Temporal variations in the diversity of airborne fungal spores in a Mediterranean high altitude site

Loretta Pace[1,2], Linda Boccacci[1], Marzia Casilli[1,2], Simone Fattorini[1]

[1]Department of Life, Health and Environmental Sciences, University of L'Aquila, Via Vetoio, 67010, Coppito, L'Aquila, Italy. E-mail: simone.fattorini@univaq.it

[2]Italian Network Monitoring on Aerobiology (R.I.M.A.®-AIA), AQ 01

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Abstract

Relationships between meteorological factors and airborne fungal spore concentrations at high altitudes are virtually unknown. We used cross-correlation analyses to test the relationships between daily variation in meteorological factors (i.e., temperature, humidity and wind speed) and airborne spore concentration, diversity (Shannon and Simpson indices) and evenness (Pielou index) in an Apennine high altitude site (Gran Sasso Massif, 2,117 m elevation). Daily average concentration of spores in this high altitude site was much lower than that observed at a low altitude site in the same area, which can be explained by the environmental conditions at high altitudes. We found that diversity and evenness tended to be negatively correlated with temperature and positively with humidity and wind speed with some delay, whereas abundance tended to be positively correlated with temperature and wind, but negatively with humidity. These relationships can be explained by the fact that rain increases turnover by removing dry spores of *Cladosporium* (the most abundant taxon) and aerosolizing wet ones. The high dominance of the most abundant taxon is a reflection of the extreme climatic conditions at high altitudes.

Keywords

Aerobiology; Humidity; Outdoor bioaerosols; Italy; Temperature; Wind speed

1 Introduction

Fungal spores constitute an important component of atmospheric aerosols. It is well known that atmospheric spore concentrations are strongly dependent on meteorological conditions, such as wind speed, humidity and temperature (Angelosante Bruno et al., 2007; Tomasetti et al., 2009; 2013). Research mainly conducted in agricultural and urban settings documented a positive correlation between air temperature and airborne spore concentration, especially of *Alternaria* spp., *Cladosporium* spp., and *Epicoccum* spp. (Langenberg et al., 1977; Aira et al., 2013; Corden et al., 2003; Grinn-Gofroń and Strzelczak, 2012; Troutt and Levetin, 2001). Also relative humidity seems to act positively on airborne fungal concentration (Webster et al., 1989; Leyronas and Nicot, 2013; Crandall and Gilbert, 2017). The influence of wind speed has been rarely investigated, but it can be postulated that an increase in wind speed should also induce higher airborne spore concentrations (see Crandall and Gilbert, 2017), although there is some evidence of a negative relationship (Fernández-Rodríguez et al., 2018).

Overall these studies indicated that airborne spore concentrations increase with air temperature, relative humidity and wind speed in the short time, but overlooked the fact that there may be some lag between variations in atmospheric conditions and spore concentration.

Moreover, most research dealt with overall or specific spore concentrations, whereas very few studies have attempted to measure airborne fungal diversity (Magyar et al. 2009; Sebők, et al. 2016; Pusz et al. 2018), and the influence of climatic factors on temporal patterns of spore diversity remains largely unknown. For example, Cáliz et al. (2018) investigated the whole airborne microbiome (bacteria, archaea, protists, and fungi) of a high altitude site in the Central Pyrenees (Spain) via high-throughput massive sequencing of 16S and 18S rRNA genes. Their analysis was however mainly based on coarse identifications, which did not allow a detailed analyses of diversity patterns. Finally, available information on the relationships between spore concentrations and meteorological conditions in Europe mainly refers to low altitude areas, whereas there is little information for high altitudes. This is a serious lack of information, because meteorological conditions at high altitudes are obviously very different from those that can be found at low altitudes and correlations between spore abundance and meteorological conditions at high altitude might be different from those observed at low altitudes.

In this paper, we present a first study of the atmospheric spore concentrations in a high altitude site from Central Italy (Gran Sasso Massif, 2,117 m elevation). Aim of our work was to test the influence of meteorological parameters (mean temperature, humidity and wind speed) on airborne spore concentration and diversity at different time lags. For this, we considered both airborne spore concentration (total abundance of spores per cubic meter of air) and diversity indices that take into account taxonomic composition and abundance.

We correlated daily spore abundance and diversity with main meteorological variables by using cross-correlation analysis, a statistical measure timing the movement and proximity of alignment between two different information sets of time series. This allowed us to explore the delay at which variations in spore concentration and diversity are related to variation in meteorological parameters. Cross-correlation analysis at different lags may be important to disclose temporal shifts in correlation between meteorological variables (temperature and humidity) and spore concentration and diversity, because fungi are expected to integrate temperature and moisture effects of a number of days. For example, moist soil and leaf conditions that persist after a rain event contribute to an increase in airborne fungal spore densities (Ganthaler and Mayr, 2015). Rain may influence spore concentration and diversity both positively, by triggering spore release in many species (Aylor and Sutton, 1992; Leyronas and Nicot, 2013; Gabey et al., 2010), and negatively, by removig fungal spores by rain-out and wash-out effects (Ingold, 1971; Lacey, 1986). Moreover, dry spore discharging fungi release spores by the flow of air or by hygroscopic twisting movements, which occur mostly when dry, warm and windy conditions prevail (Meredith, 1963; Lacey, 1986; Elbert et al., 2007).

Wind speed may produce immediate and delayed effects both directly (by promoting both the primary emission of fungal spores and a secondary presence through resuspension) and indirectly (higher winds may be correlated with increased dryness, which in turn may affect fungal physiology through desiccation). Also, after a number of days with high wind speeds, the spore supply might be exhausted, which influences spore concentrations and diversity in the atmosphere.

2 Materials and methods

Airborne spore sampling was conducted in the Alpine Botanical Garden of Campo Imperatore, at 2,117 m elevation (N 42°26'37.39'', E 13°33'29.73''), within the Gran Sasso and Laga Mountains National Park (GSML), in the Abruzzo Region (Pace et al., 2018). The environment of this site is particularly selective, due to the presence of very low temperatures, violent winds, and abundant snow from October to June (Baldoni et al., 1999; Blasi et al., 2003).

Air sampling was conducted with a 7-day recording Hirst-designed volumetric air sampler (Sadyś et al., 2014; Dananché et al., 2017; Pace et al., 2018) from 6th July to 18th September 2011 because of the continued presence of snow in the other months. Moreover, spore frequency in the winter months is strongly reduced due to the snow cover and the negative effects of low temperatures on sporulation (Grinn-Gofroń and Mika, 2008). During the study period, daily temperature varied between 5.6 and 18.6 °C (Mean ± Standard Error: 13.0±0.4); wind velocity varied between 1.9 and 13.9 m/s (Mean ± SE: 5.3±0.3 m/s); and relative humidity varied between 29.0% and 97.5% (Mean ± SE: 66.9±1.9). For instrument maintenance, the sampler was inactive in three days (7th July, 11th July and 11th September), which were therefore not considered in the analyses. Hirst's air sampler is an instrument specifically designed to assess the atmospheric concentration of fungal spores, pollen grains and other biological particles as a function of time through morphological identification. The sampler is equipped with a pump with an intake orifice through which the sampled air impacts onto a collection surface (a transparent tape coated with a silicon solution) moving at 2 mm h^{-1}. After a week the tape is cut into 48 mm long segments, representing daily samplings (Sadyś et al., 2014). Each daily segment is then scanned in four horizontal parallel transects (longer side of the slide) under a microscope at 400 x magnification to count particles, reading a surface always larger the 20% of the sampled surface. Finally, the spore counts are multiplied by an appropriate factor to give their concentrations (spores/m^3). This factor is based on the microscope setting, the air volume that went through the apparatus, the number of lines read and the size of the collection surface (see Albertini et al. 2009). Spores were classified to genus on the basis of morphological characteristics which allow unambiguous identifications.

The following parameters were recorded on a daily base from a weather station located at 50 m from the air sampler: average temperature (T, in °C), relative humidity (RH, in %) and wind speed (w, in m/s).

Analyses were restricted to identified taxa (level of genus type, Grinn-Gofroń et al., 2018). Daily diversity in spore composition was expressed by Shannon's index (*H'*), Simpson's reciprocal index (*D*) and Pielou's evenness (*J*) (e.g. Magyar et al., 2009; Sebők et al., 2016; Pusz et al., 2017).

To investigate the correlation between daily meteorological variables and spore abundance and diversity, cross-correlation analyses were performed with Spearman rank correlation coefficient, r_s (Pace et al., 2018). Cross correlation compares two time series and finds how they match up with each other, and in particular where (i.e. at which lag) the best match occurs (maximum correlation). Because the leading series are represented by the environmental variables, negative lags indicates the shifts of days on which the respective cross-correlation coefficient value was calculated. Cross-correlation analysis at different lags may be important to disclose temporal shifts in correlation between meteorological variables and spore concentration. Analyses were conducted using the software PAST version 1.89 (http://folk.uio.no/ ohammer/past) (Hammer et al., 2001). Further details on sampling procedure and data analyses are presented in Pace et al. (2018).

3 Results

The average of daily spore concentration for all the studied period was 239.061±266.245 SD spores/m^3 (max 1124.15 spores/m^3 on 2nd August). The majority of sampled spores belonged to the genus *Cladosporium*, which accounted for 92.70% of spore abundance (Table 1).

Spore abundance was particularly high in the period between 28th July and 9th August (Fig. 1). *H'*, *D* and *J* showed similar patterns, with the highest values approximatively between 17th August and 6th September (Fig. 2). Abundance was negatively correlated with *H'* (r_s = -0.366, p = 0.0015), *D* (r_s = -0.416, p = 0.0003) and *J* (r_s = -0.601, p = 0.0015), and positively with number of genera (r_s = 0.663, $p < 0.0001$). Number of genera was not correlated with *H'* (r_s = 0.145, p = 0.2256), *D* (r_s = 0.068, p = 0.5701) and *J* (r_s = -0.186, p = 0.1169). *D* was positively correlated with *H'* (r_s = 0.992, $p <$ 0.0001) and *J* (r_s = 0.930, $p < 0.0001$). Overall, these results indicate that number of genera tended to increase with abundance, but peaks in abundances were mainly due to the disproportionate increase of *Cladosporium* and *Alternaria*, which had a negative influence on *H'*, *D* and *J*. High values of *H'*, *D* and *J* in association with low abundance indicate the presence of few genera with, however, similar relative abundances (see Figure 1). *Cladosporium* peaked between 28th July and 14th August (with daily concentrations between 400 and 1087 spores/m^3); *Alternaria* had a peak on 30th August (45 spores/m^3), followed by a drop (with days with less than 20 spores/m^3), and a new,

higher peak on 12th September (57 spores/m^3); *Epicoccum* peaked on 30th August (26 spores/m^3); Stemphylium peaked on 28th July (9 spores/m^3); the other genera showed highly irregular patterns.

Table 1. Abundance (spores/m^3) and percentage of detected fungal spores.

Taxon	Abundance	%
Alternaria	733.757	4.26
Cladosporium	15955.561	92.70
Epicoccum	282.663	1.64
Helminthosporium	6.859	0.04
Pleospora	6.859	0.04
Polythrincium	31.407	0.18
Stemphylium	79.781	0.46
Torula	115.52	0.67
Total	17212.407	100.00

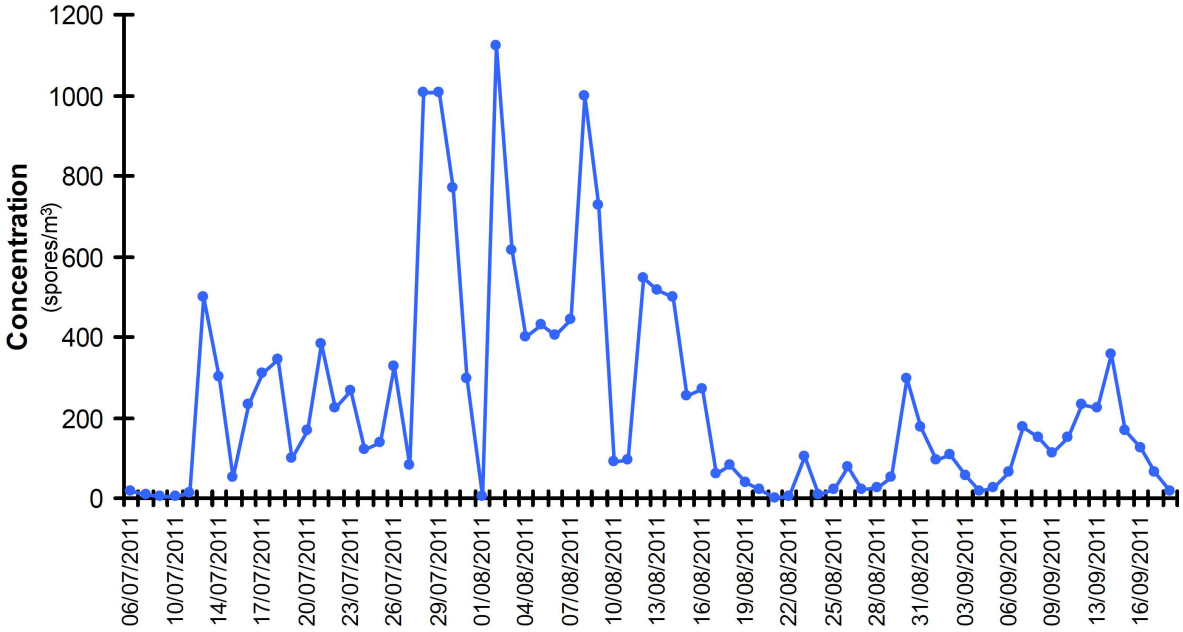

Fig. 1. Airborne spore concentration (spores/m^3) recorded in a Mediterranean high altitude site (Gran Sasso, Italy, 2,117 m elevation) from 6th July to 18th September 2011.

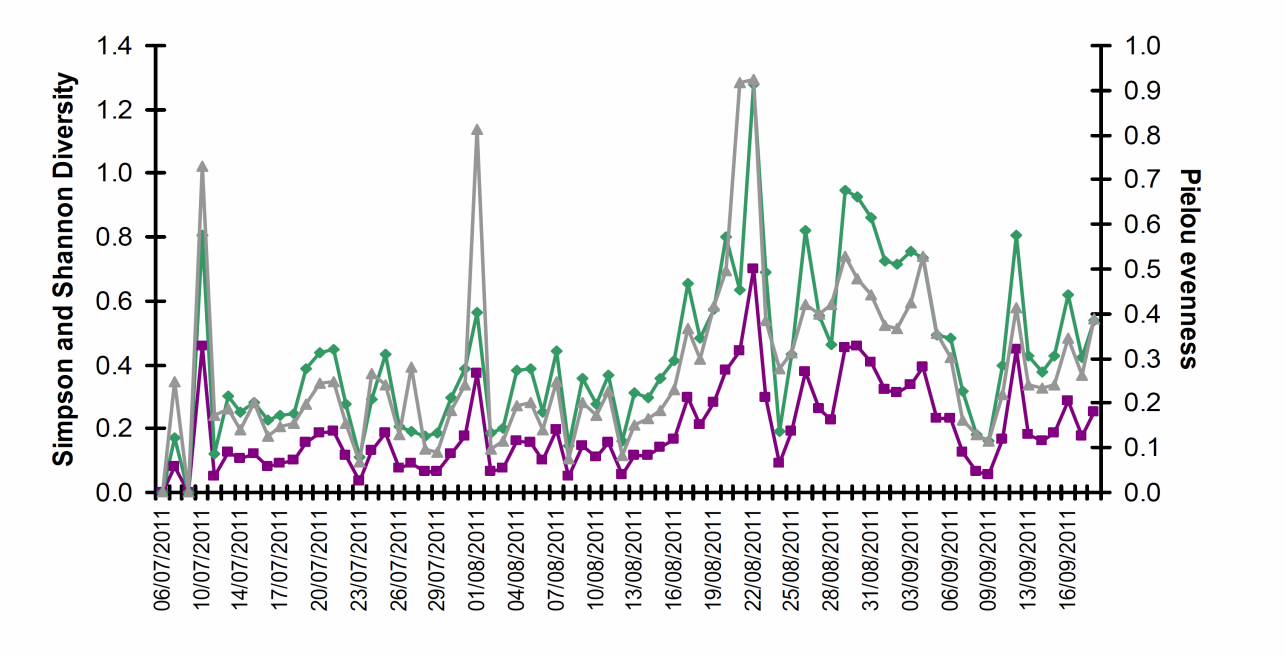

Fig. 2. Daily values of airborne spore diversity (Shannon diversity, green diamonds; Simpson diversity, violet squares; Pielou evenness, grey squares) recorded in Gran Sasso (Italy, 2,117 m elevation) from 6th July to 18th September 2011.

Cross-correlation coefficients (Table 2) indicated that increasing temperatures may increase abundance, and hence number of genera, with a certain delay, but influences negatively diversity, expressed as *D*, *H'* and *J*. Humidity may promote diversity with a certain delay, but exerts a negative effect in a shorter term (about 10 days). Wind speed correlated positively with abundance and negatively with number of genera with a relatively long delay.

Table 2. Correlation between daily meteorological variables (temperature, humidity and wind speed) and spore abundance (spores/m^3), number of genera, Simpson's diversity, Shannon's diversity and Pielou's evenness for long-term and short-term lags. *ccc* = cross-correlation coefficient (maximum significant value), *p* = *p*-values; lag = shift of days on which the respective cross-correlation coefficient value was measured.

	Daily temperature (°C)			Daily humidity (RH, in %)			Wind speed (m/s)		
	ccc	*p*	lag	*ccc*	*p*	lag	*ccc*	*p*	lag
Longer term (>15 days)									
Abundance	0.487	0.0001	-33	-0.544	0.0002	-29	0.353	0.013	-23
Number of genera	0.359	0.014	-26	-0.368	0.017	-30	-0.421	0.006	-32
Simpson's diversity	-0.650	<0.0001	-32	0.518	<0.0001	-28	0.324	0.047	-34
Shannon's diversity	-0.619	<0.0001	-32	0.511	<0.0001	-28	0.311	0.057	-34
Pielou's evenness	-0.717	<0.0001	-32	0.569	<0.0001	-28	0.362	0.028	-34
Shorter term (<15 days)									
Abundance	-0.486	<0.0001	-2	0.443	<0.001	-9	0.302	0.024	-16
Number of genera	-0.277	0.035	-14	0.315	0.018	-16	0.264	0.049	-16
Simpson's diversity	0.435	< 0.001	-2	-0.480	<0.0001	-10	-0.321	0.009	-6
Shannon's diversity	0.429	< 0.001	-2	-0.477	<0.0001	-10	-0.306	0.013	-6
Pielou's evenness	0.475	< 0.001	-2	-0.503	<0.0001	-10	-0.294	0.017	-6

4 Discussion

Although fungi play pivotal roles in most terrestrial ecosystems, aerobiological studies in high mountain areas are virtually lacking (Wojciech et al., 2017). Our research represents the first research of this type in Italy and it is particularly important because the study site is located in a protected area that hosts a unique flora (Pace et al., 2018).

The average of daily spore concentration (about 239 spores/m^3) recorded at Campo Imperatore was about half times lower than the value observed in a monitoring station located in the same area, but at a lower altitude (483 spores/m^3 at the University Monitoring Station, L'Aquila, 700 m; L. Pace, unpublished data). The low concentration of fungal spores at high altitudes may have positive implications for human health. Many allergic diseases can be caused or aggravated by fungal spores (Żukiewicz-Sobczak, 2013). In particular, airborne spores of *Alternaria*, *Cladosporium*, *Epicoccum*, *Stemphylium* and *Helminthosporium* are significant causes of allergic diseases (Burbach et al., 2009; Mari et al., 2003). Threshold concentrations for evoking allergic symptoms are estimated to be 80-100 spores/ m^3 for *Alternaria* and 2800- 3000 spores/ m^3 for *Cladosporium* (Grinn-Gofroń and Rapiejko, 2009). Thus, the lower abundance of fungal spores at high altitudes may make mountain sites important tourist destinations for people suffering from these diseases. In this context, aerobiological monitoring at high altitudes might have important health and economic implications. In particular, knowing the temporal shifts between atmospheric variations and spore abundance may be important to reduce people exposition to high concentrations.

The taxa that were found with the highest concentrations in our high-altitude site were the same that predominated in the low-altitude site. In particular, *Cladosporium* and *Alternaria* were the genera with the highest dominance in both stations (see also Pitari et al., 2014). *Cladosporium* accounted for about 93% at the high altitude site, and for 90% at the lower site; *Alternaria* accounted for about 4% at the high altitude site, and for 8% at the lower site. *Cladosporium* has been reported as the most abundant fungus in a variety of contexts (e.g., Grinn-Gofroń and Mika, 2008; Magyar et al., 2009; Grinn-Gofroń et al., 2019).

Alternaria and *Cladosporium* species live as parasites or saprophytes on a considerable number of plants (Hjelmroos, 1993). The organic substrate from arboreal and shrubby vegetation, present in considerable quantities at lower altitudes, can explain the high concentration of these fungal spores at L'Aquila, whereas the low concentrations in Campo Imperatore can be attaribuited to the scarcity of organic nutrient substrates in this high altitude site, where there are no trees.

An important finding of our study is that correlations between atmospheric parameters and spore diversity are highest with a certain lag. The delayed positive relationship between diversity and humidity can be explained by the fact that rain promotes spore discharge and increases turnover by

removing dry spores and aerosolizing wet ones (Magyar et al., 2009). Members of the dry-air spore group, such as *Cladosporium*, are known to be abundant in dry conditions (Troutt and Levetin, 2001); thus, wet conditions, reducing the abundance of this dominant taxon, increase diversity. By contrast, the short-term negative effect of humidity can be explained by the wash-out effect of precipitation. Magyar et al. (2009) found a decrease in the abundance of airborne spores of *Cladosporium* on wet days, possibly due to this effect of rains. Our cross-correlation analyses suggest that the wash-out effect, by reducing the extreme abundance of this taxon in the short period, allowed a more varied taxonomic composition of airborne spores in a longer period.

The delayed effect of wind on spore diversity was positive, possibly by increasing turnover. A negative effect on diversity in the short term can be explained by assuming that the high spore aerosolization of the most abundant taxon (*Cladosporium*) obscures the contribution of other taxa. This is in line with the conclusion of Magyar et al. (2009) that the dominance of *Cladosporium* reduces diversity of air spores.

As regards relationships between spore concentration and temperature and humidity, Cáliz et al. (2018) observed that the proportions of dominant fungal airborne communities decreased considerably in summer, possibly because of a negative effect of summer dryness on fungal dispersal. On the other hand, Troutt and Levetin (2001) found a positive correlation between temperature and average daily *Cladosporium* concentrations. Several studies found that temperature and relative humidity are the meteorological parameters most significantly influencing concentrations of *Cladosporium* and *Alternaria* spores (the most abundant taxa in our study), with temperature being positively associated and relative humidity negatively associated (Grinn-Gofroń et al., 2019).

We found a positive correlation between spore abundance and temperature, which supports the positive influence of temperature. However, we also found the negative effect of temperature on diversity and equitability. This may be explained by the fact that higher temperatures promote a very high concentration of *Cladosporium*, which become extremely dominant.

To conclude, our study suggests that the taxonomic composition of airborne spores at high altitude is an impoverished version of what can be found at lower altitudes. Dry-air spores, such as those of *Cladosporium*, are particularly favoured by high altitude conditions. When the abundance of this dominant taxon is reduced thanks to higher humidity, lower temperatures and stronger winds, there is an overall increase in diversity.

Our aerobiological analysis represents the first study of airborne spore concentrations and diversity in a Mediterranean high altitude site. Future research should be addressed to clarify if the patterns outlined in this study are consistent through years and across space. In particular, it would be

interesting to repeat the experiment annually and check for inter-annually trends, especially in consideration of the increasing effects of climate change. Finally, in our study, we assume that all spores went from the sampling sites. However, it would be interesting to perform further to determine the possible origin of the spores by combining back trajectory and/or source apportionment analyses,

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