (B)	75	4.9	14	*2.20*	*0.83*	97
	Hiroshima (D1)	80	4.2	9	*1.73*	*0.79*	100
	Hiroshima (D2)	83	4.0	9	*1.58*	*0.72*	100
	Osaka (P)	87	3.9	11	*1.85*	*0.77*	100
Grade IV (≥50%)							
	Okayama (J)	84	5.4	15	*2.21*	*0.82*	83
	Osaka (Q)	87	4.1	9	*1.68*	*0.76*	100
	Okayama (K)	93	5.1	15	*1.89*	*0.70*	88
	Himeji (N)	66	2.8	10	*1.61*	*0.70*	63
Grade III (≥25%)							
	Kurashiki (I)	74	5.9	16	*2.43*	*0.87*	100
	Tottori (M)	79	6.5	15	*2.26*	*0.84*	60
Grade II (≥10%)							
	Saijo (E)	48	2.4	9	*1.59*	*0.72*	96
	Ochi (H)	46	3.8	14	*2.05*	*0.78*	92
	Miki (L)	84	5.3	17	*2.24*	*0.79*	92
Grade + (≥1%)							
	Kobe (O)	31	2.3	12	*1.86*	*0.75*	46
	Shimanto (G)	56	4.1	11	*1.98*	*0.83*	92
	Kusatsu (S)	73	5.4	11	*2.00*	*0.83*	100
	Takashima (T1)	59	4.2	16	*2.30*	*0.83*	60
	Takashima (T2)	78	5.3	14	*2.28*	*0.86*	82
	Miyosi (F)	65	4.0	14	*2.17*	*0.82*	58
Grade + (<1%)							
	Ureshino (A)	73	3.0	8	*1.59*	*0.76*	100
	Shiihara (C)	55	3.3	16	*2.29*	*0.83*	76
	Average	71	4.3	12.5	*1.99*	*0.72*	86

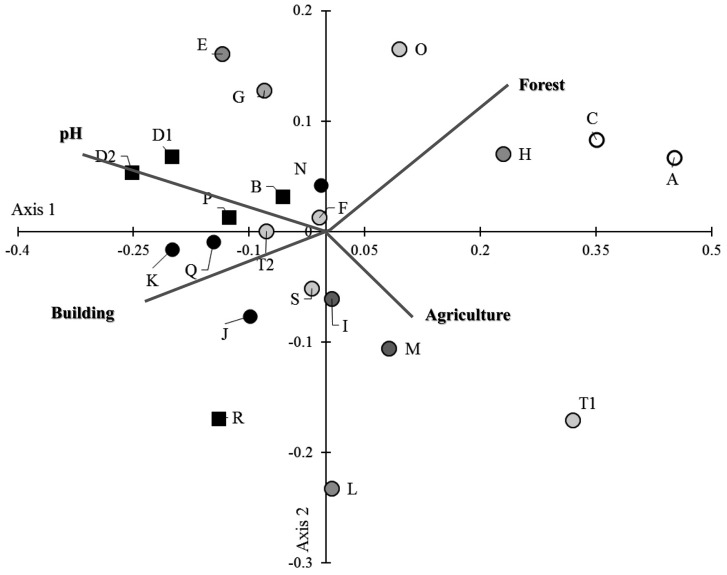

Fig. 3 – Non-metric multidimensional scaling ordination plots of myxomycete communities. The site codes are given in Table 1. Markers indicate building cover grades; ■: ≥ 75%, ●: < 75%, < 50%, < 25%, < 10%, according to the shade grade, ○: < 1%. The bars show four environmental variables in relation to the first two axes, of which the scales were relatively indicated with coefficient values on two axes in Table 5. The stress value of the NMDS was 0.181. Coefficient of determination for the first axis, $R^2 = 0.646$, and for the second axis, $R^2 = 0.131$.

exhaustive (Table 3).

A total of 44 species belonging to 16 genera were identified from 2,628 fruiting colonies (Table 4). Of these, 27 species appeared five or more abundance among samples, 17 less than five abundance, and six species only once. The most abundant species was *Diderma chondrioderma* (de Bary and Rostaf.) Kuntze (Fig. 2B) with a relative abundance of 20.1%, followed by *Perichaena vermicularis* (Schwein.) Rostaf. at 16.8%, and *Licea variabilis* Schrad. at 13.9% (Fig. 2C). Five species had a relative abundance of ≥ 3%, with 79 colonies or more: *Macbrideola confusa* Nann.-Bremek. & Y. Yamam., *Arcyria cinerea* (Bull.) Pers., *Cribraria violacea* Rex, *Licea biforis* Morgan (Fig. 2D), and *Cribraria microcarpa* (Schrad.) Pers. (Fig. 2E).

The most common species across all 20 sites were *D. chondrioderma* (100% incidence), *C. violacea* (80%), *M. confusa* (75%), *Physarum lakhanpalii* Nann.-Bremek. & Y. Yamam. (75%), and *A. cinerea* (70%). Nineteen species appeared across five sites (25% incidence), and 14 were found in only one site.

3.3. Ordination of myxomycete communities

Ordination among the 22 myxomycete communities, evaluated using NMDS analysis, is shown in Figure 3. The NMDS scores

Table 4. Myxomycete species and assemblages on the bark of *Metasequoia glyptostroboides* living trees and abundances according to the six building coverage grades. Italics indicate relative abundance (%) and frequency (%) of appearance in total sites. Species are arranged in order of characteristics at each building coverage grade and relative abundance. Characteristic species in each grade were examined by independent t-test among six assemblages of building coverage grades. Significance, $^{**}p < 0.01$.

Species	Buildings coverage grades (%)						Total abundance	Relative abundance (%)	Appearance in sites (%)
	+	I	II	III	IV	V			
Frequent species with five or more abundances									
Diderma chondrioderma (de Bary & Rostaf.) Kuntze	23	119	42	45	148 **	150	527	*20.1*	*100*
Perichaena vermicularis (Schwein.) Rostaf.	2	83	52	21	114	170 **	442	*16.8*	*95*
Licea variabilis Schrad.		69	11	20	155 **	109	364	*13.9*	*75*
Macbrideola confusa Nann.-Bremek. & Y. Yamam.	1	55 **	3	44 **	32	34	169	*6.4*	*75*
Arcyria cinerea (Bull.) Pers.	31 **	49 **	5	21	19	16	141	*5.4*	*70*
Cribraria violacea Rex		37	27 **	13	31	30	138	*5.3*	*80*
Cribraria microcarpa (Schrad.) Pers.	62 **		22 **				84	*3.2*	*15*
Licea biforis Morgan					12	67 **	79	*3.0*	*30*
Physarum crateriforme Petch		5		2	36 **	31 **	74	*2.8*	*50*
Hemitrichia minor G. Lister	2	24	4	22 **	9	11	72	*2.7*	*65*
Clastoderma debaryanum Blytt		3	46 **	6	4	10	69	*2.6*	*40*
Physarum lakhanpalii Nann.-Bremek. & Y. Yamam.		15	10	12	11	19	67	*2.5*	*75*
Paradiacheopsis rigida (Brândza) Nann.-Bremek.		18	14 **	22 **	1	1	56	*2.1*	*45*
Calomyxa metallica (Berk.) Nieuwl.	9 **	20 **		3	2	9	43	*1.6*	*55*
Cribraria confusa Nann.-Bremek. & Y. Yamam.		2	16 **	9 **	9		36	*1.4*	*40*
Licea erecta K.S. Thind & Dhillon		12	3	15 **			30	*1.1*	*15*
Physarum nutans Pers.		1	2	21 **	5		29	*1.1*	*30*
Comatricha pulchella (C. Bab.) Rostaf.	1	8	7	1	5	5	27	*1.0*	*45*
Stemonaria gracilis Nann.-Bremek. & Y. Yamam.	1	5	4	3	10	3	26	*1.0*	*60*
Licea rugosa Nann.-Bremek. & Y. Yamam.		23 **					23	*0.9*	*10*
Trichia botrytis (J.F. Gmel.) Pers.	5 **	17 **					22	*0.8*	*20*
Arcyria insignis Kalchbr. & Cooke		4	1		1	11	17	*0.6*	*30*
Ceratiomyxa fruticulosa (O.F. Müll.) T. Macbr.	4 **	10 **	2		1		17	*0.6*	*25*
Physarum roseum Berk. & Broome	17 **						17	*0.6*	*10*
Macbrideola martinii (Alexop. & Beneke) Alexop.						13	13	*0.5*	*5*
Hemitrichia calyculata (Speg.) M.L. Farr	5	1					6	*0.2*	*10*
Physarum oblatum T. Macbr.	6						6	*0.2*	*10*
Accompanied species with less than five abundances									
Physarum hagiwarae Nann.-Bremek. & Y. Yamam.		3	1				4	*0.2*	*10*
Licea operculata (Wingate) G.W. Martin		3					3	*0.1*	*5*
Macbrideola argentea Nann.-Bremek. & Y. Yamam.			3				3	*0.1*	*10*
Stemonitis smithii T. Macbr.			3				3	*0.1*	*5*
Trichia decipiens (Pers.) T. Macbr.	3						3	*0.1*	*5*
Comatricha elegans (Racib.) G. Lister				2			2	*0.1*	*5*
Comatricha laxa Rostaf.					1	1	2	*0.1*	*10*
Licea capitatoides Nann.-Bremek. & Y. Yamam.		2					2	*0.1*	*5*
Macbrideola ovoidea Nann.-Bremek. & Y. Yamam.				2			2	*0.1*	*5*
Perichaena chrysosperma (Curr.) Lister		1				1	2	*0.1*	*10*
Physarum penetrale Rex			2				2	*0.1*	*5*
Arcyria nigella Emoto			1				1	*0.04*	*5*
Hemitrichia velutina Nann.-Bremek. & Y. Yamam.					1		1	*0.04*	*5*
Licea rufocuprea Nann.-Bremek. & Y. Yamam.				1			1	*0.04*	*5*
Licea scyphoides T.E. Brooks & H.W. Keller.					1		1	*0.04*	*5*
Paradiacheopsis solitaria (Nann.-Bremek.) Nann.-Bremek.						1	1	*0.04*	*5*
Perichaena depressa Lib.	1						1	*0.04*	*5*
Total	173	589	281	285	608	692	2628	*100*	
Number of species	16	26	23	20	22	20	44		

show similarities among myxomycete communities, plotted according to the NMDS scores of the first two axes. Four primary environmental variables, forest cover, agriculture cover, building cover, and bark pH, were overlaid in relation to the community ordination for the first two axes. Sites with higher building cover were plotted on the negative side of the first axis, whereas sites with higher forest cover were plotted on the positive side. The relationships between communities as arranged in the NMDS and the environmental variables are shown in Table 5. Community arrangement had a significantly negative correlation with bark pH (r = -0.854, $p < 0.01$) and building cover (r = -0.646, $p < 0.01$) on the first axis and a positive correlation with forest cover (r = 0.646, $p < 0.01$). Geographical location was not significantly correlated with community arrangement. Although the community arrangement along the second axis had no significant correlation with the environmental variables, the second axis showed a significantly negative correlation with factors of myxomycete communities, i.e., with number of positive cultures (r = -0.550, $p < 0.01$), the mean number of species per tree (r = -0.737, $p < 0.01$) and number of colonies (r = -0.606, $p < 0.01$).

Seven abundant species found across five or more sites correlated with three environmental variables: bark pH, building coverage, and forest coverage (Table 6). Bark pH increase was significantly correlated with increasing relative abundance of *P. vermicularis* (r = 0.659, $p < 0.01$), *L. variabilis* (r = 0.871, $p < 0.01$), and *Physarum crateriforme* Petch (r = 0.613, $p < 0.01$), but negatively with abundance of *A. cinerea* (r = -0.549, $p < 0.01$), *Paradiacheopsis rigida* (r = -0.459, $p < 0.05$), and *Ceratiomyxa fruticulosa* (r = -0.438, $p < 0.05$). Increased building cover was positively correlated with the relative abundance of *P. crateriforme* (r = 0.583, $p < 0.01$) and *L. biforis* (r = 0.612, $p < 0.01$). Decreasing forest cover also increased the abundance of *P. crateriforme* (r = -0.479, $p < 0.05$). Decreasing forest cover caused an increase in the mean number of myxomycete species per tree (r = -0.557, $p < 0.01$) and positive cultures (r = -0.665, $p < 0.01$). Thus, urbanization had an impact on species incidence and abundance.

3.4. Species distribution for building grades

Grouping the identified species based on the building coverage grades (+, I, II, III, IV, and V) in Table 3 indicated that different coverage grades where associated with different Shannon species index and equitability scores, allowing the delineation of two assemblages (Fig. 4). Species diversity was higher in the assemblages of building coverage grades I–III than in grades +, IV, and V; thus, the intermediate urbanized environment was richer in species di-

Table 5. Coefficients between NMDS scores of the first two axes, environmental variables, and myxomycete occurrences. Significance, $^{**}p < 0.01$, $^{*}p < 0.05$.

	Axis 1	Axis 2
Tree traits		
DBH	0.047	0.191
Bark pH	-0.854 **	0.230
Landscape types		
Building	-0.644 **	-0.144
Green area	-0.379	-0.172
Agricultural area	0.302	-0.223
Forest	0.646 **	0.374
Water	-0.013	-0.137
Geography		
Altitude	0.535 *	0.442 *
Latitude (N°)	-0.291	-0.482 *
Longitude (E°)	-0.298	-0.440 *
Myxomycete occurrences		
Positive cultures	-0.481 *	-0.550 **
Mean number of species per tree	-0.266	-0.737 **
Number of colonies	-0.508 *	-0.606 **
Species richness	0.281	-0.479 *
Species diversity (H')	0.274	-0.504 *
Equitability (J')	0.201	-0.414

Table 6. Species distribution that significantly correlated with environmental variables such as bark pH, building, and forest covers. The correlation was calculated between relative abundance and environmental variables on species recorded at five communities or more among 22 communities. Significance, $^{**}p < 0.01$, $^{*}p < 0.05$.

	Bark pH	Building cover	Forest cover
Perichaena vermicularis	0.659 **	0.277	-0.261
Licea variabilis	0.871 **	0.375	-0.318
Arcyria cinerea	-0.549 **	-0.391	0.247
Physarum crateriforme	0.613 **	0.583 **	-0.479 *
Paradiacheopsis rigida	-0.459 *	-0.333	0.043
Licea biforis	0.422	0.612 **	-0.414
Ceratiomyxa fruticulosa	-0.438 *	-0.359	0.262

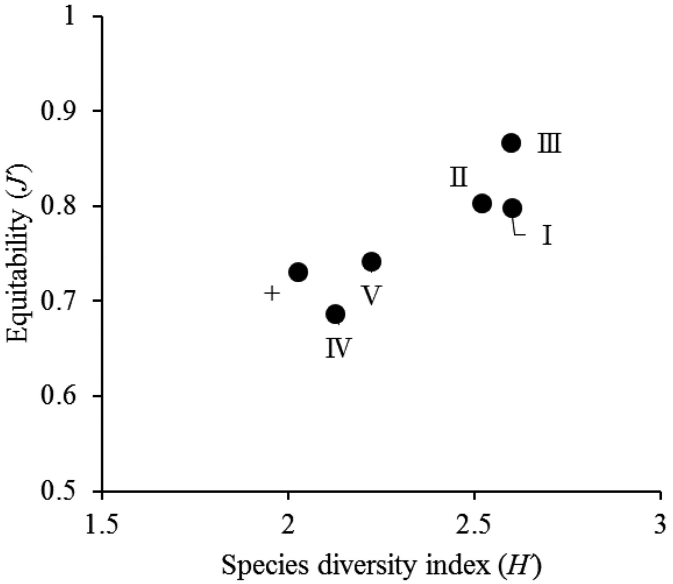

Fig. 4 – Plots of six myxomycete assemblages grouped by the building coverage grades using species diversity and equitability scores. Codes of building grades for the assemblages are provided in Table 3.

versity than were forest or urban centers.

The species associated with each building grade are shown in Table 4. Seven species were found as generalists in every building coverage grade, but their abundances varied by grade. Building coverage grade + (<1%) was dominated by six species which were absent or less abundant in the urbanized grades (III–V), consisting of *A. cinerea, Cribraria microcarpa* (Schrad.) Pers., *Calomyxa metallica* (Berk.) Nieuwl., *Trichia botrytis* (J.F. Gmel.) Pers., *C. fruticulosa*, and *Physarum roseum* Berk. & Broome. Building coverage grade I (≥1%) was dominated by four species in common with grade + and additionally by *M. confusa* and *Licea rugosa* Nann.-Bremek. & Y. Yamam. Building coverage grade II (≥ 10%) was dominated by one species in common with grade + and four other species: *C. violacea*, *Clastoderma debaryanum* Blytt., *Physarum rigida* (Brândza) Nann. -Bremek., and *Cribraria confusa* Nann.-Bremek. & Y. Yamam. Building coverage grade III (≥ 25%) was dominated by one species in common with grade I, two in common with grade II, and the three species *Hemitrichia minor* G. Lister, *Licea erecta* K.S. Thind & Dhillon, and *Physarum nutans* Pers. Building coverage grade IV (≥50%) was dominated by three species: *D. chondrioderma*, *L. variabilis*, and *P. crateriforme* (Fig. 2F). Finally, building coverage grade V (≥75%), located in central urban areas, was dominated by one species in common with grade IV and additionally by *P. vermicularis* and *L. biforis*. There were thus five distinct species that were predominant in the urbanized building grades (IV–V).

4. Discussion

Twenty species of corticolous myxomycetes of *M. glyptostroboides* have been reported for the urban green areas in western Japan (Takahashi, 2014). Fourteen of these were also found in the present study, whereas the following six were absent from our samples:

Badhamia nitens Berk., *Arcyria pomiformis* (Leers) Rostaf., *Enerthenema papillatum* (Pers.) Rostaf., *Didymium clavus* (Alb. & Schwein.) Rabenh., *Perichaena corticalis* (Batsh) Rostaf. and *Minakatella longifila* (G. Lister). The present study, however, reported 30 species for the first time, yielding a total accumulated species richness of 50 corticolous myxomycetes species on *M. glyptostroboides* bark. Under natural conditions, the fruiting bodies of these species are too small and sporadic to be detected on tree bark surfaces with naked eye. MC and microscopic observation are therefore suitable methods for observing these species (Michell, 1977). Rare species comprised with *Licea*, *Macbrideola*, *Paradiacheopsis*, and *Calomyxa* were mostly unknown ecological characteristics.

The most abundant species in the central urban areas were *P. vermicularis*, *L. variabilis*, *L. biforis*, and *P. crateriforme*, which occurred on tree barks at a pH of 6.2–6.8, but never on *Cryptomeria japonica* and *Chamaecyparis obtusa* barks with pH lower than 3.7 (Takahashi, 2014). The different characteristics of tree species influence the pH of their barks, which in turn influences myxomycete community structure. Furthermore, increase in bark pH of *C. japonica* have been shown to reduce tree vitality and alter the structure of the community on the bark surface (Takahashi & Fukasawa, 2022). The corticolous myxomycete communities on *M. glyptostroboides* were very different from those on *C. japonica*, which is endemic in Japan (30 and 32 myxomycete taxa reported in Takahashi et al. (2018) and in Takahashi (2020), respectively). This dissimilarity is supported by the high Bray-Curtis values between the samples of the present study and those of Takahashi et al. (2018; 0.850, with 16 taxa in common) and Takahashi (2020; 0.846, with 19 taxa in common). We therefore hypothesize that the bark of *M. glyptostroboides* is an important distinctive habitat for myxomycetes.

Corticolous myxomycetes have so far rarely been reported as being affected by environmental changes. Here, we provide evidence that urbanization associated with increasing residential and industrial spaces in a landscape affects myxomycete inhabitation and community structure on the bark of *M. glyptostroboides* trees. Air pollution, acid rain, and human activity have been previously reported to reduce corticolous myxomycete diversity in large European cities, such as Helsinki (Härkönen & Vänskä, 2004) and Madrid (Wrigley de Basanta, 2000). In contrast, myxomycete communities derived from several substrate types were similar in the inner city and semi-urban parks in Sydney, Australia (Hosokawa et al., 2019). This suggests that myxomycete diversity in urban parks is driven by factors at the substrate level rather than by location (inner city versus semi-urban areas). The reduction or loss of habitat and substrate decreased the abundance of myxomycete fruiting bodies in tropical forests in the Peruvian Amazon (Rojas & Stephenson, 2013). Urbanization and the consequent forest loss alter parameters such as airflow, temperature, and humidity (Rojas et al., 2022) and affect the myxomycete community structure and composition.

Conversion of forests and agricultural land to urbanized environments results in ecological simplification and fewer species in highly urbanized spaces (Beninde et al., 2015; McKinney, 2006, 2008). The forest loss in the downstream reaches of a river altered myxomycete community structures and species distribution on the tree bark of living *C. japonica* trees (Takahashi, 2021) and also caused an altered myxomycete community structure and decreased species richness around the World Heritage Site of Mt. Fuji (Takahashi & Yano, 2021). In the present study, urbanization with increased residential and industrial buildings caused forest loss (r = -0.769, $p < 0.01$) and increased bark pH ($r = 0.582$, $p < 0.01$), influencing the myxomycete community structure without loss of species diversity (Fig. 4). Our findings on *M. glyptostroboides* tree bark extend the current understanding of how urbanization with forest loss influences corticolous myxomycetes. Some species have adapted to urbanized environments, as suggested in Table 4, despite several species preferring forest environments.

Substrate pH has been connected to specific traits of trees for both the bark (Everhart et al., 2008; Takahashi, 2014) and fallen twigs of tree species (Takahashi et al., 2022). The mean pH value of *M. glyptostroboides* bark was 6.2 (5.1–7.4) in the present study, as in a previous study by Takahashi (2014), who reported the same mean over a range of 4.3 to 7.4. We found that bark pH variation occurred within this range, resulting in increasing bark pH with increasing building coverage ($r = 0.582$, $p < 0.01$) and decreasing forest cover (r = -0.513, $p < 0.05$), with significant impacts on myxomycete occurrence. The increased pH values in urban spaces compared to forest spaces affected species incidence in large cities; for example, *P. crateriforme* presence positively correlated with increasing bark pH ($r = 0.613$, $p < 0.01$) and building density ($r = 0.583$, $p < 0.01$). Our results thus suggest that the distribution of several myxomycetes is likely to be sensitive to urbanization. A correlation between bark pH and forest loss has been suggested based on the example of *C. japonica* trees, for which forest loss was associated with increasing bark pH. This decreased the occurrence of acidophilic myxomycete species; however, three species (*Comatricha pulchella* (C. Bab.) Rostaf., *Echinostelium minutum* de Bary, and *Licea kleistobolus* G.W. Martin) responded positively to increasing bark pH (Takahashi, 2021). Substrate pH affects the incidence of some species (Rincón-Marín et al., 2021); this includes effects of bark pH on the occurrence and community structure of myxomycetes.

Open-air spaces may provide opportunities for the invasion of spore-dispersed myxomycetes. The bark surface of trees in open green spaces and conurbation environments is presumed to be important in maintaining myxomycete inhabitation. Open spaces provide varied heterogeneous environments for myxomycetes that differ from those in forests, and tree bark as an available substrate may increase corticolous species diversity for airborne myxomycetes. In the present study, species diversity along a rural–urban gradient was richer in moderately urbanized locations than in rural forests and urban centers (Fig. 4). Such environments may feature increased spatial heterogeneity and consequently enhanced biodiversity, as has been reported for grassland (Yoshihara, 2019) and managed forests (Chapagain et al., 2021). Thus, increasing species richness and diversity of myxomycetes may indicate adaptation to intermediately disturbed open environments.

The increased temperature of urban areas, caused by the heat island effect, may also influence the distribution of organisms, similar to how some cicadas have adapted to the urban environment of Osaka (Moriyama & Numata, 2011). The geographical location of the Japanese island results in range of temperature regimes, influencing the distribution of corticolous myxomycete communities in the bark of *Cryptomeria japonica* trees (Takahashi et al., 2018). Since temperature changes may disturb urban ecosystems, myxomycetes may represent useful indicators for microbial ecology assessments of urban centers (Rincón-Marín et al., 2021).

An understanding of the relationship between human-altered habitats and myxomycetes requires further research on species diversity (Schnittler et al., 2017). Myxomycetes are likely to be important organisms for monitoring microbial biodiversity in urban landscapes. The degree to which urbanization affects myxomycetes may be revealed by further data collection. Myxomycetes on tree bark can be used to derive a functional interpretation of microbial dynamics along rural-to-urban gradients and are useful for monitoring urban biodiversity.

The morphology of fruiting bodies is key for this group's taxonomy, but molecular data should also be considered, as myxoamoebal trophic stages that do not favor sporocarp formation in MC can persist on the barks. Therefore, a metagenomic approach is required for elucidating the distribution of myxomycetes in diverse habitats at both large and small geographic scales (Shchepin et al., 2022). Further systematic sampling approaches should be used to generate relevant data regarding urban biodiversity monitoring and environmental change.

Disclosure

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