

# Correlations between weather conditions and airborne pollen concentration and diversity in a Mediterranean high-altitude site disclose unexpected temporal patterns

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**Abstract** Relationships between meteorological factors and airborne pollen 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 pollen concentration, diversity (number of families and Shannon and Simpson diversity indices) and evenness (Pielou index) in an Apennine high-altitude site (Gran Sasso Massif, 2117 m elevation). In contrast to

patterns observed at low altitudes, the temperature had a negative correlation with pollen abundance and diversity, whereas humidity had a positive correlation. The unexpected negative correlations with temperature can be explained with the particular position of our sampling site. Wind speed was positively correlated with pollen diversity and abundance in the short term, which can be explained by the fact that higher wind speed promotes both primary emission of pollen from the anthers and subsequent re-suspension. Evenness and wind speed were negatively correlated in the short term because of the different response of different species to meteorological conditions. In the longer term, the average concentrations of the various taxa tend to reach similar values, leading to increased values of diversity. Our finding of a decrease in pollen emission with increasing temperature has important implications for the study of the impacts of global change on high-altitude plant communities. We also detected a high abundance of Cupressaceae/Taxaceae pollen, a reflection of the expansion of thermophilic species, such as *Juniperus*, due to climate change.

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## 1 Introduction

Atmospheric pollen concentrations are determined by two sets of variables, those that mediate pollen release into the atmosphere, and those that mediate its dispersion from sources to receptors (Galán et al. 1995). Pollen release patterns reflect plant phenology, which is in turn affected by climatic factors, such as temperature (especially in trees) and humidity (especially in herbaceous plants) (Osborne et al. 2000; Hilaire et al. 2012; Galán et al. 2005; García-Mozo et al. 2009; Van Vliet et al. 2002). Dispersion, transport and deposition of aerobiological particles are influenced by meteorological parameters, among which wind speed and direction are of prominent importance (Galán et al. 1995; Jochner et al. 2012; Angelosante Bruno et al. 2007).

Wind speed is associated with both the primary emission of pollen from the anthers and with secondary emission through re-suspension. Small grains of pollen transported by wind may travel great distances and potentially pollinate receptive flowers that are located at considerable distances (Sánchez Mesa et al. 2003; Lu et al. 2005).

Relationships between meteorological factors and airborne pollen concentrations have been mostly investigated with reference to single taxa responsible for pollen-induced allergic diseases (Galán et al. 1995; Riediker et al. 2000; Bachert et al. 2004; Marshall 2004; Brauer et al. 2007; Övergaard et al. 2007). Quite surprisingly, there is a dearth of research dealing with temporal variations in airborne pollen diversity (see, e.g., Belmonte and Vilà 2004). Moreover, available information on the relationships between pollen concentrations and meteorological conditions in the Mediterranean region only refers to low-altitude areas, whereas nothing is known for high altitudes. This is a serious lack of information, because high-altitude vegetation includes rare and endemic species particularly sensitive to the global change (Theurillat 1995; Nualart 2003; Thomas et al. 2004). Also, meteorological conditions at high altitudes are obviously very different from those that can be found at low altitudes (Menzel 2003; Scheifinger et al. 2013). Thus, investigating airborne pollen concentrations at high altitudes is of paramount importance to extend our knowledge of basic aerobiological patterns.

In this paper, we present a first study of the atmospheric pollen concentrations in a high-altitude

site from Central Italy (Gran Sasso Massif, 2117 m elevation). Aim of our work was to test the influence of meteorological parameters on airborne pollen concentration and diversity. For this, we considered both total airborne pollen concentration (total abundance of pollen grains per cubic metre of air) and diversity indices that take into account taxonomic composition and abundance. We correlated daily pollen abundance and diversity with main meteorological variables (mean temperature, humidity and wind speed) 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 pollen concentration and diversity are related to variation in meteorological parameters.

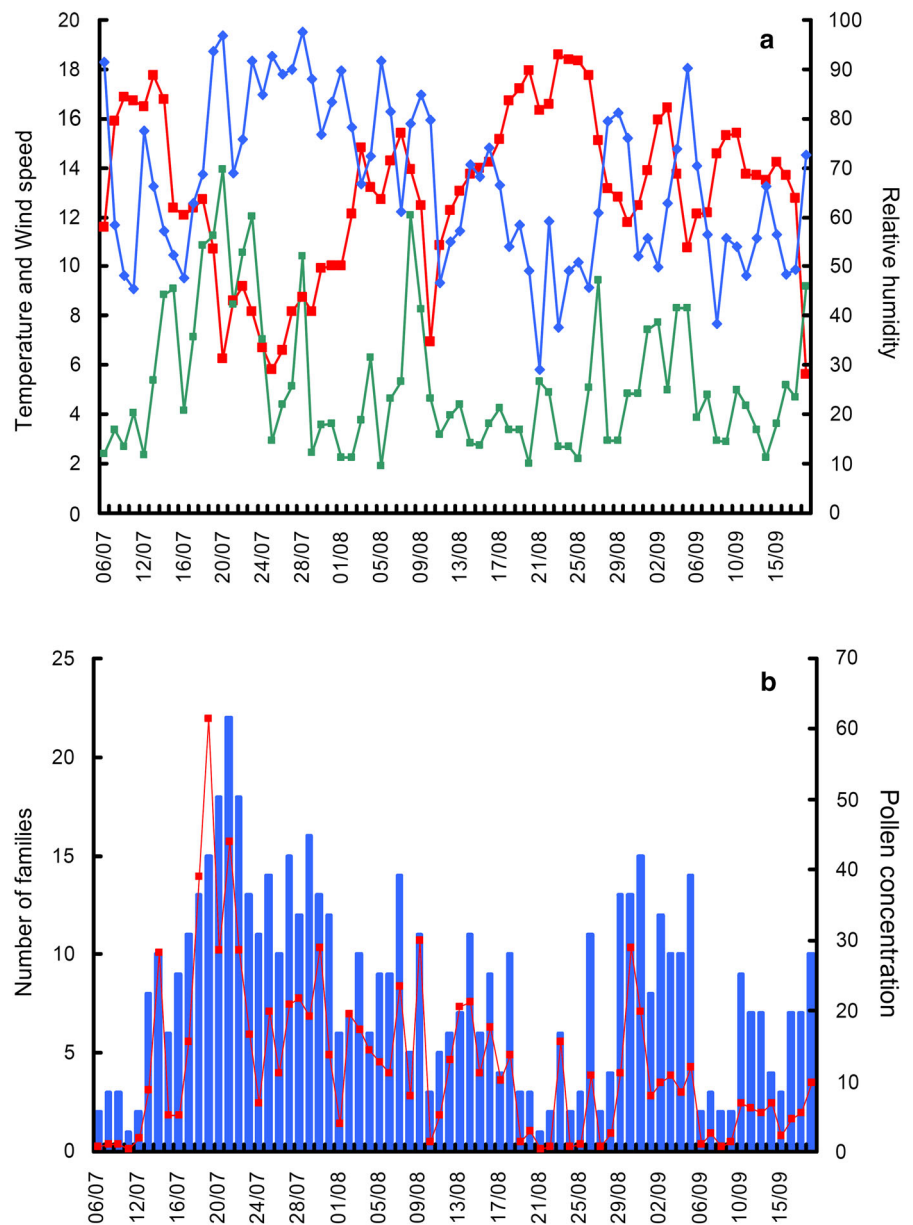
## 2 Materials and methods

### 2.1 Study area and sampling methods

Airborne pollen sampling was conducted in the Botanical Garden of Campo Imperatore, at 2117 m elevation (N42°26'37.39", E13°33'29.73"). The Alpine Botanical Garden of Campo Imperatore is situated in the heart of the Gran Sasso and Laga Mountains National Park (GSML), Abruzzo region. Abruzzo region is located in the centre of the Italian peninsula, between the Mediterranean and the European Alpine regions, thus representing a point of convergence of various climate influences, with a high frequency of northerly winds that bring cold and moist air masses and a relatively low frequency of southerly winds characterised by more temperate and dry air masses (Tammaro 1998; Cristofanelli et al. 2013). These two wind regimes are well evident in Fig. 1a, which shows the time series of temperature and relative humidity values during the study period, with some days where the maximum temperature is below 10 °C (northerly winds) and others with maximum temperature up to 18 °C (southerly winds).

The Gran Sasso massif is the highest mountain range of the Apennines (its highest peak, Corno Grande, is 2912 m high) and includes a variety of landscapes, such as rocky limestone outcrops, glacial formations and alpine meadows. The Calderone glacier, which lies just beneath the Corno Grande, is the southernmost glacier in Europe. The GSML

**Fig. 1** Meteorological parameters (a) and airborne pollen concentration and taxonomic (family) richness (b) recorded in a Mediterranean high-altitude site (Gran Sasso, Italy, 2117 m elevation) from 6 July to 18 September 2011. **a** Variations in daily mean temperature (°C, red squares), relative humidity (%), and wind speed (m/s green squares). **b** Daily values of number of families (bars) and total pollen concentration (pollen grains/m<sup>3</sup>, red squares)



(1.413 km<sup>2</sup>) is one of the largest protected areas in Europe and hosts more than 2000 species of plants, including many glacial relicts restricted to high-altitude sites. The main vegetation types occurring in the GSML include mixed oaks groves (approximately between 400 and 1000 m elevation), deciduous mesophyllitic forests (with *Quercus ilex*, *Quercus pubescens* and *Quercus cerris* in association with *Ostrya carpinifolia*, *Castanea sativa*, *Malus sylvestris*, *Pyrus pyraster*, *Populus tremula*, *Acer obtusatum*, *A.*

*campestre*, *A. pseudoplatanus*, *Populus alba*, *P. nigra*, *Salix alba*, *Carpinus betulus*, *Tilia cordata*, *Corylus avellana*, *Ligustrum vulgare*, *Fraxinus ornus*, approximately until 900 m elevation), *Fagus sylvatica* forests (approximately between 900 and 1800 m elevation) and subalpine meadows and grazing pastures (approximately between 1900 and 2300 m elevation) characterised by a high-altitude flora (with *Papaver degenii*, *Thlaspi stylosum*, *Vitaliana primuliflora*, *Matthiola italica*, *Pedicularis elegans*, *Leontopodium nivale*,

*Erigeron epiroticus*, *Aster alpinus*, *Saxifraga adscendens*, *Silene acaulis* and *Dryas octopetala*) (Conti et al. 2005; Conti and Bartolucci 2015). In general, the vegetation occurring at around 2000 m elevation is represented by primary pastures (i.e. pastures that are not derived from clearcutting) with a prevalence of Gramineae (such as *Festuca violacea* subsp. *italica* and *Festuca imperatrix*) and Cyperaceae (such as *Kobresia myosuroides*, *Carex rupestris* and *Carex kitaibeliana*). In particular, at Campo Imperatore, plant communities of grasslands and pastures can be assigned to the following alliances: *Seslerio apenninae-Dryadetum octopetalae*, *Seslerietum apenninae*, *Carici humilis-Seslerietum apenninae*, *Koelerio splendidis-Brometum erecti* (Biondi et al. 1999). In the lower areas, the observed alliances are as follows: *Poo alpinae-Festucetum circummediterranea*, *Polygalo majoris-Seslerietum nitidae*, *Luzulo italicae-Nardetum strictae*, *Poo violaceae-Nardetum strictae* and *Taraxaco apennini-Trifolietum thalii*, whereas only a small surface is covered by juniper shrubbery (Cupressaceae) (Biondi et al. 1999).

The Alpine Botanical Garden hosts high-altitude species, and specifically those restricted to the territory of the GSML, for a total of about 300 species (see Pace et al. 2005 and unpublished data). The environment of this Botanical Garden is particularly selective, due to the presence of very low temperatures, violent winds and abundant snow for a long period of the year. The growing season is very short (less than 130 days per year). During the study period, most species were flowering between June (199 flowering species) and July (141 flowering species) (see Fig. 2).

Airborne pollen sampling was conducted from 6 July to 18 September 2011. Sampling was restricted to this period for practical and biological reasons. The continued presence of snow and fierce weather made it virtually impossible to conduct aerobiological monitoring outside the summer season. Moreover, outside the study period, few species can produce pollen in the study area. Although different species have different phenological patterns, high-altitude communities of temperate zones tend to have very short flowering periods that start approximately at the end of July (Bahn and Korner 1987).

A 7-day recording Hirst-designed volumetric air sampler (Hirst 1952; Lanzoni, Bologna, Italy, catalogue code: VPPS 2000) was used to record the daily concentration of pollen grains following a standard

protocol UNI U53000810 (Mandrioli 2000). Within the study period, the sampler was inactive in only two days, on 11 July and 11 September for instrument maintenance.

The volumetric sampler used in this study has a pump that ensures a constant flow (10 l/min). Melinex tape impregnated with a 2% silicon solution was used as trapping surface. Every week, the tape was removed and cut into seven 48-mm segments, thus representing 24-h periods. The values of daily pollen concentrations (pollen grains/m<sup>3</sup>) were calculated following Albertini et al. (2009). For each daily recording, pollen grains were classified into families and pollen abundances were expressed as number of pollen grains trapped per cubic metre of air sampled per 24 h (Comtois et al. 1999). Overall concentration of pollen grains for the total study period was expressed as a seasonal pollen integral, which is the sum of the daily pollen concentration over the season. Only undetermined pollen grains were collected on 7 July, which prevented the possibility of calculating diversity indices for this day. Thus, this day and days of sampler inactivity were omitted from the analyses.

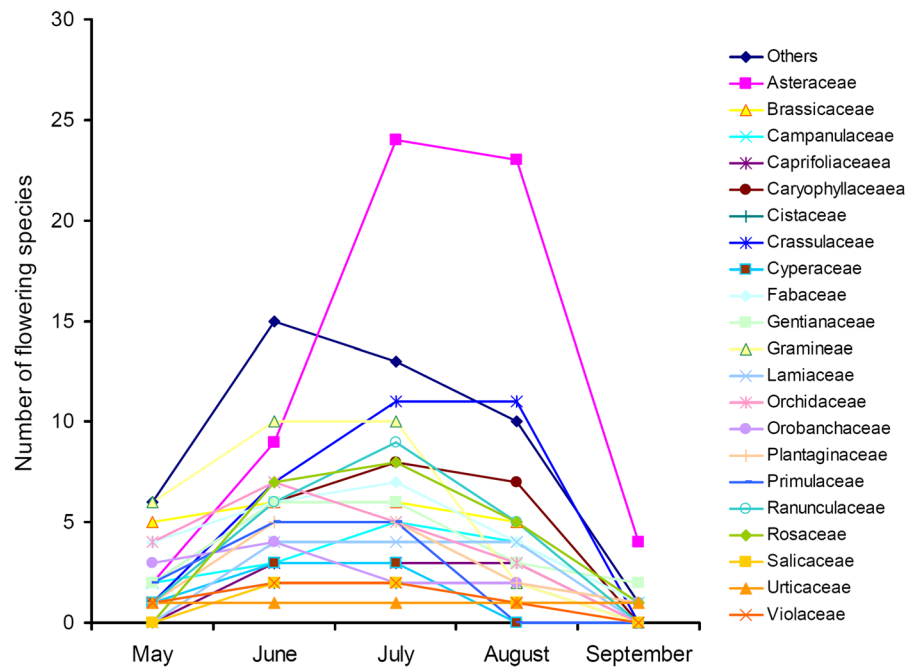
It has been observed that there is an increase in the concentration of pollen grains coming from a long distance with the increase of altitude (Markgraf 1980; Pitari et al. 2014). In our samples, we detected the presence of pollen grains of *Olea*, *Ambrosia* and Myrtaceae, all taxa that do not live in the study area. We suppose that these pollen particles come from remote sites through the movement of air masses, and we excluded them from the analyses to avoid that data regarding the true local pollen production were mingled with external pollen grains.

Meteorological data were obtained from a weather station located at 50 m from the sampling site and which is part of the regional network of the Hydrographic Service. The following parameters were recorded on a daily base: average temperature ( $T$ , in °C), relative humidity (RH, in %) and wind speed ( $w$ , in m/s).

## 2.2 Statistical analysis

Daily diversity in pollen composition was expressed by Shannon's index ( $H'$ ) and Simpson's reciprocal index ( $D$ ):

**Fig. 2** Number of flowering species recorded in the Campo Imperatore Botanical Garden (Gran Sasso, Italy) during the study period (July–September). Families with less than 10 species were lumped into the category “Others”



$$H' = - \sum \frac{p_i}{p} \ln \left( \frac{p_i}{p} \right) \tag{1}$$

$$D = 1 - \sum \left( \frac{p_i}{p} \right)^2 \tag{2}$$

where  $S$  is the total number of plant families in the air sample (day),  $p_i$  is the proportion of pollen grains that belong to the  $i$ th family and  $p$  is the total pollen concentration.

$H'$  ranges from 0 (one plant family dominates the air sample completely) to high values for samples containing pollen grains belonging to many families, each with few grains.

$D$  varies from 0 (one family dominates the air sample completely) to 1 (pollen grains from all families are equally present).

$H'$  has its foundations in information theory and represents the uncertainty about the identity of an unknown individual (in our case, a grain pollen), whereas  $D$  represents the probability that two randomly chosen individuals (i.e. grain pollens) belong to different taxa (in our case, plant families) (see Morris et al. 2014 for details). Evenness was calculated as Pielou’s index ( $J$ ), which is the ratio between  $H'$  and the logarithm of taxon richness. This measures the evenness with which individuals (i.e. pollen grains)

are divided among the taxa present, and is constrained between 0 (one plant family dominates the air sample completely) and 1 (all plant families are equally abundant).

These indices are among the most commonly used and recommended (Magurran 1988) and have been already used in aerobiological studies (e.g. Magyar et al. 2009; Sebók et al. 2016).

To investigate the correlation between daily meteorological variables and pollen abundance and diversity, cross-correlation analyses were performed (Diana et al. 2006; Nowosad et al. 2015). Cross-correlation measures the correlation between two time series and has the advantage of calculating such correlations under consideration of a time shift across their entire temporal range of overlap.

In the cross-correlation, for two time series  $x$  and  $y$ , the cross-correlation coefficient ( $ccc$ ) at lag time  $m$  is:

$$ccc_m = \frac{\sum (x_i - \bar{x})(y_{i-m} - \bar{y})}{\sqrt{\sum (x_i - \bar{x})^2 \sum (y_{i-m} - \bar{y})^2}} \tag{3}$$

The summations and the mean values are only taken over the parts where the sequences overlap for a given lag time. The equation shows that for negative lags,

features in  $x$  are leading. The  $p$  value for a given  $m$  is given by a  $t$  test with  $n - 2$  degrees of freedom, with  $n$  the number of samples that overlap

$$t = ccc_m \sqrt{\frac{n-2}{1-ccc_m^2}} \quad (4)$$

The term “lag” indicates in this study the shift of days on which the respective cross-correlation coefficient value was measured. Only lags with significant ( $p < 0.05$ ) maximum correlations were considered. The ranges of the cross-correlation functions are from  $-1$  to  $1$ . The closer the cross-correlation value is to  $1$  (or  $-1$ ), the more closely the information of the two datasets is related. Cross-correlation analysis at different lags may be important to disclose temporal shifts in correlation between meteorological variables (temperature and humidity) and pollen concentration and diversity, because plants integrate temperature and moisture effects on a number of days. Humidity may influence pollen concentration and diversity also directly, through the effects of rainfall on pollen concentration in the atmosphere. Wind speed may produce long-term effects both directly (by promoting both the primary emission of pollen from the anthers and a secondary presence through re-suspension) and indirectly (higher winds may be correlated with increased dryness, which in turn may affect plant physiology through desiccation). Also, after a number of days with high wind speeds, the pollen supply might be exhausted, which influences pollen concentrations and diversity in the atmosphere.

Data normality was checked before analyses by using Kolmogorov–Smirnov, Shapiro–Wilk and Anderson–Darling tests. Since most data deviated significantly from normality at  $p < 0.05$ , Spearman’s rank correlation coefficients were used. In cross-correlations, this was achieved by ranking variables before analyses. Diversity indices and cross-correlation analyses were conducted using the software PAST version 1.89 (<http://folk.uio.no/ohammer/past>) (Hammer et al. 2001). All other analyses were done with statistical computing environment R ([www.r-project.org](http://www.r-project.org)).

### 3 Results

As expected, temperature was negatively correlated with humidity (Spearman rank correlation test,  $r_s = -0.645$ ,  $p < 0.0001$ ,  $n = 72$ ). However, we

found that temperature was also negatively correlated, although weakly, with wind speed ( $r_s = -0.255$ ,  $p = 0.031$ ). Wind speed and humidity were not correlated ( $r_s = 0.152$ ,  $p = 0.203$ ) (Fig. 1a).

The majority of sampled pollen belongs to herbaceous plants characteristic of high-altitude habitats (Table 1). The most abundant families were Urticaceae and Gramineae, which accounted for about 47% of total pollen abundance. Pollen grains of trees and shrubs showed much lower abundances and include species that typically live at lower altitudes, such as Cupressaceae/Taxaceae, Fagaceae and Corylaceae (Table 1).

Temporal variations in pollen abundance, number of families, Simpson’s diversity and Shannon’s diversity showed similar patterns, with peaks around 19–21 July and 29 August to 1 September (Figs. 1b, 3), whereas Pielou’s evenness showed a much more irregular pattern, attaining high values also when diversity was low (Fig. 3).

Abundance was positively correlated with number of families, Simpson’s diversity and Shannon’s diversity, but negatively with Pielou’s evenness (Table 2). Number of families was positively correlated with Simpson’s diversity and Shannon’s diversity, but not with Pielou’s evenness (Table 2). Simpson’s diversity was positively correlated with Shannon diversity and Pielou’s evenness (Table 2). Shannon’s diversity was not correlated with Pielou’s evenness (Table 2). Overall, these results indicate that a higher abundance of pollen grains implied a higher diversity, but peaks in abundances were mainly due to the disproportionate increase of certain single taxa. High evenness in association with low diversity values indicates the presence of few taxa with, however, similar relative abundances.

Daily temperature was negatively correlated with pollen concentration, number of plant families, Simpson’s diversity, Shannon’s diversity and Pielou’s evenness (Table 3, Fig. 4a). This indicates that increasing temperatures affected negatively pollen abundance and diversity in the short term, whereas a longer time was needed to observe a similar effect on evenness. In general, humidity showed opposite correlations. Daily humidity was positively correlated with pollen concentration, number of plant families, Shannon’s diversity and Pielou’s evenness, but negatively with Simpson’s diversity (Table 3, Fig. 4b). However, significant correlations of comparable size

**Table 1** Concentration of pollen grains found in a Mediterranean high-altitude site (Gran Sasso, Italy, 2117 m elevation) from 6 July to 18 September 2011 expressed as a seasonal pollen integral (PI), which is the sum of the daily pollen concentration over the season, and as a percentage (%)

Family	PI	%
Betulaceae	7.9	0.9
Boraginaceae	1.8	0.2
Caprifoliaceae	1.1	0.1
Caryophyllaceae	6.1	0.7
Amaranthaceae	33.6	3.6
Compositae	33.6	3.6
Corylaceae	30.3	3.3
Crassulaceae	2.5	0.3
Cruciferae	0.4	0.0
Cupressaceae/Taxaceae	75.5	8.2
Euphorbiaceae	12.6	1.4
Fagaceae	20.2	2.2
Gramineae	151.39	16.4
Guttiferae	0.4	0.0
Hippocastanaceae	0.4	0.0
Juglandaceae	1.1	0.1
Labiatae	0.4	0.0
Leguminosae	12.3	1.3
Liliaceae	4.7	0.5
Moraceae	1.1	0.1
Oleaceae	44.0	4.8
Pinaceae	41.2	4.5
Plantaginaceae	31.0	3.4
Polygonaceae	18.8	2.0
Primulaceae	1.1	0.1
Ranunculaceae	8.7	0.9
Rosaceae	2.2	0.2
Rubiaceae	9.7	1.1
Salicaceae	19.5	2.1
Saxifragaceae	9.7	1.1
Scrophulariaceae	2.5	0.3
Umbelliferae	15.9	1.7
Urticaceae	278.7	30.2
Valerianaceae	0.4	0.0
Vitaceae	0.4	0.0
Unidentified	43.0	4.7
Total	923.8	100

but opposite sign were found at shorter lags for Simpson's index ( $ccc = 0.347$ ,  $p = 0.003$ ,  $lag = -1$ ) and Pielou's evenness ( $ccc = -0.441$ ,

$p = 0.0006$ ,  $lag = -15$ ) (Fig. 3b). This suggests that humidity may promote or reduce diversity at different lags.

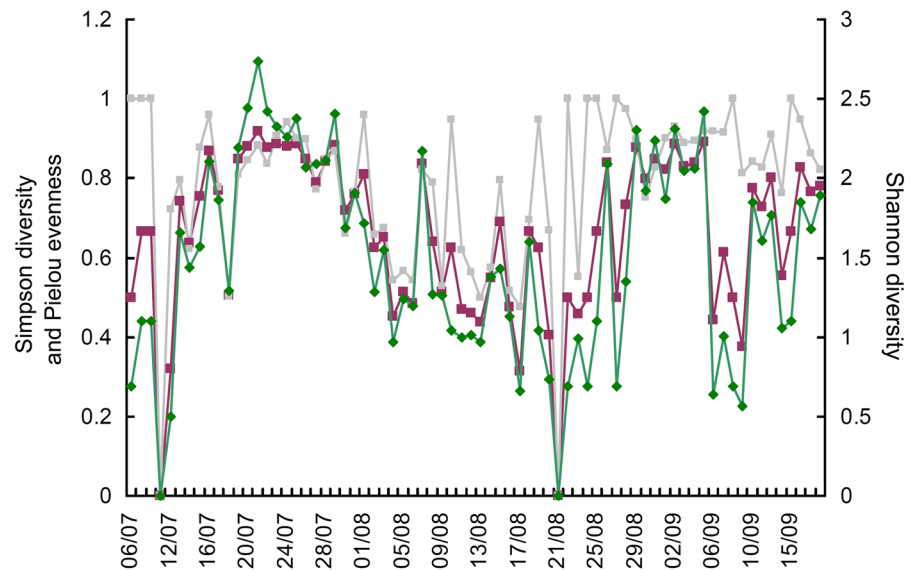
Wind speed was positively correlated with pollen abundance and number of plant families, but negatively with Simpson's diversity, Shannon's diversity and Pielou's evenness (Table 3, Fig. 4c). However, at shorter lags, wind speed showed correlation coefficients of similar size, but opposite sign, with both Simpson's diversity ( $ccc = 0.396$ ,  $p = 0.0006$ ,  $lag = -1$ ) and Shannon's diversity ( $ccc = 0.431$ ,  $p = 0.0002$ ,  $lag = 0$ ) (Fig. 4c). Overall, the general picture is that in the short term (with lags of about 0–3 days), abundance and diversity (number of families, Simpson's index and Shannon's index) tend to be negatively correlated with temperature and positively with humidity and wind speed. However, in the long term (with different lags depending on the considered index), diversity tends to be positively correlated with temperature, and negatively with humidity and wind speed. Evenness showed much more irregular patterns, being positively correlated with temperature and negatively with humidity in the medium term (with lags at around 15 days), but negatively with temperature and positively with humidity in the long term (with lags of more than 30 days). In general, wind speed was poorly correlated with evenness.

#### 4 Discussion

Weather and climate are important factors regulating pollen emission and presence in the air (Halwagy 1988; Keyynan et al. 1991; Hart et al. 1994; Puc and Wolski 2002; Bianchi and Olabuenaga 2006; Perveen et al. 2007; Alwadie 2008; Käpylä 2009; Abu-Dieyh and Ratrout 2012). However, their influence is temporally and spatially variable. As regards spatial variations, it is obvious that airborne pollen abundance and composition change according to the local weather and flora (D'Amato et al. 2011) although patterns across space remain largely unknown (see Nowosad et al. 2015 for a discussion).

In mountain ecosystems, the linear decrease in temperature with increasing elevation (approximately 0.6 °C for every 100 m in temperate regions) exerts a considerable influence on the pool of potential plant species that are able to thrive at increasingly severe climatic conditions. In general, plant species found at a

**Fig. 3** Daily values of airborne pollen diversity (Shannon diversity, green diamonds; Simpson diversity, violet squares; Pielou evenness, grey squares) recorded in Gran Sasso (Italy, 2117 m elevation) from 6 July to 18 September 2011



**Table 2** Correlation (Spearman's rank correlation coefficient) between pollen abundance (pollen grains/m<sup>3</sup>), number of families, Simpson's diversity, Shannon's diversity and Pielou's evenness

	Abundance	Number of families	Simpson's diversity	Shannon's diversity	Pielou's evenness
Abundance	–	0.864	0.389	0.600	– 0.513
Number of families	< 0.00001	–	0.744	0.892	– 0.186
Simpson's diversity	0.007	< 0.00001	–	0.957	0.419
Shannon's diversity	< 0.00001	< 0.00001	< 0.00001	–	0.170
Pielou's evenness	< 0.00001	0.118	0.0002	–	– 0.153

Values above diagonal: Spearman's  $r_s$ ; values below diagonal:  $p$  values

**Table 3** Correlation between daily meteorological variables (temperature, humidity and wind speed) and pollen abundance (pollen grains/m<sup>3</sup>), number of families, Simpson's diversity, Shannon's diversity and Pielou's evenness

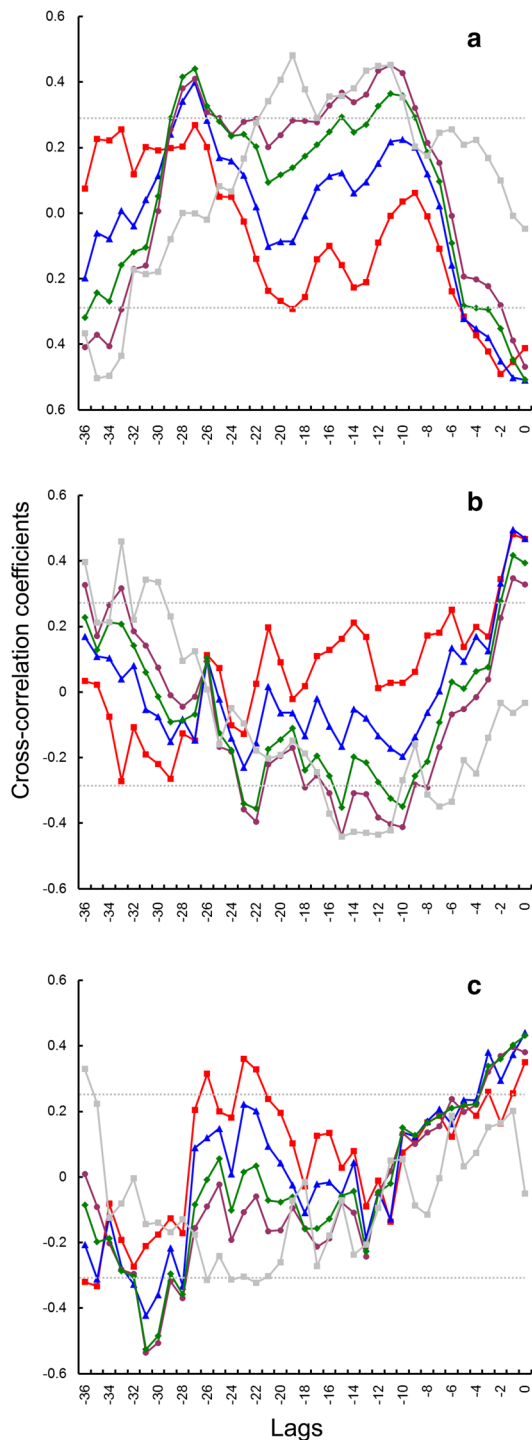
	Daily temperature (°C)			Daily humidity (RH, in %)			Wind speed (m/s)		
	<i>ccc</i>	<i>p</i>	lag	<i>ccc</i>	<i>p</i>	lag	<i>ccc</i>	<i>p</i>	lag
Abundance	– 0.491	< 0.0001	– 2	0.482	< 0.0001	– 1	0.440	0.0001	0
Number of families	– 0.509	< 0.00001	0	0.496	< 0.0001	– 1	0.350	0.003	0
Simpson's diversity	– 0.469	< 0.0001	0	0.417	0.003	– 1	– 0.536	0.0003	– 31
Shannon's diversity	– 0.508	< 0.00001	0	– 0.440	0.0006	– 15	– 0.526	0.0004	– 31
Pielou's evenness	– 0.504	0.001	– 35	0.459	0.003	– 33	– 0.323	0.022	– 22

*ccc* cross-correlation coefficient (maximum significant value),  $p$   $p$  values; lag shift of days on which the respective cross-correlation coefficient value was measured

higher elevation are mainly a subset of the broader flora of lower areas (Bruun et al. 2006). Comparing the pollen data of Campo Imperatore station with those of

a monitoring station located in the same area, but a lower altitude (University Monitoring Station AQ01, L'Aquila, 700 m), we can note that the taxa that were





**Fig. 4** Cross-correlation coefficients between meteorological parameters and airborne pollen concentration and diversity recorded in Gran Sasso (Italy, 2117 m elevation) from 6 July to 18 September 2011. **a** Cross-correlation coefficients for daily mean temperature ( $^{\circ}\text{C}$ ), **b** for humidity (%) and **c** for wind speed (m/s). Positive correlation values above the upper dashed line are significant at  $p < 0.05$ ; negative correlation values below the lower dashed line are significant at  $p < 0.05$ . Total abundance: red; number of families: blue triangles; Number of families: red squares; Shannon diversity, green diamonds; Simpson diversity, violet squares; Pielou evenness, grey squares

Bartolucci 2015; L. Pace, unpublished data). In the alpine regions of temperate zones, the flowering period of more than 70% of all species starts from the end of July (Körner 2003), to peaks of flower richness concentrated in a short period. However, even during these peaks, concentration of airborne pollen grains at high altitudes tends to be much lower than those found at lower altitudes. In the lower altitude sampling area of L'Aquila, phenological phases were anticipated by about two months and pollen concentrations were much higher (Pace et al. 2013) when compared with the Gran Sasso site.

As regards seasonal variations, because rain tends to sweep pollen from the air (Pérez et al. 2009), rainfall is typically associated with low pollen counts (Satpute et al. 1983; Ribeiro et al. 2003; Guarín et al. 2015; Hong et al. 1986). Also, according to Satpute et al. (1983), heavy rainfall does not only wash out the pollen from the atmosphere, but also lowers the pollen release. Airborne pollen composition changes through time because of differences in plant phenology, which are related to weather and climate factors (D'Amato et al. 2007). In general, available information indicates that airborne pollen abundance and diversity tend to be positively correlated with temperature, and negatively with humidity in a variety of contexts (Ribeiro et al. 2003; Guardia et al. 2006; Alwadi 2008; Abu-Dieyeh and Ratrout 2012; Rodríguez-de La Cruz et al. 2012; Perveen and Khan 2014; Camacho et al. 2016). However, Kumar (1985) found that very high temperature ( $> 40^{\circ}\text{C}$ ) and heavy rains had adverse effect on pollen incidence and Altintas et al. (2004) found positive correlations between tree pollen counts and temperature and humidity.

In contrast to these findings, we found that temperature had a negative correlation with pollen abundance and diversity (number of families, Simpson's diversity, Shannon's diversity and Pielou's

found with the highest pollen concentrations at high altitude were the same that predominated in the lower altitude samples (i.e. Cupressaceae-Taxaceae, Urticaceae and Gramineae; Conti et al. 2005; Conti and

evenness), whereas humidity had a positive correlation. Because of the strong negative correlation between temperature and humidity, we cannot exclude that the relevant factor is temperature and that correlations with humidity are in fact an indirect reflection of the correlation between temperature and humidity. This is suggested by the fact that correlations with temperature are stronger than those with humidity. The unexpected negative correlations with temperature can be explained with the particular position of our sampling site. The high-elevation environment is extreme, with large daily temperature variations, episodes of low humidity, high global and UV radiation, low precipitation amounts during summer and desiccating winds. For example, we recorded very low values of relative humidity, even around 20%. Thus, plants that live at high altitudes in the Mediterranean probably have special habits, avoiding pollen emission into the atmosphere during high temperature/low humidity phases, else they might lose their viability and could not perform the pollination (see Franchi et al. 2011).

The influence of wind speed on pollen counts is contradictory. For example, Alwadie (2008) found a negative correlation between pollen concentrations and wind velocity, whereas Kumar (1985) and Abu-Dieyeh and Ratrout (2012) found positive correlations. In fact, wind influence on pollen concentration can be either negative (by removing pollen grains locally emitted) or positive (by transporting pollen grains from other sites and/or by re-suspension processes).

We detected strong positive correlations between wind speed and pollen diversity (number of families, Simpson's diversity and Shannon's diversity) and abundance in the short term, which can be explained by the fact that higher wind speed promotes both the primary emission of pollen from the anthers of a multitude of taxa (an increase in wing velocity might increase the number of taxa from which pollen is released and maintained in the atmosphere) and a secondary presence through re-suspension. Of course, we cannot exclude that a part of the sampled pollen grains was transported by wind from other sites. However, since we have excluded all pollen grains of taxa that do not live in the study area, we are confident that most of the sampled pollen was of local origin. Negative correlations in the long term (i.e. at longer lags) reflect pollen sedimentation. Interestingly, we

found a positive correlation between wind speed and pollen evenness (Pielou's index) in the long term. This suggests that in the short term, the abundance of the airborne pollen of the different taxa is very uneven, because different taxa produce different quantities of pollen and are differently influenced by meteorological conditions. However, since pollen grains can be able to persist in the environment for relatively long periods (Brooks and Shaw 2009), in the longer term, the average concentrations of the various taxa tend to reach similar values, which reflect a sort of "average" composition.

Our aerobiological analysis represents the first study of airborne pollen concentrations and diversity in a high-altitude site. For example, Izquierdo et al. (2011) studied airborne pollen transport in a high-altitude site in Tenerife (Canary islands), but did not correlate variation in compositional parameters with meteorological factors. Also, our data refer to only one year of pollen monitoring. Thus, they cannot be compared with any other finding to assess how general they are. In particular, it would be interesting to see if the patterns outlined in this research are consistent through years by replicating the study in the same area and if they are consistent across space by performing similar analyses in the other sites. Moreover, we considered three meteorological parameters: temperature, humidity and wind speed. In the future, correlation with solar radiation and cloudiness data might shed additional light on the variation of pollen abundance and diversity. Aerobiological monitoring may provide much insight into plant biodiversity and conservation studies by linking botanical and meteorological information (Fernández-Llamazares et al. 2014). Since high-elevation areas are virtually not impacted by land use changes, and many alpine species grow slowly and are long-lived, substantial vegetational changes cannot be attributed to short-term climatic fluctuations or local anthropogenic impacts, but should be interpreted as a reflection of long-term climate change (Pauli et al. 1999).

Interestingly, we found a high abundance of pollen grains belonging to the Cupressaceae/Taxaceae group. This result can be explained by the recent expansion of *Juniperus* at high altitudes. According to Stanisci (1997), *Juniperus communis* ssp. *alpina* is entering (and in part replacing) *Seslerietum apenninae* formations, which are considered one of the most typical vegetations of the Apennine massifs. A study

conducted in Northern New Mexico revealed how an increase in aridity determined the advance of *Juniperus* at the expense of other plants already present (Allen and Breshears 1998). Shrubs like *Juniperus communis* are generally warm-adapted and tend to grow in the treeline ecotone or in the low alpine zone, which is why their expansion upwards could be attributed to increasing temperature due to climate change. Thus, it would be interesting, in future monitoring, to test if there will be an increase in the concentration of Cupressaceae/Taxaceae at high altitude as a possible reflection of climate change.

Pollen grains are important causes of allergic reactions that are among the most common chronic disorders worldwide (D'Amato et al. 2007; D'Amato et al. 2011; Ayres et al. 2009). At high altitudes, the pollen season, which is critical for allergy sufferers, is much shorter and less intense, because pollen emission is restricted to a short summer period and concentrations are lower than at low altitudes. Thus, high-altitude sites may be important tourist destinations for people suffering from pollinosis. In this context, aerobiological monitoring at high altitude might have important health and economic implications.

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