



Outdoor fungal spores and acute respiratory effects in vulnerable individuals

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ARTICLE INFO

Keywords:

Airway inflammation
Asthma
fungi
Lung function
sensitization

ABSTRACT

Background: Many outdoor fungal spores are ubiquitous, respirable and possibly allergenic. They may contribute to asthma symptoms; however, little is known about their effects on respiratory function.

Objective: To investigate if outdoor fungal spore levels were associated with lung function or airway inflammation, and whether fungal sensitization or current asthma modified any associations.

Methods: Cross-sectional associations between same day (Lag0) and cumulative 3-day lagged (Lag0-3) counts of 12 outdoor fungal spore taxa and pre-bronchodilator spirometry (FEV₁, FVC, FEF_{25%-75%}), bronchodilator response (BDR) and airway inflammation (fractional exhaled nitric oxide (FeNO) and exhaled breath condensate (EBC) nitrogen oxides (NOx) and pH were investigated in 936 Melbourne Atopy Cohort Study participants during September 2009 to December 2011. Generalized linear models were used to quantify associations with lung function, FeNO and EBC pH; generalized estimating equations for BDR; and ordinal logistic regression for EBC NOx. Models were adjusted for age, sex, height, temperature, relative humidity, grass pollen and sample storage time. Potential effect modification by fungal sensitization and current asthma were examined using interaction terms.

Results: Mixed associations were found. Higher levels of *Ustilago*/smuts were associated with lower lung function at Lag0 (FEV₁: 21ml [95%CI -36, -7]; FEF_{25%-75%}: 39ml [-65, -13]) and Lag0-3 (FEV₁: 9ml [-14, -4]; FEF_{25%-75%}: -18ml [-27, -9]). Positive BDR was associated with *Ustilago*/smuts (Lag0 OR = 1.1 [1.04, 1.2]; Lag0-3 OR = 1.04 [1.02, 1.07]), *Alternaria* (Lag0 OR = 1.3 [1.0, 1.6]) and *Drechslera* (Lag0 OR = 1.1 [1.03, 1.2]). Higher EBC NOx was associated with *Cladosporium* (Lag0-3 OR = 1.1 [1.0, 1.2]), *Alternaria* (Lag0-3 OR = 1.1 [1.0, 1.3]). No associations were found with higher FeNO. In those with fungal sensitization, *Ustilago*/smuts and *Drechslera* were associated with lower FEV₁ and FVC; *Cladosporium* was associated with increased FEV₁, FVC and FEF_{25%-75%} but also with higher FeNO and lower EBC pH. In those with current asthma, *Alternaria*, *Ustilago*/smuts and *Drechslera* were associated with lower FEV₁, FVC, FEF_{25%-75%} and EBC pH.

Conclusion: Exposure to outdoor fungal spores may be associated with lower lung function and increased airway inflammation, particularly in those with fungal sensitization and/or current asthma.

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1. Introduction

There is evidence that some fungal spore taxa are associated with a range of chronic respiratory conditions. For example, *Alternaria*, *Cladosporium*, *Aspergillus* and *Penicillium* are associated with asthma exacerbations (Pongracic et al., 2010) and asthma hospitalisations (Tham et al., 2014); and *Aspergillus* and *Cladosporium* are associated with hypersensitivity pneumonitis (Baxi et al., 2016). We and others have demonstrated that short- and long-term exposures to a range of indoor fungal spore taxa are associated with asthma symptoms (Dharmage et al., 2001) and reduced lung function in healthy adults (Hernberg et al., 2014a; Norback et al., 2011) and asthmatic adults (Dharmage et al., 2002; Hernberg et al., 2014b). Outdoor fungal spores are ubiquitous in the atmosphere and, unlike indoor fungal spores whose levels can be controlled by building remediation or other interventions, their presence is predominantly related to their symbiotic relationship with outdoor environmental organic matter and cannot be readily controlled. It is important to understand whether exposure to outdoor fungal spores may be associated with acute effects on lung function and whether some sub-groups may be more vulnerable to adverse effects. In addition, acute reductions in lung function may not cause obvious symptoms but may lead to long-term airway remodelling and chronic effects (Knutsen et al., 2012; Quanjer et al., 2012).

However, few studies have examined associations between outdoor fungal spore exposure and respiratory health, with conflicting findings. Two studies of children aged 10–12 years reported associations between outdoor fungal spore levels and reduced lung function in individuals regardless of their asthma status (Chen et al., 2014; Watanabe et al., 2016), but neither study assessed whether any reduction was due to reversible bronchoconstriction. Studies of asthmatic subjects have reported some associations between aggregated outdoor fungal spore counts and lung function decline in older adults (Rutherford et al., 2000), in a panel of 22 adults and children (Delfino et al., 1997); and increased lung function in a panel of 40 adults and children (Ross et al., 2002). Studies that demonstrated associations between outdoor fungal spore exposures and reduced lung function in healthy and asthmatic adults highlighted that the effects were not necessarily limited to individuals with asthma (Hernberg et al., 2014a; Watanabe et al., 2016), but asthma may be a potential effect modifier. Sensitization to aero-allergens, including fungal allergens, has been associated with reduced lung function (Owens et al., 2016; Vicencio et al., 2014) indicating that sensitization may also be a potential effect modifier. It is also plausible that fungal sensitization accentuates the impact of acute fungal spore exposure on clinical outcomes including lung function (acts as an effect modifier) although this has yet to be examined.

To date, no published studies have assessed whether outdoor fungal exposure induces reversible airflow obstruction or acute airway inflammation. Hence, the aim of this analysis was to investigate if levels of outdoor fungal spores, recorded over two years, were associated with lung function or airway inflammation, and whether any associations were modified by fungal sensitization or current asthma.

2. Methods

2.1. Study population

The study sample comprised 936 individuals from 264 families (probands, siblings and parents) who participated in the 18-year follow-up of the Melbourne Atopy Cohort Study (MACS) during the period September 2009 to December 2011. Participants included in this analysis underwent one session of clinical testing, at a time of their choice, to assess lung function and presence of airway inflammatory biomarkers. The MACS is a longitudinal cohort study of individuals with a family history of allergic disease. Details of the recruitment process are described elsewhere (Lowe et al., 2011) and some are provided in the Supplementary Material including a flowchart (Figure E1). Ethics

approvals were granted by The University of Melbourne and Royal Children's Hospital Human Research Ethics Committees.

2.2. Environmental factors

We used daily ambient fungal spore and pollen counts obtained between September 2009 and December 2011 for the Melbourne Air Pollen Children and Adolescent Health (MAPCAH) study with a Burkard 7-day volumetric trap located at the University of Melbourne (Tham et al., 2017). Identifiable fungal spores were classified into taxa: *Alternaria*, *Cladosporium*, *Coprinus*, *Drechslera*, *Ganoderma*, *Leptosphaeria*, *Periconia*, *Pithomyces*, *Pleospora*, *Sporormiella*, *Stemphylium* and *Ustilago*/smuts (Grant Smith, 1990).

Average daily concentrations of particulate matter up to 2.5 µm (PM_{2.5}) and 10 µm in diameter (PM₁₀), daily maximum 1-h average nitrogen dioxide (NO₂) level, daily maximum 4-h average ozone (O₃) level; daily maximum and minimum temperatures, 24-h rainfall, and daily average relative humidity were measured. Details of measurement methods are in the [Supplementary Material](#).

2.3. Potential effect modifiers

Fungal sensitization to *Alternaria*, *Penicillium* and *Cladosporium* was assessed using skin prick tests (SPT) with standardised measurement techniques (See [Supplementary Material](#)).

Current asthma was defined as: a history of doctor-diagnosed asthma and one or more of the following in the previous 12 months: (1) ≥ 1 episode of asthma, (2) ≥ 1 episode of wheeze, or (3) use of asthma medication.

2.4. Outcomes

2.4.1. Lung function and bronchodilator response

Spirometry was performed according to standardised American Thoracic Society [ATS]/European Respiratory Society [ERS] guidelines for spirometric techniques (Miller et al., 2005). The lung function indices used were forced expiratory volume at 1 s (FEV₁) – the volume expired in the first second of maximal expiration after maximal inspiration; (2) forced vital capacity (FVC) – the maximum volume of air exhaled or inspired during a maximally forced manoeuvre; (3) mid expiratory flow (FEF_{25%–75%}) – average expired flow over the middle half of the FVC manoeuvre; and bronchodilator response (BDR), defined as ≥ 12% change and ≥ 200 ml absolute change in FEV₁ from pre-bronchodilator to post-bronchodilator readings (Pellegrino et al., 2005), indicating reversibility of airflow limitation. Methods are in the [Supplementary Material](#).

2.4.2. Airway inflammation markers

Fractional exhaled nitric oxide (FeNO) was measured using a HypAir FeNO (Medisoftware, Sorinnes, Belgium) according to ATS/ERS guidelines (American Thoracic Society/European Respiratory Society, 2005). Exhaled breath condensate (EBC) samples were collected from probands only following ATS/ERS recommendations (American Thoracic Society, 2006; Horváth et al., 2005) using a glass-condensing chamber in wet ice and analyzed for nitrogen oxides (NO_x) (Aldakheel et al., 2018) and acidity (pH). Methods are in the [Supplementary Material](#).

2.5. Statistical analysis

This is a cross-sectional analysis of the 2009–2011 follow-up of the MACS cohort and their family members. Correlations between each fungal spore taxa and grass pollen were assessed using Spearman's rank correlation coefficients. Regression techniques were used to estimate effects of fungal spore taxon levels on the same day (Lag0), and cumulative 3-days prior (Lag0-3) on lung function and airway

inflammation. The fungal spore data were right skewed and the scale of interquartile ranges missed the variability between the successive days. (Baxi et al., 2016). We did not categorize the exposures into low, medium or high, as consistent threshold effects have yet to be identified (Chen et al., 2014). The odds ratio (OR) and coefficients can be interpreted as the effect per increase in fungal spore counts/m³ from the 75th to 90th percentile for each fungal taxon. Where the change in fungal spore counts/m³ from 75th to 90th percentile was zero, the coefficients/OR were expressed per one spore/m³ increase. Generalized linear models were used for FEV₁, FVC, FEF_{25–75%} (raw values and z-scores), FeNO and EBC pH. Generalized estimating equations were used to assess associations with BDR. A cluster function was used for analyses where multiple individuals from the same family contributed data. As EBC NOx was strongly right skewed, results were stratified into none (below level of detection), low (≥ 2.5 and $< 20 \mu\text{M}$) or high ($\geq 20 \mu\text{M}$) and analyzed using ordinal logistic regression. All models met the proportional odds assumption and the parallel regression assumption, as assessed using the Brant test (UCLA: Statistical Consulting Group, 2016).

Models assessing lung function included maximum temperature, relative humidity and grass pollen a priori, as these factors have been associated with fungal spore production and dispersion (Burge, 2002) and lung function (Li et al., 2014). In addition, models were adjusted for air pollutants (PM_{2.5}, PM₁₀, NO₂ or O₃) if they changed the estimated associations by $> 10\%$ or had a p-value of < 0.05 . Age, sex and height were adjusted in the models for lung function (Quanjer et al., 2012). Models examining EBC pH and NOx were adjusted for the same covariates plus storage time, to control for degradation of samples. Potential effect modification by fungal sensitization; and current asthma (yes/no) was explored using likelihood ratios, and strata specific associations were reported if the p-value for the interaction term was < 0.1 (Kirkwood and Sterne, 2003). Sensitivity analyses were conducted using multi-fungal spore taxa regression models to identify possible collinearity. All statistical analyses were performed using Stata IC 13.1 (StataCorp, College Station, Texas).

3. Results

3.1. Sample characteristics

Of the 936 participants, 55% were female. Mean ($\pm$ SD) age was 31.8 ± 16.9 years. Varying numbers of participants were tested for lung function, airway inflammation and fungal sensitization (Table 1). Numbers of participants tested each month varied with the fewest tested during winter (Supplementary Material Figures E2–E3). Approximately 21% of all participants were sensitized to any fungus (16% to *Alternaria*, 10% to *Cladosporium* and 7% to *Penicillium*); and 30% reported current asthma. Of those with current asthma who consented to SPT, 30% (71/237) were sensitized to any fungus (25% to *Alternaria*, 13% to *Cladosporium* and 13% to *Penicillium*).

3.2. Fungal spore distribution

The most prevalent fungal taxa detected were *Cladosporium* (44%), *Leptosphaeria* (14%), and *Alternaria* and *Ustilago/smuts* (11% each) of the total fungi spore count (Table 2). The remaining fungal taxa accounted for 1–7% each. Most fungal taxa showed a low to moderately positive correlation with each other (Supplementary Material Table E1), grass pollen, and maximum temperature. Fungal counts were negatively correlated with relative humidity (Supplementary Material Table E2). Fungal spore and grass pollen levels were higher during the warmer months, September to March (Figs. 1 and 2). The taxa *Periconia*, *Pleospora*, *Ganoderma*, *Pithomyces*, *Stemphylium*, *Sporormiella* were excluded from further statistical analyses due to their low concentrations and frequency.

3.3. Associations between fungal spore taxa and outcomes in adjusted models

Adjustment for air pollutants did not change these estimates by $> 10\%$ or were significant in any of these models so they were not included in the final modelling. The patterns of associations between outdoor fungal spore levels and lung function values (in millilitres) and z-scores did not change (Supplementary Table E3). However, we decided to report the raw lung function values as there was the potential for the z-scores for the reference population to be a poor fit for this high allergy risk cohort and these results were more readily interpreted in this form.

Higher levels of a few fungal taxa were associated with lower FEV₁ (*Ustilago/smuts* at Lag0 and Lag0-3; *Drechslera* at Lag0-3) (Table 3); lower FVC (*Ustilago/smuts* at Lag0 and Lag0-3) and lower FEF_{25–75%} (*Ustilago/smuts* at Lag0 and Lag0-3; *Drechslera* at Lag0-3). Conversely, increasing exposures to *Cladosporium* and total spores at Lag0 were associated with higher FEF_{25–75%} (Table 3).

Higher exposures to *Alternaria* (Lag0 only), *Ustilago/smuts* and *Drechslera* were associated with higher odds of a BDR response (Table 4). Higher levels of some fungi were associated with higher odds of elevated EBC NOx (*Cladosporium*, *Alternaria* and total spores at Lag0-3; *Drechslera* at Lag0) (Table 4). No associations were found between any fungal spore taxa and airway inflammation as assessed by lower EBC pH or higher exhaled nitric oxide using the FeNO measures (Supplementary Material Table E4). No air pollutants changed the effect estimates by $> 10\%$ or were significant in these models.

3.4. Sensitivity analyses

In multi-fungal models, the positive associations between increasing levels of *Cladosporium*, and higher lung function disappeared (Supplementary Material Table E5). The association between *Ustilago/smuts* and lower FEF_{25–75%} at Lag0 remained significant. However, the positive associations between increasing levels of *Alternaria* (Lag0 only), *Ustilago/smuts* and *Drechslera* and BDR at Lag0 and Lag0-3 disappeared in the multi-fungal models. The significant positive associations between *Drechslera* and EBC NOx at Lag0 and Lag0-3; and *Cladosporium* and *Alternaria* and EBC NOx at Lag0-3 also disappeared, but the trend toward positive associations held in most of the multi-fungal models (Supplementary Material Table E5).

3.5. Effect modification by fungal sensitization

As shown in Table 5, at Lag0, in those sensitized to at least one fungal extract, increasing exposures to *Ustilago/smuts* and *Drechslera* were associated with lower FEV₁ and FVC. Associations between *Ustilago/smuts* and lower FEF_{25–75%} were found in those without fungal sensitization. *Cladosporium* was associated with increased FEV₁, FVC and FEF_{25–75%} but also with higher FeNO and lower EBC pH in those sensitized to fungi. *Alternaria* was associated with higher odds of a positive BDR in those not sensitized.

3.6. Effect modification by current asthma

As shown in Tables 6 and 7, in those with current asthma, increasing exposure to *Alternaria*, *Ustilago/smuts* and *Drechslera* was associated with lower FEV₁, FVC, FEF_{25–75%} and EBC pH, while these effects were not observed in those without current asthma. Also, in those with current asthma *Cladosporium*, *Ustilago/smuts*, and *Drechslera* were associated with significantly lower EBC pH, than in those without current asthma.

4. Discussion

Understanding the effects of outdoor fungal spores on acute

Table 1
Participant characteristics tested during September 2009–December 2011.

	All	Probands	Mothers	Fathers	Siblings	Family unit > 1 member
n (%)	n (% of All)	n (% of All)	n (% of All)	n (% of All)	n (% of All)	
	936	264 (28%)	238 (25%)	160 (17%)	274 (29%)	254
	n (% of All)	n (% of sub-group)	n (% of sub-group)	n (% of sub-group) <	n (% of sub-group)	
Male n (%)	424 (45%)	127 (48%)	–	160	137 (50%)	–
Female n (%)	512 (55%)	137 (52%)	238	–	137 (50%)	–
Mean age years $\pm$ SD (n = 934)	31.8 $\pm$ 16.9	17.7 $\pm$ 1.5	49.8 $\pm$ 4.1	52.2 $\pm$ 5.1	17.9 $\pm$ 5.9	–
$\leq$ 25 years (% of All)	517	264 (100%)	–	–	253 (92%)	–
> 25 years	417	–	238 (100%)	160 (100%)	21 (8%)	–
Height cm	169.9 $\pm$ 10.2	172.6 $\pm$ 1.5	164.2 $\pm$ 6.0	177.7 $\pm$ 6.4	167.6 $\pm$ 11.8	–
Spirometry n (%)	873	262 (99%)	218 (92%)	146 (91%)	244 (89%)	235
FeNO tested n (%)	797	222 (84%)	205 (86%)	139 (87%)	231 (84%)	221
EBC tested n (%)	231	231 (88%)	–	–	–	8
Sensitization*						
Tested <i>Alternaria</i>	n = 905	n = 256	n = 232	n = 157	n = 260	–
<i>Alternaria</i> + n (%)	147 (16%)	25 (10%)	47 (20%)	30 (19%)	45 (17%)	–
Tested <i>Penicillium</i>	n = 907	n = 257	n = 233	n = 157	n = 260	–
<i>Penicillium</i> + n (%)	61 (6.7%)	10 (4%)	17 (7%)	16 (6%)	18 (7%)	–
Tested <i>Cladosporium</i>	n = 837	n = 240	n = 213	n = 142	n = 242	–
<i>Cladosporium</i> + n (%)	86 (10.3%)	19 (8%)	20 (9%)	20 (14%)	27 (11%)	–
Tested any	n = 907	n = 257	n = 233	n = 157	n = 260	–
$\geq$ 1 fungal reagent	191 (21%)	34 (18%)	58 (30%)	38 (20%)	61 (32%)	–
Current asthma	n = 811	n = 229	n = 207	n = 146	n = 229	–
Yes	247 (30%)	66 (29%)	74 (36%)	28 (19%)	79 (34%)	–

respiratory health is of increasing concern, especially with changing global climate where increased ambient temperatures and humidity may lead to increased atmospheric concentrations of fungal spores for longer periods of time (D'Amato et al., 2015). In this cross-sectional analysis, we observed inconsistent associations between ambient levels of various fungal spore taxa and lung function, BDR and markers of airway inflammation. Some associations were lagged; and some effects were stronger among those sensitized to fungi and those with asthma. The fungal taxa observed to be most consistently associated with lower lung function were *Ustilago*/smuts and *Drechslera* on the day of exposure and up to 3 days lag, particularly in those with current asthma or fungal sensitization.

Associations between *Alternaria*, *Ustilago*/smuts and *Drechslera* and

increased risk of BDR indicated that exposure to these fungal spores may be associated with triggering acute bronchoconstriction, further supported by the associations with reversibility of airflow limitation. However fungal spore taxa were not associated with higher FeNO or lower EBC pH, except in those with fungal sensitization. *Cladosporium*, *Alternaria*, and *Drechslera* were associated with higher levels of EBC NOx, however there were inconsistent associations with increased lung function and/or reduced EBC pH at different lags. Grass pollen appeared to act as a positive confounder between *Cladosporium* and FEF_{25%–75%}. The multi-fungal models identified possible collinearity or residual confounding that may have contributed to these results.

Fungal sensitization status is an individual characteristic that may modify the effect of exposure to potentially allergenic substances on the

Table 2
Summary statistics for fungal spore counts and other environmental factors from September 2009 to December 2011 in Melbourne.

	N (days)	Mean (SD)	Geometric mean	Minimum	25%	Median	75%	90%	Maximum	Frequency % of total
Fungal spore taxa (number of spores/m³ air tested)										
<i>Cladosporium</i>	852	12.6 (45.9)	6.3	0	0	3	8	28	907	43.8
<i>Leptosphaeria</i>	852	4.0 (13.4)	4.3	0	0	0	2	8	203	14.0
<i>Alternaria</i>	852	3.3 (8.0)	3.4	0	0	0	3	9	98	11.4
<i>Ustilago</i> /smuts	852	3.2 (14.8)	5.7	0	0	0	0	6	224	11.3
<i>Coprinus</i>	852	1.9 (6.3)	3.3	0	0	0	1	5	133	6.6
<i>Drechslera</i>	852	1.4 (4.7)	2.6	0	0	0	1	3	58	5.0
<i>Periconia</i>	852	0.7 (1.9)	2.0	0	0	0	0	2	19	2.3
<i>Pleospora</i>	852	0.5 (1.5)	2.0	0	0	0	0	2	25	1.9
<i>Ganoderma</i>	852	0.4 (2.2)	2.5	0	0	0	0	0	42	1.3
<i>Pithomyces</i>	852	0.3 (1.2)	1.7	0	0	0	0	1	23	1.0
<i>Stemphylium</i>	852	0.3 (0.9)	1.6	0	0	0	0	1	7	0.9
<i>Sporormiella</i>	852	0.1 (0.7)	1.6	0	0	0	0	0	9	0.5
Total spores	852	28.6 (65.5)	13.4	0	2	9.5	25.5	72	949	
Grass pollen	824	10.8 (27.3)	–	0	0	0	5	34	207	
Meteorological variables										
Maximum temperature (°C)	851	21.2 (5.91)	–	10.8	16.5	20.3	24.9	29.5	43.6	
Rainfall (mm)	824	2.3 (6.6)	–	0	0	0	1.4	11.8	82.4	
Relative humidity (%)	849	51.3 (15.9)	–	8	41	49	61	73	96	
Pollutant										
PM _{2.5} (µg/m ³)	831	4.9 (3.1)	–	0	2.9	4.2	6.2	8.8	20.2	
PM ₁₀ (µg/m ³)	832	17.7 (7.0)	–	3.5	12.7	16.45	21.4	26.2	60.1	
Nitrogen dioxide (ppb)	847	9.5 (4.1)	–	0.4	6.5	9	12.4	15.1	23.8	
Ozone (ppb)	816	13.7 (5.5)	–	0	10.25	13.5	17.05	20.3	32.1	

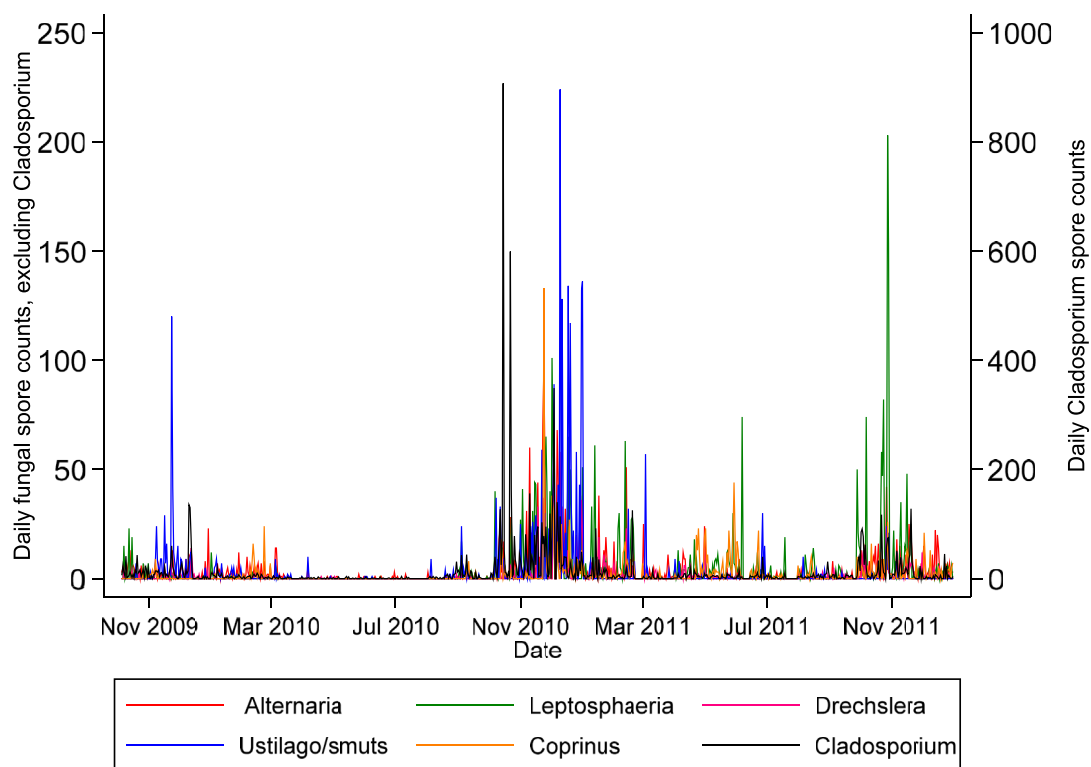


Fig. 1. Distribution of *Cladosporium*, *Leptosphaeria*, *Alternaria*, *Ustilago/smuts*, *Coprinus* and *Drechslera* fungal spores during September 2009 to December 2011 in Melbourne, Australia. Data collected using a Burkard volumetric spore trap at the University of Melbourne, Parkville, Melbourne.

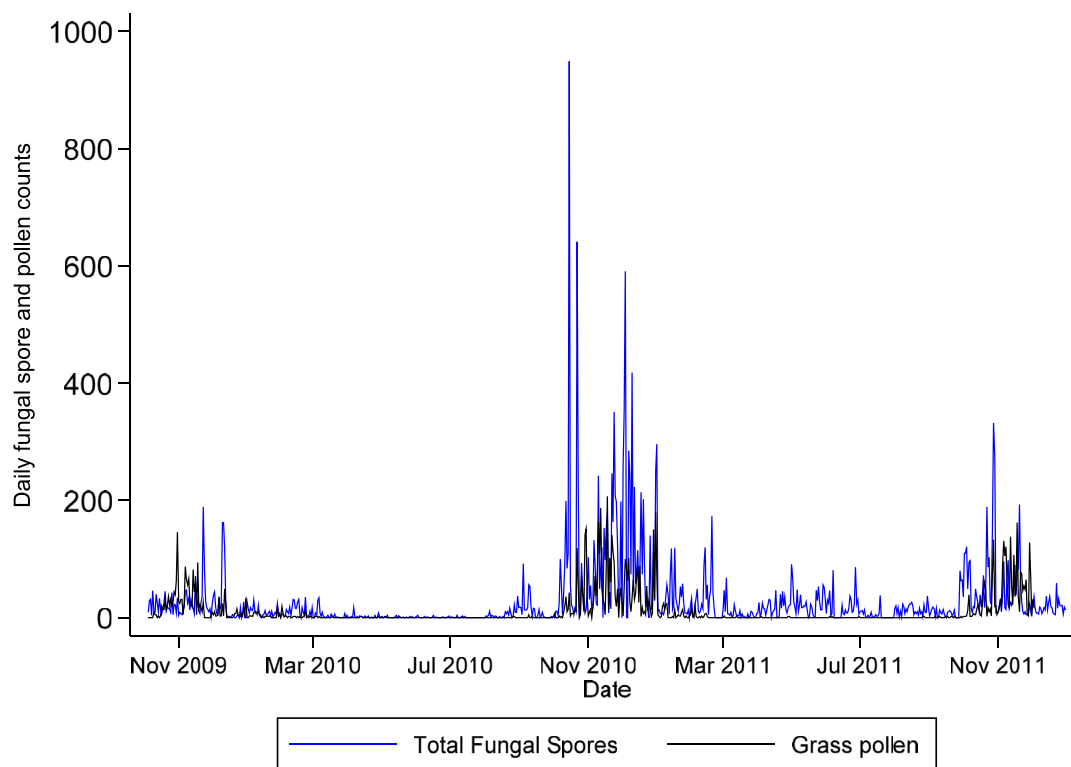


Fig. 2. Distribution of total fungal spores and grass pollen counts during September 2009 to December 2011 in Melbourne, Australia. Data collected using a Burkard volumetric spore trap at the University of Melbourne, Parkville, Melbourne.

Table 3

Adjusted associations between outdoor fungal spore taxa and lung function outcomes on same day and up to 3 days cumulative lag.

N = 866		FEV ₁ (ml/sec) coeff (95% CI) p-value		FVC (ml) coeff (95% CI) p-value		FEF _{25–75%} (ml/sec) coeff (95% CI) p-value	
Fungal spore taxa		Lag0	Lag0-3	Lag0	Lag0-3	Lag0	Lag0-3
<i>Cladosporium</i>	(A)	12 (–13, 38) .35	4 (–4, 13) .32	11 (–18, 39) .45	7 (–2, 39) .12	15 (–18, 47) .38	3 (–13, 20) .70
	(B)	12 (–16, 41) .39	5 (–6, 15) .38	11 (–22, 43) .52	7 (–3, 17) .17	18 (17, 53) .30	5 (–14, 23) .61
<i>Leptosphaeria</i>	(A)	11 (–6, 29) .20	3 (–2, 8) .28	8 (–13, 28) .46	4 (–2, 28) .17	23 (–9, 54) .16	1 (–10, 13) .81
	(B)	11 (–7, 28) .24	3 (–3, 9) .40	6 (–15, 27) .58	4 (–3, 12) .27	25 (–7, 57) .12	2 (–10, 15) .73
<i>Alternaria</i>	(A)	–17 (–59, 25) .44	5 (–13, 23) .59	–7 (–42, 28) .71	12 (–6, 28) .20	–25 (–108, 58) .55	1 (–30, 31) .97
	(B)	–33 (–80, 15) .18	4 (–17, 25) .71	–22 (–63, 20) .31	13 (13, 34) .25	–31 (–130, 68) .54	2 (–34, 38) .91
<i>Ustilago/smuts</i>	(A)	–16 (–30, –2) .03	–7 (–12, –2) .004	–7 (–17, 3) .19	–3 (–7, 3) .14	–32 (–57, –7) .01	–15 (–23, –7) < .001
	(B)	–21 (–36, –7) .004	–9 (–14, –4) < .001	–11 (–22, 0.0) .05	–4 (–8, –0.2) .04	–39 (–65, –13) .003	–18 (–27, –9) < .001
<i>Coprinus</i>	(A)	4 (–47, 56) .87	6 (–5, 18) .31	–1 (–51, 49) .96	10 (–2, 49) .10	15 (–84, 113) .77	0 (–22, 23) .97
	(B)	1 (–52, 55) .96	6 (–8, 20) .42	–7 (–59, 46) .81	10 (–5, 25) .21	–20 (–52, 12) .71	–15 (–27, –3) .87
<i>Drechslera</i>	(A)	–10 (–25, 5) .20	–5 (–11, 1) .12	–5 (–15, 5) .29	–1 (–6, 5) .63	–17 (–46, 13) .27	–11 (–21, –1) .04
	(B)	–15 (–32, 2) .09	–8 (–14, –1) .03	–9 (–21, 3) .13	–3 (–9, 3) .32	–17 (–93, 58) .22	–15 (–27, –3) .01
Total spores	(A)	2 (–34, 38) .91	0 (–12, 12) .97	7 (–28, 41) .71	5 (–5, 41) .33	–4 (–60, 53) .90	–6 (–28, 16) .58
	(B)	–1 (–46, 44) .97	–2 (–16, 13) .84	11 (–22, 43) .90	7 (–3, 17) .47	18 (17, 53) .27	5 (–14, 23) .57

Regression coefficient changes with 95%CI estimated using generalized linear models. (A) Adjusted for age, sex, height; and (B) Adjusted for age, sex, height, relative humidity, maximum temperature and grass pollen. Coefficients can be interpreted as per increase in fungal taxa counts from the 75th to 90th percentile.

Table 4

Adjusted associations between outdoor fungal spore taxa and positive bronchodilator response (BDR); and increased exhaled breath condensate nitrogen oxides (EBC NOx).

BDR n = 853				
Fungal spore taxa	Lag0	p-value	Lag0-3	p-value
	OR (95%CI)		OR (95%CI)	
<i>Cladosporium</i>	1.0 (0.9, 1.1)	.87	1.03 (0.99, 1.08)	.14
<i>Leptosphaeria</i>	1.0 (0.8, 1.2)	.86	1.01 (0.96, 1.06)	.79
<i>Alternaria</i>	1.3 (1.0, 1.6)	.06	1.09 (0.98, 1.20)	.10
<i>Ustilago/smuts</i>	1.1 (1.04, 1.2)	.003	1.04 (1.02, 1.07)	.001
<i>Coprinus</i>	1.0 (0.7, 1.4)	.79	1.03 (0.94, 1.12)	.58
<i>Drechslera</i>	1.1 (1.03, 1.2)	.009	1.06 (1.03, 1.09)	< .001
Total spores	1.1 (0.9, 1.3)	.19	1.09 (1.01, 1.16)	.02
EBC NOx (μM) n = 231				
<i>Cladosporium</i>	1.1 (0.9–1.3)	.35	1.1 (1.0–1.2)	.03
<i>Leptosphaeria</i>	0.9 (0.8–1.0)	.14	1.0 (1.0–1.1)	.55
<i>Alternaria</i>	1.3 (0.9–1.9)	.14	1.1 (1.0–1.3)	.046
<i>Ustilago/smuts</i>	1.1 (1.0, 1.2)	.20	1.0 (1.0, 1.1)	.18
<i>Coprinus</i>	1.0 (0.7, 1.2)	.96	1.1 (1.0, 1.2)	.25
<i>Drechslera</i>	1.1 (1.0–1.1)	.04	1.0 (1.0–1.1)	.05
Total spores	1.2 (0.9–1.5)	.28	1.1 (1.0–1.2)	.02

BDR associations estimated using generalized estimating equations. Adjusted for age, sex, height, relative humidity, maximum temperature and grass pollen. EBC NOx associations estimated using ordinal logistic regression. Adjusted for age, sex, height, relative humidity, maximum temperature, storage time and grass pollen.

OR (odds ratio) can be interpreted as per increase in fungal taxa counts from the 75th to 90th percentile.

Brant test results were not significant (> 0.05), so the proportional odds assumption was not violated.

respiratory system (Medrek et al., 2017). Rutherford et al. studied 53 patients with asthma in a sub-tropical region of Australia, and reported significant associations between exposure to higher levels of outdoor fungal spores (grouped together) and reduced peak expiratory flow in the fungal sensitized subgroup (Rutherford et al., 2000). Our study, in a temperate region of Australia, observed inconsistent associations when fungal sensitization was tested as an effect modifier. Increasing exposure to *Ustilago/smuts* was associated with lower lung function in those with fungal sensitization. Interestingly, *Drechslera* was not associated with lower lung function at Lag0, but fungal sensitization modified this effect, with reduced FEV₁ and FVC. Accurate interpretation of these findings may be limited by the small sample size and questionable detection of sensitization to fungal allergens due to lack of availability and standardisation of fungal extracts (Fukutomi and Taniguchi, 2015).

Although two previous studies have examined children with and without asthma (Chen et al., 2014; Watanabe et al., 2016) they did not examine whether current asthma could act as an effect modifier between exposure to outdoor fungal spores and lung function. Our findings indicated that people with current asthma may be more susceptible to both lower lung function and airway inflammation when exposed to *Alternaria*, *Ustilago/smuts* and *Drechslera*. Although our sample was younger, these findings add to those of Rutherford et al. that total outdoor fungal spores were associated with decreased peak expiratory flow in fungal sensitized adults aged > 54 years during peak spore season (Rutherford et al., 2000). In terms of comparing with the lung function parameters that we measured, peak expiratory flow rate usually correlates well with FEV₁, however it has been reported that this correlation decreases in patients with asthma as airflow diminishes (Gibson, 2000).

Overall in this analysis, *Ustilago/smuts* appeared to have the most consistent associations with lower lung function and BDR, but not increased airway inflammation. Exposures to *Ustilago/smuts* were associated with both reduced lung function and increased airway

Table 5

Associations between outdoor fungal spore taxa and pre-bronchodilator spirometry, FeNO, EBC pH and BDR at Lag0, stratified by fungal sensitization status, and interaction p-values.

Fungal spore taxa	FEV ₁ (ml/sec)			FVC (ml)			FEF _{25–75%} (ml/sec)		
	coeff (95%CI) p-value	coeff (95%CI) p-value	p-int	coeff (95%CI) p-value	coeff (95%CI) p-value	p-int	coeff (95%CI) p-value	coeff (95%CI) p-value	p-int
Sensitized ≥ 1 fungal taxa	Not sensitized n = 670 <	Sensitized n = 178		Not sensitized n = 670 <	Sensitized n = 178 <		Not sensitized n = 670	Sensitized n = 178	
<i>Cladosporium</i>	2 (–24, 30) .87	48 (22, 74) < .001	.01	0 (–30, 30) .99	48 (16, 78) .02	.01	4 (–32, 40) .81	66 (14, 116) .01	0.12
<i>Leptosphaeria</i>	3 (–18, 22) .77	53 (–0.1, 106) .05	.13	–2 (–25, 21) .86	56 (–14, 127) .11	.24	17 (–17, 52) .33	55 (–40, 149) .26	0.55
<i>Alternaria</i>	–43 (–86, 1) .06	–16 (–112, 81) .75	.65	–19 (–61, 24) .39	–40 (–131, 52) .40	.25	–84 (–184, 16) .10	103 (–68, 273) .24	0.4
<i>Ustilago/smuts</i>	–14 (–29, 0.2) .05	–35 (–59, –10) .005	.01	2 (–10, 14) .73	–35 (–55, –15) .001	< .001	–42 (–68, –15) .002	–36 (–75, 3) .07	0.79
<i>Coprinus</i>	6 (–47, 58) .84	–39 (–148, 70) .48	.25	0 (–56, 57) .99	–54 (–170, 62) .36	.28	24 (–81, 130) .65	–27 (–208, 154) .77	0.28
<i>Drechslera</i>	–11 (–24, 2) .11	–31 (–61, –0.4) .046	.02	–2 (–12, 8) .67	–35 (–59, –11) .005	< .001	–24 (–54, 7) .13	–12 (–72, 48) .69	0.83
Total spores	–9 (–42, 28) .66	14 (–98, 121) .83	.94	–2 (–42, 37) .92	1 (–107, 121) .89	.83	–19, –79, 42) .54	47 (–98, 191) .52	0.8
Sensitized ≥ 1 fungal taxa	FeNO coeff (95%CI) p-value	coeff (95%CI) p-value	p-int	EBC pH coeff (95%CI) p-value	coeff (95%CI) p-value	p-int	BDR OR (95%CI) p-value	OR (95%CI) p-value	p-int
	Not sensitized n = 670	Sensitized n = 140		Not sensitized n = 198	Sensitized n = 25		Not sensitized n = 670	Sensitized n = 178	
<i>Cladosporium</i>	–0.2 (–1.4, 1.0) .75	3.0 (2.0, 4.0) < .001	< .001	0 (0, 0) .82	–0.8 (–1.2, –0.4) < .001	.001	1.0 (0.9, 1.2) .61	0.2 (0.0, 7.0) .35	.20
<i>Leptosphaeria</i>	–0.1 (–1.8, 1.2) .90	3.6 (–1.2, 7.8) .17	.02	0 (0, 0) .002	–0.1 (–0.2, –6.0) .04	.62	1.0 (0.9, 1.2) .96	0.6 (0.1, 3.7) .58	.39
<i>Alternaria</i>	1.8 (–2.4, 6.6) .37	–1.8 (–4.8, 1.8) .37	1.00	0 (–0.1, 0) .33	(–0.1, 0.3) .18	.94	1.4 (1.1, 1.8) .01	0.1 (0.0, 3.8) .21	.08
<i>Ustilago/smuts</i>	1.2 (–3.6, 3.0) .12	–0.6 (–1.8, 0.2) .10	.25	0 (0, 0) .61	0.1 (0.0, 0.2) .17	.96	1.1 (1.1, 1.2) .001	1.8 (0.5, 6.4) .34	.39
<i>Coprinus</i>	0.8 (–4.0, 5.6) .71	3.2 (–2.4, 8.4) .29	.24	0 (0.0, 0.1) .68	0.0 (–0.2, 0.3) .66	.66	1.0 (0.7, 1.4) .98	0.4 (0.1, 2.9) .38	.33
<i>Drechslera</i>	0.8 (–0.6, 2.2) .31	–0.4 (–1.6, 0.8) .52	.96	0 (0, 0) .34	0.1 (0.0, 0.2) .13	.99	1.1 (1.0, 1.2) .003	1.0 (0.4, 2.3) .98	.31
Total spores	0.6 (–18.6, 2.8) .64	2.8 (–18.6, 9.3) .26	.08	0.0 (0.0, 0.0) .95	0.0 (–0.5, 0.5) .98	.13	1.2 (1.0, 1.4) .07	0.3 (0.0, 9.5) .46	.21

Generalized linear models were used for FEV₁, FVC and FEF_{25–75%}, FeNO and EBC pH. Generalized estimating equations were used for BDR Adjusted for age, sex, height, relative humidity, maximum temperature and grass pollen. p-int = p-value for interaction term; OR can be interpreted as per increase in fungal taxa counts from the 75th to 90th percentile.

inflammation in those with current asthma and fungal sensitization. *Ustilago/smuts* are not commonly examined as a fungal taxon associated with changes in lung function and there are currently no comparable studies. However, Atkinson and colleagues reported that *Ustilago/smuts* tended to be associated with asthma hospitalisations in London, but the relative risks were not statistically significant (Atkinson et al., 2006). Our previous case-crossover study in Melbourne did not find associations between *Ustilago/smuts* and adolescent asthma hospitalisations (Tham et al., 2017). However, associations were detected between *Ustilago/smuts* and adolescent asthma hospitalisations in our Sydney case-crossover study (OR = 1.25, 95%CI 1.03 to 1.5) (Tham et al., 2017). These differences may relate to variations in sample sizes, fungal spore levels, and other potentially confounding exposures such as differing species of grass pollen and levels of air pollutants. The risk of outdoor fungi exposure leading to adverse changes to the respiratory

system may be higher in those with fungal sensitization and/or current asthma.

Previous studies have demonstrated that fungal sensitization rates in patients with current asthma are high and range between 35 and 75% (Del Giacco et al., 2016). The fungal sensitization rate in our study population with current asthma was slightly lower, at approximately 30%. However, as sensitization to *Ustilago* is not routinely tested, we could not assess whether *Ustilago/smuts* sensitization might act as an effect modifier. There is potential for cross-reactivity between *Ustilago/smuts* and other fungal spores that contain similar proteins (Fukutomi and Taniguchi, 2015) such as *Malassezia sympodialis* (Mala s1) (Weber and Levettin, 2013) and the yeast *Cryptococcus neoformans* (Cheng et al., 2017), which may account for the effect modification observed in participants sensitized to other fungal taxa in this study.

This is the first study to combine daily outdoor fungal spore data

Table 6

Associations between outdoor fungal spore taxa, lung function outcomes at Lag0, stratified by current asthma status, and interaction p-value.

Fungal spore taxa	FEV ₁ (ml/sec)			FVC (ml)			FEF _{25–75%} (ml/sec)		
	coeff (95%CI) p-value	coeff (95%CI) p-value	p-int	coeff (95%CI) p-value	coeff (95%CI) p-value	p-int	coeff (95%CI) p-value	coeff (95%CI) p-value	p-int
<i>Current asthma</i>	No n = 708	Yes n = 134		No n = 708	Yes n = 134		No n = 708	Yes n = 134	
<i>Cladosporium</i>	7 (0, 4) .06	9 (–34, 52) .68	.40	21 (–8, 50) .15	4 (–43, 50) .88	.36	28 (–9, 65) .13	19 (–37, 76) .51	.75
<i>Leptosphaeria</i>	3 (–18, 23) .80	–8 (–138, 122) .91	.75	5 (–19, 29) .68	–36 (–158, 86) .57	.58	2 (–40, 43) .94	59 (–119, 238) .52	.64
<i>Alternaria</i>	–4 (–57, 49) .88	–124 (–217, –31) .01	.06	–7 (–66, 53) .83	–85 (–161, –10) .03	.10	1 (–100, 101) .99	–212 (–371, –52) .01	.07
<i>Ustilago/smuts</i>	–3 (–25, 19) .77	–28 (–52, –3) .03	.15	–3 (–29, 23) .82	–16 (–32, –1) .04	.26	–4 (–31, 24) .78	–47 (–86, –9) .02	.08
<i>Coprinus</i>	29 (–26, 84) .30	–132 (–289, 25) .10	.02	21 (–41, 83) .51	–83 (–233, 67) .28	.08	45 (–57, 148) .39	–243 (–463, –22) .03	.01
<i>Drechslera</i>	1 (–17, 19) .90	–30 (–56, –4) .02	.04	2 (–56, 59) .96	–19 (–38, –0.4) .045	.07	2 (–28, 32) .90	–50 (–95, –5) .03	.02
Total spores	25 (–17, 67) .25	–23 (–2, 1) .57	.09	23 (–24, 70) .34	–19 (–93, 56) .63	.09	29 (–36, 95) .38	–31 (–146, 84) .60	.22

Regression coefficient changes with 95%CI estimated using generalized linear models. Adjusted for age, sex, height, relative humidity, maximum temperature and grass pollen. p-int = p-value for interaction term; Coefficients can be interpreted as per increase in fungal taxa counts from the 75th to 90th percentile.

Table 7

Associations between outdoor fungal spore taxa and EBC pH at Lag0, stratified by current asthma status, and interaction p-value.

Fungal spore taxa	EBC pH		p-int
	coeff (95%CI) p-value	coeff (95%CI) p-value	
<i>Current asthma</i>	No n = 146	Yes n = 57	
<i>Cladosporium</i>	0.01 (–0.004, 0.03) .129	–0.04 (–0.07, –0.02) .001	< 0.001
<i>Leptosphaeria</i>	0.02 (0.008, 0.04) .004	0.1 (–0.2, 0.4) .49	0.7
<i>Alternaria</i>	–0.03 (–0.09, 0.04) .42	–0.42 (–0.61, –0.22) < .001	0.1
<i>Ustilago/smuts</i>	0.01 (–0.02, 0.04) .61	–0.05 (–0.07, –0.02) .001	0.03
<i>Coprinus</i>	0.03 (–0.034, 0.1) .33	–0.33 (–0.69, 0.04) .08	0.02
<i>Drechslera</i>	0 (–0.02, 0.02) .94	–0.06 (–0.09, –0.03) < .001	0.002
Total spores	0.04 (0.01, 0.07) .008	–0.2 (–0.3, –0.06) .003	< 0.001

Regression coefficient changes with 95%CI estimated using generalized linear models. Adjusted for age, sex, height, relative humidity, maximum temperature, grass pollen and sample storage time. p-int = p-value for interaction term; Coefficients can be interpreted as per increase in fungal taxa counts from the 75th to 90th percentile.

with objective measures of lung function, BDR and airway inflammation to assess associations between these exposures and outcomes. In addition, we were able to assess potential effect modification by fungal sensitization and current asthma status, which have not previously been reported. Our findings suggest that not all fungal spores have the same effect on the airways and when we grouped the fungal taxa into total numbers, the signals found with individual fungal taxa were not evident. This highlights the importance of not grouping fungal taxa together when exploring their health effects.

However, there are limitations associated with this analysis. We could not be certain that each participant was exposed to the same levels of fungal spores, pollen or pollution counted at a single site and exposure misclassification was likely. As we considered outdoor exposures, fungal exposure misclassification was likely to be non-differential and should have biased the risk estimates towards the null. The

absence of indoor fungal spore measurements may limit exposure assessment and contribute to exposure misclassification. However, this exposure was also likely to be non-differential and bias the results towards the null.

The sample sizes for each respiratory function test varied. The EBC was conducted only on the probands who represented approximately one-third of the total sample. Although matched in age, the small sample tested over a two-year period may have lacked adequate power to detect true associations, particularly interactions. In addition, this cross-sectional analysis could not consider daily changes in lung function and airway inflammation or repeated measures of the same individuals over time, which may provide stronger evidence of effects by fungal spore exposures. This might be particularly important if exposure to elevated or threshold levels of fungal spores is limited in duration and frequency. With multiple fungal taxa and outcomes being assessed, there was a risk of type 1 statistical errors. Hence the results should be interpreted with some caution, and replication is required in a follow-up study, especially in those at risk.

The findings from this detailed exploratory analysis suggest complex and potentially important interrelationships between outdoor fungal spores and acute adverse respiratory effects particularly in those with fungal sensitization and/or current asthma. Outdoor fungal spores are ubiquitous and cannot be readily controlled but understanding their effects on respiratory function may help predict impairment in fungal sensitized or asthmatic individuals. People who are sensitized to fungi and/or living with asthma, may benefit from minimising their exposure to elevated levels of outdoor fungal spores. However, there are a diversity of fungal spores and their levels vary by spore taxa and meteorological conditions (Mitakakis and Guest, 2001), so prediction of high-risk periods is challenging. A strategy to address this could involve including sentinel fungal spore counts in existing aeroallergen monitoring systems and providing warning information to high-risk individuals prior to high concentration days.

Funding sources

RT was funded by an Australian Government National Health & Medical Research Council (NHMRC) Research Training Program Scholarship (GNT1055754) and Centre for Air quality & health Research and evaluation (NHMRC Centre of Research Excellence) top-up scholarship. SCD and AJ Lowe are funded by the NHMRC. FMA is supported by the Department of Clinical Laboratory Sciences, College of

Applied Medical Sciences, King Saud University, Riyadh, Saudi Arabia.

The Melbourne Atopy Cohort Study (MACS) was supported by the NHMRC (APP454856), Nestec Ltd, a subsidiary of Nestlé Australia and The University of Melbourne (Melbourne, Australia). The Melbourne Air Pollen and Children and Adolescent Health (MAPCAH) study was funded by the NHMRC (APP541934).

Role of the funding source

The funding sources had no role in study design, data collection, data analysis or interpretation, or the writing of this paper. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Potential conflicts of interest

MJ Abramson holds investigator-initiated grants from Pfizer and Boehringer-Ingelheim for unrelated research. He has undertaken an unrelated consultancy for Sanofi. All other authors declare no conflict of interest.

Ethics approvals

Ethics approvals were granted by The University of Melbourne and Royal Children's Hospital Human Research Ethics Committees.

CRediT authorship contribution statement

Rachel Tham: Conceptualization, Data curation, Formal analysis, Methodology, Writing - original draft, Writing - review & editing. **Bircan Erbas:** Conceptualization, Methodology, Writing - review & editing. **Shyamali C. Dharmage:** Conceptualization, Funding acquisition, Methodology, Writing - review & editing. **Mimi LK. Tang:** Writing - review & editing. **Fahad Aldakheel:** Methodology, Writing - review & editing. **Caroline J. Lodge:** Data curation, Writing - review & editing. **Paul S. Thomas:** Writing - review & editing. **Philip E. Taylor:** Writing - review & editing. **Michael J. Abramson:** Writing - review & editing. **Adrian J. Lowe:** Conceptualization, Data curation, Formal analysis, Writing - review & editing.

Acknowledgements

We acknowledge and sincerely thank Ed Newbigin and Emma Lewis (The University of Melbourne) for collecting and analyzing the fungal spore and pollen data. FMA extends his appreciation to the College of Applied Medical Sciences Research Centre and the Deanship of Scientific Research at King Saud University.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envres.2019.108675>.

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Supplementary Material:

Outdoor fungal spores and acute respiratory effects in vulnerable individuals

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Methods

Study population

The Melbourne Atopy Cohort Study (MACS) is a longitudinal, birth cohort study comprising 620 babies (probands) enrolled prior to birth by recruiting pregnant women living in Melbourne, Australia [E1, E2]. Eligible babies had at least one first-degree family member with a history of eczema and/or asthma and/or allergic rhinitis and/or severe food allergy. Probands were born between 24 March 1990 and 1 November 1994. They were followed up monthly from birth to 15 months; at 18 months; annually from 2 to 7 years; at 12 years; and at 18 years. This study is based on the 18-year follow-up of the MACS cohort which was conducted from September 2009 to December 2011. Entire families, which included the probands, their siblings and parents, were enrolled.

Measuring outdoor fungal spores and pollen grains

Fungal spore counts were measured for the Melbourne Air Pollen Children and Adolescent Health (MAPCAH) study using a Burkard volumetric spore trap (Burkard, United Kingdom) located on the rooftop of the Earth Sciences building at the University of Melbourne, Parkville site in Melbourne, in accordance with World Allergy Organisation guidelines [E3]. Collection involved drawing 10 litres of air per minute continuously across a microscope slide coated with adhesive. Airborne particles stuck to the slide as it moved past the inlet at 2mm/hour. The fungal spores were identified and counted by a trained technician and related to the volumes of air sampled, giving concentrations per cubic metre of air, averaged over the 24-hours.

Air pollutants and weather

The 24-hour average daily concentrations of particulate matter up to 2.5µm in diameter (PM_{2.5}) and up to 10µm in diameter (PM₁₀) (µg/m³), the daily maximum one-hour average nitrogen dioxide (NO₂) level (ppb), and the daily maximum four-hour average ozone (O₃) level (ppb) were obtained from routine air quality monitoring stations in Melbourne administered by the Environment Protection Authority of Victoria. The Bureau of Meteorology (Australian Government) provided data on daily maximum and minimum temperatures (degrees Celsius), rainfall (mm), and relative humidity for Melbourne.

Fungal sensitization

Fungal sensitization was detected with a skin prick test (SPT). A single drop of *Alternaria*, *Penicillium* and *Cladosporium cladosporoides* (Hollister-Stier, Spokane, WA, USA; Alyostal, Antony, France) allergen was placed on the participant's back, and the skin was pricked with a lancet. Histamine (1mg/mL) was the positive control and saline was the negative control. Fungal sensitization was defined as a positive SPT with a wheal diameter of ≥3mm.

Lung function

Spirometry was performed according to ATS/ERS guidelines [E4] using the EasyOne Spirometer (nidd Medical Technologies Inc., Andover, MA). Participants were instructed to withhold short- and long-acting β -agonists for 4-hours and 12-hours respectively before the bronchodilator test.

Assessment of bronchodilator response

Bronchodilator response was assessed by the administration of three puffs of 100ug salbutamol using a metered dose inhaler and volumatic spacer, with each puff separated by 30 seconds. Each inhalation was held for 5 to 10 seconds. Post-bronchodilator spirometry was conducted 10 minutes after the final dose was administered.

Measuring exhaled breath condensate

Exhaled breath condensate (EBC) were collected following ATS/ERS guidelines [E5, E6] using a glass-condensing chamber in wet ice. Each sampling session obtained 0.4 to 3.0 mL of EBC. Each sample was divided into 120 μ L aliquots. The sub-samples were de-aerated with argon gas and then immediately frozen and stored at -80 °C. For analysis, EBC samples were thawed to room temperature. The total NO_x in each EBC sample was measured using a standardised protocol of enzymatic reduction of nitrate using a fluorimetric modification of the Greiss method. The limit of detection of the NO_x assay was 1.25 μ M. A micro pH-meter (LAQUA Twin pH Meter, Spectrum Technologies, USA) was used to measure pH of the EBC samples.

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Supplementary Material: Tables and Figures

Table E1: Correlations between fungal spore taxa from the MAPCAH study: September 2009 to December 2011 in Melbourne (p-values below correlation values)

Table E2: Correlations between fungal spore taxa and grass pollen, maximum temperature and relative humidity from the MAPCAH study: September 2009 to December 2011 in Melbourne (p-values below correlation values)

Table E3: Associations between outdoor fungal spore taxa and z-scores of lung function outcomes on same day and up to 3 days cumulative lag.

Table E4: Adjusted associations between fungal spore counts and exhaled nitric oxide using FeNO measures.

Table E5: Multi-fungal models of associations between outdoor fungal spore taxa and FEV₁ preBD, FVC preBD, FEF_{25-75%} preBD, BDR and EBC NOx on same day and up to 3 days lag. Adjusted for age, sex, height, relative humidity, maximum temperature and grass pollen. EBC NOx also adjusted for storage time.

Figure E1: Flowchart of the Melbourne Atopy Cohort Study (MACS) recruitment and follow-up testing

Figure E2: Distribution of lung function testing of MACS participants by month

Figure E3: Distribution of exhaled breath condensate testing of MACS participants by month

Table E1: Correlations between fungal spore taxa from the MAPCAH study: September 2009 to December 2011 in Melbourne (p-values below correlation values)

	<i>Cladosporium</i>	<i>Leptosphaeria</i>	<i>Alternaria</i>	<i>Ustilago/smuts</i>	<i>Coprinus</i>	<i>Drechslera</i>	<i>Periconia</i>	<i>Pleospora</i>	<i>Ganoderma</i>	<i>Pithomyces</i>	<i>Stemphylium</i>	<i>Sporormiella</i>	Total spores
<i>Cladosporium</i>	1												
<i>Leptosphaeria</i>	.462 <.001	1											
<i>Alternaria</i>	.492 <.001	.258 <.001	1										
<i>Ustilago/smuts</i>	.337 <.001	.131 <.001	.28 <.001	1									
<i>Coprinus</i>	.252 <.001	.409 <.001	.269 <.001	.063 <.001	1								
<i>Drechslera</i>	.459 <.001	.294 <.001	.441 <.001	.215 <.001	.269 <.001	1							
<i>Periconia</i>	.315 <.001	.216 <.001	.286 <.001	.212 <.001	.212 <.001	.302 <.001	1						
<i>Pleospora</i>	.245 <.001	.394 <.001	.166 <.001	.129 <.001	.262 <.001	.145 <.001	.111 <.001	1					
<i>Ganoderma</i>	.025 <.001	.182 <.001	.062 <.001	-.025 <.001	.245 <.001	.041 <.001	.047 <.001	.104 <.001	1				
<i>Pithomyces</i>	.14 <.001	.201 <.001	.219 <.001	.088 <.001	.186 <.001	.238 <.001	.175 <.001	.123 <.001	.034 <.001	1			
<i>Stemphylium</i>	.316 <.001	.177 <.001	.291 <.001	.086 <.001	.147 <.001	.284 <.001	.26 <.001	.156 <.001	.177 <.001	.113 <.001	1		
<i>Sporormiella</i>	.156 <.001	.205 <.001	.131 <.001	-.053 <.001	.176 <.001	.126 <.001	.036 <.001	.09 <.001	.145 <.001	.096 <.001	.164 <.001	1	
Total spores	.829 <.001	.626 <.001	.665 <.001	.404 <.001	.495 <.001	.557 <.001	.413 <.001	.38 <.001	.186 <.001	.259 <.001	.36 <.001	.198 <.001	1

Table E2: Correlations between fungal spore taxa and grass pollen, maximum temperature and relative humidity from the MAPCAH study: September 2009 to December 2011 in Melbourne (p-values below correlation values)

	Grass pollen	Maximum temperature	Relative humidity
<i>Cladosporium</i>	.501 <.001	.217 <.001	-.173 <.001
<i>Leptosphaeria</i>	.162 <.001	-.034 .330	.067 .056
<i>Alternaria</i>	.492 <.001	.336 <.001	-.120 .001
<i>Ustilago/smuts</i>	.492 <.001	.213 <.001	-.141 <.001
<i>Coprinus</i>	.202 <.001	.083 .017	.061 .081
<i>Drechslera</i>	.375 <.001	.223 <.001	-.090 .01
<i>Periconia</i>	.243 <.001	.158 <.001	-.071 .043
<i>Pleospora</i>	.056 .109	-.047 .182	.022 .525
<i>Ganoderma</i>	-.007 .834	-.101 .004	.049 .159
<i>Pithomyces</i>	.068 .050	.087 .013	.028 .430
<i>Stemphylium</i>	.193 <.001	.069 .047	-.019 .597
<i>Sporormiella</i>	.074 .033	.019 .579	.004 .903
Total spores	.507 <.001	.237 <.001	-.112 .0013
Grass pollen	1.000	.456 <.001	-.223 <.001
Max temperature		1	-.482 <.001
Relative humidity			1

Table E3: Associations between outdoor fungal spore taxa and z-scores of lung function outcomes on same day and up to 3 days cumulative lag.

N=866		FEV ₁ z-score coeff (95% CI) p-value		FVC z-score coeff (95% CI) p-value		FEF _{25-75%} z-score coeff (95% CI) p-value	
Fungal spore taxa		Lag0	Lag0-3	Lag0	Lag0-3	Lag0	Lag0-3
<i>Cladosporium</i>	(A)	.001 (-.001,.003) .46	.0003 (-.001, .001) .44	.0006 (-.001, .003) .55	.0004 (-.0002, .001) .20	.001 (-.001, .002) .43	.0002 (-.001, .001) .67
	(B)	.001 (-.002, .003) .47	.0003 (-.001, .001) .46	.0006 (-.002, .003) .62	.0004 (-.0002, .001) .24	.001 (-.001, .003) .33	.0003 (-.0007, .001) .60
<i>Leptosphaeria</i>	(A)	.002 (-.004, .007) .59	.0000 (-.002, .002) .93	.00004 (-.006, .006) .99	.0004 (-.001, .002) .64	.004 (-.002, .009) .18	.0002 (-.002, .002) .82
	(B)	.001 (-.004, .007) .59	-.0000 (-.002, .002) .87	-.0003 (-.006, .005) .91	.0002 (-.002, .002) .854	.004 (-.001, .009) .14	.0004 (-.002, .003) .75
<i>Alternaria</i>	(A)	-.003 (-.019, .012) .68	.001 (-.005, .007) .78	.001 (-.009, .012) .79	.003 (-.002, .008) .26	.004 (-.02, .01) .65	.0002 (-.006, .006) .96
	(B)	-.006 (-.025, .013) .52	.001 (-.007, .007) .87	-.0001 (-.013, .013) .99	.003 (-.002, .009) .26	-.004 (-.02, .02) .69	.0006 (-.006, .007) .85
<i>Ustilago/smuts</i>	(A)	-.004 (-.009, -.0001) .046	-.002 (-.003, -.001) .002	-.0009 (-.003, .001) .421	-.0005 (-.001, .0003) .233	-.005 (-.01, .0002) .06	-.002 (-.004, -.0007) .005
	(B)	-.006 (-.01, -.001) .009	-.003 (-.004, -.001) <.001	-.002 (-.004, .0008) .187	-.001 (-.002, .0002) .11	-.006 (-.01, -.001) .03	-.003 (-.005, -.001) .002
<i>Coprinus</i>	(A)	.004 (-.02, .03) .77	.003 (-.003, .009) .29	-.0005 (-.02, .02) .96	.004 (-.0004, .009) .07	.009 (-.02, .03) .50	.001 (-.005, .008) .67
	(B)	.004 (-.02, .03) .79	.004 (-.004, .01) .31	-.002 (-.02, .02) .87	.005 (-.001, .011) .13	.01 (-.01, .04) .41	.002 (-.004, .009) .50
<i>Drechslera</i>	(A)	-.007 (-.02, .009) .40	-.004 (-.009, .002) .16	-.001 (-.009, .007) .81	-.0002 (-.004, .003) .92	-.01 (-.02, .01) .45	-.005 (-.01, .001) .12
	(B)	-.009 (-.03, .007) .26	-.006 (-.01, -.0002) .04	-.003 (-.01, .007) .56	-.001 (-.005, .003) .60	-.007 (-.02, .01) .43	-.007 (-.013, .0003) .06
Total spores	(A)	.0001 (-.001, .001) .99	.0001 (-.001, .0004) .92	.0002 (-.0009, .001) .73	.0001 (-.0002, .0005) .37	-.0001 (-.001, .001) .96	-.0001 (-.001, .0004) .725
	(B)	-.0001 (-.002, .002) .95	-.0001 (-.001, .0005) .78	-.0001 (-.001, .001) .89	.0001 (-.0003, .0005) .48	.0001 (-.001, .001) .93	-.0001 (-.001, .0005) .73

Regression coefficient changes with 95%CI estimated using generalized linear models. (A) Unadjusted; and (B) Adjusted for relative humidity, maximum temperature and grass pollen.

Table E4: Adjusted associations between fungal spore counts and exhaled nitric oxide using FeNO measures.

FeNO n=797	Lag0		Lag0-3	
Fungal spore taxa	coeff (95%CI)	p-value	coeff (95%CI)	p-value
<i>Cladosporium</i>	0.6 (-0.6,1.8)	.35	0.3 (-0.2, 0.9)	.28
<i>Leptosphaeria</i>	0.3 (-1.3, 1.9)	.68	0.2 (-0.3, 0.7)	.29
<i>Alternaria</i>	1.9 (-1.3, 5.1)	.24	0.5 (-0.6, 1.5)	.87
<i>Ustilago/smuts</i>	0.9 (-0.2, 1.9)	.11	0.4 (0.0, 0.8)	.25
<i>Coprinus</i>	2.1 (-2.2, 6.4)	.34	0.9 (-0.2, 1.9)	.52
<i>Drechslera</i>	0.6 (-0.4, 1.7)	.24	0.3 (-0.1, 0.8)	.64
Total spores	1.3 (-0.8, 3.4)	.21	0.6 (-0.2, 1.3)	.29

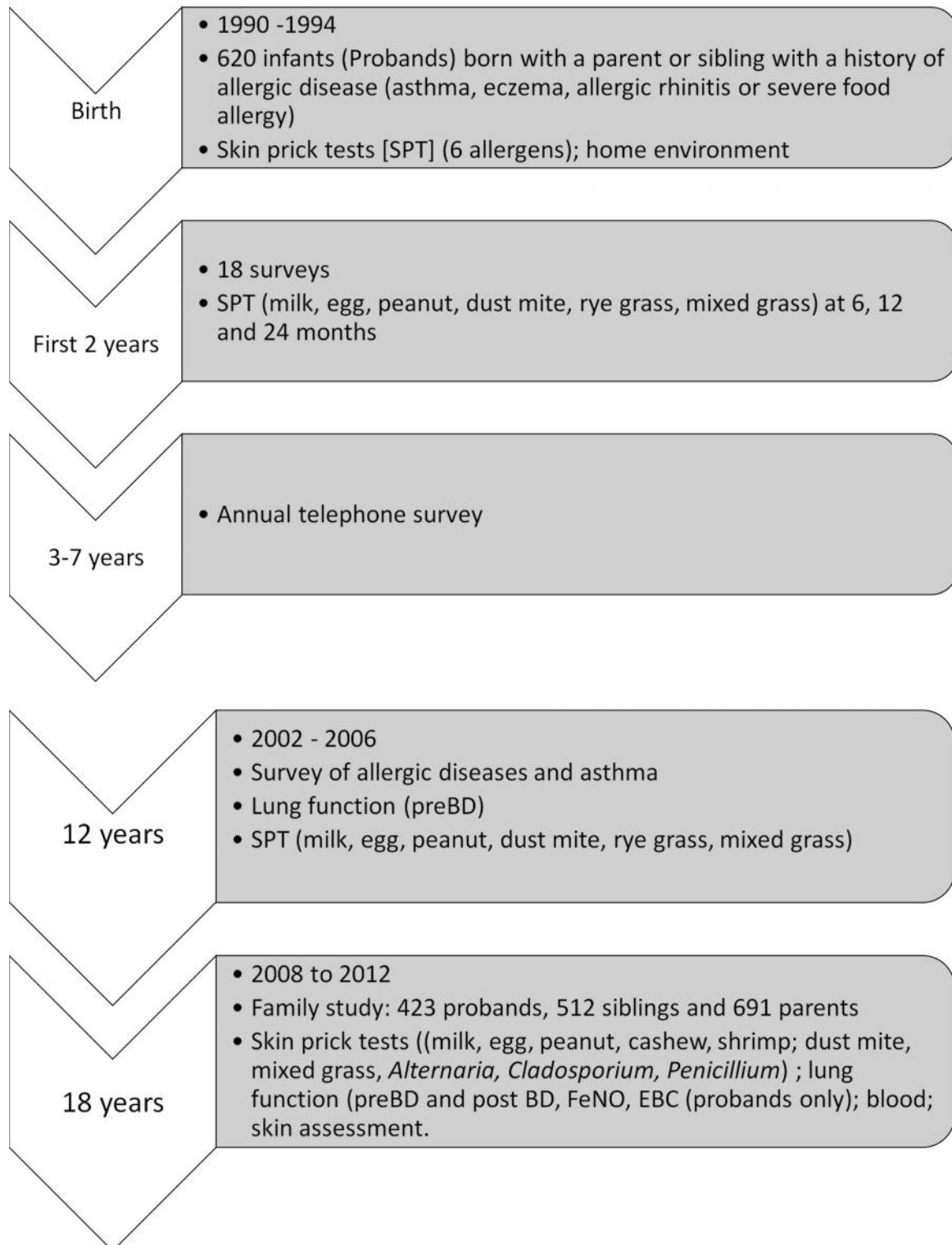
Associations estimated using generalized linear models, to account for clustering of exposures and outcomes within family units. Adjusted for age, sex, height, relative humidity, maximum temperature, and grass pollen. Coefficients can be interpreted as per increase in fungal taxa counts from the 75th to 90th percentile.

Table E5: Multi-fungal models of associations between outdoor fungal spore taxa and FEV₁ preBD, FVC preBD, FEF_{25-75%} preBD, BDR and EBC NOx on same day and up to 3 days lag.

FEV₁ PreBD (ml/sec)	Lag0 (Coeff 95% CI)	p-value	Lag0-3 (Coeff 95% CI)	p-value
<i>Cladosporium</i>	23 (-15, 61)	.23	9 (-5, 22)	.23
<i>Leptosphaeria</i>	6 (-18, 30)	.64	-2 (-14, 9)	.69
<i>Alternaria</i>	-24 (-93, 45)	.50	7 (-21, 35)	.63
<i>Ustilago/smuts</i>	-16 (-42, 10)	.23	-6 (-18, 6)	.35
<i>Coprinus</i>	24 (-38, 86)	.45	18 (-3, 38)	.09
<i>Drechslera</i>	-8 (-45, 28)	.66	-9 (-26, 8)	.30
FVC PreBD (ml)	Lag0 (Coeff 95% CI)		Lag0-3 (Coeff 95% CI)	
<i>Cladosporium</i>	21 (-22, 64)	.34	8 (-6, 23)	.26
<i>Leptosphaeria</i>	3 (-24, 30)	.84	-4 (-15, 8)	.52
<i>Alternaria</i>	-15 (-88, 59)	.70	14 (-14, 42)	.31
<i>Ustilago/smuts</i>	-1 (-26, 24)	.92	-1 (-14, 12)	.90
<i>Coprinus</i>	10 (-53, 74)	.75	18 (-5, 40)	.12
<i>Drechslera</i>	-14 (-53, 26)	.50	-10 (-29, 9)	.30
FEF_{25-75%}PreBD (ml/sec)	Lag0 (Coeff 95% CI)		Lag0-3 (Coeff 95% CI)	
<i>Cladosporium</i>	22 (-29, 73)	.40	12 (-12, 36)	.32
<i>Leptosphaeria</i>	17 (-28, 63)	.45	-3 (-28, 22)	.83
<i>Alternaria</i>	-16 (-149, 116)	.81	7 (-43, 58)	.77
<i>Ustilago/smuts</i>	-50 (-98, -2)	.04	-18 (-42, 5)	.13
<i>Coprinus</i>	35 (-92, 162)	.59	18 (-18, 54)	.32
<i>Drechslera</i>	9 (-49, 66)	.77	-6 (-38, 27)	.73
BDR (OR 95%CI)	Lag0 (OR, 95%CI)		Lag0-3 (OR, 95%CI)	
<i>Cladosporium</i>	0.95 (0.81, 1.12)	.54	1.02 (0.96, 1.08)	.62
<i>Leptosphaeria</i>	1.0 (0.82, 1.21)	.99	0.97 (0.89, 1.06)	.49
<i>Alternaria</i>	1.09 (0.71, 1.66)	.70	1.05 (0.90, 1.22)	.53
<i>Ustilago/smuts</i>	1.04 (0.90, 1.20)	.58	0.99 (0.92, 1.06)	.73
<i>Coprinus</i>	0.73 (0.44, 1.21)	.22	0.93 (0.8, 1.09)	.40
<i>Drechslera</i>	1.10 (0.89, 1.36)	.37	1.08 (0.98, 1.19)	.13
EBC NOx (OR 95%CI)	Lag0 (OR, 95%CI)		Lag0-3 (OR, 95%CI)	
<i>Cladosporium</i>	1.04 (0.87, 1.26)	.65	1.05 (0.97, 1.14)	.24
<i>Leptosphaeria</i>	0.92 (0.80, 1.06)	.25	0.97 (0.90, 1.04)	.38
<i>Alternaria</i>	1.03 (0.62, 1.73)	.90	1.09 (0.92, 1.30)	.33
<i>Ustilago/smuts</i>	0.95 (0.79, 1.14)	.57	0.96 (0.87, 1.06)	.40
<i>Coprinus</i>	0.91 (0.55, 1.52)	.73	1.01 (0.87, 1.17)	.93
<i>Drechslera</i>	1.17 (0.90, 1.51)	.24	1.08 (0.94, 1.25)	.26

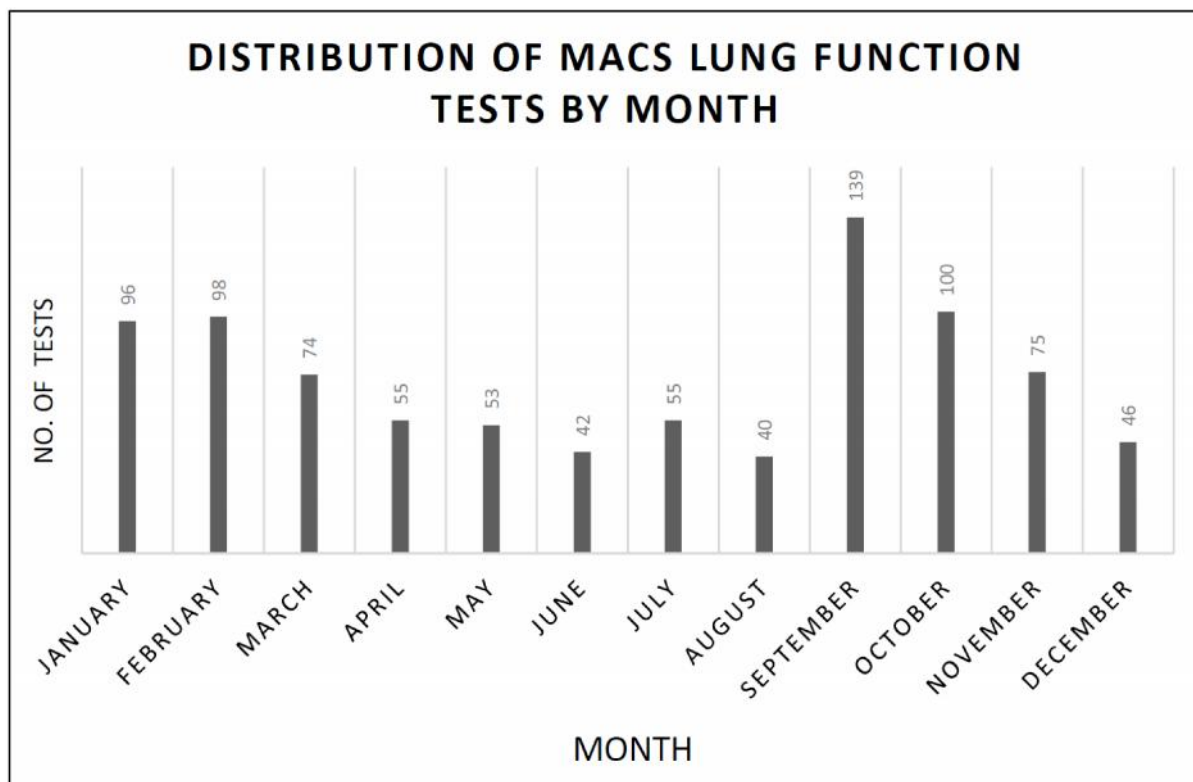
Adjusted for age, sex, height, relative humidity, maximum temperature and grass pollen. EBC NOx also adjusted for storage time. Coefficients and odds ratios (OR) can be interpreted as per increase in fungal taxa counts from the 75th to 90th percentile.

Figure E1: Flowchart of the Melbourne Atopy Cohort Study (MACS) recruitment and follow-up testing



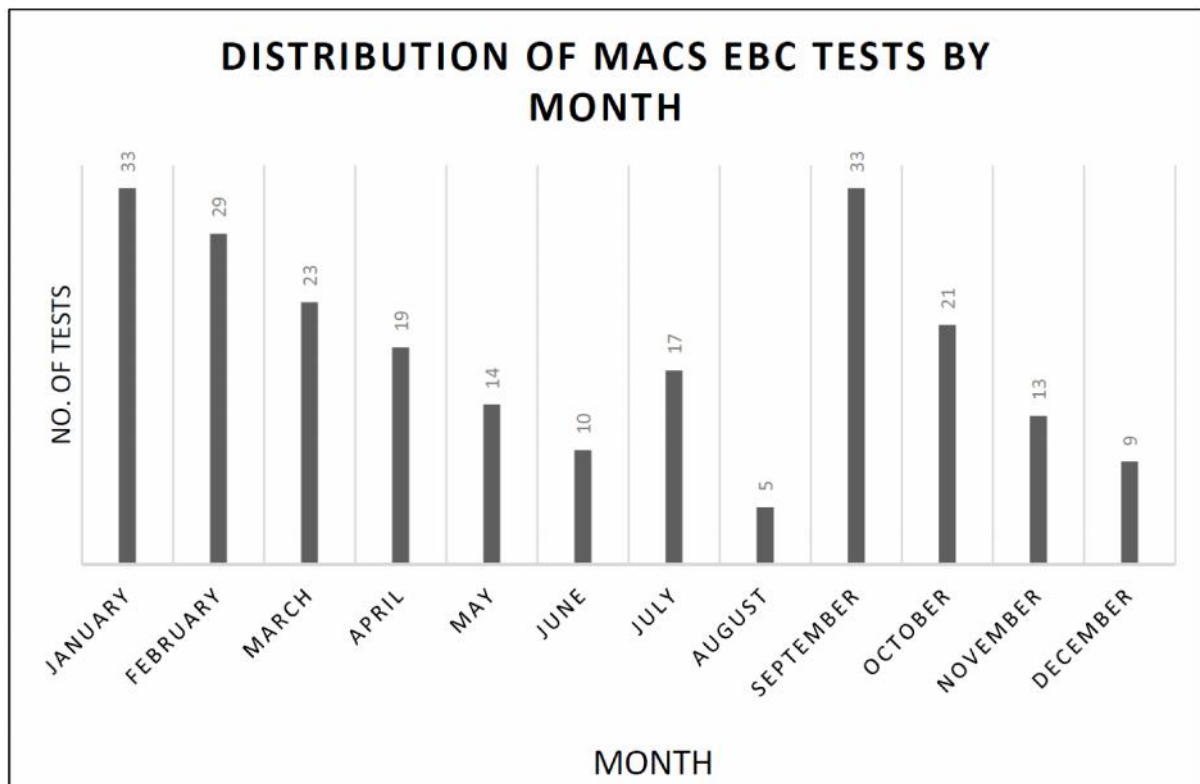
BD = bronchodilator; FeNO = Fractional exhaled nitric oxides; EBC = exhaled breath condensate; SPT = skin prick tests.

Figure E2: Distribution of lung function testing of MACS participants by month



Summer: December to February; Autumn: March to May; Winter: June to August; Spring: September to November

Figure E3: Distribution of exhaled breath condensate testing of MACS participants by month



Summer: December to February; Autumn: March to May; Winter: June to August; Spring: September to November