

A survey of the aeromycota of Sydney and its correspondence with environmental conditions: grass as a component of urban forestry could be a major determinant

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Received: 26 January 2015 / Accepted: 13 May 2015
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Abstract A comprehensive survey of airborne fungi has been lacking for the Sydney region. This study determined the diversity and abundance of outdoor airborne fungal concentrations in urban Sydney. Monthly air samples were taken from 11 sites in central Sydney, and culturable fungi identified and quantified. The genus *Cladosporium* was the most frequently isolated fungal genus, with a frequency of 78 % and a mean density of 335 CFU m⁻³. The next most frequently encountered genus was *Alternaria*, occurring in 53 % of samples with a mean of 124 CFU m⁻³. Other frequently identified fungi, in decreasing occurrence, were as follows: *Penicillium*, *Fusarium*, *Epicoccum*, *Phoma*, *Acremonium* and *Aureobasidium*. Additionally, seasonal and spatial trends of airborne fungi were assessed, with increases in total culturable fungal concentrations experienced in the summer months. The correspondence between a range of key environmental variables and the phenology of airborne fungal propagules was also examined, with temperature, wind speed and proximal greenspace having the largest influence on fungal propagule density. If the greenspace was comprised of grass, stronger associations with fungal behaviour were observed.

Keywords Airborne fungi · Bioaerosols · Seasonal · Asthma · Allergy · Risk assessment · Public health

1 Introduction

Airborne fungal propagules are ubiquitous and constitute a large proportion of the biological matter that is present in the air (Bauer et al. 2008). Exposure to this fungal material can elicit an array of health complications, ranging from exacerbating asthma (Tham et al. 2014) to allergic alveolitis (Bush 2009), and even systemic disease (Crook and Burton 2010).

In Australia, *Cladosporium* and *Alternaria* are the two most common airborne fungal genera, and the volume of material inhaled from either genus can be very high, even during non-conductive outdoor conditions, with individuals inhaling up to 794 *Cladosporium* spores h⁻¹ and 396 *Alternaria* spores h⁻¹ in urban environments (Mitakakis et al. 2000). Such exposure can induce allergic rhinitis symptoms in sensitized individuals (Andersson et al. 2003).

As these organisms pose a potential human health risk, documentation of variations in the numbers and taxonomic composition of the mycobiota is of importance to public health (Denning et al. 2006). The propagule density and species composition of airborne fungi vary substantially amongst studies made in different parts of the world (Fröhlich-Nowoisky et al. 2012; Kemp et al. 2003; Oliveira et al. 2009), thus making local surveys imperative. It is thought that

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variations amongst geographical locations occur because of local environmental conditions, varying growth substrates and human activities (Gots et al. 2003). These contributing variables can differ greatly from region to region, relating to human population, usage and climatic influences and differences between urban and rural areas (Simeray et al. 1993). Thus, the relevance of each survey is exclusive to the area from which samples were collected and any associated site-specific environmental and temporal conditions.

In Australia, the diversity of airborne fungi has been relatively well documented for most cities with studies in Brisbane (Hargreaves et al. 2003; Rutherford et al. 1997), Melbourne (CSIRO 2010; Derrick and McLennan 1963; Dharmage et al. 1999; Mitakakis et al. 1997; Mitakakis and Guest 2001), Adelaide (Gaskin et al. 2012) and Perth (Kemp et al. 2003), as well as rural studies in Northern NSW (Green et al. 2006), Moree and Wagga Wagga (Mitakakis and McGee 2000; Mitakakis et al. 2001) and the La Trobe valley (Garrett et al. 1997).

A comprehensive survey of airborne fungi is lacking for the Sydney region (Burton and Katelaris 2014), although a number of studies have been undertaken on specific spore types. Stennett and Beggs (2004) documented peak atmospheric *Alternaria* spore concentrations in late summer periods for Sydney, with positive associations detected between *Alternaria* spores and increased temperature as well as air pressure. Bass and Morgan (1997) similarly noted that *Alternaria* spores in Western Sydney were mainly prevalent in late spring and summer.

Only three studies on the diversity of airborne fungi have been conducted in Sydney. The first, Frey and Durie (1960), addressed the species composition of airborne moulds in Sydney by sampling agar plates left about in open areas. However, it has since been established that more buoyant fungal spores, including potential pathogens such as *Aspergillus* and *Penicillium* spp. (Bunse and Merk 1992), do not penetrate the agar's boundary layer (Dix and Webster 1995) and thus will be underestimated by this method. Accordingly, these genera, and an unknown number of others, may have been severely underestimated by Frey and Durie (1960). Burton and Katelaris (2014) surveyed airborne fungal spores with a focus on *Didymella* spp. and *Coprinus* spp.; however, their investigation was limited to an area 54 km from the city's centre. In a third Sydney study, Torpy et al. (2013) assessed the

relationship between indoor plants and indoor aeromycota. Seasonal indoor air samples from the offices of two buildings in Sydney were compared to offices in the same buildings with plants present. Outdoor air samples were taken in order to act as reference samples only; thus, this study's conclusion is limited to the locations of the buildings rather than establishing a general survey of the density and community structure of the aeromycota in Sydney.

The research presented here aimed to provide a comprehensive survey of the ecology and phenology of the aeromycota of inner Sydney. The objectives of this study, therefore, were to:

- Determine the diversity and abundance of outdoor airborne fungal concentrations for urban Sydney
- Examine seasonal trends of airborne fungi in urban Sydney
- Investigate the spatial variability of the aeromycota across urban Sydney
- Determine the correspondence between a range of key environmental variables and the diversity and abundance of airborne fungi

2 Methods and materials

2.1 Study area

Sydney, Australia, is a city with a population of 4.5 million and lies on a coastal lowland plain between the Pacific Ocean and elevated sandstone tablelands. The climate for Sydney is warm and temperate. Days in which rainfall events occur are evenly distributed throughout the year for Sydney; however, rainfall volume is maximal in autumn (March–May).

2.2 Sample sites

All samples were taken between September 2013 and August 2014. Monthly samples were collected across eleven sites within the City of Sydney Local Government Area (LGA). Sites were selected to provide a range of different conditions with respect to population and traffic density and usage, and greenspace/urban forest density (Fig. 1) and are detailed below:

- *Newtown* (−33.9108, 151.2074) is a medium-density residential and parkland area. The quantity

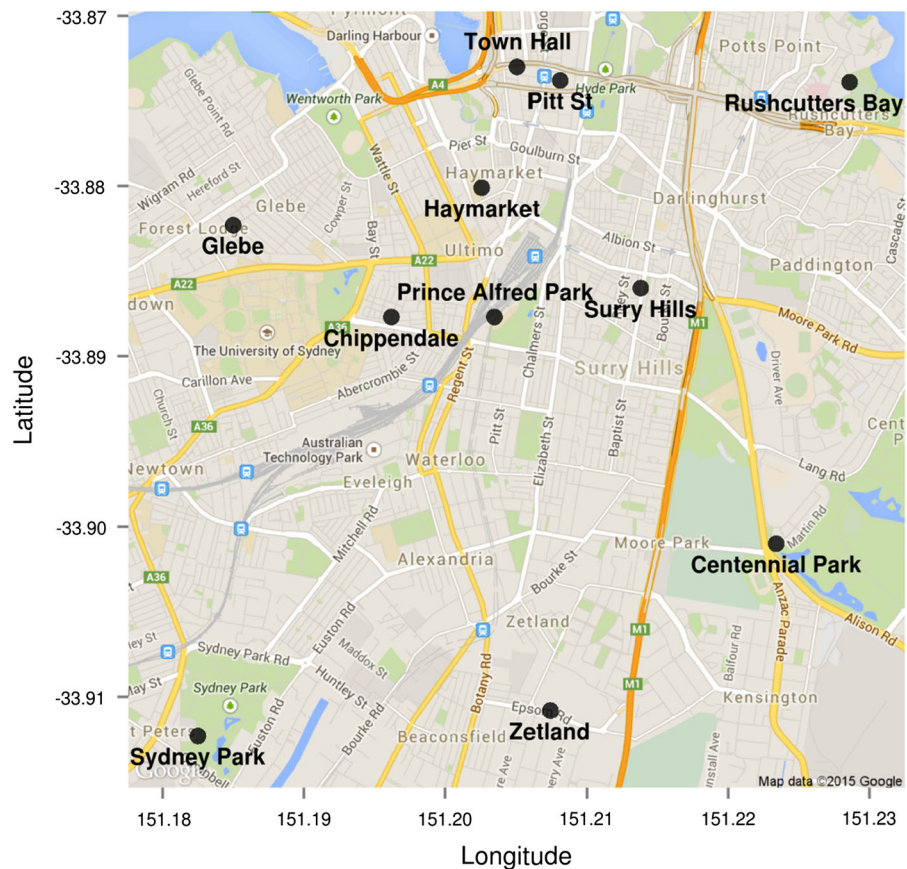
of greenspace of Newtown is relatively high, with >20 % greenspace cover. The greenspace at this site was comprised mainly of grassed lawns and a combination of the following tree species: *Casuarina* spp., *Corymbia maculata*, *Angophora costata* and various eucalypt species, e.g. *Eucalyptus sideroxylon*.

- **Centennial Park** (−33.9010, 151.2234) is a medium-density residential and parkland area. The quantity of greenspace of Centennial Park is relatively high and is comprised of grassed lawns, pond side vegetation and a combination of the following trees: *Ficus macrophylla*, *Araucaria cunninghamii* and various eucalypts, e.g. *Eucalyptus saligna* and *Melaleuca quinquenervia* and *Liquidambar styraciflua*.
- **Rushcutters Bay** (−33.8739, 151.2286) is a medium-density residential harbour side area. The quantity of greenspace in Rushcutters Bay is high due to highly planted residential areas and is mainly comprised of *Lophostemon confertus*,

Ficus macrophylla, *Jacaranda* spp., *Eucalyptus sideroxylon* and *Eucalyptus saligna*.

- **Prince Alfred Park** (−33.8884, 151.2035) is a medium-density residential and parkland area. The quantity of greenspace in Prince Alfred Park is moderately high and is comprised of *Lophostemon confertus*, *Melaleuca quinquenervia* and Australian figs (*Ficus* spp.)
- **Surry Hills** (−33.886, 151.2138) is a medium-density residential and commercial area. The quantity of greenspace in Surry Hills is moderately high due to highly planted residential areas and is mainly comprised of *Lophostemon confertus*, *Liquidambar styraciflua*, *Platanus × acerifolia* and *Populus nigra*.
- **Chippendale** (−33.8877, 151.1962) is a medium-density residential and commercial area. The quantity of greenspace in Chippendale is moderate and is comprised of the street trees: *Lophostemon confertus*, *Liquidambar styraciflua*, *Platanus × acerifolia*.

Fig. 1 Map of Sydney, Australia, depicting the geographical locations of the sampling region and the sites of the eleven sample sites. Figure made with the R packages ggplot2 and ggmaps, utilizing static maps from Google Maps



- *Glebe* (−33.8823, 151.185) is a medium-density residential area, with moderate greenspace. The street trees present are *Callistemon* spp., *Lophostemon confertus*, *Platanus* × *acerifolia*, *Jacaranda* spp.
- *Haymarket* (−33.8801, 151.2026) is a high-density residential and commercial area, with relatively low greenspace density. Only street trees are present and are predominantly *Platanus* × *acerifolia*.
- *Zetland* (−33.9108, 151.2074) is a mixed industrial and high-density residential and commercial area. The greenspace in Zetland is low and is dominated by a small number of *Lophostemon confertus* street trees.
- *Town Hall* (−33.873, 151.2051) is a high-density commercial area, with very low greenspace. There are a small number of isolated street trees only, comprised of *Platanus* × *acerifolia* and *Populus nigra*.
- *Pitt St* (−33.8738, 151.2081), is a high-density commercial area, with very low greenspace. Street trees only were present, and were only of the species *Platanus* × *acerifolia* and *Populus nigra*.

Monthly samples were taken on a single day, midweek, between 1000 and 1400 in an effort to eliminate temporal variations associated with time of day, and some rainfall effects (see below). A temporarily independent design was implemented, where single air samples were collected from different randomly selected points within the 100 m radius of the site centres each month, to negate the requirement for repeated-measures analysis. No samples were taken whilst it was raining, and a minimum wait of 2 days was given after any rain event before the collection of samples. These procedures were necessary as rainfall has been shown to ‘wash’ spores and other particle matter out of the atmosphere (Nishihara et al. 1989), and we wished to eliminate the short-term influence of this effect from our data set (Table 1).

2.3 Environmental conditions

It was predicted a priori that plant material may have an influence on aeromycota behaviour. Greenspace at the sites was approximated across a 500-m-radius circle from the geographical centre of each sample site (Table 2). These areas were further subdivided into 250- and 100-m-radius areas, forming areas of 3.14,

Table 1 Sampling dates

Month	Date
September	28.09.2013
October	22.10.2013
November	15.11.2013
December	11.12.2013
January	19.01.2014
February	23.02.2014
March	19.03.2014
April	18.04.2014
May	17.05.2014
June	20.06.2014
July	19.07.2014
August	8.09.2014

16.5 and 59.0 ha, respectively. The proportion of these areas under tree cover, grass cover and also total greenspace was calculated using Daft Logic Google Maps Distance Calculator (2013) from the satellite images available in Google Maps. The zoom capability of the Google Maps allowed accurate estimates of greenspace cover to be made. As it has been demonstrated that cropping activities (Mitakakis et al. 2001) and the mowing of lawn grasses can aerosolize fungal spores (Comtois et al. 1995), no sampling was undertaken when these events were in progress.

Real-time environmental samples were collected for 5 min per site, apart from the fungal air samples which are described below, from a height of ~170 cm to replicate the breathing space of an average human. Temperature and relative humidity were recorded using a Multifunction Environment metre (Digitech, China). A Turbometer Davis anemometer (Davis Instruments Corp. Hayward, California, USA) was used to measure wind speed and direction specific to the locations sampled.

General meteorological data such as relative humidity (daily average), rainfall (previous week, month and days since last rain event), temperature (daily and weekly maxima and minima) and prevailing wind direction (strength, direction over 24 h, 48 h and weekly time scales) were obtained from the Australian Bureau of Meteorology (2013–14).

2.4 Fungal air samples

Airborne fungal samples were collected using a Reuter centrifugal air sampler, which operates on the

Table 2 Greenspace cover composition (area %) at the sample sites within 100, 250 and 500 m radii. Canopy cover is comprised of tree and shrub species

Site	100 m radii			250 m radii			500 m radii		
	Canopy	Grass	Combined	Canopy	Grass	Combined	Canopy	Grass	Combined
Newtown	2.1	84.9	87.0	7.3	72.4	79.7	4.3	42.0	46.2
Centennial Park	35.4	37.2	72.6	13.6	54.2	67.8	13.6	58.9	72.5
Rushcutters Bay	40.5	27.1	67.5	30.5	13.8	44.3	11.8	12.3	24.2
Prince Alfred Park	20.0	7.1	27.1	16.7	8.6	25.3	8.6	5.6	14.2
Surry Hills	21.1	2.6	23.7	12.5	0.7	13.2	8.7	1.6	10.3
Chippendale	21.5	2.0	23.5	12.6	0.8	13.4	4.2	12.7	16.8
Glebe	17.9	0.3	18.2	13.9	0.4	14.3	11.1	1.9	13.0
Haymarket	11.0	0.0	11.0	7.4	0.0	7.4	8.6	1.1	9.7
Zetland	8.5	1.8	10.3	4.0	2.1	6.1	7.3	1.2	8.5
Town Hall	3.6	0.0	3.6	5.7	0.2	5.9	1.6	0.8	2.4
Pitt St	1.9	0.0	1.9	8.1	0.6	8.8	18.4	4.5	22.9

Combined cover is the sum of canopy and grass cover

principle of impacting particles from the sample air onto an agar medium coating a plastic backing strip by centrifugal force (Laflamme and Miller 1992). Each air sample (per site per month) was 80 l in volume (air samples were taken over a 2-min duration). Sabouraud's dextrose agar (SDA; Biotest AG, Germany) was used as the fungal growth medium, as its low pH inhibits most bacterial growth. On return to the laboratory, the medium was incubated for 7 days at 23 °C. Mould colonies were identified to genus level using gross microscopic morphology and expressed as colony-forming units (CFUs), utilizing the descriptions and keys of Alexopoulos et al. (1996), Ellis et al. (2007), Klich and Pitt (1988) and Larone (2002). If colonies were then unidentifiable, they were subcultured onto new Sabouraud's dextrose agar and re-incubated until sporulation, or deemed sterile mycelia after a minimum period of 21 days. Special attention was paid to *Aspergillus*, as species within this genus can cause a range of saprophytic, invasive and allergic diseases, some with high mortality (O'Gorman 2011). Thus, colonies of *Aspergillus* were further identified to species level.

2.5 Data analysis

Univariate analysis of the data was conducted utilizing SPSS version 21.0 (SPSS Inc., USA) and multivariate analysis using PRIMER version 6.1.6 (Primer- E Ltd, 2006).

Differences in fungal CFU concentrations across sites and months were examined by general linear model analyses of variance (ANOVA), and *P* values <0.05 were considered significant. Fungal community differences across months were compared using analyses of similarities (ANOSIM) using a Euclidean distance similarity matrix. Similarity percentages (SIMPER) analysis was also used to identify the genera that were responsible for differences across groups (sites and months). Linear associations between fungal density and diversity with individual environmental conditions were examined by computing Pearson correlation coefficients. Stepwise multiple linear regression was carried out to determine the relative influence of the environmental variables on the variance in fungal levels.

3 Results

Table 3 shows the frequency at which the various fungal genera were detected in the 132 air samples taken during the study, along with their average propagule density (as colony-forming units: CFUs). The genus *Cladosporium* was the most frequently isolated fungal genus, with a frequency of 78 % and a mean density of 335 CFU m⁻³. The next most frequently encountered genus was *Alternaria*, occurring in 53 % of samples with a mean of 124 CFU m⁻³. Other frequently identified fungi, in decreasing occurrence,

Table 3 Total frequency (% incidence in samples), mean and range (CFU/m³), for genera identified in outdoor air samples across Sydney, Australia

Genus	Frequency (%)	Mean (CFU/m ³)	Range (CFU/m ³)	Genus	Frequency (%)	Mean (CFU/m ³)	Range (CFU/m ³)
Total	100	952.6	75-9200	<i>Paecilomyces</i>	6.1	5.0	ND-250
<i>Cladosporium</i>	78.0	335.1	ND-741	<i>Bipolaris</i>	3.8	3.0	ND-100
<i>Alternaria</i>	53.0	124.7	ND-2750	<i>Sepdonium</i>	3.0	1.9	ND-100
Sterile Mycelia	51.5	65.5	ND-550	<i>Trichothecium</i>	3.0	1.5	ND-50
<i>Penicillium</i>	42.4	59.3	ND-950	<i>Madurella</i>	2.3	1.5	ND-100
Yeasts	32.6	46.9	ND-700	<i>Scopulariopsis</i>	2.3	1.1	ND-50
<i>Fusarium</i>	28.0	21.7	ND-300	<i>Trichoderma</i>	2.3	1.1	ND-50
<i>Epicoccum</i>	26.5	56.5	ND-1300	<i>Ulocladium</i>	2.3	1.3	ND-50
<i>Phoma</i>	22.0	27.7	ND-350	<i>Wallemia</i>	2.3	2.3	ND-150
<i>Acremonium</i>	21.2	15.1	ND-250	<i>Botrytis</i>	1.5	1.3	ND-100
<i>Aureobasidium</i>	20.5	21.8	ND-450	<i>Chaetomium</i>	1.5	1.5	ND-100
<i>Cladophialphora</i>	17.4	14.1	ND-250	<i>Chrysonilia</i>	1.5	1.5	ND-100
<i>Aspergillus</i>	15.2	15.3	ND-215	<i>Cunninghamella</i>	1.5	0.6	ND-50
<i>Curvularia</i>	14.4	16.6	ND-950	<i>Dreschlera</i>	1.5	1.9	ND-200
<i>Pithomyces</i>	9.8	9.8	ND-150	<i>Geotrichum</i>	1.5	0.8	ND-50
<i>Beauveria</i>	9.1	9.1	ND-150	<i>Absidia</i>	0.8	0.4	ND-50
<i>Malbranchea</i>	8.3	8.3	ND-200	<i>Blakeslea</i>	0.8	0.4	ND-50
<i>Rhizopus</i>	8.3	8.3	ND-450	<i>Fonsecea</i>	0.8	0.4	ND-50
<i>Stemphylium</i>	8.3	8.3	ND-750	<i>Gliocladium</i>	0.8	0.4	ND-50
<i>Veronea</i>	8.3	8.3	ND-150	<i>Scytalidium</i>	0.8	0.6	ND-50
<i>Verticillium</i>	8.3	8.3	ND-100	<i>Syncephalastrum</i>	0.8	0.4	ND-25
<i>Nigrospora</i>	6.8	7.4	ND-200				

ND not detected

were as follows: *Penicillium*, *Fusarium*, *Epicoccum*, *Phoma*, *Acremonium* and *Aureobasidium*. All other genera were encountered in <20 % of samples.

3.1 Seasonal patterns of airborne fungi

Total airborne propagule densities showed temporal patterns across the study period (Fig. 3), with higher concentrations during the spring and summer, and lower numbers during winter. The mean total number of CFUs throughout the year ranged from 425 CFU m⁻³ in August (midwinter) to 1531 CFU m⁻³ in February (late summer; Fig. 2). This seasonal periodicity was expected, as the factors that we proposed a priori that might influence the proliferation of moulds (humidity, warm temperature and rainfall) also fluctuate throughout the year, and it was predicted that various fungi would have preferences for specific environmental conditions. Thus, the relationship between total airborne propagule

density and a range of climatic and weather variables was tested statistically (see below).

The number of genera of fungi encountered was less variable through time, with mean diversity ranging from 4 to 15 genera per sample over the study period, and no detectable temporal trend was present.

Cladosporium was the most frequent genus encountered and was detected in high concentrations across all sampling months relative to the other fungi encountered with greatest numbers appearing in late spring and lowest numbers between midsummer and early autumn (Fig. 4). *Alternaria* and sterile mycelia were somewhat less frequently identified and occurred more frequently and comprised a larger percentage of total fungi during the summer months.

When comparing airborne fungal communities across months, ANOSIM indicated that the only significant pattern in the data set was that the community structures of fungi identified in the summer months (December,

Fig. 2 Average total number of fungal CFU/m³ encountered and average number of genera encountered during the year

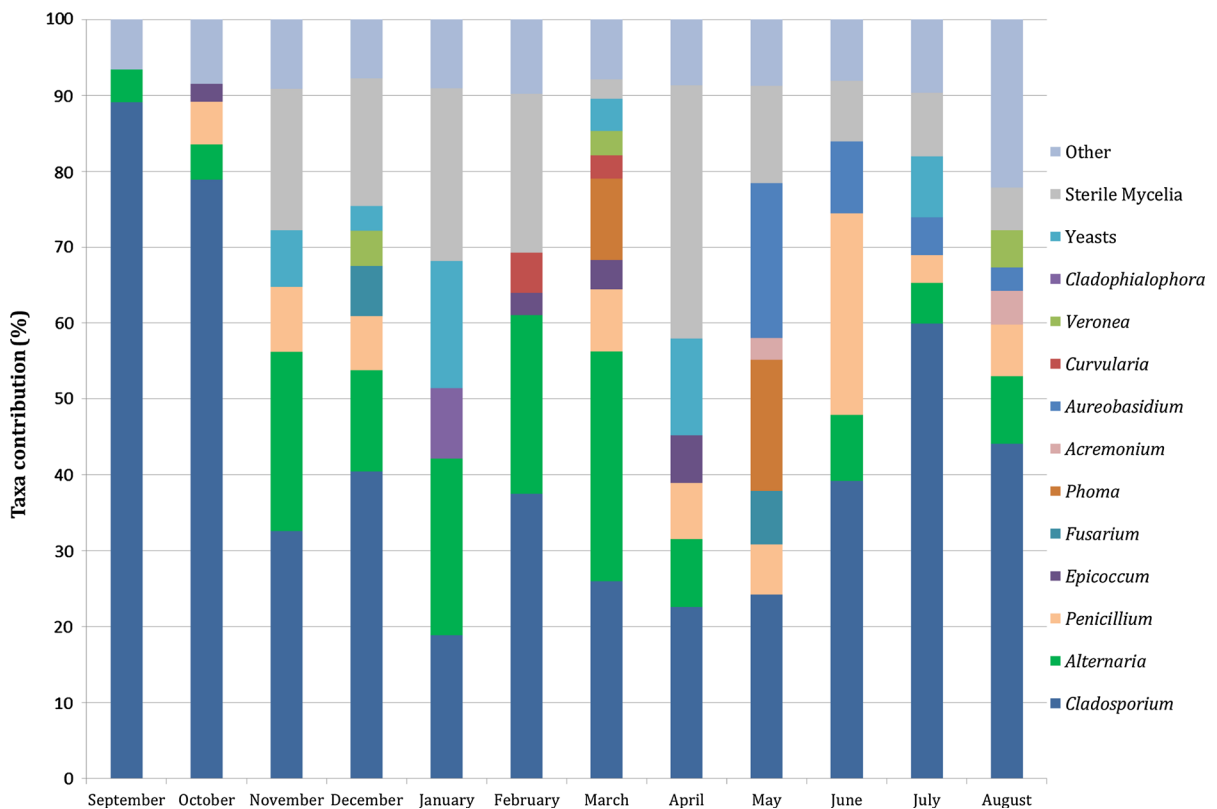
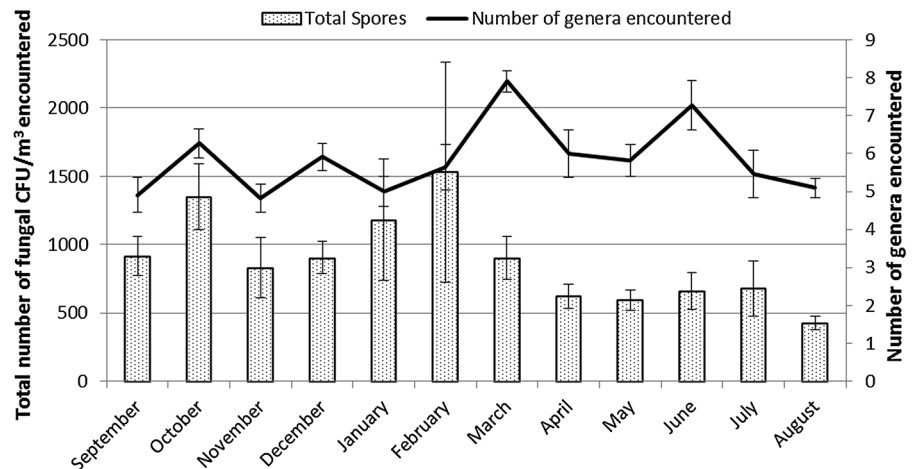


Fig. 3 Temporal relative abundances of fungal genera encountered in Sydney, Australia, averaged across sites

January and February) were significantly different from all other months (Global $R = 0.175$, $P = 0.01$). In all cases, SIMPER analysis identified increased concentrations of *Cladosporium* as the primary change differentiating the summer samples from the other months,

contributing to 23.8–45.7 % of the between-month differences. The only other taxa that contributed more than 10 % of the seasonal differences were increased *Alternaria* and *Epicoccum*, contributing 11.60 and 10.52 % of the between season differences, respectively.

3.2 Spatial patterns of airborne fungi

The mean total number of fungal colony-forming units identified at the sites ranged from 346 CFU m⁻³ in the highly urban Haymarket to 2250 CFU m⁻³ in the Newtown site, which was located in Sydney Park, a large, predominantly grassed parkland (Fig. 4).

Amongst sites, the Newtown samples had significantly higher mean numbers of fungal genera, followed by another parkland site, Centennial park (GLM ANOVA both $P < 0.05$, compared to all other sites).

The other sites, which all had far lower levels of greenspace than the Newtown and Centennial Park sites, all supported statistically similar fungal densities (GLM ANOVA; $P > 0.05$), apart from the Haymarket sample site, which had significantly lower concentrations on average than all the other sites (GLM ANOVA; $P < 0.05$, Fig. 4).

Locations with high fungal densities also supported higher diversity of fungal genera (Pearson's $r = 0.302$, $P < 0.000$).

Whilst several genera showed considerable variation throughout the year, few genera demonstrated trends between the different sites, with some quantitatively minor exceptions: significantly, more *Epicoccum* was found in Newtown (GLM ANOVA; $P < 0.05$), and yeasts were frequently found in higher numbers in Centennial park (GLM ANOVA; $P < 0.05$). At a community level, ANOSIM did not

detect differences in community structure across sites (Global $R = 0.008$, $P = 0.329$).

3.3 Identification of pathogens

Few pathogenic fungi were identified in the samples, and the densities of the all allergenic groups of most concern, including *Alternaria*, were within an acceptable range for human health (Fig. 5).

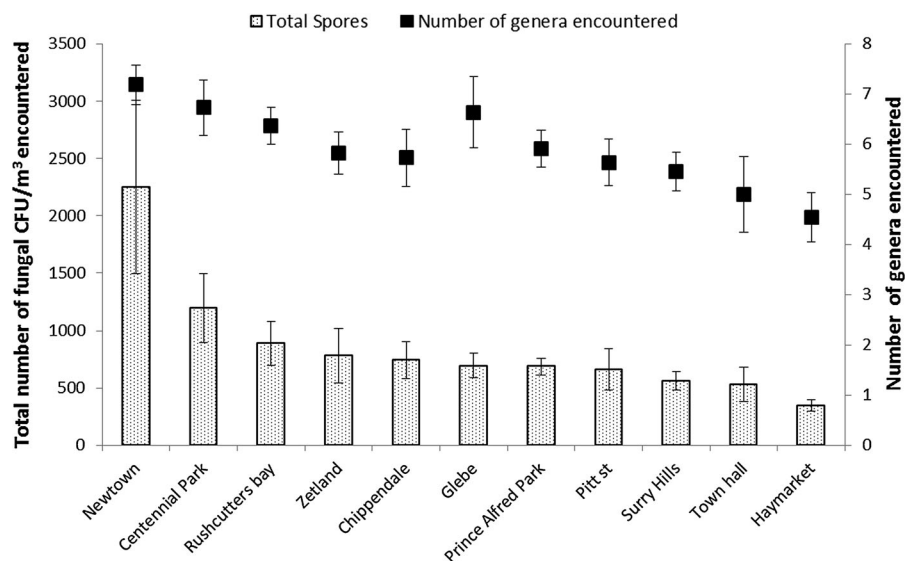
A small number of potentially opportunistic organisms of human health concern were encountered: for example, several *Aspergillus* were documented; however, no dimorphic or systemic pathogens were detected, nor were any dermatophytes. Yeast isolates were tested with a capsule stain to determine whether there was a possibility of *Cryptococcus* spp; however, none were found.

Seven species of *Aspergillus* were identified throughout the entire study, with *Aspergillus versicolour* being the most prevalent, occurring in 6.8 % of samples (Table 4). *Aspergillus fumigatus*, the species of most concern (O'Gorman 2011), was detected much less frequently, occurring in 1.5 % of samples.

3.4 Environmental predictors of aerosolized fungi

Whilst many correlations between environmental conditions and aeromycota were statistically significant, values of the correlation coefficients obtained were fairly low in all cases (none exceeded 0.6).

Fig. 4 Average total number of fungal CFU/m³ encountered and average number of genera encountered across sampling sites



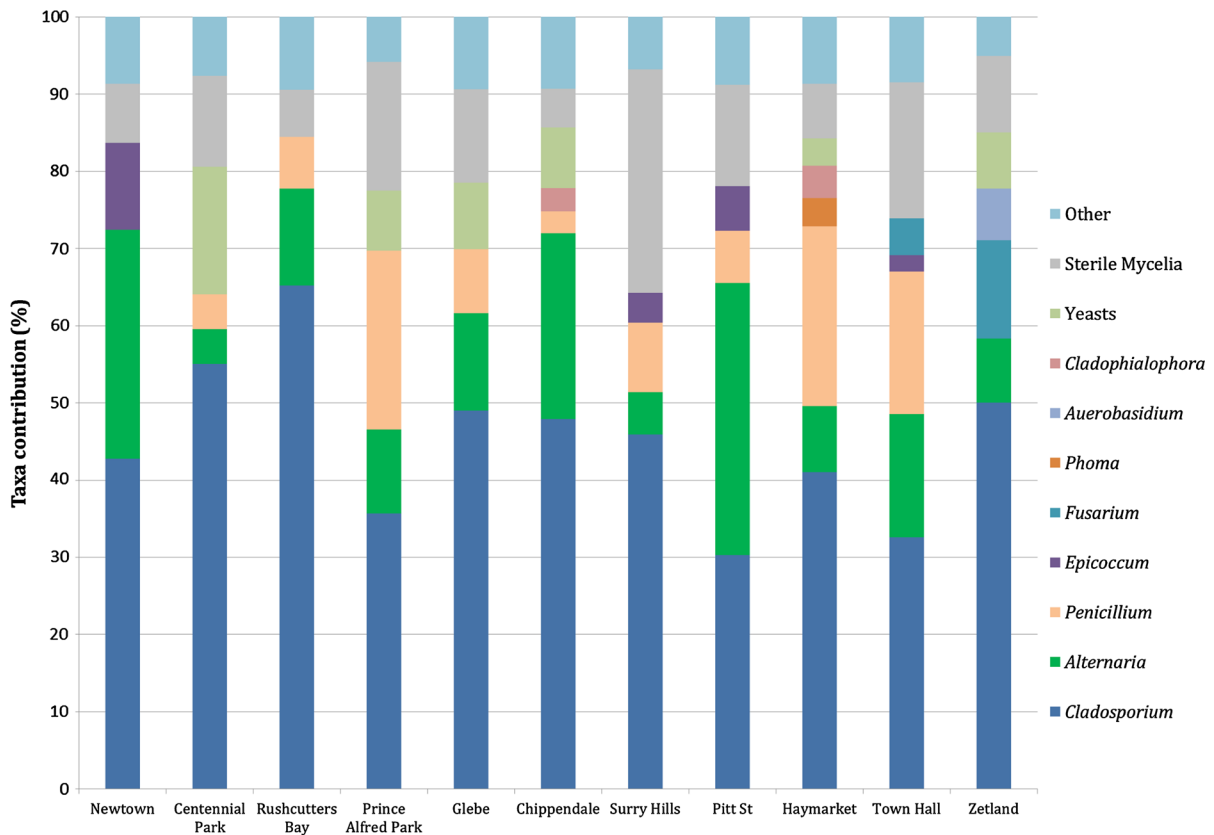


Fig. 5 Spatial relative abundances of fungal genera encountered in Sydney, Australia, averaged across months

Table 4 Total frequency (% incidence in samples), mean and range (expressed as CFU/m³), for *Aspergillus* species identified in outdoor air samples across Sydney, Australia

Genus	Frequency (%)	Mean (CFU/m ³)	Range (CFU/m ³)
Total <i>Aspergillus</i> spp.	15.2	15.3	ND-215
<i>Aspergillus versicolour</i>	6.8	6.8	ND-150
<i>Aspergillus niger</i>	6.8	6.8	ND-200
<i>Aspergillus glaucus</i>	2.3	0.9	ND-50
<i>Aspergillus terreus</i>	2.3	1.5	ND-100
<i>Aspergillus fumigatus</i>	1.5	0.8	ND-25
<i>Aspergillus flavus</i>	1.5	0.6	ND-25
<i>Aspergillus parasiticus</i>	0.8	0.4	ND-25

ND not detected

Total airborne fungal CFU was significantly correlated with wind speed measured at the time of sampling ($r = 0.563$, $P = 0.000$) and temperature ($r = 0.233$, $P = 0.007$). Total CFU was also correlated with percentage greenspace at all levels of measurement (100, 250 and 500 m radii), where increasing greenspace was positively and significantly correlated with increases in total fungal density ($r \geq 0.351$, $P < 0.001$ in all cases).

Number of genera was significantly correlated with wind speed measured at the time of sampling ($r = 0.451$, $P = 0.000$), total rain in the past month ($r = 0.187$, $P = 0.032$), total rain in the last week ($r = 0.179$, $P = 0.049$) and negatively correlated with temperature ($r = -0.211$, $P = 0.015$) and time since last rain event ($r = -0.228$, $P = 0.009$). Like total CFU, number of genera was also positively correlated with percentage greenspace at all levels of

Table 5 Multiple linear regression model results with corresponding Pearson correlation coefficients for total airborne fungal CFUs and number of taxa encountered

Air quality variable	Predictors ranked in order of predictive power		
	First	Second	Third
Total airborne fungal CFUs	Grass cover within 100 m radii	Wind speed (m/s)	Total rainfall in the past month (mm)
Regression	$R^2 = 35.55, P = 0.000$	$R^2 = 14.72, P = 0.000$	$R^2 = 4.65, P = 0.000$
Correlation	$r = 0.509, P = 0.000$	$r = 0.563, P = 0.000$	$r = 0.302, P = 0.000$
Cumulative regression R^2	35.55	50.27	54.92
Number of taxa encountered	Wind speed (m/s)	Temperature ($^{\circ}\text{C}$)	Total rainfall in the past month (mm)
Regression	$R^2 = 28.33, P = 0.000$	$R^2 = 4.42, P = 0.000$	$R^2 = 4.26, P = 0.000$
Correlation	$r = 0.451, P = 0.000$	$r = -0.211, P = 0.000$	$r = 0.187, P = 0.000$
Cumulative regression R^2	28.33	32.75	37.01

measurement (100, 250 and 500 m radii; $r \geq 0.273$, $P < 0.001$ in all cases).

To test the effects of different greenspace components, percentage greenspace was split between canopy coverage (i.e. trees and shrubs) and grass coverage. When analysed, only grass cover was found to be significantly correlated with increases in total fungi and number of genera at all levels of measurement (100, 250 and 500 m radii; $r \geq 0.424$, $P < 0.001$ in all cases), whilst no relationships were found between tree canopy coverage and total fungi/generic diversity at all three levels of measurement ($P > 0.05$ in all cases).

Multiple stepwise linear regression analysis was used to determine which environmental variables were the strongest predictors of the fungal bioparticle air quality variables as per Green et al. (2003). The analysis ranked the variables in order of predictive power for total propagule concentration, with backward elimination performed to check the significance of each variable.

The analysis indicated that only three predictors were worthy of consideration, and adding further predictors to the model made little contribution ($< 2\%$) to its overall explanatory power.

For total concentration of airborne fungi, the grass cover within a 100 m radius was the largest contributor to the overall variation in the model, explaining 35.55 % of the linear pattern in the fungal data ($R^2 = 35.55$). Adding wind speed to the model explained an additional 14.72 % of the variation, and adding total rain in the past month added 4.65 %

explanatory power. The three-variable model thus explained 54.87 % of the variability in the data set (Table 5) and is expressed in Eq. 1:

$$\begin{aligned} \text{Total airborne fungi (CFU m}^{-3}\text{)} \\ = 16.6a + 138b - 1.9c + 370.1 \end{aligned} \quad (1)$$

where a = percentage grass cover within 100 m radius, b = wind speed (m s^{-1}), c = total rainfall in the past month (mm).

Similarly, when the number of genera encountered was used as the response variable, again only three predictors explained the majority of the variation within the model: with wind speed explaining 28.33 % of the linear pattern in the fungal data ($R^2 = 28.33$). Adding mean maximum temperature for the month to the model explained an additional 4.42 % of the variation, and adding total rain in the past month added 4.26 % explanatory power. The three-variable model thus explained 37.01 % of the variability in the data set (Table 5) and is expressed in Eq. 2:

$$\begin{aligned} \text{Number of genera encountered (per 80 L sample)} \\ = 0.218a - 0.207b + 0.0065c + 9.8 \end{aligned} \quad (2)$$

where a = wind speed (m s^{-1}), b = temperature ($^{\circ}\text{C}$), c = total rainfall in the past month (mm).

4 Discussion

The current study provides the most comprehensive assessment to date on the density and prevalence of

culturable airborne fungi in the outdoor air of urban Sydney.

Cladosporium, *Alternaria* and *Penicillium* were common across both spatial and temporal samples. The distribution of genera encountered in this study not only resembles the composition of fungi found in other major city centres in Australia (Garrett et al. 1997; Rutherford et al. 1997; Mitakakis and Guest 2001) but also other regions that have comparable climates: Santa Fe City, Argentina (Basilico et al. 2007), Porto, Portugal (Sousa et al. 2008) and Granada, Spain (Sabariego et al. 2000). Similarly, previous work has also identified increases in fungal density and diversity during summer months. These could be due to changes in meteorological factors occurring in summer, as we detected positive associations between variables that increase during summer in Sydney, such as temperature and rainfall, and many fungal genera. Most fungi had positive associations with wind speed, which is logical as wind is known to be a major vector of spore dispersal (Lin and Li 2000) and is also higher in the summer months for Sydney.

Much like the aeromycology of Brisbane, a more northerly Australian city that has been previously studied, *Cladosporium* was the dominant airborne fungal genus, whilst *Alternaria* became consistently more numerous during summer (Rutherford et al. 1997). However, the total spore counts in Brisbane reached a maximum in late summer and early autumn, whereas we detected peak density in Sydney to occur several weeks earlier. Sydney is further south than Brisbane and thus more temperate climate, which is the most probable explanation for these observations. Another study conducted in Brisbane (Hargreaves et al. 2003) noted that the most frequently isolated fungal genera were *Cladosporium*, *Curvularia*, *Alternaria*, *Fusarium* and *Penicillium*; fungal genera were also the most common in our study. Correspondingly, in a Melbourne study utilizing a Burkard bioparticle sampler, (Mitakakis and Guest 2001), *Cladosporium* was the most prevalent fungal type, followed by numerous basidiospores and sexual ascospores. The latter spore types, if encountered in the current study, may have been deemed sterile mycelia, as they may not have produced identifiable structures on the media we used. Alternatively, the Burkhardt method does not permit easy identification of many genera that produce small conidia (Mitakakis

and Guest 2001), such as *Penicillium* and *Aspergillus*, thus making detailed comparison of bioparticle and culturable fungi studies difficult. Further, the current findings are very similar to the only other study to address fungal taxonomic composition in urban Sydney (Torpy et al. 2013); except that we found the concentration of total fungi to be much higher in spring than Torpy et al. (2013) recorded. The variations in numbers and diversity of fungi encountered in this study could potentially be attributed to spatial differences in microclimate and vegetation, as Torpy et al. (2013) only studied a single site which was only directly comparable to the Haymarket site in the current work.

Interestingly, there were no differences in overall community assemblage (i.e. the relative frequency of the different genera) across sites; however, some individual genera did show trends; *Epicoccum* was frequently found in Newtown. As *Epicoccum* is a known plant endophyte and involved in the early stages of plant decomposition (Thormann et al. 2003), there may be a potential urban forestry association with this genus for this particular site, which had substantial greenspace density. Yeasts were frequently found in higher numbers in Centennial Park. Yeasts are ubiquitous and abundant in the air and are often phylloplane organisms (Fonseca and Inácio 2006), thus being encountered in an area with relatively high density of greenspace comes as no surprise; however, the high frequency at Centennial park compared to other planted areas requires further investigation.

Amongst sites, the Newtown samples had significantly higher mean numbers of fungal genera, followed by another parkland site, Centennial park, indicating that greenspace, especially the grass component, could be a major determinant of the density of airborne fungi in urban areas. As hypothesized by Mitakakis et al. (2001) and Hargreaves et al. (2003), elevated mould spores in urban areas could be a result of proximal vegetation. In the current study, total fungal CFU, the number of fungal genera and the densities of *Cladosporium*, *Alternaria* and *Epicoccum* all showed positive correlations with greenspace. One of the more noteworthy findings of this work is the finding that grass coverage consistently showed stronger associations with the fungal density and diversity than tree and shrub canopy coverage. It was previously unknown whether urban forestry type would have a quantifiable influence on fungal

phenology. One potential reason as to why grass contributed to more fungi is the way in which the streets are maintained: trees tend to line fully paved surfaces in Sydney, and these street surfaces are cleaned regularly, as opposed to grass cover, which exists in parks, and may harbour greater quantities of decaying organic material; which, if the fungi are saprophytes, could proliferate and then produce aerosolized spores. It is plausible that saprobic colonization and sporulation of grass endophytic fungi could become unrestrained when their host plant tissue dies (Vázquez de Aldana et al. 2013). Additionally, turf grass in urban areas is regularly mown: potentially mechanically aerosolizing endophytic and epiphytic fungi (Streifel 1988) and exposing damaged plant tissues and leaking protoplasm, which may encourage fungal growth. Comtois et al. (1995) demonstrated that the act of mowing lawns can aerosolize fungal spores, increasing the exposure of lawn mowing workers to aeroallergens. Similarly, previous work by Mitakakis et al. (2001) showed that crops and the harvest of crops increase aeromycota concentrations in proximal towns (Mitakakis et al. 2001).

Fungal propagule concentrations in the air and their taxonomic compositions are known to fluctuate, a consequence of the interaction between biological and environmental factors including geographical location, human activity and local source of vegetation (Lacey 1981). Further, the effects of meteorological changes on fungal occurrence in the air have been highlighted by many authors for many different regions (e.g. Grinn-Gofron and Bosniacka 2015). Total airborne fungal CFUs and number of genera encountered were significantly correlated with a number of meteorological conditions, including wind speed and temperature. This corresponds with the findings of many previous studies (Goncalves et al. 2010; Grinn-Gofron and Bosniacka 2015; Li and Kendrick 1995; Oliveira et al. 2009; Stennett and Beggs 2004; Rutherford et al. Rutherford et al. 1997). When environmental variables were analysed for their combined effects on total airborne fungal CFUs, percentage grass cover within 100 m radius, wind speed and total rainfall in the past month explained 54.87 % of the variation. Remarkably, percentage grass cover within 100 m radius explained more variability in total CFUs in the multiple stepwise linear regression model than any meteorological variable measured. Considerable research has found associations with wind and

precipitation with airborne fungal propagules (Li and Kendrick 1995; Lin and Li 2000; Trimmer et al. 1998), however rarely has any study incorporated a potential source like urban vegetation into their analysis.

The diversity of culturable airborne fungi was associated with wind speed as well as several precipitation variables; however, higher temperatures were associated with decreased generic diversity. Many of the higher temperature days were coupled with rain events. Whilst higher temperatures facilitate fungal sporulation, thus increasing diversity of aerosolized fungal propagules, precipitation potentially removes some spore types from the atmosphere, reducing diversity. Nonetheless, some studies do find positive associations with some spore types and precipitation (Kurkela 1997), potentially a result of increasing humidity inducing sporulation (Quintero et al. 2010) or rain drops facilitating the dispersion of these spores from vegetation (Levetin and Horner 2002). This could be due to the fact that higher temperatures facilitate fungal sporulation, thus increasing diversity, whilst precipitation potentially removes some spore types from the atmosphere, reducing diversity. Surprisingly, no associations were found with humidity, possibly because Sydney's humidity levels do not fluctuate throughout the year as much as other geographical regions. *Cladosporium* and *Alternaria*, the most dominant taxa identified in this study, have been found to be most prevalent during midday when the humidity is lower and the wind is drier than other times of the diurnal cycle (Burch and Levetin 2002), as they require relatively low humidity to sporulate. We only collected samples from the middle of the day, which may have further reduced any humidity-associated pattern for these genera, and led to their overrepresentation in our data set relative to the full diurnal cycle.

It is evident that the associations between airborne fungal propagule density and meteorological variables are complex (Oliveira et al. 2009). With the additional influences of associations with substrate, habitat preference and other environmental variables, it is unsurprising that all research has attempted to model airborne fungi find weak patterns. The current study, by adding an assessment of several environmental variables into these models, provides some additional understanding of the complexity of airborne fungal dispersal.

Few human pathogens were identified in the survey: *Aspergillus* species were detected in 15.2 %

of samples, however accounted for <0.8 % of the total fungal propagules detected, which is comparable to the ambient background frequencies recorded throughout the year in New York by Recer et al. (2001). *Aspergillus fumigatus* was found in <1.5 % of samples taken, with a mean concentration of 0.8 CFU m⁻³. There are no universally recommended threshold limits on airborne *A. fumigatus*; however, the Spanish Society of Infectious Diseases and Clinical Microbiology recommends concentration maxima for hospitals at 0.5 CFU m⁻³ for operating rooms and 25 CFU m⁻³ for other parts of the hospital (Ruiz-Camps et al. 2011). As we only took outdoor air samples, the concentrations of aspergilli detected in the current work were thus of an acceptable level for human health.

Associations have been made with increases in fungal exposure and allergenic symptoms, incidence of asthma and unscheduled hospital visitations (Pongracic et al. 2010). In the current study, the highest concentrations of airborne fungi were observed in summer. Asthma hospitalizations tend to increase in summer periods (AIHW 2013), a pattern that is potentially related to increases in allergens at this time of year (Sharpe et al. 2015). This trend is less distinct in northern Australia, where the seasonal differences are less defined (Sharpe et al. 2015). No study is yet to find a relationship between allergic symptoms or hospital admissions and airborne fungal concentrations in any Australian city (Hanigan and Johnston 2007; Tham et al. 2014); however, positive associations have been demonstrated in studies from the USA (Pongracic et al. 2010), UK (Atkinson et al. 2006) and Canada (Dales et al. 2004).

Further work that utilizes molecular methods for the characterization of fungal diversity such as the DNA sequencing of sampled fungi could be utilized to better identify the sterile mycelia encountered in culturable fungal studies (Kausrud et al. 2005; Lang-Yona et al. 2012). Similarly, work that considers the concentrations of specific urban plant species in urban areas and their potential associations with the proximal aeromycota would be of value.

5 Conclusions

The work here presents a comprehensive survey of the culturable aeromycota of Sydney by determining the diversity and abundance of outdoor fungal propagule

concentrations for urban Sydney. Seasonal trends of airborne fungi in urban Sydney were observed, with increases in fungal concentrations experienced in summer months. The environmental variables that made the largest detectable influence to the diversity and abundance of fungal propagule loads were temperature, wind speed and proximal greenspace. If the greenspace was comprised of grass, stronger associations with the aeromycota were observed.

Acknowledgments Peter Irga conducted majority of this work with the support of an Australian Postgraduate Award, funded by the Australian Government and a grant from the City of Sydney's Environmental Grants Program. The authors would like to thank Margaret Burchett, Angela Dacunto, Gemma Armstrong and Gabe O'Reilly for their assistance.

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