

1 **Airborne *Alternaria* and *Cladosporium* fungal spores in**  
2 **Europe: forecasting possibilities and relationships with**  
3 **meteorological parameters**

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#### 41 **Abstract**

42 Airborne fungal spores are prevalent components of bioaerosols with a large impact on  
43 ecology, economy and health. Their major socioeconomic effects could be reduced by  
44 accurate and timely prediction of airborne spore concentrations. The main aim of this study  
45 was to create and evaluate models of *Alternaria* and *Cladosporium* spore concentrations  
46 based on data on a continental scale. Additional goals included assessment of the level of

47 generalization of the models in space and description of the main meteorological factors  
48 influencing fungal spore concentrations.

49 Aerobiological monitoring was carried out at 18 sites in six countries across Europe over 3 to  
50 21 years depending on site. Quantile random forest modelling was used to predict spore  
51 concentrations values. Generalization of the *Alternaria* and *Cladosporium* models was tested  
52 using (i) one model for all the sites, (ii) models for groups of sites, and (iii) models for  
53 individual sites.

54 The study revealed the possibility of reliable prediction of fungal spore levels using gridded  
55 meteorological data. The classification models also showed the capacity for providing larger  
56 scale predictions of fungal spore concentrations. Regression models were distinctly less  
57 accurate than classification models due to several factors, including measurement errors and  
58 distinct day-to-day changes of concentrations. Temperature and vapour pressure proved to be  
59 the most important variables in the regression and classification models of *Alternaria* and  
60 *Cladosporium* spore concentrations.

61 Accurate and operational daily-scale predictive models of bioaerosol abundances contribute to  
62 the assessment and evaluation of relevant exposure and consequently more timely and  
63 efficient management of phytopathogenic and of human allergic diseases.

64

#### 65 **Keywords**

66 Advanced statistical models; aerobiology; bioaerosols; biometeorology; continental scale;  
67 molds

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#### 69 **Highlights**

- 70 • No operational forecasting model for allergenic fungal spore exposure exists in Europe
- 71 • Potential exposure in Europe was assessed and predicted for 2 major allergenic fungi
- 72 • Random forest modelling was applied to >7,000 daily time series
- 73 • Air temperature and vapour pressure were the most significant variables
- 74 • Classification models showed higher capacity for large-scale spore predictions

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## 77 **1. Introduction**

78 Fungal spores are one of the most prevalent components of bioaerosols, found across a  
79 wide range of biogeographic regions over long time periods each year. The primary source of  
80 spore emissions are the substrates on which fungi grow, such as plants, soil and decaying  
81 organic matter. This means the majority of fungal spores in the air originate from farms, forest  
82 stands and decomposing plant material (Bowers et al., 2013). They will remain airborne for  
83 variable amounts of time and will be transported over distances ranging from a few  
84 centimeters to hundreds of kilometers (Ansari et al., 2015; Heald and Spracklen, 2009;  
85 Stockmarr et al., 2007).

86 *Alternaria* and *Cladosporium* are ubiquitous asexually reproducing fungal genera that  
87 produce spores, known as conidia, which are readily airborne. Both genera contain plant  
88 pathogens (Carlile et al., 2007; Chaerani and Voorrips, 2006; Lee et al., 1997; Nowicki et al.,  
89 2012; Thomma et al., 2005), many of which also produce phytotoxic metabolites that affect  
90 mammalian cells (De Lucca, 2007; Friesen et al., 2008; Mamgain et al., 2013). Fungal spores  
91 are also important aeroallergens, causing adverse health effects (Krouse et al., 2002).

92 Aerobiological surveys reported *Alternaria* as one of the most prevalent airborne  
93 fungal types and an important aeroallergen (Budd, 1986; Mitakakis et al., 2001). Also,  
94 *Cladosporium* is frequently reported as the most abundant aeroallergen and the second most

95 allergenic fungal type worldwide (Tariq et al., 1996). Allergy to fungi from both genera has  
96 been responsible for hospital admissions due to severe asthma attacks in sensitized  
97 individuals, particularly among children (Bush and Prochnau, 2004; Dales et al., 2000). Due  
98 to their ubiquitous nature (Damialis et al., 2017; Jędryczka, 2014) exposure to these  
99 aeroallergens is literally inevitable during their dispersion season.

100         It is important to assess the potential risks of the atmospheric presence of fungal  
101 spores, and more research is needed to evaluate this (Beggs, 2004; Crameri et al., 2014).  
102 There is limited data on long-term trends in airborne fungal spore abundance, in part due to a  
103 lack of representative fungal spore data sets longer than a decade across the globe. Airborne  
104 fungal spores as allergens have received comparatively less attention than pollen, and  
105 associated public health consequences are likely to have been underestimated (Damialis et al.  
106 2015).

107         Sporulation and dispersion of fungi are influenced by several meteorological factors,  
108 including air temperature, relative humidity, precipitation, atmospheric turbulence, wind  
109 speed, and UVB radiation (Al-Subai, 2002; Carlile et al., 2007; Cecchi et al., 2010; Straatsma  
110 et al., 2001). However, the exact influence of climatic variability on fungal ecology at a larger  
111 scale is still not understood. There are indications that changing climate may lead to  
112 alterations in phenology (Corden et al., 2003; Gange et al., 2007; Kausrud et al., 2010) and  
113 dynamics of fungal communities (Gange et al., 2011).

114         This complexity of biological and ecological processes represent one of the key  
115 problems of modelling biological systems. There are inconsistencies regarding what drives  
116 and controls the distribution of fungal bioaerosols both at a local and regional scale. Multiple  
117 meteorological factors may alter the spatiotemporal distribution of *Alternaria* and  
118 *Cladosporium* spores (Corden et al., 2003; Damialis and Gioulekas, 2006; De Linares et al.,  
119 2010; Escudero et al., 2011; Iglesias et al., 2007; Recio et al., 2012; Sindt et al., 2016; Skjøth

120 et al., 2016). The relationships between fungal development and environmental factors,  
121 including major climatic variables, are often the only component used for disease forecasting  
122 systems (van Maanen and Xu, 2003).

123 Atmospheric dispersion models have been used to describe the spatiotemporal  
124 dispersal of fungal pathogens (Burie et al., 2012; Oteros et al., 2015; Stockmarr et al., 2007;  
125 van Leuken et al., 2016). Additionally, descriptive, predictive or conceptual modelling of  
126 airborne fungal spores concentration is another promising tool, albeit challenging (Grinn-  
127 Gofroń and Bosiacka, 2015; Grinn-Gofroń et al., 2018; Iglesias et al., 2007; Jędryczka et al.,  
128 2015). At a minimum, high-resolution data on meteorological and geographic variables should  
129 be included in such models. It is important to include and analyse all associated factors, so  
130 that the resulting models can accurately describe the complex environmental inter-  
131 dependencies.

132 Given the widespread, ecological, economical and health impact of spores from  
133 *Alternaria* and *Cladosporium*, the main goal of the present study was to build models that  
134 generalize beyond the observed data and are capable of estimating the spatiotemporal  
135 distribution and concentration of fungal spores on a broad scale and to address the factors that  
136 influence the fungal spatiotemporal patterns. Created models could help to answer several  
137 questions - (i) is it possible to make a reliable prediction of fungal spore concentrations using  
138 only one model for all sites, (ii) is there a difference in quality between one model for all the  
139 sites, models for the groups of sites, and models for individual sites, (iii) what are the main  
140 meteorological factors influencing fungal spore concentrations, and (iv) do these factors differ  
141 between created models (analysed sites)?

## 142 **2. Materials and Methods**

### 143 **2.1. Aerobiological data**

144 The taxa selected for this study were those of *Alternaria* and *Cladosporium*. These comprise  
145 some of the most well studied fungal taxa worldwide due to their allergenic and  
146 phytopathogenic properties. In the current research, the spore abundance ranges greatly from  
147 >15% to over 96% of the total annual spore index per site (Table 2). This variation alone  
148 challenges for the elaboration of a universal forecasting model in Europe. But even if we had  
149 added the rest of the fungal diversity per site, this would have not most probably provided any  
150 additional insight to either ecological/biological processes or health impacts, as responses of  
151 different fungi are highly individualistic and their sensitivity (or not) to environmental stress  
152 (i.e. climate change) would not be reflected reliably (e.g. Damialis et al. 2015). Also, in most  
153 stations in the world, mainly these two types alone are counted. So as to obtain similar data in  
154 such a large-scale spatial study design and consequently comparable results, we did not  
155 extend to the investigation of additional fungal taxa.

156 Fungal spores were collected in the frame of long-term aerobiological monitoring in  
157 18 sites from six countries across Europe and for a time span of 1987-2015 (Fig. 1, Table 1).  
158 *Alternaria* spore concentrations were measured at all sites and *Cladosporium* spore  
159 concentrations at 15 out of the 18 (Table 1).

160 In brief, for this research, all data providers have been inquired regarding major  
161 changes in methodological procedures and no significant alteration has been reported.  
162 Moreover, in all participating countries, microscopic identification of airborne fungal spores  
163 has been conducted by experts with long-standing experience in such techniques. Therefore,  
164 in all stations, the same method of collection and analysis was used. Standard sampling,  
165 processing and analysis techniques were followed, according to the recommendations of the  
166 European Aerobiology Society (Frenguelli, 2003) and the British Aerobiology Federation  
167 (1995). At each location, a Hirst-type volumetric spore trap was used to sample airborne  
168 fungal spores (Hirst, 1952), which is considered the gold-standard device for sampling

169 airborne particles of biological origin (Galán et al., 2014). Samples were collected and  
170 analysed weekly, applying standard methods for sample processing and microscopic  
171 identification (e.g. British Aerobiology Federation, 1995; Galán et al., 2014; Grant Smith,  
172 1984). Final measurements referred to daily resolution and were expressed as concentrations  
173 of fungal spores per cubic meter of air on a given date.

## 174 **2.2. Meteorological data**

175 Eight meteorological factors were included as co-factors in the data analysis, namely  
176 maximum temperature, minimum temperature, average temperature, vapour pressure, sum of  
177 precipitation, potential evaporation from a free water surface, potential evapotranspiration  
178 from a crop canopy, and total global radiation. These were acquired from the AGRI4CAST  
179 Interpolated Meteorological Database (Baruth et al., 2007).

180 Based on maximum ( $T_{max}$ ) and minimum ( $T_{min}$ ) temperature, an additional ninth  
181 parameter was included, growing degree days (GDD). Cumulative GDD is an indicator  
182 measuring a heat accumulation, and as a proxy, can represent plant and fungal development.

183 GDD value is calculated as follows:

$$184 \quad GDD = \frac{T_{max} + T_{min}}{2} - T_{base}$$

185 Values of GDDs were accumulated starting from January 1. GDD do not accumulate when the  
186 daily mean temperature ( $(T_{max} + T_{min})/2$ ) is lower or equal to the base temperature. Value of  
187  $T_{base}$  was set to 5. No relevant previous information on base temperatures for fungal spores  
188 existed, so we assumed that these would be similar to later-flowering plant species (being  
189 abundant mainly during May-August) and which could be found across a variety of latitudes  
190 and climates in Europe; therefore, we set the base temperature at 5, in a similar manner to  
191 studies on grass pollen (Emberlin 1993; Frenguelli et al., 1989).

192 All the the abovementioned factors were considered either based on their availability,  
193 or because of the focus of the current study, or based on previous literature on the topic. For  
194 instance, relative humidity data are not available via AGRI4CAST as these cannot be  
195 corrected for altitude differences among sites within the target climatic grid cell. Regarding  
196 other factors, like wind vectors (speed, persistence and direction), these were excluded from  
197 this analysis, as this would make more sense in a smaller-scale temporal data processing,  
198 when the intermittent nature of wind would be possible to take into account. Finally, there  
199 have been previously published reports of particular meteorological factors in site-specific  
200 studies, included in the current analysis, proven to be the most decisive for obtaining accurate  
201 and reliable forecasts (e.g. Damialis and Gioulekas, 2006).

### 202 **2.3. Models**

203 Partial autocorrelation function (Durbin, 1960) was applied independently to  
204 *Alternaria* and *Cladosporium* daily counts to check for the temporal autocorrelation of spore  
205 data. This summarizes the relationship between an observation  $x_t$  and observations with  
206 lagged time steps (days) removing the impact of the values at all shorter lags.

207 Redundancy among the lagged values of meteorological predictors was explored using  
208 principal coordinates analysis (PCA) (Jolliffe, 1986). PCA transforms a number of correlated  
209 variables into a smaller set of uncorrelated variables. The role of PCA in this study was to  
210 reduce the dimension of the data, reduce computational time of models building, minimize  
211 spurious effects of single lags and ease interpretation of the final models.

212 Two main types of modeling techniques were used, regression and classification. The  
213 Quantile Random Forest method was used to create regression models. This is a  
214 generalization of Random Forests that infer the full conditional distribution of a response  
215 variable (Li et al., 2011). We decided not to predict mean value, but rather a median value,  
216 because of a non-symmetrical distribution of fungal spore values. Daily spore concentrations

217 of *Alternaria* or *Cladosporium* were used as dependent variables (model output) in regression  
218 models. Breiman's Random Forest (Breiman, 2001) was used in classification models. So as  
219 to eliminate high levels of statistical noise, daily spore concentrations were divided into two  
220 levels, low and high, according to the thresholds of *Alternaria* and *Cladosporium* allergens to  
221 evoke allergic symptoms (Gravesen, 1979). *Alternaria* values lower than 100 spores were  
222 considered as 'low' and beyond that threshold they were characterized as 'high'. For  
223 *Cladosporium*, this threshold was 3,000 spores. As airborne fungal spore measurements could  
224 still exhibit a huge disparity in the frequencies of the observed classes, we adopted an  
225 optimizing probability threshold technique (Kuhn and Johnson, 2013; Nowosad, 2016). In this  
226 approach, alternative cutoffs for the predicted probabilities were determined using  
227 resampling. Sensitivity (Sens), specificity (Spec), positive predictive value (Ppv), and  
228 negative predictive value (Npv) were calculated for 20 different threshold values. For each  
229 model, optimal threshold value was established minimizing the distance between Sens, Spec,  
230 Ppv, Npv and the value of 1. This value indicates the best possible performance.

231         Attempting to also reduce the spatial variability among monitoring sites across  
232 Europe, analyzed sites were divided into three groups based on the annual temporal changes  
233 of fungal spore concentrations and on data availability (Table 1), as follows: northeastern  
234 Spain (Barcelona, Bellaterra, Girona, Lleida, Manresa, Roquetes-Tortosa, Tarragona, Vielha),  
235 western Spain (Don Benito, Plasencia, Zafra) and non-Spanish sites (Derby, Leicester,  
236 Szczecin, Thessaloniki, Timisoara Vinnytsia, Worcester). The grouping was based on the  
237 seasonality and multi-modality of airborne spore concentrations, i.e. the first group exhibited  
238 the highest seasonality and normality of data, with longer seasons and fewer outliers. The  
239 third group presented a bi- or multi- modal yearly pattern, whilst the second group included  
240 those sites with only *Alternaria* spore measurements.

241 The modeling was performed in three ways: 1) per site, 2) per group of sites as  
242 mentioned above, and 3) an integrated model for all sites combined. This resulted in 22  
243 combinations for *Alternaria* (18 sites, 3 groups of sites, and 1 whole dataset) and 18  
244 combinations for *Cladosporium* (15 sites, 2 groups of sites, and 1 whole dataset) generating a  
245 total of 40 regression and 40 classification models.

#### 246 **2.4. Validation metrics**

247 The accuracy of all models was assessed using a repeated k-fold cross-validation  
248 (Kuhn and Johnson, 2013). Regression models were evaluated using the Symmetric Mean  
249 Absolute Percentage Error (SMAPE). Thus, accuracy measure was based on relative errors  
250 instead of standard metrics like Root Mean Square Error (RMSE) or Mean Absolute Error  
251 (MAE), which was particularly important in our study because of high among-site data  
252 variability. Classification modelling results were characterized using balanced accuracy. This  
253 metric is calculated as sensitivity + specificity/2. It is a better measurement in the case of  
254 imbalanced datasets as it gives the same weight for correctly predicted cases with low  
255 concentration and correctly predicted cases with high concentration. Influence of the  
256 predictors was determined using a scaled permutation importance (mean decrease in  
257 accuracy) (Breiman, 2001).

### 258 **3. Results**

#### 259 **3.1. Spatiotemporal variability of spore concentrations**

260 Fungal spore concentrations differed between *Alternaria* and *Cladosporium*, between  
261 sites, and between years of measurements (Table 2, Figs. 2, 3). The annual sum of *Alternaria*  
262 daily concentrations varied between 907 (Vielha) and 67,166 (Lleida) and had a mean value  
263 of 13,448. In addition to Lleida having the highest average annual sums of *Alternaria*, the  
264 values here were also the most variable between years. Other sites with substantial changes

265 between years were Derby, Thessaloniki, Vielha, and Girona. The annual sum of the daily  
266 concentrations of *Cladosporium* varied between 24,637 (Thessaloniki) and 1,500,699 (Derby)  
267 and had a mean value of 405,237. Annual spore concentrations in Lleida were also the most  
268 changeable for *Cladosporium*. Overall, the order of average values for all sites was similar for  
269 *Alternaria* and *Cladosporium* with two exceptions, Worcester and Thessaloniki. The annual  
270 sum of the daily concentrations of *Alternaria* in Worcester were relatively small and  
271 invariable, whilst *Cladosporium* values were high and changeable. In Thessaloniki *Alternaria*  
272 levels were moderate and varied substantially, whilst *Cladosporium* annual values were small  
273 and more consistent.

274 For *Alternaria*, sites could be split into two groups based on the time course of the  
275 season: (i) sites with period of high concentration and period of lower concentrations (such as  
276 Barcelona, Lleida, etc.), (ii) sites with one period of high concentrations and period with  
277 absence of *Alternaria* spores (such as Szczecin, Thessaloniki and Vielha) (Fig. 2). Time  
278 course of *Cladosporium* spore concentrations is more heterogeneous, with probably two main  
279 groups: (i) Spanish sites with period of high concentration and period of moderate  
280 concentrations, (ii) sites with one period of high concentration and period with low  
281 concentrations or absence of spore concentrations (such as Szczecin, Derby, Vinnytsia).  
282 Additionally, a third group, consisting of Don Benito, Plasencia, and Zafra, was separated due  
283 to missing data of *Cladosporium* (Fig. 3).

284

### 285 **3.2. Predictor variables**

286 Based on the spatiotemporal analysis, lagged daily values of nine meteorological parameters  
287 between 1-15 days were created. A principal component analysis (PCA) for each of the  
288 parameters was run and the results of the PCA gave an insight into the variability of  
289 predictors. In most of the cases, the two first components explained the majority of variations.

290 The cumulative value was between 0.85 for radiation and 0.94 for average temperature in  
291 these variables. The first component expressed the value of a given parameter (large values of  
292 loadings for all of the lags), whilst the second parameter expressed the temporal changes of a  
293 given parameter (the largest, positive value for the first lag, and the lowest, negative value for  
294 the last lag). In case of cumulated GDD, we used only the first component, which explained  
295 0.95 of variation. Precipitation values were the most changeable, and the first two components  
296 had a cumulative variance of 0.22. Therefore based on the autocorrelation plot, we used raw  
297 values of precipitation for lags between 1 and 4 days.

298 The final group of predictors consisted of 19 variables - the first principal component  
299 of cumulated GDD, one to four day lags of precipitation, and the first two principal  
300 components of the rest of the meteorological parameters (Table 3).

### 301 **3.3. Regression models**

#### 302 **3.3.1. Performance of the models**

303 The final 40 regression models (22 for *Alternaria* and 18 for *Cladosporium*) were built  
304 and results compared using Symmetric Mean Absolute Percentage Error (SMAPE) (Table 4).  
305 These values ranged between 0.56 and 0.90 for *Alternaria* (average value of 0.69), and 0.53  
306 and 0.73 for *Cladosporium* (average value of 0.61). For all of the sites, the models gave a  
307 value of 0.76 for *Alternaria* and a value of 0.73 for *Cladosporium*. Those values were slightly  
308 higher than values for separate groups and distinctly higher than values for most of the  
309 individual sites. Similarly, most of the models for the site groups performed worse than the  
310 models for the individual sites. *Alternaria* model had a SMAPE value of 0.73 for the first  
311 group (average for the models for the individual sites in this group was 0.67), 0.75 for the  
312 second group (average of 0.74), and 0.69 for the third group (average of 0.61). These  
313 differences were higher in *Cladosporium* models with SMAPE of 0.67 for the first group

314 (average of 0.60) and 0.71 for the second group (average of 0.59). Only in four *Alternaria*  
315 models, Derby, Worcester, and Vielha, values of SMAPE were higher than values for models  
316 of groups of sites.

### 317 **3.3.2. Variable importance**

318 The same set of variables seemed to influence the models of both taxa (Fig. 4). The  
319 most important predictors were the first principal coordinates of vapor pressure and  
320 temperatures (minimum, average, maximum and cumulated GDD). They were followed by  
321 the first principal coordinates of evapotranspiration, evaporation, and radiation. The second  
322 principal coordinates of those parameters had small importance. The smallest values were  
323 observed for lagged values of precipitation.

## 324 **3.4. Classification models**

### 325 **3.4.1. Performance of the models**

326 The final classification models were compared using the balanced accuracy metric  
327 (Table 4). Average value of balanced accuracy were 0.78 for *Alternaria* and 0.73 for  
328 *Cladosporium*. These values varied among sites between 0.50 and 0.90 for *Alternaria* and  
329 between 0.50 and 0.99 for *Cladosporium*. Models for all the sites gave similar results of 0.80  
330 and 0.78. The *Alternaria* model gave a balanced accuracy value of 0.77 for the first group of  
331 sites compared to the average of individual site's models of 0.75, a value of 0.85 for the  
332 second group of sites comparing to the average of individual site's models of 0.81, and a value  
333 of 0.80 for the third group of sites compared to the average of individual site's models of 0.71.  
334 Values of a balanced accuracy for the models of groups of sites were also higher for  
335 *Cladosporium*. The first group had a value of 0.68 (average for individual sites was 0.66), and  
336 the second group had a value of 0.85 (average for individual sites was 0.80). About 44% (15

337 of 34) models for individual sites gave worse values of balanced accuracy than models for  
338 groups of sites.

### 339 **3.4.2. Variable importance**

340 Values of variable importance for classification models were more diverse than for  
341 regression models. *Alternaria* and *Cladosporium* classification models were influenced  
342 mostly by the same predictors (Fig. 4). Temperature (cumulated GDD) was the most  
343 important variable, followed by the first and second principal coordinate of vapor pressure.  
344 The rest of the predictors showed moderate to low importance. Similarly to regression  
345 models, predictors with values of precipitation had the smallest importance.

346

## 347 **4. Discussion**

348 The present study revealed that wide-scale, accurate, operational modeling of fungal  
349 spore abundances is feasible with one universal model, which answers the first research  
350 question. Of course, there are restrictions because of annual and spatial variability, which  
351 result in varying performance of the obtained models. Based on the model used, these  
352 individualistic responses (also based on the fungal taxon examined) can be decreased to an  
353 extent. We found that specific meteorological factors significantly contributed to the  
354 forecasting power, with air temperature playing the leading role. Consequently, the current  
355 research highlights the possibility and need for developing universal predictive models of  
356 airborne fungal spore abundances, something currently lacking and therefore making this  
357 study novel.

358 In the current research, an integrative approach of the variables affecting the  
359 distribution of these bioaerosols was adopted, on a local or a regional scale, providing a

360 deeper comprehension of the dynamics of these fungal taxa. The survey revealed distinct  
361 relationships between spore concentrations and sites and years of sampling. The overall spore  
362 frequency and the annual sum of the daily concentrations of *Cladosporium* was higher than  
363 *Alternaria*'s, a finding also reported previously in different regions of the world. In Cartagena  
364 (Spain), *Cladosporium* represented 62.2% of the total spore count and *Alternaria* only 5.3%,  
365 however, *Alternaria* was still the second most abundant fungal type (Elvira-Rendueles et al.,  
366 2013). Likewise, in Bursa (Turkey), *Cladosporium* represented 88.1% of the total spore count  
367 followed by *Alternaria* at 4.9% (Ataygul et al., 2007). A similar trend was found in  
368 Thessaloniki (Greece) by Gioulekas et al. (2004) and in Madrid (Spain) by Sabariego et al.  
369 (2007).

370 Two types of models, regression and classification, were built as a part of this study.  
371 The goal of the first one was to predict values of the fungal spore concentration in the studied  
372 sites, while the classification models were created to predict high levels of the fungal spore  
373 concentrations. Classification models were more accurate than regression models. This is due  
374 to a number of factors. Concentration values of *Alternaria* and *Cladosporium* were classified  
375 into two groups prior to the modelling. This procedure generalises the fungal spore values  
376 and, therefore, changes in values. As a result, it gives more general trends instead of showing  
377 the local trends and day-to-day differences. Moreover, the obtained values of concentration  
378 are an estimation of the whole population and are prone to random and systematic (bias)  
379 errors (Comtois et al., 1999; Oteros et al., 2013). The benefit of using a classification method  
380 is that it reduces the influence of methodological differences between sites, such as the  
381 relative position of samplers and the heights of buildings, or instrumental and human errors.

382 The life cycles of many fungal pathogens are strongly determined by weather. The  
383 airborne spore concentrations are affected by biological factors (reproduction and survival),  
384 weather parameters, land use, resource availability and competition (Boddy et al., 2014).

385 Dispersal and circulation are highly influenced by wind and rainfall, while germination and  
386 infection rates are often dependent upon liquid water on the plant surface (sometimes high  
387 relative humidity) and species-specific optimal temperature ranges. In this study the most  
388 important meteorological variables were the same for both *Alternaria* and *Cladosporium*. This  
389 shows that despite the differences in the values of spore concentration, both taxa are mostly  
390 affected by the same meteorological factors, such as temperature (minimum, average,  
391 maximum and cumulated GDD) and vapour pressure, which determines the water content in  
392 the air (related to air humidity).

393 In many aerobiological studies, temperature and relative humidity of the air are  
394 meteorological parameters that significantly influence concentrations of *Cladosporium* and  
395 *Alternaria* spores, with temperature being positively associated and relative humidity  
396 negatively associated (e.g. Grinn-Gofroń and Strzelczak 2009; O'Connor et al. 2014; Sadyś et  
397 al. 2016; Ianovici 2016; Almeida et al. 2018)

398 Other variable - global radiation includes both the direct solar radiation and the diffuse  
399 radiation resulting from reflected or scattered sunlight, and can be considered as a function of  
400 temperature (Meza and Varas 2000). One of the solar radiation components, UVB radiation,  
401 is reported to affect the survival of airborne fungal spores during movement over long  
402 distances through the atmosphere (Al-Subai 2002). This predictor showed moderate  
403 importance in our models.

404 Rain has been often cited as being one of the most influential factors in reducing  
405 airborne pollen (e.g. Damialis et al., 2005). However, precipitation was identified as the least  
406 important factor in our models. There are two main reasons behind the small effect of  
407 precipitation on the fungal spore models. Firstly, variation of daily precipitation values does  
408 not closely correlate with the values and levels of *Alternaria* and *Cladosporium*  
409 concentrations, both of which are regarded as dry weather spores. For example, it is possible

410 to have a rainless day and a high fungal spore concentration (the middle of the season) and to  
411 have a rainless day without airborne fungal spores (off-season). Secondly, precipitation is  
412 characterized by high variability, which could not be captured in a daily timescale (sum of  
413 precipitation). To better understand the effects of rainfall on fungal spore diversity and  
414 abundance, and on circulation patterns, finer resolution data (hourly scale), with different  
415 statistical techniques (i.e. artificial intelligence models) would be required.

## 416 **5. Conclusions**

- 417 • Classification models were more accurate than regressions for *Alternaria* and  
418 *Cladosporium* fungal spores.
- 419 • Regression models gave better results for individual sites compared to grouped sites,  
420 resulting potentially from strong effects from local meteorological conditions.
- 421 • Classification models gave better results in grouped sites rather than for individual  
422 sites, thus, displaying the capacity for accurately providing larger scale predictions of  
423 fungal spore concentrations (compared to the more localized regression models).
- 424 • Temperature (in the form of minimal, average, maximum temperature, and cumulated  
425 GDD) and vapour pressure were the most important variables in models of *Alternaria*  
426 and *Cladosporium*, while radiation and daily sum of precipitation had a smaller impact  
427 on the models.

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612 **Figure captions:**

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614 **Figure 1.** Location of measurement points against the background of biogeographical  
615 conditions in Europe (according to European Environmental Agency: [www.eea.eu.int](http://www.eea.eu.int)  
616 modified)

617

618 **Figure 2.** Daily concentrations of airborne *Alternaria* spores by day of year for all of the  
619 analyzed sites on a logarithmic scale ( $\log(1+x)$ ). Black lines are cubic spline smoothers for  
620 each site, whereas colored lines represent the actual data. Variable coloration of Figures  
621 represents different timespan of data, with darker colors indicating lack of more recent spore  
622 data.

623

624 **Figure 3.** Daily concentrations of airborne *Cladosporium* spores by day of year for all of the  
625 analyzed sites on a logarithmic scale ( $\log(1+x)$ ). Black lines are cubic spline smoothers for  
626 each site, whereas colored lines represent the actual data. Variable coloration of Figures  
627 represents different timespan of data, with darker colors indicating lack of more recent spore  
628 data.

629

630 **Figure 4.** Variable importance of each input variable for *Alternaria* and *Cladosporium* -  
631 regression models (top) and classification models (bottom). The variables are showed by the  
632 mean value of variable importance for all of the taxa in descending order

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