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Slimes in the city: The diversity of myxomycetes from inner-city and semi-urban parks in Sydney, Australia

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ABSTRACT

Natural and agricultural areas are rapidly becoming urbanised, causing changes in habitat structure and diversity. Although the effect of urbanisation on the diversity of terrestrial plants and animals has been well studied, there is a significant gap in our understanding of how urbanisation impacts diversity in protists. Here, we measure the diversity of plasmodial slime moulds (a group of large, macroscopic protists also known as myxomycetes) in inner-city and semi-urban parks. We studied the impact of a range of environmental characteristics (pH, temperature, canopy cover, area of green space and substrate type) on species richness and composition of myxomycetes. We also examined the influence of different degrees of urban development surrounding these parks. Species composition was significantly different between substrate types but not between inner-city and semi-urban parks. Temperature was the only environmental characteristic that affected diversity, having a negative effect on myxomycete presence. Our findings suggest that myxomycete diversity in urban parks is driven by factors at the substrate level, and not by the park's location within the city (inner city or semi-urban).

1. Introduction

Urbanisation leads to increased habitat fragmentation and dramatic changes in biodiversity amongst a range of taxa (Grimm et al., 2008). Generally, the conversion of natural and agricultural land into urbanised environments leads to ecological simplification, with fewer species being present in highly urbanised spaces (Beninde et al., 2015). However, cities are a highly heterogeneous environment, ranging from inner-city to remnant bushlands to outer suburbs. These varying landscape types can have different impacts on biodiversity (Beninde et al., 2015). As the human urban population is expected to reach 70% by 2050 (United Nations, 2012), it is important to understand how biodiversity can change in response to urbanisation.

Most studies of urban diversity are focused on macroscopic taxa such as birds, arthropods and vascular plants, with very little research on protists (Faeth et al., 2011). Protists are a large and important group of organisms, filling key ecological roles of decomposition and nutrient cycling (Feest, 1987). Given the important role protists play in the environment, understanding how they are impacted by different degrees of urbanisation is important, as changes in protist assemblages could impact ecosystem functioning. However, the small size of most protists makes collection and identification difficult, thus limiting studies on their biodiversity, particularly in urban environments.

Unlike most protists, myxomycetes (commonly known as 'slime moulds') produce macroscopic fruiting bodies that are relatively easy to collect and identify (Stephenson and Stempen, 1994). While the specific ecological roles of most myxomycete species are unknown, they are generally thought to function as modulators of decomposition via their predation on bacteria and fungi (Feest and Madelin, 1985). There are approximately 1000 known morphologically discernible myxomycete species as of 2017 (Lado and Eliasson, 2017). Myxomycete distribution is widespread and they have been found in a range of environments including temperate forests (Takahashi and Hada, 2009; Kazunari, 2010), tropical rainforests (Dagamac et al., 2012), dryland ecosystems (Estrada-Torres et al., 2009) and the tundra of north-central Siberia (Novozhilov et al., 1999). Myxomycetes are thought to reside primarily on decaying plant matter, but they have also been found on a range of substrates such as tropical inflorescences (Schnittler and Stephenson, 2002; Wrigley de Basanta et al., 2008), algae (Smith and Stephenson, 2007), and herbivore dung (Eliasson, 2013).

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Myxomycete diversity has also been investigated in soils as part of the greater protozoan community (Feest and Madelin, 1985; Kamono et al., 2009). These studies found that the abundance of myxomycetes was highest in grassland and agricultural soils (Feest and Madelin, 1985). Myxomycete diversity has also been well studied at the microhabitat level. Air litter substrates, which are leaves still attached to plants, yield more species of myxomycetes than ground litter (Rojas and Stephenson 2008, 2013; Dagamac et al., 2012). Groups of myxomycete species specialise in certain substrates such as bark, herbivore dung, and melting snowbanks (Wrigley de Basanta, 2000; Eliasson, 2013; Shchepin et al., 2014).

Despite urbanisation being one of the most ubiquitous forms of disturbance, there have been few studies on the urban ecology of myxomycetes (Ing, 1998). Myxomycetes indeed appear to be sensitive to a range of disturbance types. For example, forest fragmentation and habitat loss generally decreased myxomycete diversity in the Amazon rainforest (Rojas and Stephenson, 2013). Disturbance also decreased richness and the overall diversity of myxomycete species in an island habitat following a typhoon and earthquake (Macabago et al., 2017). Acid rain affects species

Fig. 1. A. Map of sampling sites in Sydney.
B Location of Sydney Inner City and Ku-ring-gai Council. Our sites for inner-city parks were in the Sydney Inner City area (blue). Our sites for semi-urban parks are in the Ku-ring-gai council area (red).

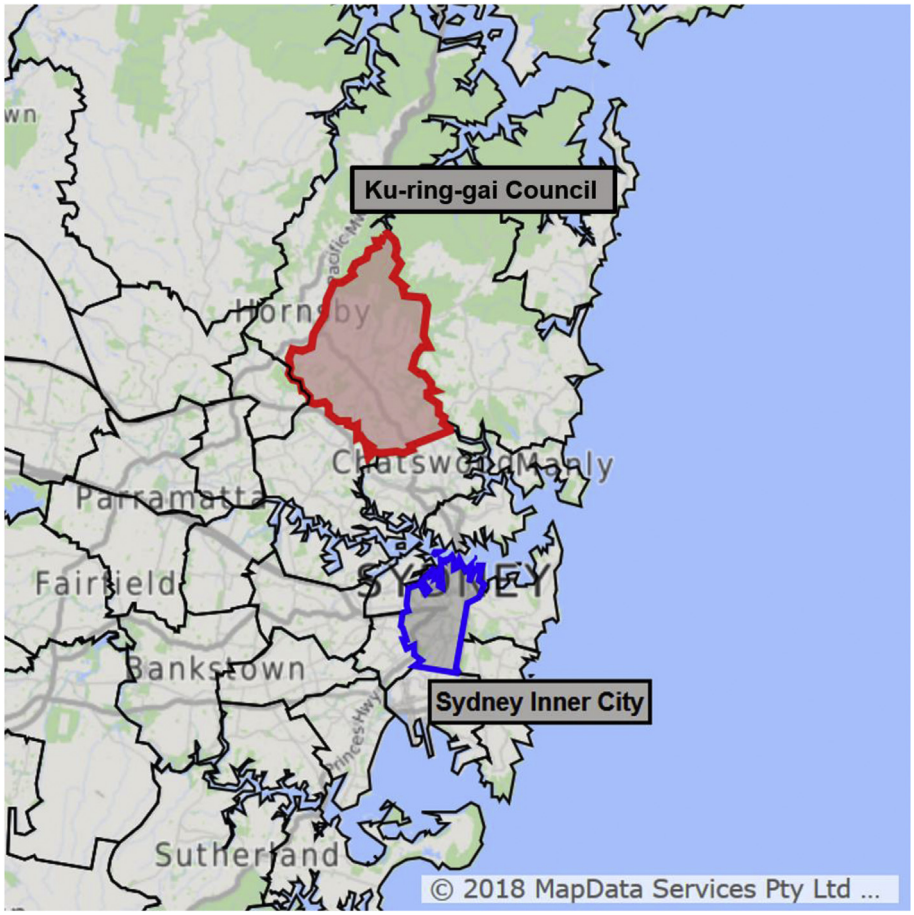

Fig. 1. (*continued*).

composition of bark-dwelling myxomycetes by shifting the assemblage to favour acid tolerant species (Wrigley de Basanta, 2000).

In the present study, we aimed to determine if myxomycetes were influenced by different degrees of urbanisation in Sydney, Australia. Specifically, we focused on the diversity of myxomyctetes within inner-city and semi-urban parks. We chose to study urban parks as they are key habitat refuges for many terrestrial species (Nielsen et al., 2014). While the vegetation of parks tends to be consistent across sites, the matrix in which they are embedded differs strongly from inner-city parks surrounded by a high degree of impervious surfaces, to semi-urban parks surrounded by bushland and large, tree-filled yards. We, therefore, determined whether the amount of green space surrounding each park (a proxy for urbanisation) influenced myxomycete species richness. We also examined the impact of a range of environmental characteristics (temperature, canopy cover, pH of substrate, substrate type) on myxomycete diversity.

2. Materials and methods

2.1. Site descriptions

Thirty-two parks were selected within the Sydney Basin bioregion in New South Wales, Australia (Fig. 1). The underlying geology is of horizontal sandstones and shales dating from the Permian to Triassic age (NSW Office of Environment and Heritage, 2016). Vegetation mainly consists of eucalypt forests, woodlands and heaths but also includes coastal landscapes such as cliffs, beaches and estuaries (NSW Office of Environment and Heritage, 2016). The climate is described as humid subtropical (NSW Office of Environment and Heritage, 2016).

We chose half our sites from the inner-city area and the other half from a semi-urban area. We characterised an inner-city area as having a high proportion of medium to high density housing with limited green space, whereas a semi-urban area had a lower proportion of medium to high density housing and consisted mainly of low density housing including large vegetated yards. We chose 16 sites from the inner-city area of Sydney (City of Sydney, Inner-west and Randwick councils) where a high proportion (78%) of residential properties were classed as medium to high density housing, including flats, apartments, townhouses and terrace houses (Australian Bureau of Statistics, 2016). We chose the remaining 16 sites from the Ku-ring-gai council area, which we classified as 'semi-urban' as a low proportion (6%) of the residential area consisted of medium to high density housing (Australian Bureau of Statistics, 2016). Ku-ring-gai council is located 16 km north of Sydney and is also surrounded by three national parks: Garigal National Park, Lane Cove National Park and Ku-ring-gai Chase National Park.

2.2. Sampling procedure

We sampled from two 1 m^2 plots in 16 parks from the inner-city

area of Sydney and 16 parks from the semi-urban Ku-Ring-Gai council (Fig. 1). We classified a park as a publicly available green space with an area larger than 500 m^2. Most of the parks had large expanses of grass surrounded by trees. Hence, a random selection process would bias our plot selection towards the grass substrate category, which often contained too little material for sampling. To avoid this issue, we used a two-step process to select suitable 1 m^2 plots. We first allocated a centre point for each park which served as the starting point and then randomly selected a compass direction between 1 and 360°. Using the compass direction as a heading, we walked until we came to an area with one or more trees. If no trees were available in the randomly selected direction, we chose again. Once we had decided on a suitable patch containing trees, we randomly generated a second compass direction and a number of steps between one and thirty to decide where to place the 1 m^2 sampling quadrat.

Within each 1 m^2 plot, we measured air temperature using a thermometer (Townson and Mercer), and estimated canopy cover by grading the percentage of light that was blocked directly above each plot. Rankings of 1–4 corresponded to increasing 25% increments of blocked light, with 1 being the lowest (full sun) and 4 being the highest (full shade).

We used 'area of surrounding green space' as a proxy for urbanisation. Area of surrounding green space was measured in a radius of 125 m around the centre point of each site using ImageJ and Google Earth images. We used the colour thresholding function in ImageJ to select and calculate the area within the radius that was green in colour (Schneider et al., 2012).

We collected a volume of 500 ml of four types of substrates at each plot: ground litter, dried twigs, leaves, and bark/branches. Ground litter consisted exclusively of leaf matter on the ground, whereas dried twigs were always branches or bark on the ground, depending upon what was available in the sample plot. Leaves and bark/branches were collected directly from a tree. In most cases, we selected the tree that was closest to the 1 m^2 plot. In the case that a tree was not present within 5 m of the plot, the closest shrub was chosen. Leaf and bark/branch samples were taken from between ground level and 170 cm. Bark was preferentially sampled from trees as previous diversity studies have shown high occurrence of myxomycete species in bark (Stephenson, 1989). However, many native Australian trees do not have easily detachable bark so when bark was unavailable we collected samples of branches instead. Samples were kept in paper bags and transported to the laboratory.

Samples were collected from April–July 2017.

2.3. Moist chamber cultivation

We measured myxomycete species richness from the four types of substrates using the moist chamber method (Stephenson and Stempen, 1994). Substrate samples were split evenly between two 145 mm petri dishes lined with filter paper. This meant we had a maximum of 16 moist chambers for each site (total $n = 426$), although some sites had fewer as they were missing certain substrate types or did not have enough substrate samples to fill two Petri dishes. Unless the substrates in the plot were sparse (ie. grass patches) there was enough substrate to cover the entire base area of the Petri dish. Petri dishes were filled with tap water and left closed for 24 h, after which we poured off the excess water and measured its pH. Previous studies have shown a high correlation between pH of the excess water and myxomycete diversity (Stephenson, 1989). Moist chambers were kept at constant conditions of 25 °C and 12 h of light per day, for 6 weeks. We misted the moist chambers with distilled water as required and checked for plasmodia or fruiting bodies every 2–3 d. Each fruiting body was carefully removed, dried and kept in matchboxes for later identification. If plasmodia were found, we transferred a section of the plasmodium to a 1% agar petri dish and fed them with oat flakes (Woolworths Homebrand, Bella Vista, NSW) and kept them in dark conditions. If the plasmodium established on the agar dish, it was exposed to sunlight to stimulate production of a fruiting body to allow identification. Fruiting bodies collected in this investigation are stored in the Invertebrate Ecology and Behaviour laboratory in the Heydon Laurence building (A08), University of Sydney.

2.4. Identification

Fruiting bodies were used to identify specimens to the species level where possible. We examined the morphological features of the fruiting bodies using a biological microscope (Olympus CH) and a stereomicroscope (Leica MZ7.5). To prepare slides for microscopic examination, we lightly crushed the fruiting body and fixed it to the slide using ethanol. We then applied 3% KOH solution so that the cell features would expand to a larger size. This allowed us to see the details of the spores and capillitium, which are the main features used to differentiate between species within a genus. We used keys from Martin and Alexopoulos (1969) to identify our specimens. We cross-referenced identifications with images from Discoverlife (https://www.discoverlife.org), an online database which includes myxomycete species (Stephenson, 2017). For species that were particularly difficult to identify, we sent images to an expert on Australian myxomycetes (Sarah Lloyd, pers. com.). Some plasmodia did not develop fruiting bodies. We recorded these instances as myxomycete presence for the sites but did not attempt to identify them as plasmodial morphology is a poor indicator of species identity.

2.5. Statistical analysis

We calculated myxomycete species richness (total number of species) for each site. We used a poisson regression model to analyse the effect of environmental characteristics on species richness in our sites. The model included temperature, canopy cover, pH of substrate, substrate type, and the area of surrounding green space around each site. We checked for goodness of fit by looking at the residual deviance. We used a binomial generalised linear model with a logit link function to analyse the effect of environmental characteristics on the presence or absence of myxomycetes. We used the Hosmer and Lemeshow goodness of fit test to check the goodness of fit of the model (Lele et al., 2017). Unless otherwise specified, we used the Rcommander package (Fox, 2005) in R to perform statistical analyses (R Core Team, 2013).

To assess the impact of urbanisation and substrate type on myxomycete species composition, we compared the similarities between species composition of inner-city and semi-urban parks, as well as between our four substrates: ground litter, dried twigs, bark or branches and leaves. We used a Bray-Curtis matrix of dissimilarity to analyse differences in species composition between inner-city and semi-urban parks, as well as between substrates. Bray-Curtis calculates dissimilarity on a spectrum where a value less than 0.5 shows similarity and a value greater than 0.5 shows dissimilarity. Similarity analyses were conducted using the vegan package in R (Oksanen et al., 2007).

3. Results

3.1. Diversity

We found myxomycetes in 203 out of 426 moist chambers. We were able to identify 123 specimens as fruiting bodies down to the species level. The remaining 80 myxomycete specimens were

Table 1
Effect of environmental characteristics on species richness using poisson regression models. Significant values are highlighted in bold. Substrate type key DT: dried twigs. GL: ground litter. B: Bark/branches.

	Estimate	Std. Error	$\chi 2$	Pr(>$\chi 2$)
area of green space	-1.618×10^{-5}	1.15×10^{-5}	1.91	0.1669
canopy cover	−0.03	0.05	0.23	0.63
pH of substrate	4.581×10^{-3}	0.09	0	1
substrate[DT]	0.074	0.09	1.78	0.62
substrate[GL]	0.017	0.105		
substrate[B]	0.128	1.08		
air temperature	2.076×10^{-2}	0.019	1.13	0.287
urban[city]	−0.96	0.11	0.72	0.393

plasmodia that could not be identified because they did not form fruiting bodies in either the moist chamber or in the agar plate.

None of the environmental characteristics we assessed significantly affected species richness of myxomycetes in our sites (all $P > 0.05$, Table 1). The only environmental characteristic that affected presence or absence of myxomycetes in our sites was temperature such that higher temperatures in sites significantly lowered the presence of myxomycetes ($P = 0.015$, Table 2). The mean temperature recorded in our sites was 16.8 °C ± 0.15. The minimum temperature was 10 °C and the maximum temperature was 23 °C.

3.2. Species composition

We identified 23 distinct species of myxomycetes - 16 to the species level and 7 to the genus level. Our species were distributed among five families: Acryciaceae (1), Didymiaceae (15), Physaraceae (9), Stemonitidaceae (6) and Reticulaceae (1) (Table 3). Species composition between inner-city and semi-urban parks were similar (Bray-Curtis: 0.398). Species composition between substrates showed dissimilarity (Figs. 2 and 3). The species composition of myxomycetes found on leaves was dissimilar from all other substrates, whereas species composition on dried twigs and bark/branches were similar. Ground litter substrates also had a dissimilar myxomycete species composition compared to all other substrates, although the dissimilarity was not as strong.

4. Discussion

We found no evidence that the diversity or assemblage composition of myxomycetes differed between inner-city and semi-urban parks. Our finding agrees with previous work on urban assemblages of soil-dwelling organisms which did not find differences in diversity between different degrees of urbanisation (Kaye et al., 2005; Pavao-Zuckerman and Coleman, 2007). However, at least one study found an increased microbial biomass in more urban sites (Kaye et al., 2005). Although we did not detect an impact of the degree of urbanisation on myxomycete diversity, it is possible that urbanisation could impact the abundance of myxomycetes. Abundance is difficult to assess via the moist chamber method as one plasmodium can form multiple fruiting bodies. Other techniques, such as culturing and counting 'plasmodium forming units' would be a better approach for studies aiming to assess the effect of urbanisation on abundance.

Table 2
Effect of environmental characteristics on presence or absence using a binomial generalised linear model with a logit link function. Significant values are highlighted in bold. Substrate type key DT: dried twigs. GL: ground litter. B: Bark/branches.

	Estimate	Std. Error	$\chi 2$	Pr(>|$\chi 2$|)
area of green space	-3.903×10^{-5}	2.749×10^{-5}	2.02	0.156
canopy cover	0.103	0.13	0.63	0.425
pH of substrate	−0.11	0.21	0.33	0.559
substrate[DT]	0.08	0.23	7.14	0.068
substrate[GL]	−0.19	0.45		
substrate[B]	0.55	0.23		
air temperature	−0.09	0.04	4.65	**0.032**
urban[semi-urban]	4.635×10^{-1}	5.338×10^{-1}	0.745	0.385

Table 3
Species list of both inner-city and semi-urban parks in the Sydney Basin bioregion. Known distributions of the species are denoted in the following codes: "Aus" shows presence in Australian species list, "NSW" shows presence in New South Wales species list.

Species	Family	Order	Distribution
Arcyria cinerea	Arcyriaceae	Trichiales	NSW, Aus
Badhamiopsis ainoae	Physaraceae	Physarales	NSW, Aus
Colloderma oculatum	Stemonitidaceae	Stemonitales	–
Comatricha longipila	Stemonitidaceae	Stemonitales	Aus
Diachea leucopodia	Didymiaceae	Physarales	NSW, Aus
Diachea megalospora	Didymiaceae	Physarales	–
Diachea spp 1	Didymiaceae	Physarales	n/a
Dictydiaethalium plumbeum	Reticulariaceae	Liceales	NSW, Aus
Diderma effusum	Didymiaceae	Physarales	NSW, Aus
Diderma spp 1	Didymiaceae	Physarales	n/a
Didymium intermedium	Didymiaceae	Physarales	Aus
Didymium iridis	Didymiaceae	Physarales	NSW, Aus
Didymium nigripes	Didymiaceae	Physarales	NSW, Aus
Didymium ovoideum	Didymiaceae	Physarales	–
Didymium squamulosum	Didymiaceae	Physarales	NSW, Aus
Didymium verrucosporum	Didymiaceae	Physarales	NSW, Aus
Didymium spp 1	Didymiaceae	Physarales	n/a
Didymium spp 2	Didymiaceae	Physarales	n/a
Didymium spp 3	Didymiaceae	Physarales	n/a
Didymium spp 4	Didymiaceae	Physarales	n/a
Hemitrichia calyculata	Trichiaceae	Trichiales	Aus
Lamproderma cucumer	Stemonitidaceae	Stemonitales	NSW, Aus
Lamproderma scintillans	Stemonitidaceae	Stemonitales	NSW, Aus
Macbrideola spp	Stemonitidaceae	Stemonitales	NSW, Aus
Physarum bivalve	Physaraceae	Physarales	NSW, Aus
Physarum compressum	Physaraceae	Physarales	NSW, Aus
Physarum leucopus	Physaraceae	Physarales	NSW, Aus
Physarum melleum	Physaraceae	Physarales	NSW, Aus
Physarum nicaraguense	Physaraceae	Physarales	NSW, Aus
Physarum nudum	Physaraceae	Physarales	Aus
Physarum pusillum	Physaraceae	Physarales	NSW, Aus
Stemonitis virginiensis	Stemonitidaceae	Stemonitales	Aus
Willkommlangea reticulata	Physaraceae	Physarales	NSW, Aus

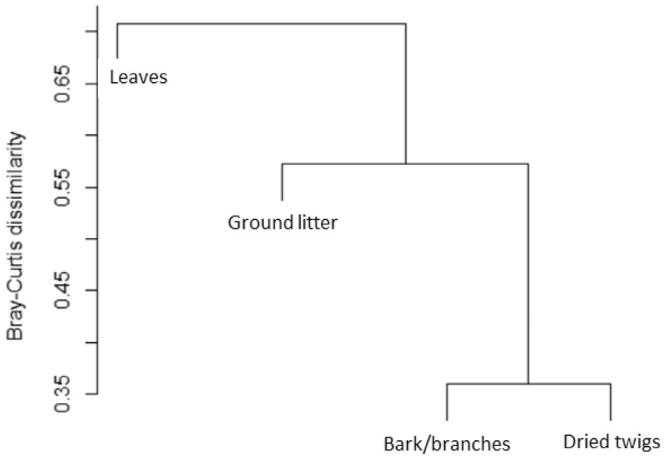

Fig. 2. Bray-Curtis dissimilarity dendrogram of species composition between substrate types. Bray-Curtis dissimilarity of 0 shows complete similarity between species composition and a value of 1 shows complete dissimilarity.

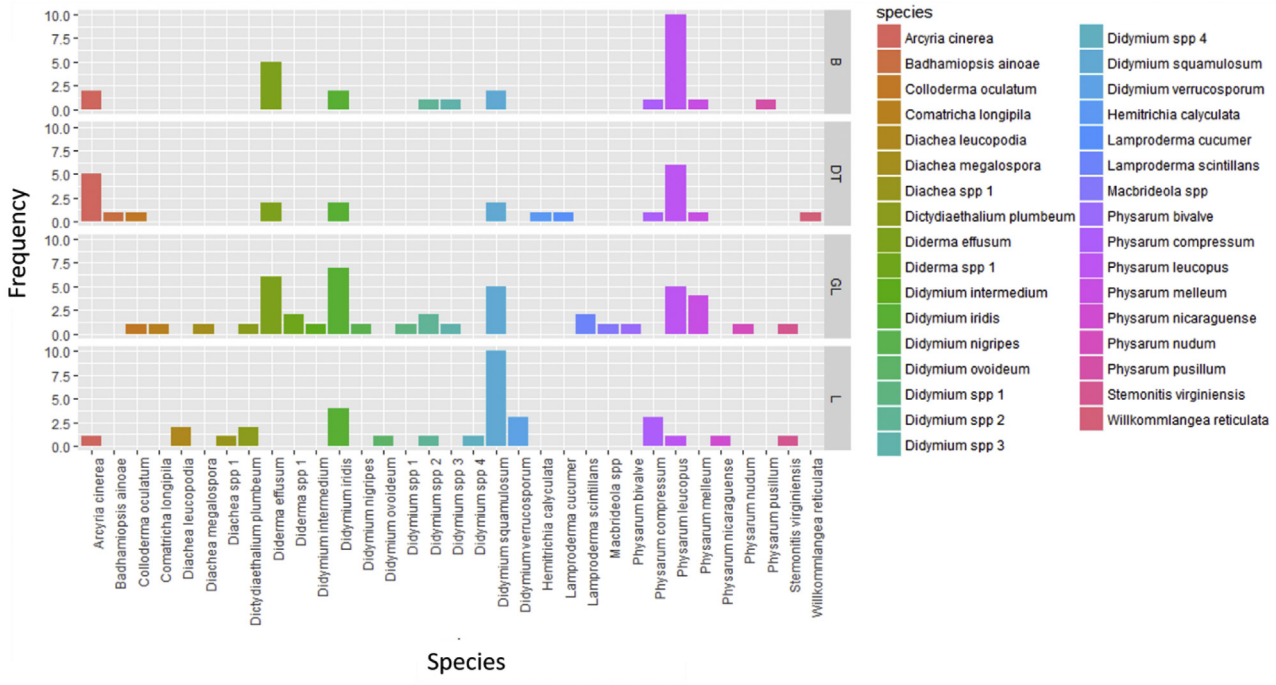

Fig. 3. Species compositions on different substrates. B = bark, DT = dried twigs, GL = ground litter and L = leaves.

We chose to compare inner-city parks to semi-urban parks because doing so allowed us to assess the effect of urbanisation whilst largely controlling for site-level differences in vegetation. While it would be interesting to examine differences between protected areas and urbanised sites, the vegetation differences would be significant, and this could obscure the effects of urbanisation per se (fragmentation, increased impervious surfaces, higher levels of air and soil pollution). Future studies might compare the diversity of myxomycetes found within the city to other landscape types such as agricultural fields and protected areas.

We found dissimilarities in myxomycete species composition between our substrates with leaves and ground litter having unique assemblages, while barks, twigs and branches had similar assemblages. Leaves and ground litter were dominated by *Didymium squamulosum, Didymium iridis* and *Dictydiaethalium plumbeum* while bark, twigs and branches were dominated by *Physarum leucopus, Diderma effusum* and *Arcyria cinerea* (Fig. 3). The different species compositions on different substrate types in an urban environment agree with the established patterns seen in natural environments (Stephenson, 1989; Stephenson et al., 2008; Rojas et al., 2014). Substrates are often viewed as microhabitats, with certain species showing preferences to particular substrate types (Novozhilov et al., 2006). Whether or not the species composition patterns we observed are the result of active habitat selection by slime mould plasmodia is unknown. Alternatively, different species compositions on different substrates could occur if spores settle randomly, but some species have higher substrate-dependent mortality rates than others. The mechanism underlying species composition on different substrates would be an excellent avenue for future research.

Despite differences in myxomycete species composition at the substrate level, the environmental characteristics we measured (pH of substrates, air temperature, canopy cover, area of surrounding greens space and substrate type) did not affect species richness. Air temperature, however, had a significant effect on myxomycete presence such that higher numbers of myxomycetes were collected from cooler places than from hotter places. Annual temperature and air temperature has also been used to explain the patterns of succession of lignicolous myxomycete species assemblages (Kazunari, 2010).

There is evidence that seasonality affects the abundance of myxomycetes. Moist chamber yield was significantly higher in dry seasons compared to rainy seasons in tropical environments (Tran et al., 2008; Dagamac et al., 2012). In temperate environments, moist chamber yield was highest in late winter (Stephenson, 1988). Our sampling took place during Austral winter, when temperatures are at their lowest. It is possible that diversity might change throughout the year, and future studies could address the seasonal variation in myxomycete species within urban areas.

It is interesting to note that the pH of substrates did not affect myxomycete diversity in our study. pH is widely accepted to be a main driver in myxomycete species distribution and abundance (Stephenson, 1989; Schnittler and Stephenson, 2000). Species composition can depend on the pH of substrate, with acid-tolerant species being favoured among corticolous types (Wrigley de Basanta, 2000). Species-specific pH ranges have also been described in species such as *Arcyria cinera* which has a wide optimum pH range (Kilgore et al., 2009). Since most of our samples tended to be acidic (see Table 4), it is possible that the substrates did not have a sufficiently large pH range to pick up differences in the myxomycete community.

The overall yield of myxomycetes in our study (40% of moist chambers yielded plasmodia) was lower than has been previously reported for studies in temperate environments. Tropical environments have been found to have a lower diversity of myxomycetes on bark than do temperate environments climates (Stephenson, 2011) which could partially explain our low yields. Indeed, our yields are higher than that of Dagamac et al. (2012) from a tropical forest in the Philippines but are considerably lower than results from tropical Northern Queensland in Australia (90% yield; Black et al., 2004). The reasons for variation in moist chamber yields are unclear and further research is needed to determine whether low

Table 4
Summary of moist chamber yields and pH between substrate types: leaves, ground litter, dried twigs and bark/branches.

Substrate type	No. of cultures	Positive (%)		No. of species	pH	
		Plasmodia	Fruiting Bodies		Mean	Range
Leaves	100	45	33	13	6.56	5.35–7.34
Ground Litter	115	63	38	17	6.01	4.22–7.51
Dried Twigs	110	34	22	13	6.22	4.65–7.30
Bark/branches	101	51	27	9	6.4	3.78–7.56

yields reflect methodological differences or are a true reflection on lower myxomycetes abundance in certain sites. It is possible that the low yield we experienced was due to the urbanised nature of our field sites, all of which were within Greater Sydney metropolitan area. There has been little investigation of urban myxomycete diversity (especially in the subtropics), so it is unclear whether our yield is unusual. Future research could determine if this was the case by comparing myxomycetes diversity along an urbanisation gradient.

Our results could have been influenced by the collection techniques we used to sample myxomycetes diversity (moist chambers). The moist chamber method is the most widely used approach to investigate myxomycete diversity. It has the benefit of causing spores to germinate so that samples can be collected even when there are no obvious fruiting bodies present. However, it is constrained by the need to get plasmodia to fruit to identify individuals to the species level. It has been pointed out that ecological studies of myxomycetes are constrained by our inability to identify non-fruiting individuals so that moist chamber studies and field collections likely only show a small fraction of actual myxomycete diversity (Novozhilov et al., 2017). In our study, 70% of plasmodia collected in August failed to fruit compared to 36% that failed to fruit in June and the 39% that failed to fruit in July. The low fructification rate thus limited our ability to identify plasmodia to species. Given that all cultures were kept under the same light regime, temperature and humidity, the reasons for the different fruiting rates are unclear but could perhaps be related to seasonal differences in plasmodial behaviour.

Recently, more biodiversity studies have used molecular methods to investigate diversity. These have the considerable benefit of not requiring plasmodia to fruit for identification. Although rapidly improving, molecular methods are not yet able to identify the majority of myxomycete morphospecies. Methods of molecular barcoding using SSU rRNA gene sequences are being developed for myxomycetes, however, due to the large divergence between bright-spored and dark-spored species, a universal primer has not been developed for myxomycetes (Borg Dhal et al. 2018a). Near universal primers for dark-spored myxomycetes are available and have been used in several recent diversity studies using environmental sampling (Kamono et al., 2012; Fiore-Donno et al. 2016; Borg Dhal et al. 2018b). There is only reliable information on the partial SSU sequences of a minor proportion of myxomycete species, which makes it difficult to understand the species diversity of myxomycetes solely through environmental sampling techniques (Schnittler et al., 2017). Until more molecular data is collected on a larger number of myxomycete species, the moist chamber method remains one of the most reliable and effective methods of investigating species richness of myxomycetes. Nevertheless, using a combination of methods (e.g. moist chamber + field sampling) may help overcome some of the limitations of the moist chamber method.

Of the 23 species of myxomycetes we found, three had never before been recorded in Australia (*Colloderma oculatum, Diachea megalospora,* and *Didymium ovoideum*) and five had never been recorded in NSW (*Comatricha longipila, Didymium intermedium, Hemitrichia calyculata, Physarum nudum,* and *Stemonitis virginiensis*). Myxomycete diversity in Australia is relatively understudied. The most recent comprehensive species list for Australia includes 177 species with data collected from Queensland, Northern Territory, South Australia, Tasmania and Western Australia (McHugh et al., 2009). Species lists for several sites in NSW have also been published, but none are from the Sydney Basin bioregion (Stephenson and Shadwick, 2009; Leontyev et al., 2014; Wellman, 2017). It is likely that the diversity of myxomycete species is higher as approximately 40% of myxomycete samples could not be identified because they did not form fruiting bodies. Future studies might use molecular tools to allow identification of plasmodia which do not produce fruiting bodies as shown in Shchepin et al. 2017. Field surveys may also be of interest to detect larger fruiting bodies that cannot develop through the moist chamber technique. Further research is desperately needed to understand the biodiversity of myxomycetes in Australia, particularly in the less-studied urbanised areas, so that we can accurately assess the human impact on these important micro-organisms.

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References

Australian Bureau of Statistics, 2016. 2016 census QuickStats. http://www.abs.gov.au/websitedbs/D3310114.nsf/Home/census. (Accessed 10 October 2017).
Beninde, J., Veith, M., Hochkirch, A., 2015. Biodiversity in cities needs space: a meta-analysis of factors determining intra-urban biodiversity variation. Ecol. Lett. 18, 581–592.
Black, D.R., Stephenson, S.L., Pearce, C.A., 2004. Myxomycetes associated with the aerial litter microhabitat in tropical forests of northern Queensland, Australia. Systematics and Geography of Plants 74, 129–132.
Borg Dahl, M., Brejnrod, A.D., Unterseher, M., Hoppe, T., Feng, Y., Novozhilov, Y.K., Sørensen, S.J., Schnittler, M., 2018a. Genetic barcoding of dark-spored myxomycetes (Amoebozoa) – identification, evaluation and application of a sequence similarity threshold for species differentiation in NGS studies. Molecular Ecology Resources 18 (2), 306–318.
Borg Dahl, M., Shchepin, O.N., Schunk, C., Menzel, A., Novozhilov, Y.K., Schnittler, M., 2018b. The fruits reflect the forest – a four year survey reveals a coherent pattern between distribution of fruit bodies and soil amoebae populations for nivicolous myxomycetes in the German Alps. Sci. Rep. 8, 11662.
R Core Team, 2013. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing. http://www.R-project.org/.
Dagamac, N.H.A., Stephenson, S.L., Dela Cruz, T.E.E., 2012. Occurrence, distribution and diversity of myxomycetes (plasmodial slime moulds) along two transects in Mt. Arayat National Park, Pampanga, Philippines. Mycology 3, 119–126.
Eliasson, U., 2013. Coprophilous myxomycetes: recent advances and future research directions. Fungal Divers. 59, 85–90.
Estrada-Torres, A., Wrigley De Basanta, D., Conde, E., Lado, C., 2009. Myxomycetes associated with xerophyllous scrubland of the Tehuacán-Cuicatlán valley biosphere reserve, Mexico. Fungal Divers. 36, 17–56.
Faeth, S.H., Bang, C., Saari, S., 2011. Urban biodiversity: patterns and mechanisms. Ann. N. Y. Acad. Sci. 1223, 69–81.
Feest, A., 1987. The quantitative ecology of soil mycetozoa. Prog. Protistol. 2, 331–361.
Feest, A., Madelin, M., 1985. A method for the enumeration of myxomycetes in soils

and its application to a wide range of soils. FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Ecol. 1, 103–109.
Fiore-Donno, A.M., Weinert, J., Wubet, T., Bonkowski, M., 2016. Metacommunity analysis of amoeboid protists in grassland soils. Sci. Rep. 6, 19068.
Fox, J., 2005. Getting started with the R commander: a basic-statistics graphical user interface to R. J. Stat. Software 14, 1–42.
Grimm, N.B., Faeth, S.H., Golubiewski, N.E., Redman, C.L., Wu, J., Bai, X., Briggs, J.M., 2008. Global change and the ecology of cities. Science 319, 756–760.
Ing, B., 1998. Corticolous myxomycetes from central London. Lond. Nat. 77, 83–90.
Kamono, A., Matsumoto, J., Kojima, H., Fukui, M., 2009. Characterization of myxomycete communities in soil by reverse transcription polymerase chain reaction (RT-PCR)-based method. Soil Biol. Biochem. 41, 1324–1330.
Kamono, A., Meyer, M., Cavalier-Smith, T., Fukui, M., Fiore-Donno, A.M., 2012. Exploring slime mould diversity in high-altitude forests and grasslands by environmental RNA analysis. FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Ecol. 84, 98–109.
Kaye, J., McCulley, R., Burke, I., 2005. Carbon fluxes, nitrogen cycling, and soil microbial communities in adjacent urban, native and agricultural ecosystems. Global Change Biol. 11, 575–587.
Kazunari, T., 2010. Succession in myxomycete communities on dead *Pinus densiflora* wood in a secondary forest in southwestern Japan. Ecol. Res. 25, 995–1006.
Kilgore, C.M., Keller, H.W., Ely, J.S., 2009. Aerial reproductive structures of vascular plants as a microhabitat for myxomycetes. Mycologia 101, 305–319.
Lado, C., Eliasson, U., 2017. Taxonomy and systematics: current knowledge and approaches on the taxonomic treatment of myxomycetes. Myxomycetes. Elsevier 205–251.
Lele, S.R., Keim, J.L., Solymos, P., Solymos, M.P., 2017. ResourceSelection: resource selection (probability) functions for use-availability data. R package version 0.3-2. https://CRAN.R-project.org/package=ResourceSelection.
Leontyev, D.V., Stephenson, S.L., Schnittler, M., 2014. A new species of alwisia (myxomycetes) from New South Wales and Tasmania. Mycologia 106, 1212–1219.
Macabago, S.A.B., Dagamac, N.H.A., Dela Cruz, T.E.E., Stephenson, S.L., 2017. Implications of the role of dispersal on the occurrence of litter-inhabiting myxomycetes in different vegetation types after a disturbance: a case study in Bohol Islands, Philippines, 104. Nova Hedwigia, pp. 221–236.
Martin, G.W., Alexopoulos, C.J., 1969. The Myxomycetes, vol. 61. University of Iowa Press, Iowa City.
McHugh, R., Mitchell, D.W., Brims, M.H., Stephenson, S.L., 2009. New additions to the myxomycota of Australia. Australasian Mycologist 28, 56–64.
Nielsen, A.B., Bosch, M., Maruthaveeran, S., Bosch, C.K., 2014. Species richness in urban parks and its drivers: a review of empirical evidence. Urban Ecosyst. 17, 305–327.
Novozhilov, Y.K., Schnittler, M., Stephenson, S.L., 1999. Myxomycetes of the Taimyr peninsula (north-central Siberia). Karstenia 39, 77–97.
Novozhilov, Y.K., Zemlianskaia, I.V., Schnittler, M., Stephenson, S.L., 2006. Myxomycete diversity and ecology in the arid regions of the lower Volga river basin (Russia). Fungal Divers. 23, 193–241.
Novozhilov, Y.K., Schnittler, M., Erastova, D.A., Shchepin, O., 2017. Myxomycetes of the Sikhote-alin State Nature Biosphere Reserve (Far East, Russia), vol. 104. Nova Hedwigia, pp. 183–209.
NSW Office of Environment and Heritage, 2016. Sydney Basin bioregion. http://www.environment.nsw.gov.au/bioregions/SydneyBasinBioregion.htm. (Accessed 15 August 2017).
Oksanen, J., Kindt, R., Legendre, P., O'Hara, B., Stevens, M.H.H., Oksanen, M.J., Suggests, M., 2007. Vegan: community ecology package. R package version 2.5-2. https://CRAN.R-project.org/package=vegan.
Pavao-Zuckerman, M.A., Coleman, D.C., 2007. Urbanisation alters the functional composition, but not taxonomic diversity, of the soil nematode community. Appl. Soil Ecol. 35, 329–339.
Rojas, C., Stephenson, S., 2008. Myxomycete ecology along an elevation gradient on Cocos Island, Costa Rica. Fungal Divers. 29, 117–127.
Rojas, C., Stephenson, S.L., 2013. Effect of forest disturbance on myxomycete assemblages in the southwestern Peruvian Amazon. Fungal Divers. 59, 45–53.
Rojas, C., Rollins, A.W., Stephenson, S.L., 2014. Distribution of myxomycetes among the microhabitats available for these organisms in tropical forests. In: Fungi from Different Substrates. CRC Press, Boca Raton, pp. 126–143.
Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of image analysis. Nat. Methods 9, 671–675.
Schnittler, M., Stephenson, S.L., 2000. Myxomycete biodiversity in four different forest types in Costa Rica. Mycologia 92, 626–637.
Schnittler, M., Stephenson, S.L., 2002. Inflorescences of Neotropical herbs as a newly discovered microhabitat for myxomycetes. Mycologia 94, 6–20.
Schnittler, M., Shchepin, O., Dagamac, N.H.A., Borg Dahl, M., Novozhilov, Y.K., 2017. Barcoding myxomycetes with molecular markers: challenges and opportunities. Nova Hedwigia 104, 323–341.
Shchepin, O., Novozhilov, Y., Schnittler, M., 2014. Nivicolous myxomycetes in agar culture: some results and open problems. Protistology 8 (2), 53–61.
Shchepin, O.N., Dagamac, N.H., Sanchez, O.M., Novozhilov, Y.K., Schnittler, M., Zemlyanskaya, I.V., 2017. DNA barcoding as a tool for identification of plasmodia and sclerotia of myxomycetes (Myxogastria) appearing in moist chamber cultures. Mycosphere 8 (10), 1904–1913.
Smith, T., Stephenson, S.L., 2007. Algae associated with myxomycetes and leafy liverworts on decaying spruce logs. Castanea 72, 50–57.
Stephenson, S.L., 1988. Distribution and ecology of myxomycetes in temperate forests. I. Patterns of occurrence in the upland forests of southwestern Virginia. Can. J. Bot. 66, 2187–2207.
Stephenson, S.L., 1989. Distribution and ecology of myxomycetes in temperate forests. II. Patterns of occurrence on bark surface of living trees, leaf litter, and dung. Mycologia 81, 608–621.
Stephenson, S.L., 2011. From morphological to molecular: studies of myxomycetes since the publication of the Martin and Alexopoulos (1969) monograph. Fungal Diversity 50, 21–34.
Stephenson, S.L., 2017. Myxomycetes - identification guide. http://www.discoverlife.org/20/q?guide=Myxomycetes. (Accessed 25 August 2017).
Stephenson, S.L., Shadwick, J.D., 2009. Nivicolous myxomycetes from alpine areas of south-eastern Australia. Aust. J. Bot. 57, 116–122.
Stephenson, S.L., Stempen, H., 1994. Myxomycetes: a Handbook of Slime Molds. Timber Press, Portland.
Stephenson, S.L., Schnittler, M., Novozhilov, Y.K., 2008. Myxomycete diversity and distribution from the fossil record to the present. Biodivers. Conserv. 17, 285–301.
Takahashi, K., Hada, Y., 2009. Distribution of Myxomycetes on coarse woody debris of *Pinus densiflora* at different decay stages in secondary forests of western Japan. Mycoscience 50, 253–260.
Tran, H.T., Stephenson, S.L., Hyde, K.D., Mongkolporn, O., 2008. Distribution and occurrence of myxomycetes on agricultural ground litter and forest floor litter in Thailand. Mycologia 100, 181–190.
United Nations, 2012. World Urbanisation Prospects, the 2011 Revision. Final Report with Annex Tables. United Nations Department of Economic and Social Affairs, New York, NY.
Wellman, P., 2017. Distinctive corticolous myxomycete assemblages of tropical, temperate and arid regions of Australia. Cunninghamia 16, 101–114.
Wrigley de Basanta, D., 2000. Acid deposition in Madrid and corticolous myxomycetes. Stapfia 73, 113–120.
Wrigley de Basanta, D., Stephenson, S., Lado, C., Estrada-Torres, A., Nieves-Rivera, A., 2008. Lianas as a microhabitat for myxomycetes in tropical forests. Fungal Divers. 28, 109–125.