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Airborne culturable fungi in naturally ventilated primary school environments in a subtropical climate

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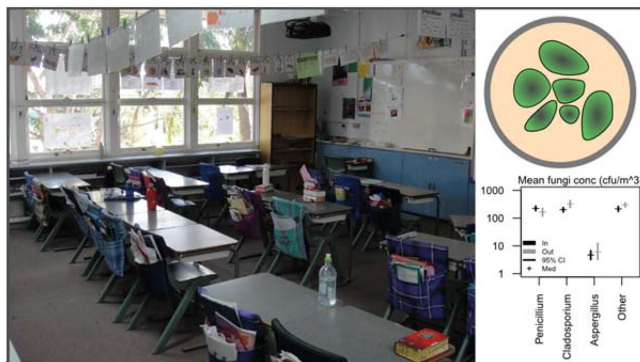
Graphical abstract



Abstract

There is currently a lack of reference values for indoor air fungal concentrations to allow for the interpretation of measurement results in subtropical school settings. Analysis of the results of this work established that, in the majority of properly maintained subtropical school buildings, without any major affecting events such as floods or visible mould or moisture contamination, indoor culturable fungi levels were driven by outdoor concentration. The results also allowed us to benchmark the "baseline range" concentrations for total culturable fungi, *Penicillium* spp., *Cladosporium* spp. and *Aspergillus* spp. in such school settings. The measured concentration of total culturable fungi and three individual fungal genera were estimated using Bayesian hierarchical modelling. Pooling of these estimates provided a predictive distribution for concentrations at an unobserved school. The results indicated that "baseline" indoor concentration levels for indoor total fungi, *Penicillium* spp., *Cladosporium* spp. and *Aspergillus* spp. in such school settings were generally ≤ 1450, ≤ 680, ≤ 480 and ≤ 90 cfu/m^3, respectively, and elevated levels would indicate mould damage in building structures. The indoor/outdoor ratio for most classrooms had 95% credible intervals containing 1, indicating that fungi concentrations are generally the same indoors and

outdoors at each school. Bayesian fixed effects regression modeling showed that increasing both temperature and humidity resulted in higher levels of fungi concentration.

Keywords: Culturable fungi, School environment, Subtropical area, Concentration, Fungal flora

1. Introduction

The association between moisture damage in school buildings, microbial growth due to excess moisture and the adverse health outcomes of the occupants, such as respiratory illnesses and allergy, has been reported in many studies (Aydogdu et al., 2005; Hussin et al., 2011; Meklin et al., 2002). However, there are no health-based guideline values for indoor dampness or microbes (Karvala, 2012; WHO, 2009). Measured microbiologic agents have shown less consistent association with health effects than qualitative assessment like visible dampness or mold odor (Karvala, 2012). In buildings with moisture and mould damage, indoor sources of fungi can be significant, and the overall mycobiota indoors may be extensive (Gutarowska and Piotrowska, 2007; Meklin et al., 2003).

Generally, there are no uniformly accepted, or validated, quantitative environmental sampling methods to assess exposure to mould and other agents associated with damp indoor environments (ACGIH, 2009; Frankel et al., 2012 a). Andersen Impactor and Biotest RCS High Flow air samplers are widely used to detect and quantify bioaerosols, identify bioaerosol release from sources, assessment of human exposure to biological agents, and monitor the effectiveness of control measures (Li, 2011; Saldanha and Manno, 2008). It should be noted that although cultivation methods are convenient, being able to identify major fungal species with simple equipment and analysis techniques, they are slow and always selective and therefore underestimate the total fungal counts (ACGIH, 1999) and may ignore some clinically relevant moulds (Baxi et al., 2013; Holme et al. 2010).

Although, microbial levels by themselves should not be used as an indicator of a health risk, reference values for viable culturable fungi concentrations are needed in order to identify abnormal sources of microbes in different indoor environments in different climate regions.

Generally, the majority of the indoor airborne fungal population is derived from outdoor sources and is transferred inside through windows and doors (Burge et al., 2000; Levetin, 1995; Shelton et al., 2002). Fungal populations depend significantly on outdoor climatic conditions and meteorological factors, such as temperature and humidity (Bartlett et al., 2004; Frankel et al., 2012b; Wu et al., 2007). Nevertheless, when suitable conditions are present indoors, fungi may also grow on indoor building structures (Górny, 2004; Meklin et al., 2002). In such buildings, moisture and mould problems may manifest in elevated levels and/or altered types of culturable fungi in dust and air (Meklin et al., 2002; WHO, 2009). In addition, several other factors, such as the age of the building and presence of occupants and pets (Bartlett et al., 2004; Lehtonen et al., 1993) may cause variations in indoor fungal levels and explain differences between studies.

72 Indoor/outdoor (I/O) ratios are a direct numerical comparison of indoor fungal levels with outdoor
73 levels, and can be used to determine if indoor spaces are contaminated with airborne microorganisms
74 (Kim and Kim, 2007). Although it is generally accepted in the literature that, in non-damaged
75 buildings, the microbiological concentration in indoor air is similar to outdoor air (I/O ratio is close to
76 1) (Bartlett et al., 2004), there are very few data available about I/O ratios in naturally ventilated non-
77 moisture-damaged school buildings.

78 For properly maintained school structures and classrooms using natural ventilation in subtropical
79 urban environments, this study aimed to (a) test the study's hypothesis that, under normal conditions,
80 and without any major affecting events (e.g. floods) or visible mould or moisture contamination, the
81 indoor culturable fungi concentrations is mostly driven by outdoor concentrations; (b) benchmark
82 "baseline range" concentrations (and I/O ratios) of total culturable fungi, *Penicillium* spp.,
83 *Cladosporium* spp. and *Aspergillus* spp.; and (c) investigate the prevalence of fungal species and the
84 effect of temperature and relative humidity on fungal levels.

85 It should be noted that the choice of the term "baseline" was not a straightforward one, however it
86 was deemed the most appropriate, as it had to represent a situation when only the "normal" or "typical"
87 sources were present, without any unusually high contributing sources. Such sources therefore
88 contribute to the "baseline" situation.

89

90 ## 2. Materials and methods

91

92 ### *2.1. Study design, location and classroom characteristics*

93 This cross-sectional study was carried out in 25 randomly selected primary schools (S01-S25) in
94 the Brisbane Metropolitan Area, Australia. The participating school classrooms were naturally
95 ventilated, using open windows. Some of the classrooms were equipped with ceiling fans, which were
96 operated occasionally to improve thermal comfort. The selection criteria for schools, and detailed
97 information about the classrooms are described in our other paper (Salonen et al., 2013) and are also
98 available in the supplementary information (SI) . This study was conducted during teaching periods at
99 S18-S21 in autumn (March-May), S08-S12 and S22-S25 in winter (June-August), S01-S03 in spring
100 (September-November) and S04 in summer (December-February), regardless of the weather condition
101 (e.g. rainy days).

102 Prior to sampling, a "walk-through" assessment was carried out to determine indoor and outdoor
103 sampling locations. Room characteristics, with regards to moisture damage, cleanliness, floor type,
104 and other possible bioaerosol sources, were also assessed in each studied classroom visually as well as
105 via a questionnaire and information form (Appendix S1 in the SI). The classrooms had carpeted floors
106 and there were no animals or pot plants inside the classrooms. Our inspections and interviews with
107 school maintenance and management personnel showed that there was no visible moisture or mould
108 in building structures at the time of the measurements, nor was there a history of moisture or mould

109 problems (except at schools S09 and S10). Schools S09 and S10 were affected by the Brisbane flood
110 in 2011, six months before the measurements were conducted. However, major clean-up and
111 renovation works were conducted in the affected building structures and classrooms immediately after
112 the flood waters receded. All of the wet material in these two affected schools were completely
113 removed and replaced with new material and furniture. In other schools, no renovations were
114 conducted during last two years. The classrooms were located in the ground level or one level above
115 the ground.
116 The daily cleaning schedule during the measurement period included carpet vacuum cleaning
117 and desk wiping in each classroom. Vacuum cleaning was conducted before or after school hours and
118 desk wiping was often done once a week. Children were undertaking their normal classroom activities
119 (reading and writing) during the sampling. Windows were mainly open (42 out of the 50 classrooms)
120 during the measurements (generally the case during school hours in classrooms in) and there was no
121 rainy (except mild rain in one school) or windy weather during sampling.
122
123
124 *2.2. Sampling and instrumentations*
125
126 *2.2.1. Culturable fungi*
127 Culturable fungi were collected at two indoor locations (classrooms A and B) and one central
128 outdoor location (location C) within each school setting. All measurements were conducted during
129 regular school hours and normal room activities (reading and writing) to reflect the conditions to
130 which students and staff are exposed between the hours of 9 am and 3 pm. Measurements were taken
131 at a height of 1.0 m above floor level, which is representative of the children's breathing zone.
132 Outdoor "control" samples (2-5 samples) were collected at a height of 1.2 m above ground level at the
133 central location within the school grounds. The indoor sampling of fungi was conducted in the middle
134 of the classrooms and no fans or ceiling fans were in operation at any point during the measurements.
135 Two samples were collected from each classroom at the first nine schools and five samples were
136 collected at the remaining 16 schools. For each sampling location, all samples (2-5) were collected
137 within a 30 minutes time period during school hours and all measurements were conducted within
138 three hours at each school. Agar media blanks were taken into the field but not opened (Bartlett et al.,
139 2004).
140 The sampling device used for fungi monitoring was the Biotest RCS High Flow (Biotest Hycon,
141 Art. No. 940210, Ser. No. 30709), which is known as an effective instrument for bioaerosol sampling
142 and is widely used around the world (Yao and Mainelis. 2007; Reponen et al. 2001; Saldanha and
143 Manno 2008). The RCS High Flow instrument has a particle diameter cut off size (d50) of 2-5 µm
144 (Millipore, 2003) which meets the cut off size requirements for most of the fungal spores in indoor
145 environments (2 to 4 µm in aerodynamic diameter) (Reponen et al. 2001). The sampling volume used

146 was 20 L (50L in first three schools). Rose bengal agar strips were used for recovery and incubated at
147 25°C for 7 days prior to counting and partial identification of the culturable fungal colonies
148 (*Penicillium* spp., *Cladosporium* spp. and *Aspergillus* spp., plus “other fungal colonies” to a genus
149 level). The media blanks were incubated in the same way as the culture strips. The Biotest RCS High
150 Flow was calibrated on a regular basis by the manufacturer.
151
152 *2.2.2. Temperature and humidity*
153 Outdoor and indoor temperature was measured 24/7 at the three sampling locations in each school.
154 Measurements were conducted using a pSENSE portable CO_2 Metre and a TSI IAQ Monitor (Model
155 8551) at the indoor locations. Relative humidity and temperature at the outdoor location were also
156 measured 24/7 using a Monitor Sensors µSmart Series weather station.
157
158 *2.3. Statistical analysis*
159 All statistical analysis was performed in R(R Core Team, 2012) using the rjags package
160 (Plummer, 2012). All models were checked for convergence after a burn-in of between 1000 and
161 10000 samples, depending on model complexity. Every tenth (out of 10000) was retained.
162 Bayesian multilevel regression modeling (Gelman and Hill, 2007) was performed to examine
163 differences in total fungi concentrations within, and across, schools, based on 75 unique locations
164 (Indoor A and B, Outdoor C at each school). The distribution of fungi concentrations at a location at
165 an unobserved school (labeled school 26) can be modelled by a log-Normal distribution whose mean
166 is drawn from the relevant school level mean distribution and whose precision is a weighted average
167 (by number of observations) of the other schools’ precisions. A Bayesian log-Normal regression
168 model was fit to model the effects of temperature and humidity (and their interaction) on the total
169 culturable fungi concentrations. The relationship between indoor and outdoor total fungi
170 concentrations was characterized by examining, within each school, the 95% credible interval of the
171 distribution of the difference between the posterior samples of the means of the log-Normal
172 distributions which represent the fungi concentrations. In order to estimate the proportion of each of
173 the four fungal groups, *Penicillium* spp., *Cladosporium* spp., *Aspergillus* spp., and “other fungal
174 colonies”, a hierarchical multinomial model with exchangeable Dirichlet priors was fit. Pooling of
175 estimates in the multilevel model is necessary when attempting to predict at an unobserved school,
176 and the individual variances are assumed completely independent. A meta-analysis, including the
177 effect size and its precision, provides an estimate of the average proportion from the prevalence model
178 presented in SI (Appendix S2). For each of the fungal genera and the total fungi concentration, the
179 effect of seasonality was modelled by including a random effect level in the hierarchy, such that the
180 schools are nested within one of the four seasons (Summer = 1, Autumn = 2, Winter = 3, Spring = 4).
181 Detailed information about statistical tests are presented in Appendix S2.
182

183

184 # 3. Results and discussion

185

186 *3.1. Concentrations and I/O ratios of total culturable fungi*

187

188 The posterior predictive mean and median total culturable fungi concentrations in indoor air
189 (mean: 800 cfu/m^3; median: 727 cfu/m^3) were lower than in outdoor air (mean 1344 cfu/m^3; median:
190 799 cfu/m^3). The maximum recorded total culturable fungi concentration outdoors was about 2.5
191 times greater than that of the indoor observations. The lowest fungal concentrations in outdoor and
192 indoor air were measured in school S02 and S22, respectively. Schools S22, S10 and S19 showed the
193 highest median culturable fungi concentrations in outdoor air, while schools S09-S11 had the highest
194 fungi concentrations in indoor air. Quantiles of the posterior predictive distribution of total fungi
195 concentration, and concentration of the four fungal genus groups of interest for both indoor and
196 outdoor locations at all schools calculated from the hierarchical linear model, are presented in Table 1.

197

198 **Table 1**. Quantiles of the posterior predictive distribution of total fungi concentration (cfu/m^3) and
199 concentration of the four fungal groups of interest for both indoor and outdoor locations at all schools,
200 calculated from the hierarchical linear model.

	Total		*Penicillium* spp.		*Cladosporium* spp.		*Aspergillus* spp.		Other	
	Indoor	Outdoor	Indoor	Outdoor	Indoor	Outdoor	Indoor	Outdoor	Indoor	Outdoor
5%	375	159	78	56	81	125	0	0	62	89
10%	443	230	96	72	98	155	0	1	80	112
20%	532	356	127	95	124	199	1	1	113	156
30%	602	488	157	117	150	236	2	2	142	194
40%	667	635	188	142	174	276	3	3	176	239
50%	734	807	222	170	193	315	4	5	209	286
60%	807	1031	258	203	225	361	7	9	249	351
70%	895	1334	305	242	257	413	11	16	303	427
80%	1015	1848	394	297	306	479	20	28	388	576
90%	1229	2844	520	378	370	634	48	61	536	827
95%	1452	4123	677	479	477	791	94	127	725	1069

201
202

203 We frequently found high - over 1000 cfu/m^3- total culturable fungi outdoor levels throughout
204 the year, including the subtropical winter time, which explains the consequent high fungal levels in
205 indoor air, despite there being no mould or moisture damage in studied school buildings. Based on
206 this finding, we suggest that in subtropical areas, the concentration of culturable fungi in outdoor air
207 should be always taken concurrently with indoor air concentrations.

208 The outdoor and indoor air concentrations of total culturable fungi from the predictive model
209 provided estimates of the credible intervals of concentrations in each of the three locations (two
210 indoor and one outdoor) at each of the 25 schools and an estimate of the credible intervals at an
211 average, unobserved school (labeled school 26) (Figure S1). The median and 95% credible intervals
212 of the predictive distributions at school 26 were: indoors – 730 (320, 1710) and outdoors – 790 (130,
213 5200) cfu/m^3. The posterior predictive distribution of the indoor and outdoor culturable fungi
214 concentrations at the unobserved school (S26) was estimated by pooling the precisions from each of
215 the other schools, weighted by sample size, and drawing the mean concentration from the hierarchical
216 prior (Figure S2). The mean was approximately equal for both indoor and outdoor predictive
217 concentrations, but the predictive distribution for indoor concentrations had a higher precision, as
218 outdoor concentrations are more variable. The results indicated that "baseline" indoor concentration
219 levels (≤ 95th percentile) for indoor total fungi, *Penicillium* spp., *Cladosporium* spp. and *Aspergillus*
220 spp. in a naturally ventilated subtropical school setting, with no visible mould or moisture
221 contamination, were generally ≤ 1450, ≤ 680, ≤ 480 and ≤ 90 cfu/m^3 (≤ 95th percentile), respectively.
222 Elevated levels would indicate mould damage in building strictures.
223 Analysis of the difference in means between indoor and outdoor locations calculated from the
224 hierarchical Bayesian model showed that most classrooms had an I/O ratio whose 95% and 90%
225 credible interval contained one (Figure S3). A credible interval strictly less than one implies that
226 outdoor culturable fungi concentrations are higher than those indoors. The 95% and 90% credible
227 intervals were akin to seeking statistical significance with p values of 0.05 and 0.1, respectively. The
228 classrooms in which the indoor mean total culturable fungi concentration was lower than the outdoor
229 mean were (at 95% credibility, an asterisk, *, represents 90% credibility) 17A, 17B, 18A, 18B, 19A,
230 19B, 20A, 20B, 22A and 22B. The lowest median I/O ratio was at S22, 22A – 0.04 (95% CI 0.01,
231 0.34), 22B – 0.08 (95% CI 0.04, 0.37), which resulted from a combination of low indoor counts and
232 very high outdoor counts, compared to those counts seen at other schools. Classrooms whose I/O ratio
233 had a 95% credible interval strictly greater than one (or a 90% credible interval strictly greater than
234 one if marked with an asterisk) are 9A*, 9B*, 14B*, 16A*, 16B* and 21B. The highest median I/O
235 ratio was at school 9 in both classrooms, 9A* – 2.66 (90% CI 1.02, 4.56) and 9B* – 3.41 (90% CI
236 1.17, 5.59). School S09 was affected by the Brisbane flood six months before the measurements.
237 Although wet materials were removed within one week of the flood, and the building structures in the
238 school subsequently cleaned and dried, the high indoor air concentration and I/O ratio for total fungi
239 indicate the abnormal indoor source and the need for additional investigations. The total outdoor fungi
240 concentrations at schools 9, 14, 16 and 21 are comparable to those at the other schools and therefore
241 not unusually low. These results indicate that in addition to school 9 (which was affected by the flood)
242 classrooms 14A, 14B, 16A, 16B and 21B may have indoor sources of fungi. While the means for
243 classrooms at school 3 are higher than 1 the credible intervals are quite wide, owing to the low

244 number of measurements made at that school, and so an indoor source of fungi cannot credibly be
245 suggested.
246 In the present study, the I/O ratios for culturable fungi were generally higher than I/O-ratios
247 reported from mechanically ventilated buildings in colder climatic regions (Bartlett et al., 2004;
248 Cheong et al., 2004; Jo and Seo, 2005). In warmer areas higher indoor fungal levels and I/O ratios
249 were reported, where a greater reliance on natural ventilation results in similar or higher indoor than
250 outdoor fungal levels and composition (Bartlett et al., 2004), and where an abundant and diversified
251 microflora is present (De Aquino Neto and De Goes Sigueira, 2000).
252
253 *3.2. Concentrations and I/O ratios of fungal flora*
254
255 The concentration and occurrence of indoor air mycoflora in the present study largely
256 reflected the fungal flora present in the outdoor air, which was congruent with earlier studies
257 conducted in different climate regions (Cheong and Neumeister-Kemp, 2005; Li and Kendrich, 1996;
258 Shelton et al., 2002): *Cladosporium,* the most dominant genus with the highest concentrations in
259 outdoor air, both in present study, as well as in other studies, also seems to be one of the most
260 common fungal genera indoors around the world. As well as the other universally common genus in
261 outdoor air, *Penicillium* was the fungal genus also most frequently found in indoor air, in accordance
262 with previous studies (Burge, 2002; Meklin et al., 2002; Verhoeff et al., 1992). It has been reported
263 that although *Penicillium* spp. are among those fungi whose concentration increases indoors in the
264 presence of high levels of moisture, *Penicillium* is also known to grow in house dust in buildings
265 without obvious moisture problems and can be detected indoors at level greater than those detected
266 outdoors (Adkinson et al. 2003; Bush et al. 2006; Codina et al. 2008), as illustrated by the results of
267 this study.
268 Our study agrees with earlier studies that *Aspergillus* is also a commonly found fungal genus
269 in both outdoor and indoor air, but its occurrence is generally significantly lower than the occurrence
270 of *Cladosporium* spp. and *Penicillium* spp. (Hargreaves et al., 2003; Meklin et al., 2003; Salonen et al.,
271 2012).
272 In outdoor air, the mean and median concentrations of *Cladosporium* spp. (mean 385 cfu/m^3;
273 median 324 cfu/m^3) were higher than the concentrations of *Penicillium* spp. (mean 202 cfu/m^3;
274 median 164 cfu/m^3), while in indoor air, the mean and median concentrations of *Penicillium* spp.
275 (mean 277 cfu/m^3; median 224 cfu/m^3) were higher than the concentrations of *Cladosporium* spp.
276 (mean 229 cfu/m^3; median 197 cfu/m^3). The mean outdoor and indoor concentrations of
277 *Cladosporium* spp. and *Penicillium* spp. in this study were comparable to *Cladosporium* spp. and
278 *Penicillium* spp. concentrations previously reported in the same climate area (Cheong and
279 Neumeister-Kemp, 2005), and much higher than the reported concentrations in colder climate areas
280 (Bartlett et al., 2004; Jo and Seo, 2005; Meklin et al., 2003). For example, in Finland the reported

281 winter mean (GM) indoor concentrations of *Cladosporium* spp. and *Penicillium* spp. were much lower
282 (Meklin et al., 2002; Meklin et al., 2003), than those found in present study. In the present study, open
283 windows and doors were one important factor which may explain the variation in prevalence and
284 concentration of *Penicillium* spp. and *Cladosporium* spp.

285 Although the mean and median concentration of *Aspergillus* spp. were much lower in this
286 study than the concentrations of *Penicillium* spp. and *Cladosporium* spp. in outdoor (mean 36 cfu/m^3;
287 median 6 cfu/m^3), as well as in indoor air (mean 30 cfu/m^3; median 5 cfu/m^3), the indoor
288 concentration was substantially higher than the reported *Aspergillus* spp. concentration in colder areas,
289 which varied from 0.6 to 1.9 cfu/m^3 (Meklin et al., 2002; Meklin et al., 2003). We found that the
290 concentration of *Aspergillus* spp. in outdoor and indoor air was almost equal, which is consistent with
291 earlier findings (Bartlett et al., 2004; Ramachandran et al., 2005). Different local factors, such as
292 climate and meteorology, affect fungal levels and explain differences between studies. In subtropical
293 areas, high relative humidity and mild temperature contribute to high outdoor (as well as indoor)
294 concentrations of *Aspergillus* spp., as well as other studied fungi (Bartlett et al., 2004; Hargreaves et
295 al., 2003).

296 The mean fungal concentration for each fungal group in the hierarchical linear model showed
297 some variation both across all, and within each school (Figure S4). For each fungal group, the
298 location-within-school level effect was centered around a location level (indoor, outdoor) mean across
299 all schools. The proportional prevalence of each of the fungal group at each of the schools is
300 discussed below, as is the I/O ratio for each classroom at each school for each of the groups.
301 *Cladosporium* spp. and "Other fungal colonies" had I/O ratios strictly less than one (Figure S5) when
302 summarized across all schools, whereas *Penicillium* spp. and *Aspergillus* spp. had 95% CIs that
303 covered a ratio of one.

304 At the individual school level, higher *Penicillium* spp. levels were found in classrooms 12B,
305 13B, 16AB, 21AB, 23A, 24AB and 25AB than outdoors. High outdoor levels were found in 9B and
306 22. At school 22, the high observed counts of *Aspergillus* spp. (1900-6950 cfu/m^3) and *Penicillium*
307 spp. (450-900 cfu/m^3) are much higher than the indoor concentrations of *Aspergillus* spp. (50-200
308 cfu/m^3) and *Penicillium* spp. (50-150 cfu/m^3), and this is what is responsible for the low I/O ratio of
309 overall fungi concentration. The overall *Cladosporium* spp. levels were higher outdoors than indoors.
310 Indoor levels of *Cladosporium* spp. were higher in 7B, 9B* (* = 90% credibility) and 25B.
311 *Aspergillus* spp, which occurs at quite low concentrations both indoors and outdoors, typically has an
312 I/O ratio whose 95% credible interval contains one. At schools 4A*, 8B*, 18A, 19AB and 24AB the
313 outdoor concentrations were higher than those indoors. Indoor concentrations were higher in 14B*,
314 16B and 21B*.

315 The broad category of other fungal colonies had higher concentrations outdoors than indoors,
316 when summarized across schools. At individual schools, the I/O ratio usually contained one in its 95%

credible interval. There was no classroom for which the indoor mean was higher than the outdoor mean. Outdoor means were higher than indoor means in 4A, 16B 17B and 22AB.

There was a high amount of variation in the relative proportions of each fungal group across the 25 schools (Figure S6). This finding supports earlier studies conducted e.g. in Minnesota (Ramachandran et al., 2005), Kansas City, Santa Fe and Orlando (Levetin et al., 1995). As schools 1 to 3 had no group level counts, the estimates of the proportions at these schools represent a prior belief of the expected proportions at an unobserved school. The expected proportions and their 95% credible intervals were: *Penicillium* spp. – 29.1% (8.7, 55.7), *Cladosporium* spp. – 30.9% (9.3, 58.2), *Aspergillus* spp. – 6.8% (0.1, 25) and other fungal colonies – 33.2% (11.4, 60.6). The proportions given by simulating from the (Dirichlet-Gamma) prior were 25% (95% CI 0, 82.5); the inclusion of data from other schools narrowed the credible interval of these estimates, especially *Aspergillus* spp.

A Bayesian meta-analysis of the calculated proportions at each school, where the means of the proportions from the multinomial model were centred around a pooled mean, yield mean and 95% credible intervals of the percentage proportion of each groups as follows: *Penicillium* spp. – 28.9 (26.0, 31.9), *Cladosporium* spp.– 31.3 (27.4, 35.3), *Aspergillus* spp. – 5.13 (3.27, 7.12) and other – 34.4 (30.8, 37.9). The meta-analysis credible intervals were centred around similar values to the posterior estimates for schools 1 to 3, but were much narrower, as they were a pooling of the estimated proportions rather than a distribution from which the proportions were drawn. These results confirmed the study's hypothesis that in buildings without microbe sources in their structures, outdoor air is the main source of microbes, and it affects the microbial flora and concentrations indoors (Burge, 1990; Levetin, 1995; Shelton et al., 2002). Based on the earlier findings (Hyvärinen, 2002; Meklin et al. 2003; Salonen, 2009), it is expected that in schools with mold growth, the microbial levels indeed are higher. In addition to elevated concentrations of indicator microbes (e.g. *A. Versicolor* and *Stachybotrys* spp.), moisture damage may cause increased concentrations of species commonly encountered in non-damaged buildings (e.g. *Penicillium* spp., *Cladosporium* spp., and *Aspergillus* spp.)

3.3. The effect of temperature, relative humidity and seasonality on fungal concentrations

In the studied school settings, the 24-hour average temperature ranged from 8°C to 27°C. The 24-hour averaged relative humidity (RH) ranged from 42% to 91% (Figure S7). Regression modelling revealed that fungi concentration was lowest when the average temperature was high (above 25 degrees) (Figures S8a and S8b) ~~in SI)~~ and the 24 hour average relative humidity was low (below 50%). When the humidity was high and the temperature was low, the fungi concentrations were also low. Fungal concentrations were at their highest when humidity and temperature were either low or high. Several earlier studies support these findings. For example, studies have found that the levels of ambient fungi were associated positively with temperature (Burch and Levetin, 2000; Lin and Li,

354 2000; Wu et al., 2007). It should be noted that the RH of the air affects the water content of materials
355 in the room. However, it is the available moisture in the substrate, not the RH of the room air, which
356 limits the growth of microorganisms in or on the materials. In fact, no microorganisms found in
357 indoor air are able to grow if the equilibrium RH of the material is below 65% (Flannigan and Morey,
358 1996).

359 For the total fungi concentration of all fungi genera at each of the schools, the 95% credible
360 interval of δ_s, being the difference between the mean of season *s* and the all-seasons mean, contained
361 zero for all seasons (SI, page S7). This indicates that there was no seasonal variation in the fungi
362 concentrations. The number of schools measured in each of the four seasons was one in summer, 7 in
363 autumn, 9 in winter and eight in spring. The difference between school means and mean of their
364 respective seasons' did vary, such that there was an observable school-level effect, but these indicate
365 school to school differences rather than seasonality. A model that contains both seasonality (as treated
366 here) and temperature and humidity was not fit, as temperature and humidity vary both seasonally and
367 spatially (i.e. school to school variation) and such a model would have problems with identifying
368 whether the effect was due to seasonality or spatial variation.

369 A limitation of this study was that each school was only measured during one season and only
370 6-15 samples per each school were collected, limiting the depth of analysis which we were able to
371 undertake, such as the ability to investigate seasonality. School buildings were ventilated via opened
372 windows and doors and the air exchange rates were not known. For example, the turbulence induced
373 may have influenced fungi concentrations. Simultaneous effects of different local factors add
374 complexity and more studies during different seasons and geographical locations are needed to
375 identify additional effects.

376

377

378 4. Conclusions

379 Tools to evaluate and characterize the microbial status of school buildings are needed,
380 because moisture and mold in building structures can cause adverse health effects among students and
381 teachers. Statistical analysis of the collected data in this study established that in the majority of
382 properly maintained subtropical school buildings, without any major affecting events or visible mould
383 or moisture contamination, indoor culturable fungi levels were driven by outdoor concentration. We
384 suggest that additional investigations are needed if the concentrations for total culturable fungal
385 spores, *Penicillium* spp., *Cladoporium* spp. and *Aspergillus* spp. exceed 1450 cfu/m^3, 680 cfu/m^3, 480
386 cfu/m^3 and 90 cfu/m^3, respectively. These levels are applicable to urban naturally ventilated school
387 buildings in subtropical areas. Elevated fungal concentrations may indicate mould damage in building
388 structures, and require additional environmental investigations. For most classrooms in this study
389 (except those at S22), the 95% credible intervals for the I/O ratios contained 1, indicating that fungi
390 concentrations are generally the same indoors and outdoors at each school. Bayesian fixed effects

391 regression modeling showed that increasing both temperature and humidity resulted in higher levels
392 of fungi concentration.
393
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406
407 **Supplementary information available** free of charge via the Internet at http://..
408
409 **References**
410
411 ACGIH, 1999. Bioaerosols: Assessment and control. American Conference of Governmental
412 Industrial Hygienists, Cincinnati.
413 ACGIH, 2009. Threshold limit values (TLVs) for chemical substances and physical agents and
414 biological exposure indices (BEIs). American Conference of Governmental Industrial
415 Hygienists (ACGIH), Cincinnati, USA, pp. 223-226.
416 Adkinson, N.F., Yunginger, J.W., Busse, W.W., Bochner, B.S., Holgate, S.T., Simons, F.E.R.
417 (editors), 2003. Allergy Principles and Practice. Sixth edition. Philadelphia: Mosby Co., 2003.
418 p.529-555.
419 Aydogdu, H., Asan, A., Otkun, M.T., Ture, M., 2005. Monitoring of fungi and bacteria in the indoor
420 air of primary schools in Edirne city, Turkey. Indoor and Built Environment 14, 411-425.
421 Bartlett, K., Kennedy, S.M., Brauer, M., Van Netten, C., Dill, B., 2004. Evaluation and a predictive
422 model of airborne fungal concentrations in school classrooms. The Annals of occupational
423 hygiene 48, 547-554.
424 Baxi, S. N., et al., 2013. Exposures to molds in school classrooms of children with asthma. Pediatric
425 allergy and immunology 24, 697–703.
426 Burch, M., Levetin, E., 2000. Effects of meteorological conditions on spore plumes. International
427 journal of biometeorology 46, 107-117.
428 Burge, H.A., 1990. Bioaerosols: Prevalence and health effects in the environment. The journal of
429 allergy and clinical immunology 86, 687-701.
430 Burge, H.A., 2002. An update on pollen and fungal spore aerobiology. The journal of allergy and
431 clinical immunology 110, 544-552.
432 Burge, H.A., Pierson, D.L., Groves, T.O., Strawn, K.F., Mishra, S.K., 2000. Dynamics of airborne
433 fungal populations in a large office building. Current microbiology 40, 10-16.
434 Bush, R. K., et al., 2006. The medical effects of mold exposure. Position paper. Journal of Allergy
435 and Clinical Immunology 117, 326-333.

436 Cheong, C.D., Neumeister-Kemp, H.G., 2005. Reducing airborne indoor fungi and fine particulates in
437 carpeted Australian homes using intensive, high efficiency HEPA vacuuming. JEHR 4.
438 Cheong, C.D., Neumeister-Kemp, H.G., Dingle, P.W., Hardy, G.S.J., 2004. Intervention study of
439 airborne fungal spora in homes with portable HEPA filtration units. JEM 6, 866-873.
440 Codina, R., et al., 2008. Typical levels of airborne fungal spores in houses without obvious moisture
441 problems during a rainy season in Florida, USA. Journal of Investigational Allergology and
442 Clinical Immunology 18(3): 156-162.
443 De Aquino Neto, F.R., De Goes Sigueira, L.F., 2000. Guidelines for indoor air quality in offices in
444 Brazil, Proceedings of the sixth international conference on Healthy Buildings., Espoo,
445 Finland, pp. 549-554.
446 Flannigan, B., ;, Morey, P.R., 1996. Control of moisture problems affecting biological indoor air
447 quality. International Society of Indoor Air Quality and Climate, Ottawa, Canada, p. 3.
448 Frankel, M., Timm, M., Hansen, E.W., Madsen, A.M., 2012a. Comparison of sampling methods for
449 the assessment of indoor microbial exposure. Indoor Air 22, 405–414.
450 Frankel, M., Bekö, G., Timm, M., Gustavsen, S., Hansen, E.W., Madsen, A.M., 2012b. Seasonal
451 variation of indoor microbial exposures and their relations to temperature, relative humidity
452 and air exchange rates. Applied and Environmental Microbiology 78, 8289-8297.
453 Gelman, A., Hill, J., 2007. Data Analysis Using Regression and Multilevel/Hierarchical Models.
454 Cambridge University Press.
455 Górny, R.L., 2004. Filamentous microorganisms and their fragments in indoor air - a review. Annals
456 of Agricultural and Environmental Medicine 11, 185-197.
457 Gutarowska, B., Piotrowska, M., 2007. Methods of mycological analysis in buildings. Building and
458 Environment 42, 1843-1850.
459 Hargreaves, M., Parappukkaran, S., Morawska, L., Hitchins, J., He, C., Gilbert, D., 2003. A pilot
460 investigation into associations between indoor airborne fungal and non-biological particle
461 concentrations in residential houses in Brisbane, Australia. Science of the Total Environment
462 312, 89-101.
463 Holme, J., et al. 2010. Culturable mold in indoor air and its association with moisture related
464 problems and asthma and allergy among Swedish children. Indoor Air 20, 329-340.
465 Hussin, N.H.M., Sann, L.M., Shamsudin, M.N., Hashim, Z., 2011. Characterization of bacteria and
466 fungi bioaerosol in the indoor air of selected primary schools in Malaysia. Indoor and Built
467 Environment 20, 607–617.
468 Jo, W.-K., Seo, Y.-J., 2005. Indoor and outdoor bioaerosol levels at recreation facilities, elementary
469 schools, and homes. Chemosphere 61, 1570-1579.
470 Karvala, K., 2012. Asthma in damp indoor work environments. People and work. Research reports 97.
471 [Dissertation]. Helsinki, Finnish Institute of Occupational Health. 236p.
472 Kim, K.Y., Kim, C.N., 2007. Airborne microbiological characteristics in public buildings of Korea.
473 Building and Environment 42, 2188–2196.
474 Lehtonen, M., Reponen, T., Nevalainen, A., 1993. Everyday activities and variation of fungal spore
475 concentrations in indoor air. International Biodeterioration & Biodegradation 31, 25-39.
476 Levetin, E., 1995. Fungi, in: Burge, H.A. (Ed.), Bioaerosols. CRC Press, Boca Raton (FL), pp. 87-120.
477 Levetin, E., Shaughnessy, R., Fisher, E., Ligman, B., Harrison, J., Brennan, T., 1995. Indoor air
478 quality in schools: exposure to fungal allergens. Aerobiologia 11, 27-34.
479 Li, D.-W., Kendrich, B., 1996. Functional and causal relationships between indoor and outdoor
480 airborne fungi. Canadian Journal of Botany 74, 194-209.
481 Li, K., 2011. Molecular comparison of the sampling efficiency of four types of airborne bacterial
482 samplers. Science of the Total Environment 409, 5493-5498.
483 Lin, W.-H., Li, C.-S., 2000. Associations of fungal aerosols, air pollutants and meteorological factors.
484 Aerosol Science and Technology 32, 359-368.
485 Meklin, T., Husman, T., Vepsäläinen, A., Vahteristo, M., Koivisto, J., Halla-Aho, J., Hyvärinen, A.,
486 Moschandreas, D., Nevalainen, A., 2002. Indoor air microbes and respiratory symptoms of
487 children in moisture damaged and reference schools. Indoor Air 12, 175-183.
488 Meklin, T., Hyvärinen, A., Toivola, M., Reponen, T., Koponen, V., Husman, T., Taskinen, T., Korppi,
489 M., Nevalainen, A., 2003. Effect of building frame and moisture damage on mikrobiological
490 indoor air quality in school buildings. AIHAJ. 64, 108-116.

491 Millipore., 2003. Monitoring airborne microorganisms during food and beverage processing.
492 Comparison of four microbial air samplers. Technical brief; Millipore: Billerica, MA.; pp 1-4.
493 Plummer, M., 2012. rjags: Bayesian graphical models using MCMC. http://cran.r-
494 project.org/web/packages/rjags/index.html
495 R Core Team, 2012. R: A Language and Environment for Statistical Computing. Vienna, Austria.
496 http://www.r-project.org/.
497 Ramachandran, G., Adgate, J.L., Banerjee, S., Church, T.R., Jones, D., Fredrickson, A., Sexton, K.,
498 2005. Indoor air quality in two urban elementary schools--measurements of airborne fungi,
499 carpet allergens, CO2, temperature, and relative humidity. Journal of Occupational and
500 Environmental Hygiene 2, 553-566.
501 Reponen, T.; Willeke, K.; Grinshpun, S.; Nevalainen, A., 2001. Biological Particle Sampling. In
502 Aerosol measurement: Principles, Techniques, and Applications. Second Edition, Baron, P.
503 A.; Willeke, K., Eds. Wiley-Inter Science, Inc; pp 751-776.
504 Saldanha, R., Manno, M., 2008. The influence of sampling duration on recovery of culturable fungi
505 using the Andersen N6 and RCS bioaerosol samplers. Indoor Air 18, 464–472.
506 Salonen, H., 2009. Indoor air contaminants in office buildings. Ph.D. Dissertation. Finnish Institute of
507 Occupational Health, Helsinki.
508 Salonen, H., Duchaine, C., Létourneau, V., Mazaheri, M., Clifford, S., Morawska, L., 2013.
509 Endotoxins in indoor air and settled dust in primary schools in subtropical climate.
510 Environmental Science & Technology 47, 9882-9890.
511 Salonen, H., He, C., Ling, X., Jayasundra, N., Cheung, J., Huygens, F., Hargreaves, M., Morawska, L.,
512 2012. Exposure to airborne fungi and bacteria in Brisbane houses after the 2011 flood,
513 Proceedings of the 10th International Conference on Healthy Buildings, Brisbane, Australia.
514 Shelton, B.G., Kirkland, K.H., Flanders, W.D., Morris, G.K., 2002. Profiles of airborne fungi in
515 buildings and outdoor environments in the United States. AEM J. 68, 1743-1753.
516 Verhoeff, A.P., Vanwijnen, J.H., Brunekreef, B., Fischer, P., van Reenen-Hoekstra, E.S., Samson,
517 R.A., 1992. Presence of viable mold propagules in indoor air in relation to house damp and
518 outdoor air. Allergy. 47, 83-91.
519 WHO, 2009. WHO guidelines for indoor air quality: dampness and mould. World Health
520 Organization, Copenhagen, p. 248.
521 Wu, Y.-H., Chan, C.-C., Rao, C.Y., Lee, C.-T., Hsu, H.-H., Yueh-Hsiu Chiu, Y.-H., Chao, H.J., 2007.
522 Characteristics, determinants, and spatial variations of ambient fungal levels in the subtropical
523 Taipei metropolis. Atmospheric Environment 41, 2500–2509.
524 Yao, M.; Mainelis, G., 2007. Use of portable microbial samplers for estimating inhalation exposure to
525 viable biological agents. Journal of Exposure Science and Environmental Epidemiology 17,
526 (1), 31-38.
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580 # SUPPLEMENTARY INFORMATION

581 # APPENDIXES S1-S2

582 # FIGURES S1-S8

583

584

585 **MANUSCRIPT TITLE:**

586 **Airborne culturable fungi in naturally ventilated primary school**
587 **environments in a subtropical climate**

588

589 **AUTHORS:**

590 HEIDI SALONEN, CAROLINE DUCHAINE, MANDANA MAZAHERI, SAM CLIFFORD, LIDIA
591 MORAWSKA

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596

597 **Study Design**

598

599 This was a cross-sectional study, which was carried out between October 2010 and August 2012, in a
600 total of 25 randomly selected primary schools (S01−S25) in the Brisbane Metropolitan Area,
601 Australia, as part of a large epidemiological project titled "Ultrafine Particles from Traffic Emissions
602 and Children's Health (UPTECH)" (www.qut.edu.au/research/research-projects/uptech). According
603 to the selection criteria for the schools, there were no major local air pollution sources, including
604 infrastructure projects such as roads, tunnels, and building construction in the vicinity of the schools
605 other than road traffic. All selected schools were built more than 10 years ago, constructed of
606 concrete or wood, with no central air-conditioning system. The school buildings were ventilated
607 primarily via opened windows and doors. Two classrooms used by 8−11 year old children from each
608 school were selected for the measurements.

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619 **APPENDIX S1**

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621

622 **Questionnaire and Information form for Air Quality assessment in**
623 **schools-Bioaerosols**

624
625

626 **School name** ____________________________________

627 **Room number** ______________________________

628 **Date completed** _____________________________________

629 **Signature** ____

630
631

632 **When filling the observation questionnaire, check when the item is observed. If not checked,**
633 **means that this observation was not made. If not applicable, write NA. Write details where line**
634 **provided.**

635

636 **1. General Cleanliness**
637 **1.1. Dust on surfaces** ☐
638 **1.2. Presence of full rubbish bins** ☐
639 **1.3. Presence of food wastes** ☐
640 **1.4. Signs of pests (droppings, insects)** ☐
641 **2. Spills or water damages**
642 **2.1. Traces of water damages on surfaces (discoloration may indicate leak history)** ☐
643 **2.2. Visible moisture on surfaces (condensation)**
644 **2.2.1. Window** ☐
645 **2.2.2. Window frames** ☐
646 **2.2.3. Cold water pipes** ☐
647 **2.2.4. Indoor surface of exterior walls** ☐
648 **2.3. Water in drain traps** ☐
649 **2.4. Smell from drain traps** ☐
650 **2.5. Leaks from classroom sink (around and under)** ☐
651 **2.6. Leaks in classroom lavatories** ☐
652 **3. Animals**
653 **3.1. Presence of animals in classroom** ☐
654 **3.1.1. Cleanliness of cage** ___________
655 **3.1.2. Odours** _______
656 **3.1.3. Droppings** ____________
657 **4. Pot plants**
658 **4.1. Presence** ____________
659 **4.2. number** _____________
660 **5. Ventilation**
661 **5.1. Passive** ☐
662 **5.1.1. Windows open or close at the moment of sampling** ☐
663 **5.1.2. Door open or close at the moment of sampling** ☐
664 **5.2. Mechanical ventilation** ☐
665 **5.2.1. Ventilation rate** ______________

666 **5.2.2. Air filtration** __________

667 **5.2.3. Air supply and return vent location** ___________

668 **6. Odours**

669 **6.1. Food/kitchen smell** ☐

670 **6.2. Chemical smell** ☐

671 **6.3. Mould/mildew smell** ☐

672 **6.4. Vehicle exhaust smell** ☐

673 **6.5. Other** ______________

674

675 # APPENDIX S2

676

677 ## Statistical analysis

678 All statistical analysis was performed in R(R Core Team, 2012) using the rjags package
679 (Plummer, 2012). All models were checked for convergence after a burn-in of between 1000 and
680 10000 samples, depending on model complexity. Every tenth (out of 10000) was retained.

681

682 *Predictive multilevel modeling of mean fungi concentrations*

683 Bayesian multilevel regression modeling(Gelman and Hill, 2007) was performed to examine
684 differences in total fungi concentrations within, and across, schools, based on 75 unique locations
685 (Indoor A and B, Outdoor C at each school). For each sampling replicate, k, in location j at school i,
686 the fungi concentration was log-normally distributed with mean μ_{ij} and precision (the inverse of
687 variance) τ_{ij}. Within each school, the means for each classroom were assumed exchangeable, and
688 were given a prior centered around a school level indoor mean. These school level indoor means were
689 assumed exchangeable across all 25 schools and were given a prior which was centered around a
690 mean for indoor locations within schools. The mean of the distribution of school level indoor means
691 was given a weakly informative Normal prior with a mean of zero and a small precision. Similarly,
692 the outdoor means were assumed exchangeable and were drawn from a distribution of outdoor means
693 which was centered around a mean for outdoor locations within schools. All precisions were given
694 weakly informative Gamma distributions with a mean of 1 and a variance of 1000.

695

696
$$
\begin{aligned}
y_{ijk} &\sim \ln \mathcal{N}\left(\mu_{ij}, \tau_{ij}\right) \\
\mu_{ij} &\sim \begin{cases} \mathcal{N}\left(\mu_{Ii}, \tau_{Ii}\right) & \text{for indoor } (j=1,2) \\ \mu_{Oi} & \text{for outdoor } (j=3) \end{cases} \\
\tau_{ij} &\sim \begin{cases} \Gamma(0.001, 0.001) & \text{for indoor } (j=1,2) \\ \tau_{Oi} & \text{for outdoor } (j=3) \end{cases} \\
\mu_{Ii} &\sim \mathcal{N}\left(\mu_I, \tau_I\right) \\
\mu_{Oi} &\sim \mathcal{N}\left(\mu_O, \tau_O\right) \\
\tau_{Ii}, \tau_{Oi} &\sim \Gamma(0.001, 0.001) \\
\mu_I, \mu_O &\sim \mathcal{N}\left(0, 10^{-6}\right) \\
\tau_I, \tau_O &\sim \Gamma(0.001, 0.001)
\end{aligned} \qquad (1)
$$

697

698 The distribution of fungi concentrations at a location at an unobserved school (labeled school 699 26) can be modelled by a log-Normal distribution whose mean is drawn from the relevant school level 700 mean distribution and whose precision is a weighted average (by number of observations) of the other 701 schools' precisions. The indoor/outdoor concentration ratios for total fungi and genera for the 702 classrooms at each school can be determined by similar multilevel modelling. In the model below, the 703 variances of all observations were assumed equal and the school level means for indoor and outdoor 704 concentration were exchangeable, as before.

$$
\begin{aligned}
y_{ijk} &\sim \ln \mathcal{N}\left(\mu_{ij}, \tau_y\right) \\
\mu_{ij} &\sim \mathcal{N}\left(\alpha_j, \tau_j\right) \\
\alpha_1, \alpha_3 &\sim \mathcal{N}\left(0, 10^{-6}\right) \\
\alpha_2 &= \alpha_1 \\
\tau_y, \tau_j &\sim \Gamma(0.001, 0.001).
\end{aligned}
\qquad (2)
$$

705

706 The differences between the indoor and outdoor means, $\mu_{i1}-\mu_{i3}$ and $\mu_{i2}-\mu_{i3}$, indicate 707 whether the mean fungi concentration is higher outdoors or indoors, as above.

708

709 *Effect of humidity and temperature on fungi concentration*

710 A Bayesian log-Normal regression model was fit to model the effects of temperature and 711 humidity (and their interaction) on the total viable fungi concentrations. The covariates were 712 standardized by subtracting their mean (e.g., $\overline{T}$) and dividing by their standard deviation (e.g., s_T). The 713 coefficients were given weakly informative Normal priors with a small precision. The precision of the 714 observations, τ_y, was given a non-informative Gamma prior with a mean of 1 and a variance of 1000. 715 The regression model is given as:

$$
\begin{aligned}
y_i &\sim \ln \mathcal{N}\left(\mu_i, \tau_y\right) \\
\mu_i &= \beta_0 + \beta_T\left(\frac{T-\overline{T}}{s_T}\right) + \beta_H\left(\frac{H-\overline{H}}{s_H}\right) + \beta_{TH}\left(\frac{T-\overline{T}}{s_T}\right)\left(\frac{H-\overline{H}}{s_H}\right) \\
\beta_0, \beta_T, \beta_H, \beta_{TH} &\sim \mathcal{N}\left(0, 10^{-6}\right) \\
\tau_y &\sim \Gamma\left(0.001, 0.001\right)
\end{aligned}
\qquad (3)
$$

716

717 The temperature exhibits seasonal variation, resulting in the inability to separate the 718 variability from temperature and the variability from measuring at a given school. As such, a random 719 effects mean at the school (or location within school) level was omitted. For classrooms where the 720 temperature record was missing, the 24 hour averaged temperature was estimated by regressing the 721 available temperature data for that room on the temperature data from the other classroom. If this was 722 unavailable, the outdoor temperature values were used.

723

724 *Indoor/Outdoor ratio*

725 The relationship between indoor and outdoor total fungi concentrations can be characterized
726 by examining, within each school, the 95% credible interval of the distribution of the difference
727 between the posterior samples of the means of the log-Normal distributions which represent the fungi
728 concentrations. Exponentiation of this difference provides a ratio of the means of the indoor and
729 outdoor fungi concentrations. If the 95% credible interval (CI) of the difference in means (on the log
730 scale) contains zero, the 95% CI of the ratio will contain 1 and it can be concluded that there is no
731 difference between indoor and outdoor fungi concentrations.

732

733 *Prevalence of species*

734 In order to estimate the proportion of each of the four fungal groups, *Penicillium* spp.,
735 *Cladosporium* spp., *Aspergillus* spp., and "other fungal colonies", a hierarchical multinomial model
736 with exchangeable Dirichlet priors was fit.

$$\begin{aligned} y_{ijk} &\sim \text{Multinomial}\left(\theta_{ij}\right) \\ \theta_{ij} &\sim \text{Dirichlet}\left(\alpha_j\right) \\ \alpha_j &\sim \Gamma\left(2,2\right) \end{aligned} \quad (4)$$

737

738 This regression model assumes that for the k replicate counts of fungal groups j at school i,
739 the proportion, θ, of each genera varies around an "all schools" proportion. The Dirichlet-Gamma
740 prior assumes, a priori, that the proportion of each fungal group is equal and the collected data updates
741 that belief. The Gamma prior for the Dirichlet parameters has a mean of 1 and a variance of 0.5 and is
742 weakly informative. Fungal group (genus) information was not available for schools 1 to 3, so the
743 estimates of the proportions were based on the hierarchical prior only.

744

745 *Bayesian meta-analysis*

746 Pooling of estimates in the multilevel model is necessary when attempting to predict at an
747 unobserved school and the individual variances are assumed completely independent. A meta-analysis,
748 including the effect size and its precision, provides an estimate of the average proportion from the
749 prevalence model in section 0. The meta-analysis model for effects β_i with precision τ_i, which vary
750 around the meta-analysis estimate with mean θ and precision τ_θ, is:

$$\begin{aligned} \beta_i &\sim \mathcal{N}\left(\mu_i, \tau_i\right) \\ \mu_i &\sim \mathcal{N}\left(\theta, \tau_\theta\right) \\ \theta &\sim \mathcal{N}\left(0, 10^{-6}\right) \\ \tau_\theta &\sim \Gamma\left(0.001, 0.001\right). \end{aligned} \quad (5)$$

751

752

753 *Seasonality*

754 For each of the fungal genera and the total fungi concentration, the effect of seasonality may
755 be modelled by including a random effect level in the hierarchy, such that the schools are nested
756 within one of the four seasons (Summer = 1, Autumn = 2, Winter = 3, Spring = 4). The hierarchical
757 model is therefore:

$$
\begin{aligned}
y_{ijk} &\sim \text{Poisson}\left(\lambda_{ijk}\right) \\
\log \lambda_{ijk} &= \gamma_i \\
\gamma_i &\sim \mathcal{N}\left(\theta_{s_i}, \tau_\gamma\right) \\
\theta_s &\sim \mathcal{N}\left(\alpha_0, \tau_\theta\right) \\
\alpha_0 &\sim \mathcal{N}\left(0, 10^{-6}\right) \\
\tau_\gamma, \tau_\theta &\sim \Gamma\left(0.001, 0.001\right)
\end{aligned}
\quad (6)
$$

758

759 where the γ_i are the means at each school, centred around the season-level mean θ_s. Weakly
760 informative priors are used for the precisions of the random effects and for the all-seasons mean α_0.

761 The Poisson likelihood has been used here as the log-Normal likelihood does not admit zero values of
762 the response and some fungi genera have a zero count at some schools.

763 To determine whether or not seasonal variation exists, the derivable quantity $\theta_s - \alpha_0$
764 represents the difference between the mean of season *s* and the all-seasons mean. Where the credible
765 interval contains zero, the season can not be said to have a mean which is distinguishable from the
766 overall mean.

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776 **FIGURE S1**

777

778 **Figure S1.** Predicted fungi concentration for (from left to right within each group) Indoor A, Indoor B
779 and Outdoors at each of the 25 primary schools and Indoors and Outdoors for an unobserved school
780 (26).

781

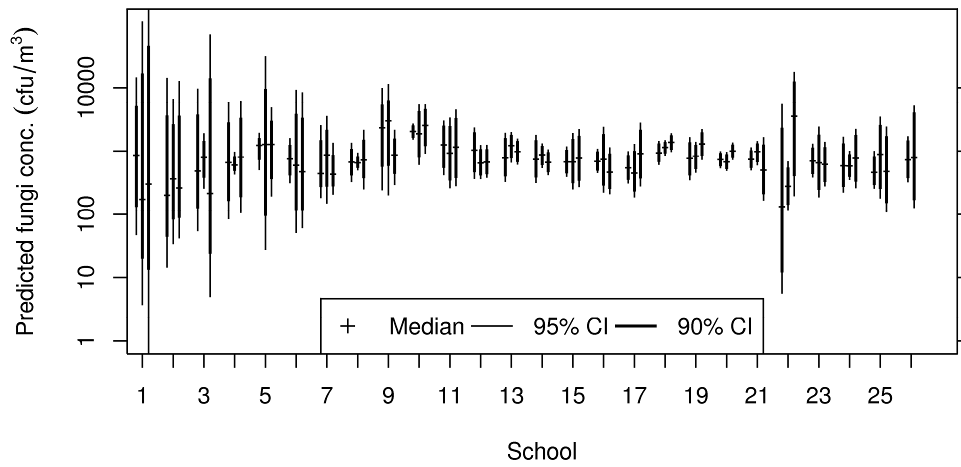

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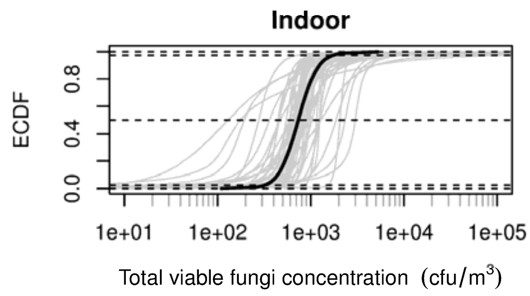
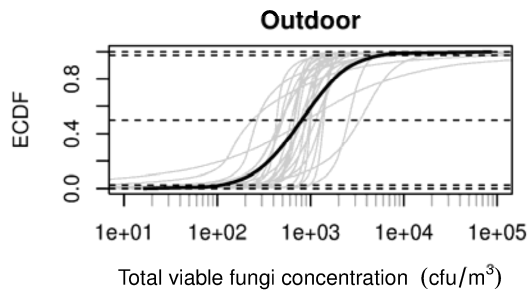
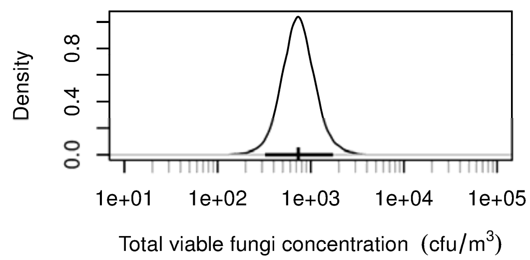
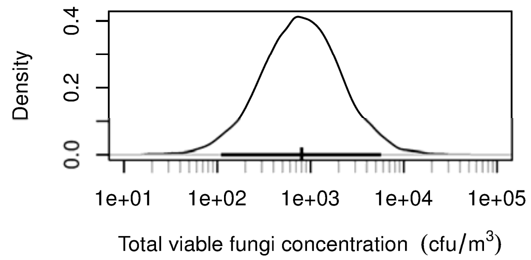
FIGURE S2.

Figure S2. Indoor and outdoor predictive distributions of total viable fungi concentration. Empirical cumulative density functions (ECDFs) of individual locations are shown as thin grey lines, the solid black ECDF (and EPDFs below) represent the estimates from pooling precisions.









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809 # FIGURE S3

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811 **Figure S3.** Means and 95% and 90% credible intervals of the ratio of the means of indoor and outdoor
812 fungal concentrations (Indoor A is on the left in every group of two at each school).

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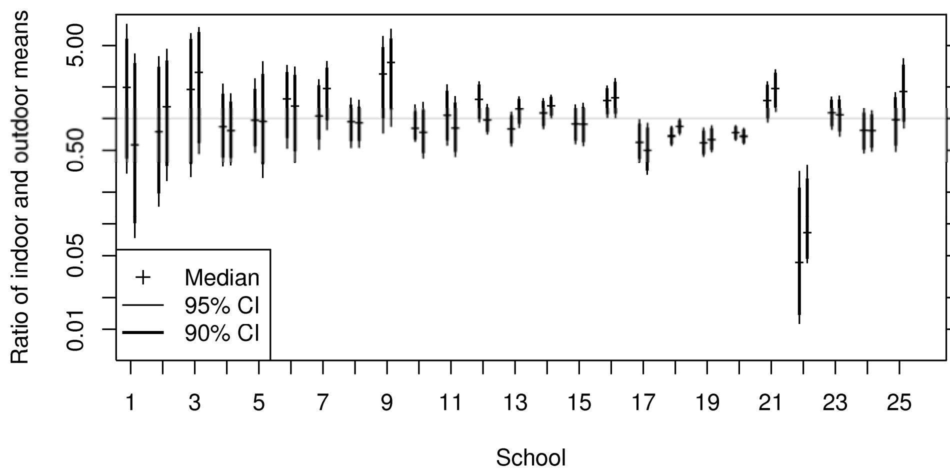

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833 **FIGURE S4**
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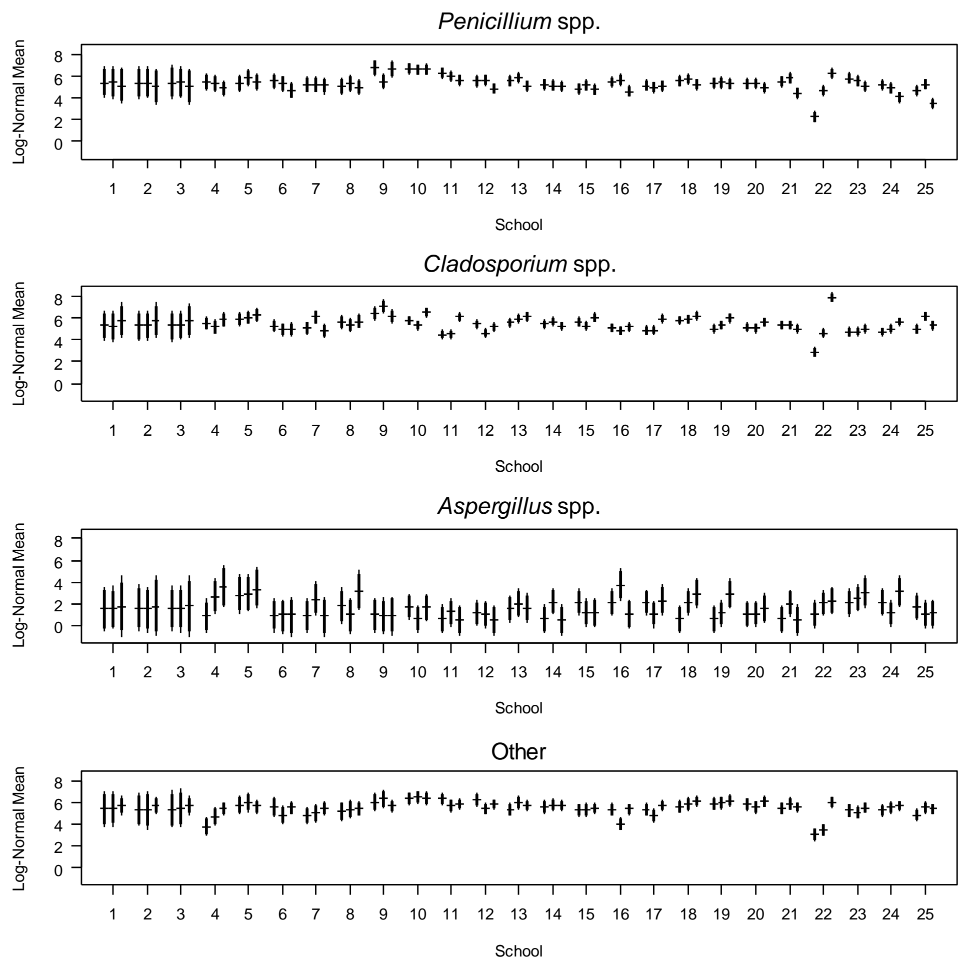
835 **Figure S4.** Mean parameters from the log-Normal Bayesian hierarchical linear model fit to the
836 concentrations of each of the four fungal groups.



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839 **FIGURE S5**

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841 **Figure S5.** Posterior summary of the mean fungi concentrations for each of the four fungal groups
842 (top). Difference between all-school outdoor and indoor mean parameters (from the log-Normal
843 regression model) for the four fungi groups (bottom). Horizontal lines represent the median and the
844 thin and thick vertical lines represent the 95% and 90% credible intervals, respectively.

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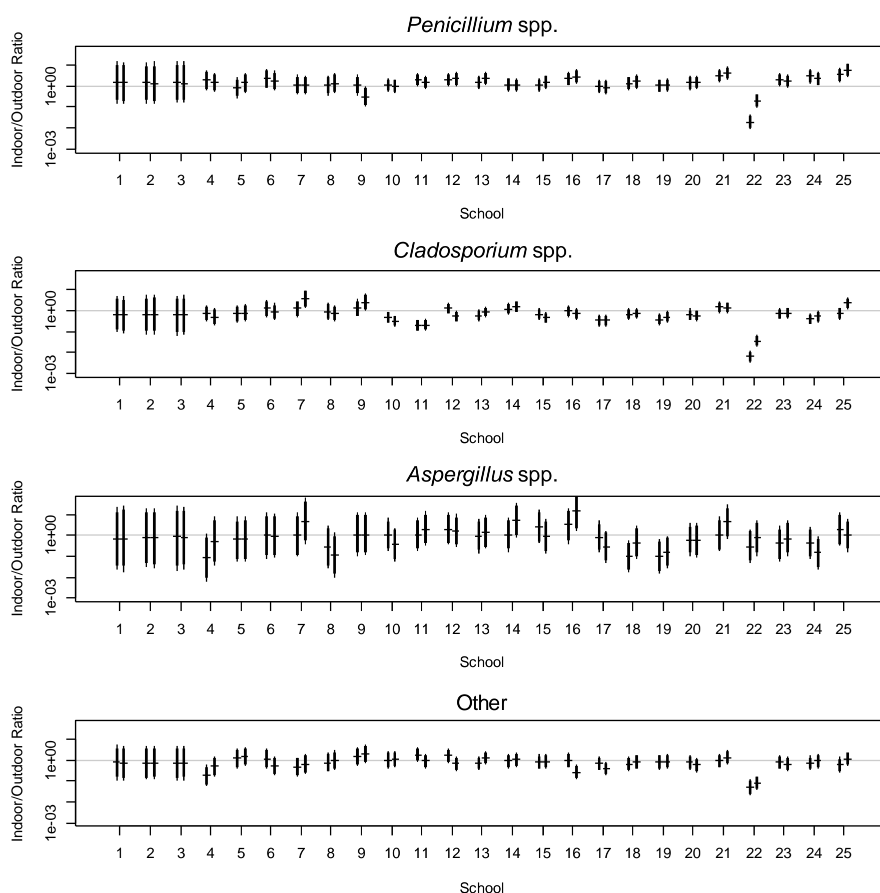

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856 **FIGURE S6**

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858 **Figure S6.** Proportion of each fungal groups at each of the 25 schools. The medians are shown as
859 horizontal lines and the 95% credible interval as vertical lines. Dots represent the data.

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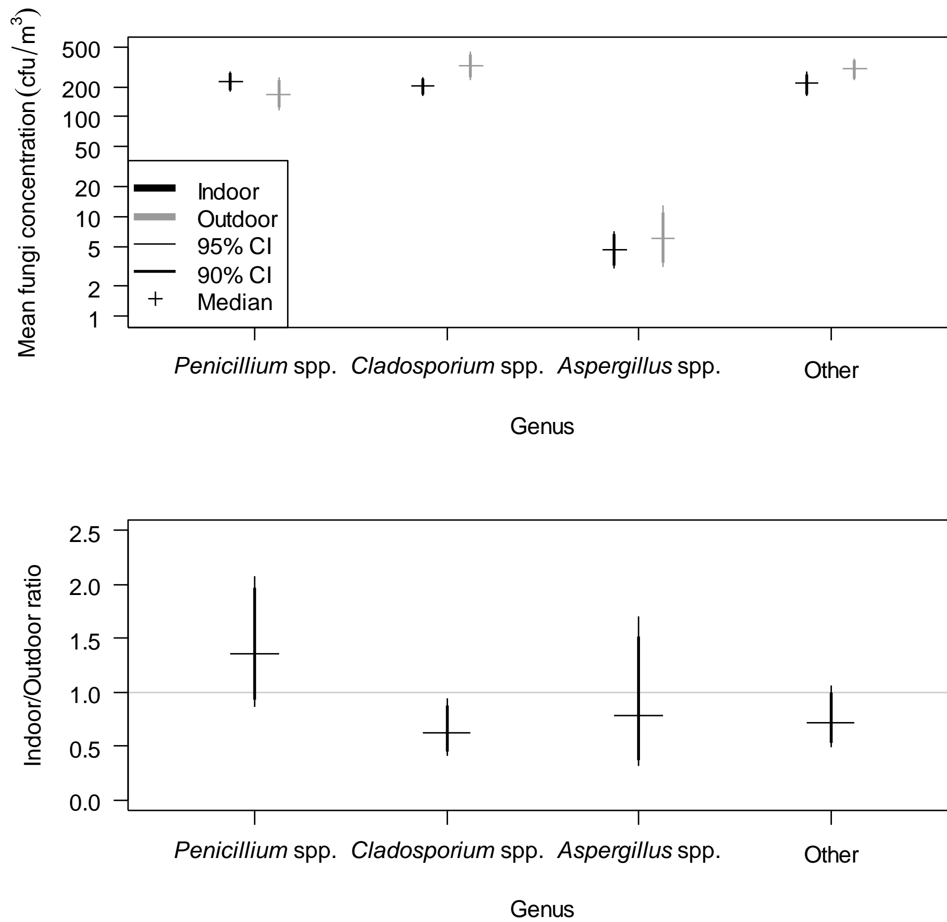

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864 # FIGURE S7

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866 **Figure S7.** Temperature and relative humidity (RH) measured for the 25 schools. Records are not
867 available for both temperature and humidity during the fungi experiments at schools 3, 4, 6, 18 and 25.

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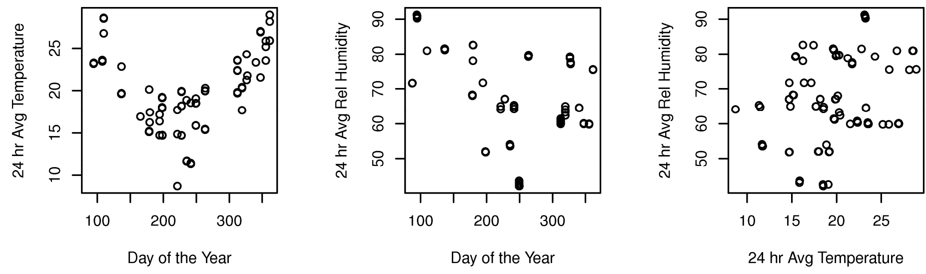

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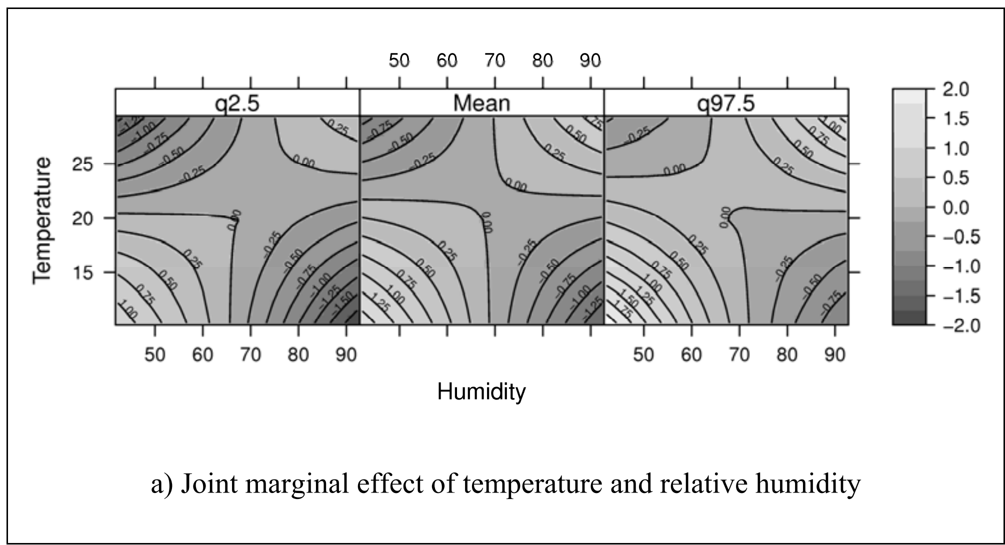
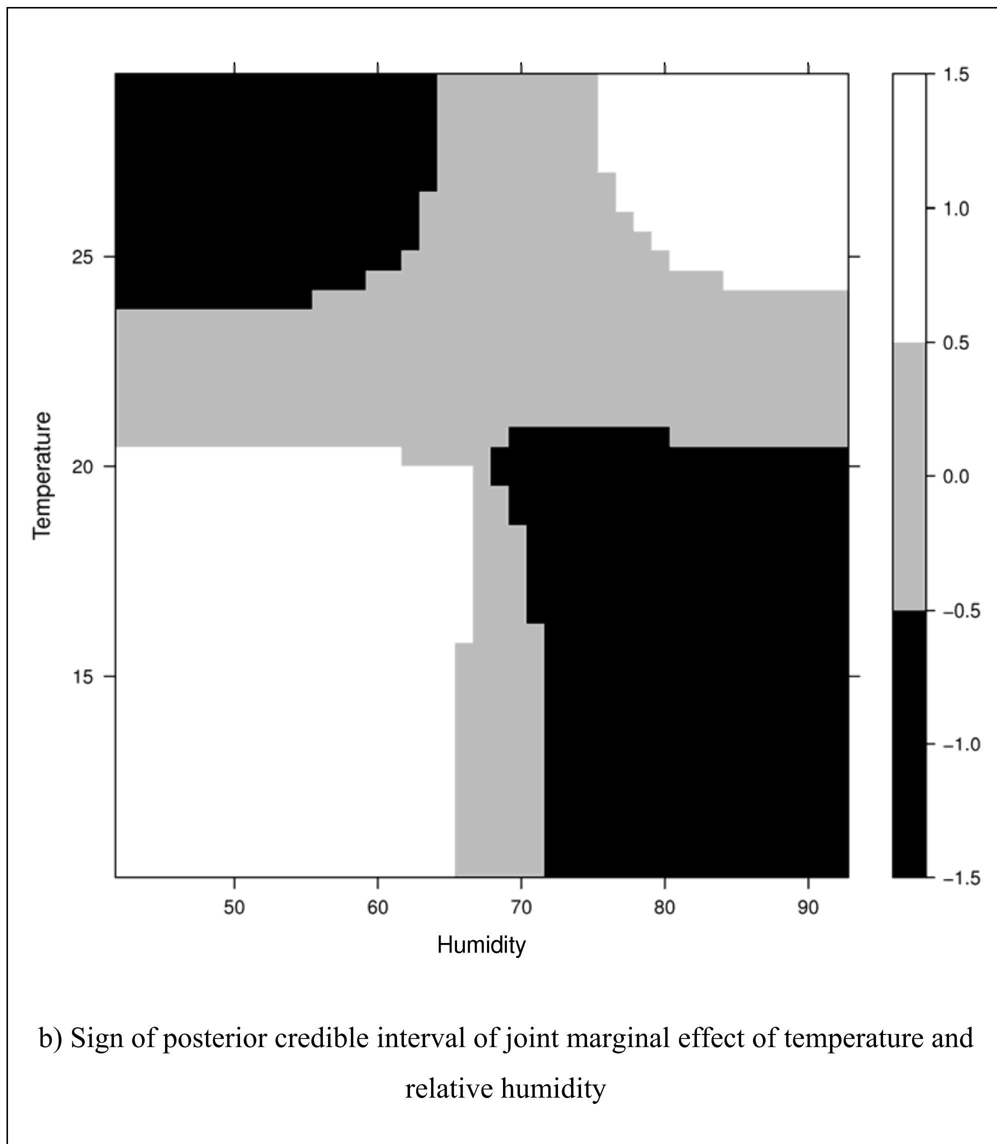
FIGURE S8

Figure S8. a) Contour plot of posterior joint marginal effect of humidity and temperature on total viable fungi concentration. The 95% posterior credible interval is represented by grey dashed lines, points represent the observed temperature and humidity data b) Sign of 95% credible interval of the joint marginal effect. White regions are strictly positive, black are strictly negative, grey contain zero.



a) Joint marginal effect of temperature and relative humidity



b) Sign of posterior credible interval of joint marginal effect of temperature and relative humidity

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902 **Gelman, A., Hill, J., 2007. Data Analysis Using Regression and Multilevel/Hierarchical Models.**
903 **Cambridge University Press.**

904 **Plummer, M., 2012. rjags: Bayesian graphical models using MCMC. http://cran.r-**
905 **project.org/web/packages/rjags/index.html**

906 **R Core Team, 2012. R: A Language and Environment for Statistical Computing. Vienna, Austria.**
907 **http://www.r-project.org/.**

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