

Airborne fungal spore load and season timing in the Central and Eastern Black Sea region of Turkey explained by climate conditions and land use

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ABSTRACT

The widespread fungal aeroplankton comprises numerous plant pathogens and allergenic components. Here, we present the first study describing the airborne spore composition in the Central and Eastern Black Sea region of Turkey against the background of meteorological variables and land use. This region is climatically diverse and there are large differences in altitude and land cover. Using multivariate statistical techniques, we assessed the combined effects of the main weather factors on the airborne spore count and temporal patterns of spore season for 30 fungal taxa in five provinces with three different climate types. Moreover, we combined meteorological and land use data to search for potential source areas of airborne spores recorded at the study sites.

Spore season start and peak dates substantially varied between sites (maximally 130 days between western and eastern part of the study area – for *Boletus* mean start date), however for most of the taxa investigated the season ended at a similar time at different sites. All the meteorological variables included in redundancy analysis accounted for 10.8–48.9% of the total variance in the fungal spore data, with the highest value in sites with continental climate. Daily mean air temperature was the most important variable and significantly correlated with the daily count of all the spore types ($0.11 \leq r_s \leq 0.84$). However, when temperature range was calculated for three large, percentile-based spore count clusters it turned out that between 17% (subtropical climate) and 56% (continental climate) of the taxa showed no difference in temperature between low and high counts. Finally, based on combination of wind conditions and land use data we identified grasslands, croplands and coniferous forests as the main potential sources of fungal spores in the study area, and suggested that spores from the forests may be transported over longer distances than from open areas.

1. Introduction

Due to their documented and potential pathogenic and allergenic properties as well as their ability to spread and survive over a wide spectrum of conditions, fungal spores have attracted the attention of researchers all over the world. Meteorological conditions belong to the most important environmental factors that affect concentration of the airborne spores, their release, presence, composition, movement and viability. Most analyses have focused on air temperature, relative humidity, precipitation, wind speed and direction, as those factors have

the largest effect on the airspora occurrence (e.g. Pakpour et al., 2015; Sadyś et al., 2015; Grinn-Gofroń et al., 2018). So far, the effects of different land cover types as possible source areas for airborne spores have very rarely been studied (Skjøth et al., 2012; Fernandez-Rodriguez et al. 2015; Olsen et al., 2019).

In aerobiological studies, the greatest attention was paid to *Alternaria* and *Cladosporium* spores (e.g. Artaç et al., 2014; Sindt et al., 2016; Skjøth et al., 2016; Grinn-Gofroń et al., 2019). These are types of fungi with strong allergenic properties (Gabriel et al., 2016; Pomés et al., 2016), as well as species causing serious crop diseases,

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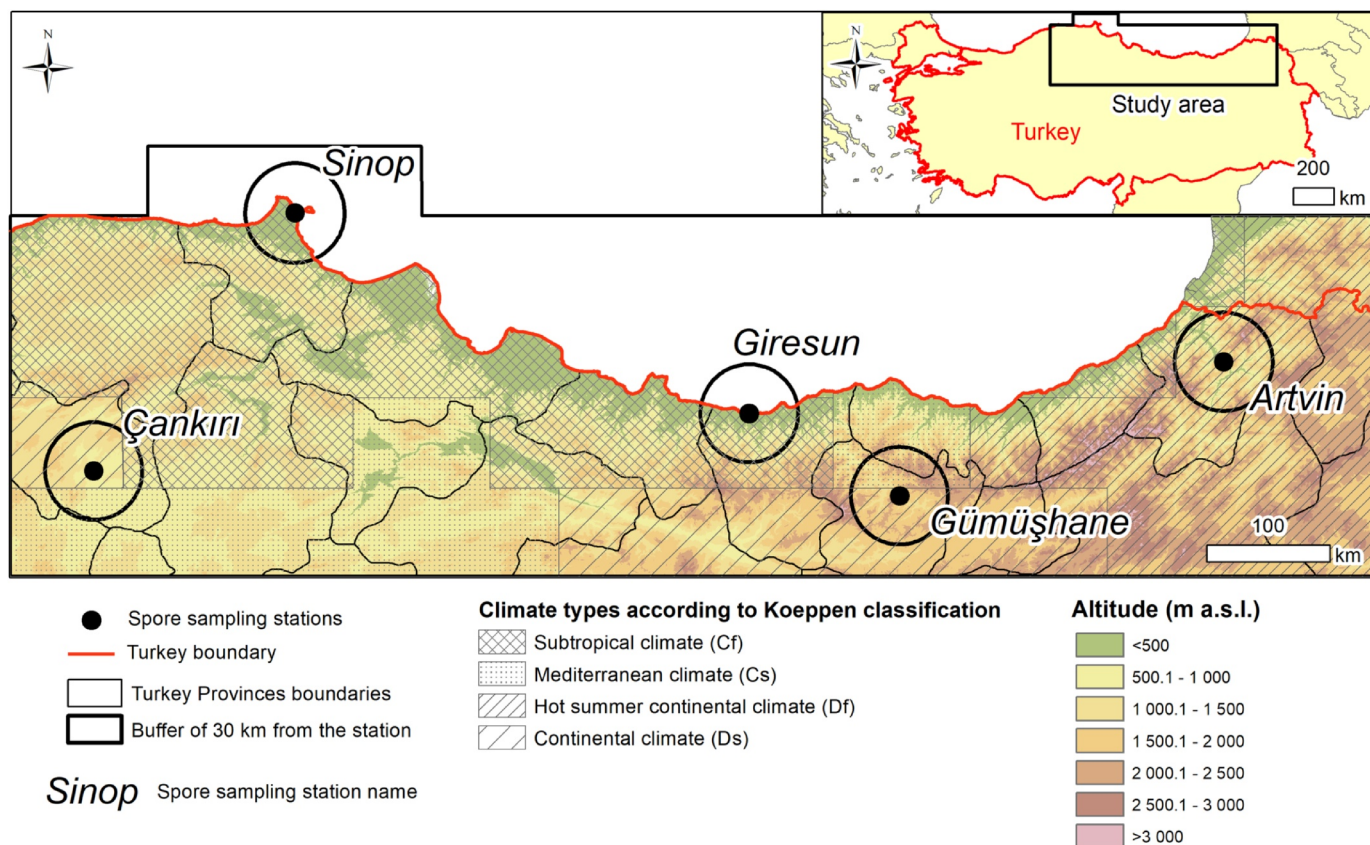


Fig. 1. Location of the study area in relation to the Turkish borders, climate types and altitude.

resulting in significant economic losses, e.g. *Cladosporium fulvum* infecting tomatoes (Thomma et al., 2005), *Alternaria solani* causing potato early blight (Abuley and Nielsen, 2017), and *A. brassicicola* responsible for dark spot disease in most species of the Brassicaceae family (Nowakowska et al., 2019).

Many other types of fungal spores are rarely considered in the monitoring of a wider spectrum of aeroplankton components (e.g. Magyar et al., 2009; Akgül et al., 2016; Grinn-Gofroń et al., 2018; Antón et al., 2019; Ščevková and Kováč, 2019). However, they also comprise plant pathogens and potentially allergenic types. Jędryczka et al. (2016) found allergenic properties of *Leptosphaeria* spores similar to those of *Alternaria* and *Cladosporium*. Recently, positive skin prick tests were obtained in patients when tested against *Curvularia*, *Fusarium*, *Epicoccum* or *Nigrospora* extracts (Dey et al., 2019). Among plant pathogens, species of *Botrytis* are responsible for heavy losses in a number of economically important horticultural and floral crops, e.g. *Botrytis cinerea* can infect tomato, grapevine, strawberry and flax (Filingier and Elad, 2016); the *Ganoderma* genus includes wood-decaying fungi causing white rot of trees (Schwarze and Ferner, 2003; Loyd et al. 2018, Jędryczka et al., 2015); *Leptosphaeria maculans* is the causal agent of blackleg disease of Brassicas, mainly on oilseed rape (West et al., 2001); *Pleospora betae* is an important fungal pathogen of table beet and sugar beet, causing foliar disease, damping-off and root decay (Pethybridge et al., 2018); some species of *Fusarium* cause plant diseases affecting cereals and various species of tropical crops, e.g. *Fusarium oxysporum* causes vascular wilt disease in tomato (Armstrong and Armstrong, 1981); the genera *Aspergillus* and *Penicillium* include postharvest pathogens that attack various fruit, e.g. *Penicillium digitatum* and *P. expansum* cause green and blue mold in harvested fruit, generating high economic losses in citrus fruit tree crops (Garrigues et al., 2016); *Puccinia triticina* causes leaf rust in bread wheat and durum wheat growing areas (Bolton et al., 2008); *Ustilago maydis* infects maize, leading to severe economic losses worldwide (Martinez-

Espinoza et al., 2002).

The first aerobiological study in Turkey was conducted by Özkaragöz (1969) who, using the open Petri dish technique, identified the atmospheric fungal spores in Ankara. In subsequent years, the number of aerobiological studies was consistently increasing and atmospheric fungal spores were identified employing different methods (Asan et al., 2004; Okten et al., 2005; Ataygul et al., 2007; Erkarar et al., 2008, 2009; Çeter and Pinar, 2009; Kalyoncu, 2010; Bülbül et al., 2011; Yükselen et al., 2013; Artaç et al., 2014; Akgül et al., 2016; Sevindik and Akgül, 2018). However, studies using volumetric methods, comparable to ours in Turkey, have been relatively scarce. Some of them are just monitoring studies, others use simple statistical methods and few environmental variables. In addition, there have been no reports on fungal spores in the air from the area we examined.

We selected the Central and Eastern Black Sea region of Turkey for our aerobiological research. This region is climatically diverse and there are large differences in altitude and land use. Due to these conditions, we were able to set the following objectives of the study: (1) to calculate the spore season severity and timing, (2) to determine the relationship between the occurrence of fungal spores in the air and basic meteorological factors, (3) to identify land use types that have the greatest impact on the occurrence of spores, and (4) to assess the combined effects of meteorological factors and land use types on the composition of fungal airborne spores in different climates.

2. Material and methods

2.1. Study area

Turkey is located in the large-scale geographical region of the Mediterranean and the Black Sea. The landscape diversity, and particularly the mountains running parallel to the coasts, results in significant differences in climatic conditions between the regions of the

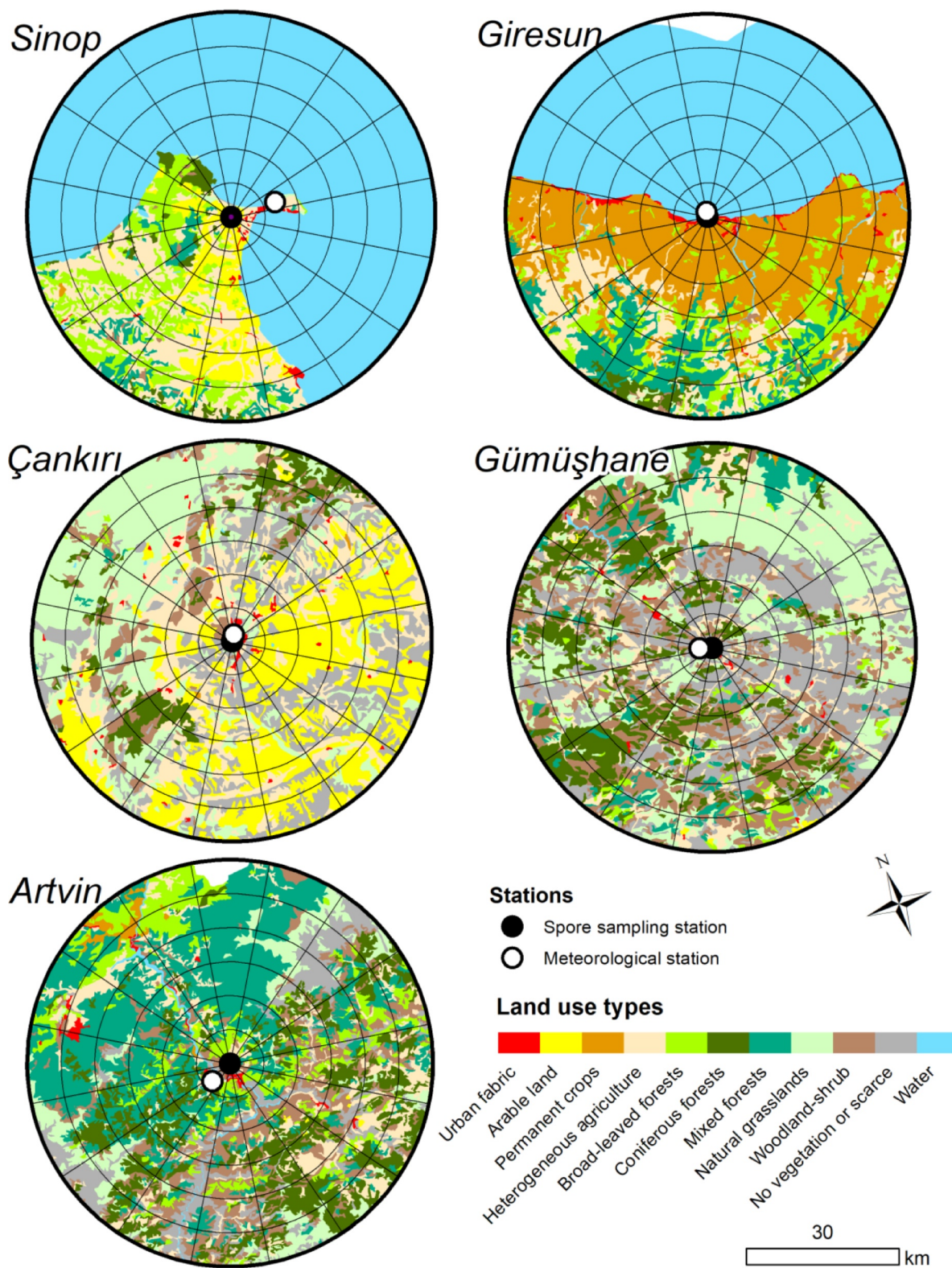


Fig. 2. Land use types overlapped with wind-direction sector boundaries at five study sites.

country. The Black Sea region is bordered by the Marmara Region to the west, the Central Anatolia Region to the south, the Eastern Anatolia Region to the southeast, the Republic of Georgia to the northeast, and the Black Sea to the north. Aerobiological studies were carried out in five provinces of the Central and Eastern Black Sea region, characterised by three different types of climate: continental climate (Çankırı, Gümüşhane), hot summer continental climate (Artvin) and

subtropical climate (Giresun, Sinop). In addition, there are large differences in hypsometric features of the region – the terrain from the coast rises sharply and reaches almost 4000 m over a distance of 50 km. More details about the study sites are given in Tables 1 and S1, and Figs. 1 and 2.

2.2. Aerobiological, meteorological and land use data

Aerobiological data were collected during a two-year period (from 1 July 2010 to 31 June 2012, 731 days) in five provinces of the Central and Eastern Black Sea region of Turkey (Fig. 1). Spores were collected using a Hirst-type volumetric trap and identification procedures followed the recommendations of the Spanish Aerobiological Network (REA: Red Española de Aerobiología; Galán et al., 2007) and are described in detail in Grinn-Gofroń and Bosiacka (2015). Samplers were placed at 10 m (Sinop, Giresun) or 15 m (Çankırı, Gümüşhane, Artvin) above ground level. Their locations met the minimum requirements for aerobiological monitoring (Galán et al., 2007; 2014). Initially, more than 50 spore types were identified (Fig. S1, Supplementary materials). However, after the validation of the sample identification, we limited the spore types only to those recorded at the genus (type) level and with the highest possible reliability. As a result, 30 spore types were taken into account: *Agrocybe*, *Alternaria*, *Aspergillus/Penicillium*, *Boletus*, *Botrytis*-type, *Chaetomium*, *Cladosporium*, *Coprinus*, *Curvularia*, *Didymella*, *Drechslera*-type, *Epicoccum*, *Fusarium*, *Ganoderma*, *Leptosphaeria*-type, *Nigrospora*, *Paraphaeosphaeria*, *Periconia*, *Peronospora*-type, *Pithomyces*, *Pleospora*, *Puccinia* (Urediniospores), *Spegazzinia*, *Sporormiella*, *Stemphylium*, *Tilletia*, *Torula*, *Ustilago*-type, *Venturia*, *Xylaria*-type (phaeoamero-spores with a germination slit). Altogether, we obtained 731 daily spore samples per site with only several missing values and a species-specific number of zero values (when a particular spore type was not recorded in the air). After removing the zero values, the number of samples ranged from single samples containing *Tilletia* and *Spegazzinia* spores to 725 samples containing *Cladosporium* spores in Artvin (Table S2). This means that *Tilletia* was detected in Artvin on only one day and *Cladosporium* was detected almost every day throughout the study period.

Meteorological data in hourly or 3-hourly resolution (depending on the site) were obtained for the study area from the OGIMET database using the *climate* R package (Czernecki et al., 2020). Meteorological stations were located close to the spore sampling sites, except for Artvin and Sinop, where they were located 5 km and 9.2 km from a spore trap, respectively. On the basis of hourly data, the following daily meteorological variables were calculated: mean air temperature (TME; °C), mean relative humidity (RH;%), total precipitation (PRECIP; mm), and mean wind speed (WINDME; m s⁻¹). The minimum, maximum and mean values of the selected meteorological variables for each province during the study period are presented in Table 2. In addition, a frequency approach was used based on the hourly wind data to assess possible directions of spore transport to the traps and to compile the number of spores according to wind direction. In this case, we assigned 24 or eight values of wind speed and direction to one daily value of spore count, similarly as in Bogawski et al., 2019a. Bivariate polar plots showing the relationship between the spore count and wind conditions (speed and direction) were prepared using the *openair* R package (Carslaw and Ropkins, 2012).

Land cover data were obtained from the Corine Land Cover database (Copernicus 2012), version for 2012, because this dataset perfectly matches the study period. We modified the number of land use types by aggregating similar classes, eventually distinguishing urban areas, arable land, permanent crops, heterogeneous agricultural areas (complex of different crops and natural vegetation), broad-leaved, coniferous and mixed forests, woodland-shrub vegetation, natural grasslands as well as areas with scarce vegetation and water. The land cover data were clipped to an area within a 30 km radius from the study sites as this is a typical distance that reflects regional spore concentration when a spore trap is placed on the rooftop (Olsen et al., 2019).

2.3. Data processing and statistics

The abundance of spores at different sites was first analysed by calculating the following descriptive statistics: median, the 1st and 3rd

Table 1
Description of the study sites.

Province	Coordinates of spore sampling station	Coordinates of meteorological station	Mean altitude above the sea level	Dominant type of climate	Weather conditions
Çankırı	40°35'44.78"N 33°36'28.29"E	40°36'18.14"N 33°36'40.19"E	820 m a.s.l.	Continental climate	The summers are warm and dry (in the height of the summer, the midday temperatures usually exceed 25 °C, and summer nights tend to become cool due to the high elevation); the winters are cold and snowy (temperatures usually plummet to -10 °C and occasionally become even as low as -20 °C); spring and early summer are the wettest seasons
Gümüşhane	40°27'31.61"N 39°29'4.12"E	40°27'31.06"N 39°27'45.97"E	1210 m a.s.l.		
Artvin	41°11'54.30"N 41°50'58.46"E	41°10'30.90"N 41°49'5.62"E	980 m a.s.l.	Hot summer continental climate	The summers are usually hot and dry (the warmest month are July or August and tend to be in the high 20 °C or low 30 °C; the winters are cold (average January afternoon temperatures are near or well below freezing); the wettest winter month is December with an extreme amount of precipitation, August being the driest summer month
Giresun	40°54'53.84"N 38°23'19.79"E	40°55'17.28"N 38°23'13.91"E	<500 m a.s.l.	Subtropical climate	The summers are warm and humid (the average daytime high of 26 °C; the temperature, however, rarely exceed 30 °C); the winters are cool and wet (with the average temperature oscillating around 5 °C); the heaviest precipitation occurs in autumn and spring; a week- or fortnight-long snowfall is quite common between December and March
Sinop	42°0'35.77"N 35°4'34.31"E	42°1'46.92"N 35°9'16.37"E	<500 m a.s.l.		

Table 2

Values of selected meteorological parameters in the provinces of the Central and Eastern Black Sea region of Turkey during the sampling period (01.07.2010–31.06.2012).

Meteorological parameters	Provinces					
	Çankırı	Gümüşhane	Artvin	Giresun	Sinop	
Daily mean air temperature TME [°C]	mean	10.5	10.6	13.1	15.6	14.8
	max	30.9	29.3	30.4	28.8	28.2
	min	−10.6	−13.7	−2.2	−0.5	−1.6
Daily mean relative humidity RH [%]	mean	66.5	61.9	60.9	67.8	72.4
	max	99.0	95.1	96.0	95.3	99.2
	min	26.0	25.7	19.3	18.2	26.3
Daily mean wind velocity WINDME [m s ⁻¹]	mean	1.3	1.5	2.1	1.6	2.9
	max	5.0	5.8	5.4	5.4	11.2
	min	0.5	0.1	0.3	0.1	0.9
Daily amount of precipitation PRECIP [mm]	mean	1.6	1.4	1.8	3.2	2.3
	max	73.7	33.8	68.8	102.8	72.6
	min	0	0	0	0	0

quartiles, extreme values and outliers. In addition, the percentage contribution of each spore taxa to the total spore pool counted at each site during the study period was calculated. The timing of the spore season was determined on the basis of four spore season parameters, calculated for 16 taxa that mostly contributed to the total spore pool in each year and at each site: start, peak and end date of the season and season duration. The start and end of the spore seasons were calculated according to the 90% method (Nilsson and Persson 1981). This method indicates the start date of the spore season on the day when 5% of the total spore count is exceeded and the end of the spore season – on the day when 95% of the total daily spore count is reached. This method is accepted as the most appropriate to calculate season parameters for airborne spores (Sadyś et al., 2016; Skjøth et al., 2016; Olsen et al., 2019). In this study, collection of samples started on 1 July 2010 and finished on 31 June 2012. For the sake of presentation clarity, we combined the data from 2010 and 2012 to complete two years of data collection together with 2011, when sampling was performed continuously. In addition, we used these combined data to prepare airborne spore calendars for the study sites, as this could be useful to readers when interpreting the results. The spore season parameters and spore calendars were calculated using R software v. 3.3.1 (R Core Team 2018) and *AeRobiology* R package (Rojo et al., 2019).

The normality of the spore count and meteorological variable distributions was tested with the commonly used Kolmogorov–Smirnov and Chi-square tests. Since most of the data did not fit a normal distribution ($p < 0.05$), the statistical significance of differences between weather variables was assessed with the non-parametric Kruskal–Wallis test and *post-hoc* Dunn's multiple comparison test. Spearman's rank correlation test was applied to examine the effect of selected weather parameters on daily counts of selected airborne fungal spores.

To identify meteorological parameters that have the greatest impact on counts of airborne fungal spores and their distribution patterns, we decided to apply multivariate statistics. One of the advantages of using multivariate statistics is that we simultaneously assess several different meteorological variables that may affect the daily spore count and deduce which variable is the most important one, without neglecting other variables. First, we performed Detrended Correspondence Analysis (DCA) that detected a linear structure in the spore data. To this end, the spore taxa distribution pattern in relation to selected meteorological variables was determined by the Redundancy Analysis (RDA). The data were not transformed. The significance of the first and all canonical axes was tested for the statistical assessment of the relationship between the fungal spore taxa composition and the meteorological variables (Monte Carlo test: 499 permutations under reduced model). Canonical axes proved significant in all the cases ($p = 0.002$). The Monte Carlo permutation test was further applied to test the

statistical significance of meteorological variables in explaining the fungal spore taxa composition. For this purpose, the stepwise “forward selection” of explanatory variables was used. The analyses were run with the software package CANOCO v. 5.0 (Šmilauer © 2012–2019, Šmilauer and Lepš, 2014). More detailed information on the multivariate methods used, the stepwise forward selection and tests of significance can be found in Grinn-Gofroń and Bosiacka (2015).

In addition to the combined effect of different meteorological variables on spore counts, we focused on temperature and wind conditions to prepare recommendations on when (under which weather conditions) high or low spore counts can be recorded. First, we calculated the 50th (median) and 90th percentile of spore counts for all 30 taxa. Then, mean temperature was calculated for all days when spore counts exceeded the median value (but not the 90th percentile) and separately for days with spore counts exceeding the 90th percentile. As a result, we determined thermal conditions for low (< 50 th percentile), moderate (> 50 th percentile and < 90 th percentile) and high airborne spore counts (≥ 90 th percentile). The statistical significance of differences in thermal conditions of airborne spore occurrence was assessed with the non-parametric Kruskal–Wallis test and Dunn's *post-hoc* test. The P-value was adjusted by using the Benjamini–Hochberg correction. To examine the relationship between the spore count and wind conditions, we also plotted daily spore counts of eight most abundant fungal taxa (separately at each station) against wind speed and direction using bivariate polar plots.

We assumed that, apart from meteorological variables, land use may also have a great impact on the composition and count of airborne spores. This is due to the fact that fungal taxa may have different habitat (host) preferences. Therefore, we proposed a novel modification of the Concentric Ring Method (Oteros et al., 2015) to assess the effect of different land use types on the spore count. Similar to Oteros et al. (2017) and Bogawski et al., 2019a, the circular area surrounding a sampling site (here: 30 km from a trap) was divided into non-overlapping 5 km intervals (rings). At each study site, these six rings were divided into 96 (16 x 6) sectors according to 16 world directions (N, NNE, NE, etc.; Fig. 2). Then, the proportion of each land use type within each sector was calculated (summary in Table S1). Moreover, daily spore counts were assigned to 16 wind directions and totalled, using a frequency approach as in polar plots (Bogawski et al., 2019a; Grewling et al., 2020). In this approach, we assigned hourly wind direction values to one daily spore count. Consequently, the frequency of wind directions per day (daily spore count) was determined. Considering that wind direction can change substantially during the day, this method increases the reliability of wind data because of using all available wind data. Finally, the wind-direction-aggregated spore sum was correlated with the proportions of different land use types at



Fig. 3. Airborne spore contribution to the total spore pool of 30 investigated taxa at five study sites. For better visibility of low-count taxa, y axis scale has been square root transformed.

different distances from a spore trap by using Spearman's correlation coefficient.

3. Results

3.1. Airborne fungal spore loads

Cladosporium, *Leptosphaeria* type, *Alternaria*, *Aspergillus/Penicillium*, *Pleospora* and *Ustilago* spores contributed most to the total load of airborne fungal spores in the Central and Eastern Black Sea region of Turkey (34.4–72.8%, 4.3–11.7%, 2.1–5.7%, 2.6–29.0%, 0.5–3.9% and 0.8–2.5%, respectively). Considering individual provinces, Giresun was characterised by greater (compared to other sites) contribution of spores of saprotrophic fungi of the genus *Coprinus* (25.2%). In turn, Artvin may be distinguished by greater than at other sites contribution of spores of forest mycorrhizal fungi of the genus *Boletus* (5.1%) and saprotrophic fungi of the genus *Xylaria* (9.9%). A relatively large contribution of *Aspergillus/Penicillium*, *Botrytis*, *Epicoccum*, *Periconia*, *Fusarium* was characteristic for the Sinop station. In Gümüşhane and Çankırı, a relatively high contribution of *Pleospora* spores was observed (Fig. 3). The highest and the lowest spore counts were recorded in Sinop (more than 410,000 spores per year) and Gümüşhane (less than 112,000 spores per year), respectively. The daily median spore counts rarely exceeded 100 spores: median > 100 occurred only in the case of *Cladosporium* in Çankırı, Sinop and Giresun, and the *Leptosphaeria* type in Sinop (excluding 0 cases). Sporadically, a daily value of 100 spores was reached at all sites for the *Leptosphaeria* type, *Alternaria*, *Aspergillus/Penicillium*, *Coprinus* and *Didymella*. The following taxa rarely reached 100 spores per day and only at selected sites: *Epicoccum*, *Periconia*, *Fusarium*, *Peronospora*, *Puccinia*, *Pleospora*, *Xylaria*, *Torula*, *Stemphylium*, *Ustilago*, *Boletus* and *Botrytis* (Fig. 4).

3.2. Fungal spore season timing

In general, spore seasons started at the earliest in Çankırı (44% of the investigated taxa) and Giresun (38%), and at the latest in Sinop (38%) and Artvin (25%; Fig. 5A and B). Spore season timing varied regionally, depending on the species. For some types of spores, the spore season started earlier in Çankırı and Gümüşhane compared to the other sites (e.g. *Boletus*: 83 Day of the Year (DOY) and 113 DOY, the *Leptosphaeria* type: 90 DOY and 107 DOY, *Stemphylium*: 91 DOY and 105 DOY, *Xylaria*: 67 DOY and 95 DOY for Çankırı and Gümüşhane, respectively). At these two sites, the peak day of *Xylaria*, *Boletus*, the *Leptosphaeria* type and *Epicoccum* also occurred earlier than at the other sites, resulting in a very short pre-peak period in Çankırı and Gümüşhane. The *Fusarium* pre-peak period in Çankırı and Gümüşhane was also very short but, unlike the previous spore types, occurred much later than at the other sites. In general, Çankırı and Gümüşhane were characterised by a similar pattern of spore season timing – only the peak date of *Pleospora* was much later in Gümüşhane compared to Çankırı. The peak day of *Pleospora* was strongly affected by longitude – from west to east, the peak day of *Pleospora* was delayed by 126 days – from Çankırı (128 DOY) through Sinop (153 DOY), Giresun (194 DOY), Gümüşhane (238 DOY) to Artvin (254 DOY; Fig. 5A and B), which roughly gives a delay of 16 days per 1° longitude. For most of the taxa investigated, spore seasons ended at a similar time at different sites in the study area (late September to late October). The largest differences were recorded for *Boletus* (136 days between Çankırı and Artvin), *Puccinia* (60 days between Çankırı and Giresun), *Coprinus* (56 days between Çankırı and Giresun) and *Torula* (50 days between Çankırı and Giresun; Fig. 5A and B). Spore calendars were also used to show the temporal pattern of the spore season and its intensity for all the studied spore types (Fig. S2–S6).

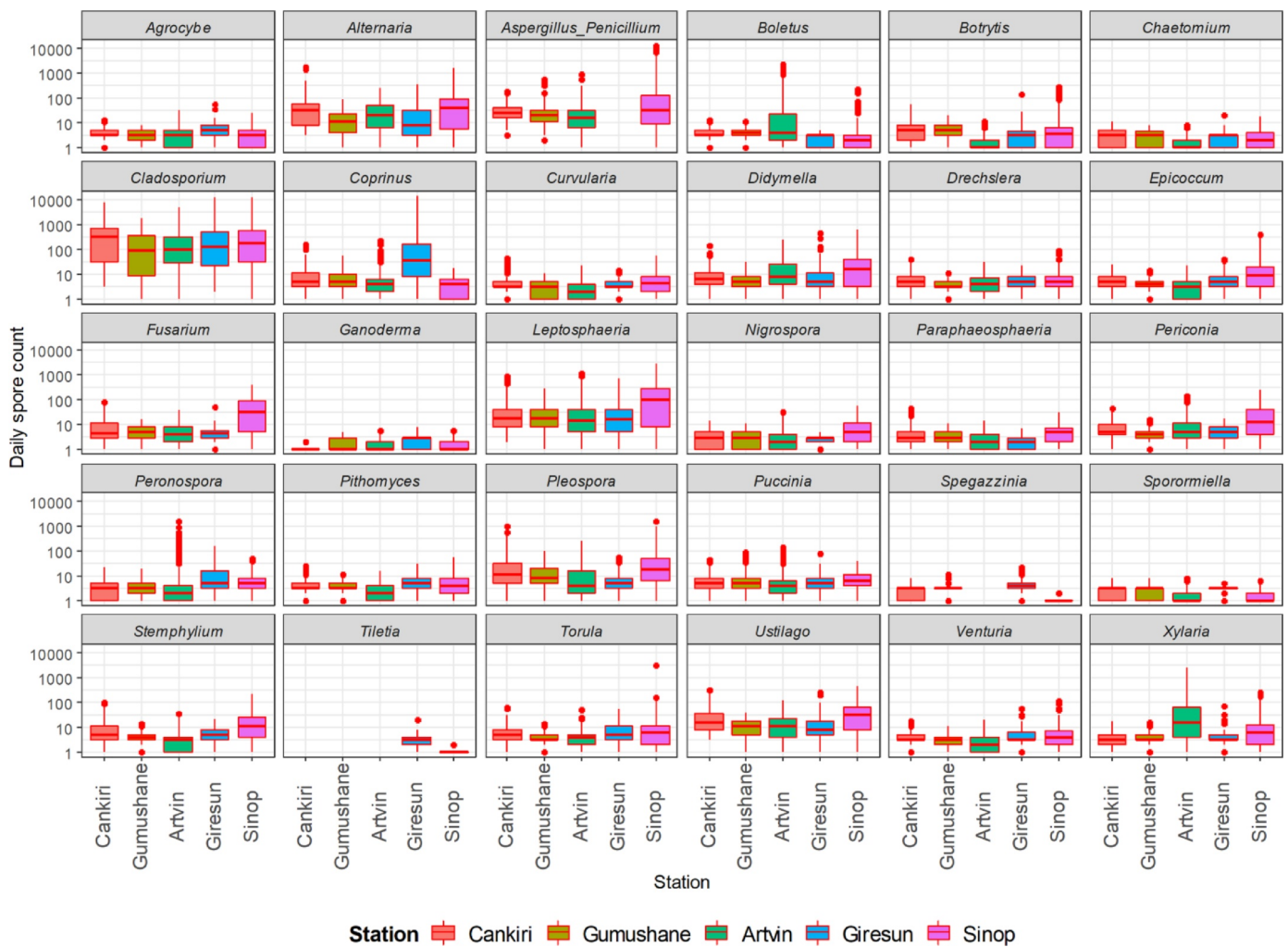


Fig. 4. Mean daily spore counts at five study sites.

3.3. Relationship between the spore count and meteorological conditions

The mean daily humidity, precipitation and wind speed generally varied significantly between individual sites (Tables 2 and 3). However, thermal conditions in two provinces with the continental climate – Çankırı and Gümüşhane – were similar, and the same was true for two provinces with the subtropical climate: Giresun and Sinop. TME significantly positively ($p < 0.05$) correlated with the spore counts for all spore types at all sites. The highest correlations were observed for *Alternaria*, *Aspergillus/Penicillium* and *Cladosporium* ($r_s > 0.7$). Such high correlations were also observed for spores of *Agrocybe*, *Puccinia* in Çankırı, and the *Drechslera* type, *Epicoccum*, *Periconia*, *Pithomyces*, *Puccinia*, *Stemphylium*, *Torula* and *Ustilago* spores in Sinop. Correlations between the daily spore counts and the remaining meteorological variables were significantly lower (Table 4). All the meteorological variables listed in Table 4 were included in RDA and accounted for 10.8–48.9% of the total variance in the fungal spore data in individual provinces, with the highest value in Çankırı and Gümüşhane, at sites with continental climate (Table 5, Fig. 1). The results of stepwise forward selection of explanatory variables revealed three variables to be statistically significant: TME (explaining 9–30% of the total variance in the spore composition, the highest contribution to the explained variance), RH (1–3%) and WINDME (1%, in two provinces only; Table 6). Ordination plots for each site confirmed the importance of temperature for the occurrence of spores in the air (Fig. 6). The positions of fungal spore types in the ordination space result from the impact of all main

meteorological variables together and the vertical projection of points of individual spore types on the TME arrow does not reflect a simple correlation with temperature, but is a result of a conditional effect. The spore type positions in the RDA diagrams generated for Çankırı and Gümüşhane (with continental climate) and for Artvin (with hot summer continental climate) were related to the temperature range of 12–17 °C, whereas temperature ranges for Giresun and Sinop (with subtropical climate) referred to higher temperatures (15–19 °C and 16–20 °C, respectively). In addition, different spore types were assigned to high or low TME at different sites, suggesting that the behavior of airborne spores of the same type may vary depending on the climate conditions (Fig. 6).

Given that temperature was the most important factor, we prepared recommendations when high spore counts may be recorded. High (> 90th percentile) and moderate (> 50th percentile) daily spore counts were clearly associated with significantly higher temperature than low counts at all sites for the following taxa: *Alternaria*, *Aspergillus/Penicillium*, *Cladosporium*, the *Drechslera* type, the *Leptosphaeria* type and *Periconia* (Fig. 7; Chi-squared and p-values presented in Table S3). *Xylaria* spore counts increased with increasing temperature at all sites except Çankırı. On the other hand, *Paraphaeosphaeria*, *Curvularia*, *Coprinus*, *Sporormiella* and *Chaetomium* spore counts appear to be constant regardless of temperature (Fig. 7, Table S3). The median air temperature during high *Cladosporium* counts was up to 16 °C higher than during low-count days (Çankırı and Sinop; Fig. 7). Similarly, *Alternaria* spores were present in large numbers when median temperature

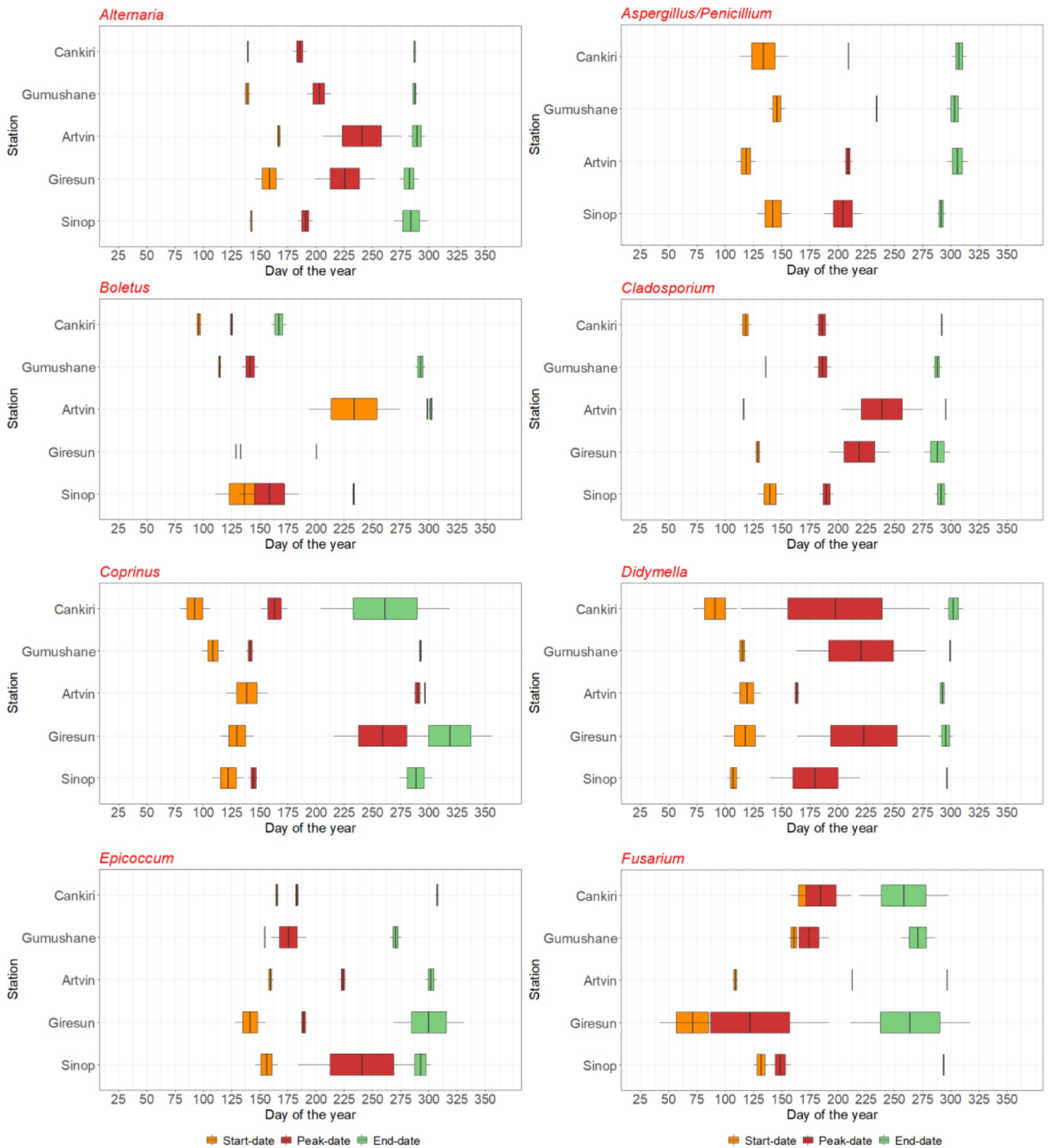


Fig. 5. (A, B). Spore season timing for selected 16 most abundant spore types in the study area.

increased by 10 °C. In Çankırı and Gümüşhane, *Epicoccum*, *Fusarium*, *Pithomyces* and *Sporormiella* spore counts decreased with increasing temperature. Whereas high spore counts of *Stemphylium*, *Ustilago* and *Venturia* were recorded at lower temperatures compared to moderate spore counts. The most significant temperature drop along with an increase in spore counts was observed for *Peronospora* in Artvin (by 7 °C lower for high spore count compared to low spore count). Artvin also showed an inversely proportional spore count-temperature pattern for

taxa that increased with increasing temperature at other sites, e.g. *Sporormiella*, *Puccinia*, *Peronospora* or *Ganoderma* (Fig. 7, Table S3).

3.4. Searching for source areas of airborne spores

The local source of spores seems to be very important in Giresun and Gümüşhane, where the very low wind speed contributes to high spore counts. However, when winds blow from SE in Gümüşhane, no spores

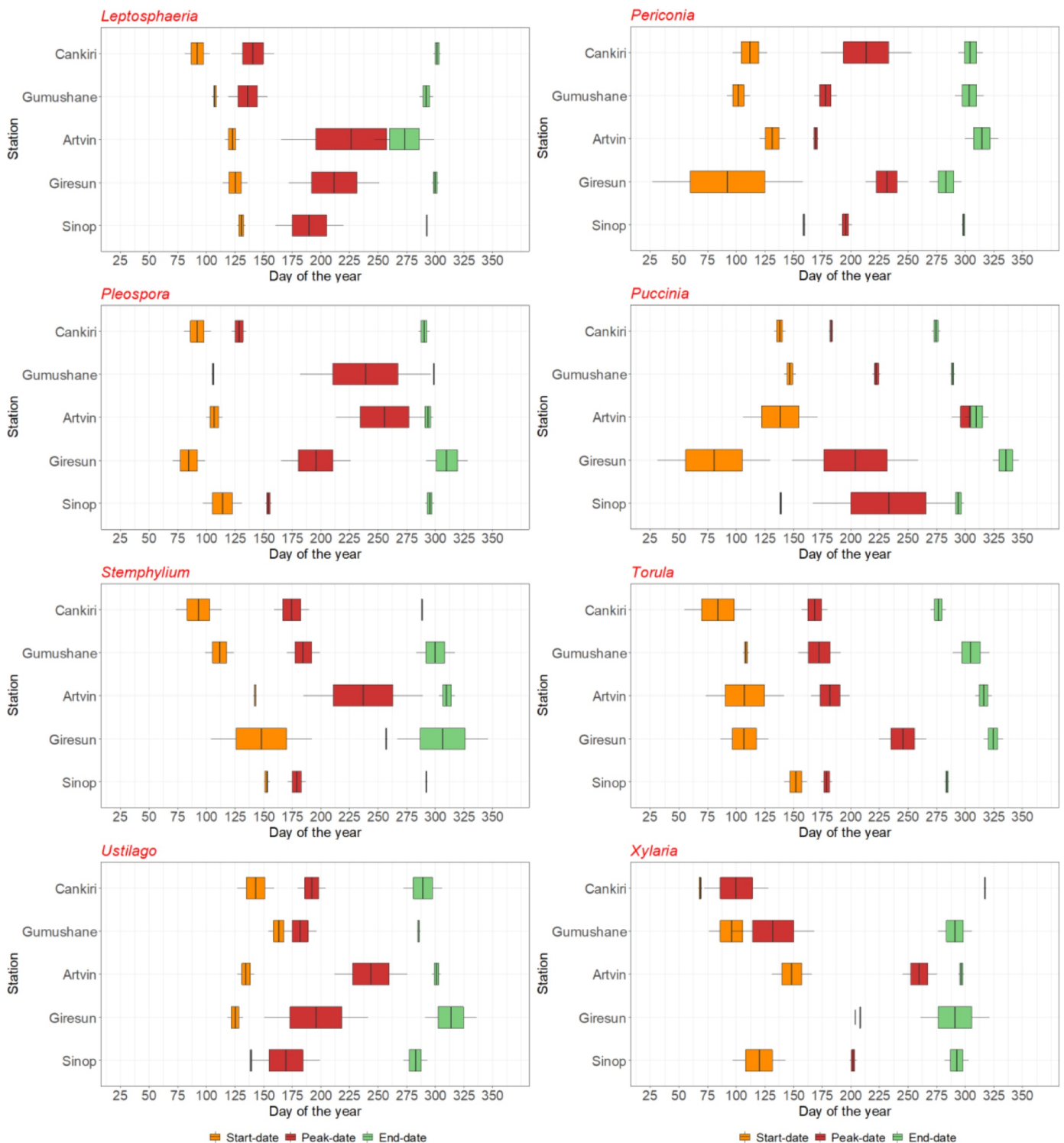


Fig. 5. (continued)

are recorded at the site (Fig. 8). This can be explained by land use because bare soil, rocks and areas with scarce vegetation are located in SE, so there are no hosts (plants) for fungi (Fig. 2). The majority of spore types at Sinop, Çankırı and Artvin are likely to originate from remote sources, because high wind speed is necessary to record high spore counts at these sites. In Çankırı, *Cladosporium*, *Alternaria*, *Didymella* and *Aspergillus/Penicillium* spores probably originate mainly from the same source area – the arable land located N, NE, E of the spore trap, which is confirmed by significant correlations (r_s from 0.5 to 0.6, $p < 0.05$) between the spore count and the area of arable land at a

distance of 0–5 km from the trap (Fig. 2, Fig. 9, Table S1). Another source of airborne spores for Çankırı may be grasslands that cover large areas, especially in N–NW–W directions and are positively correlated with the spore count (maximum 0.684, $p < 0.01$ for *Puccinia* – 15–20 km from the trap; Fig. 2, Fig. 9, Table S1). On the other hand, the main source of airborne spores in Artvin is located northwest of the city (Fig. 8). It is likely that mixed forests (or some specific types of forests within them) are the primary sources of the main fungal spore types in Artvin (Fig. 2 and 8), even though no significant correlations with land use were determined (Fig. 9). In Sinop, grasslands (0–5 km from the

Table 3
Results of Kruskal-Wallis test and post hoc Dunn's multiple comparisons test, showing significance of differences in meteorological variables at studied stations during the sampling period.

Variables	Kruskal-Wallis test		Dunn's multiple comparisons test									
	H	p-value	Artvin-Çankırı	Artvin-Giresun	Artvin-Gümüshane	Artvin-Sinop	Çankırı-Giresun	Çankırı-Gümüshane	Çankırı-Sinop	Giresun-Gümüshane	Giresun-Sinop	Gümüshane-Sinop
TME	147.7363	0.000*	0.015873*	0.000000*	0.009750*	0.001543*	0.000000*	1.000000	0.000000*	0.000000*	0.051252	0.000000*
RH	556.8278	0.000*	0.000128*	0.000000*	0.242703	0.00*	0.093683	0.000000*	0.00*	0.000000*	0.00*	0.00*
PRECIP	56.94362	0.000*	0.057408	0.010166*	0.482673	1.000000	0.000000*	1.000000	0.15060	0.000001*	0.001804*	1.000000
WINDME	776.0903	0.000*	0.000000*	0.000000*	0.000000*	0.00*	1.000000	1.000000	0.00*	0.543041	0.00*	0.00*

TME, daily mean air temperature; RH, daily mean relative humidity; PRECIP, daily amount of precipitation; WINDME, daily mean wind velocity.

* $p < 0.05$ (significance level).

trap) and coniferous forests (5–15 km from the trap) appear to be the main source areas for fungal spores, especially areas located northwest of the trap (Fig. 2 and 8). Correlations between the grassland area and spore counts reached the maximum value of 0.783 ($p < 0.01$ for *Pithomyces*), whereas in the case of coniferous forests and spore counts, r_s was 0.746 ($p < 0.01$ for *Epicoccum*) (Fig. 9). In general, grasslands significantly correlated with spore counts at all sites at different distances, but not in Artvin (where grasslands covered only 7.6% of a 30 km buffer; Table S1).

4. Discussion

This is the first study dealing with the combined meteorological-land use impact on 30 airborne fungal spore types commonly occurring in the Central and Eastern Black Sea region of Turkey. Using multivariate statistical techniques, we were able to comprehensively assess the combined effects of the main weather variables on the airborne spore count. We showed that the response of the airborne spore count to the most important factor – temperature – is species-specific: the same thermal conditions resulted in different spore counts for various spore types. Moreover, by combining the land use data with the meteorological data (especially wind direction and speed), we successfully identified possible source areas for airborne spores recorded at the study sites.

4.1. Spore abundance and timing of spore seasons

Cladosporium spores were the most abundant in the atmosphere of the entire study area, like in many other studies (Almaguer et al., 2014; Sadyś et al., 2015; Akgül et al., 2016; Grinn-Gofroń et al., 2018; Antón et al., 2019; Ščevková and Kováč, 2019), where they were reported to account for 30–85% of all spores recorded. Together with *Alternaria* (also a common spore type in our study), they are the best-known causative agents of allergic reactions and plant diseases (Bavbek et al., 2006; Abuley and Nielsen, 2017; Nowakowska et al., 2019). Another common spore type in our study (*Leptosphaeria* type) includes plant phytopathogens and can potentially seriously contribute to asthma. *Leptosphaeria* species produce proteins that are highly similar (above 90% of similarity) to well-known fungal allergens, such as Alt a 5, Alt a 3, Cla h 6 (*Alternaria* and *Cladosporium* allergens, respectively; Jędrzycka et al., 2016). Spores of *Aspergillus/Penicillium*, also considered to be clinically and phytopathologically important (Oliveira et al., 2010; Garrigues et al., 2016), were the fourth most abundant airborne spore type in the study area.

Little is known about the timing of the sporulation season worldwide. The present study fills this knowledge gap for mid-latitudes of Western Asia. Compared with Northern Europe (Sadyś et al., 2016, using the same 90% method), *Alternaria*, *Epicoccum* and *Puccinia* start at almost the same time in the Central and Eastern Black Sea region of Turkey (end of May, beginning of June, April to May, for *Alternaria*, *Epicoccum* and *Puccinia*, respectively). *Cladosporium*, *Didymella*, *Stemphylium* spore seasons start in the studied area of Turkey earlier than in Northern Europe (Sadyś et al., 2016). On the other hand, the *Pleospora* spore season starts much later in Turkey (March–April vs January–February in the UK). The timing of the spore season can often be assessed roughly graphically by spore calendars. Spore calendars increase the applicability of the results as the spore timing and load are clearly visible without specific aerobiological knowledge. So far, spore calendars are the most effective way of communicating the aerobiological results to agricultural and forestry practitioners and the general public. In the present study, we included spore calendars in the supplementary materials (due to space limitations) for each site separately (Fig. S2-S6). Such spore calendars were also prepared, for example, in Melbourne, Australia (Mitakakis and Guest, 2001), Cuba, North America (Almaguer et al., 2014), Slovakia and Central Europe (Ščevková and Kováč, 2019), Kolkata, India (Dey et al., 2019) or Spain,

Table 4
The results of Spearman's rank correlation test between daily numbers of fungal spores and selected meteorological parameters in the provinces of the Central and Eastern Black Sea region of Turkey.

Taxon	TME					RH					WINDME					PRECIP				
	Çankırı	Gümüşhane	Artvin	Giresun	Sinop	Çankırı	Gümüşhane	Artvin	Giresun	Sinop	Çankırı	Gümüşhane	Artvin	Giresun	Sinop	Çankırı	Gümüşhane	Artvin	Giresun	Sinop
<i>Agrocbe</i>	0.83	0.19	0.54	0.16	0.49	-0.53	-0.09	0.20	0.23	0.14	0.34	0.18	0.10	-0.10	0.08	0.08				
<i>Alternaria</i>	0.15	0.80	0.75	0.74	0.81	-0.36	-0.14	0.12	0.09	0.08	0.08	0.18	0.18	-0.14	0.16	-0.14	-0.21	-0.18	-0.23	
<i>Aspergillus/Penicillium</i>	0.70	0.72	0.26		0.77			-0.14			0.25					-0.19			-0.15	
<i>Boletus</i>	0.16	0.17	0.67	0.32	0.45	-0.32	-0.11	0.10	0.16	0.08	0.08	0.11	0.11	-0.10	0.12	-0.14			-0.12	
<i>Botrytis</i>	0.54	0.50	0.29	0.28	0.64	-0.24	-0.11	0.15	0.08	0.09	0.19	0.10	0.10	-0.09	0.13	-0.14			-0.08	
<i>Chaetomium</i>	0.37	0.28	0.48	0.35	0.54	-0.53	-0.11	0.13	0.15	0.19	0.31	0.18	0.18	-0.15	0.10	-0.10			-0.20	
<i>Cladosporium</i>	0.80	0.84	0.76	0.71	0.76	-0.09	-0.11	0.15	0.08	0.19	0.08	0.08	0.08	-0.11	0.09	-0.10	0.08	-0.10	-0.20	
<i>Coprinus</i>	0.25	0.30	0.62	0.16	0.49	-0.09	-0.11	0.15	0.26	0.15	0.13	0.18	0.18	-0.10	0.10	0.09			-0.12	
<i>Curvularia</i>	0.49	0.32	0.47	0.34	0.63	-0.30	-0.10	0.14	0.13	0.15	0.13	0.14	0.14	-0.13	0.13	-0.13	0.11	0.13	-0.13	
<i>Didymella</i>	0.43	0.39	0.52	0.30	0.58	-0.22	-0.12	0.19	0.17	0.11	0.12	0.18	0.18	-0.10	0.10	-0.13			-0.16	
<i>Drechslera type</i>	0.69	0.39	0.68	0.58	0.74	-0.44	-0.12	0.11	0.09	0.17	0.14	0.18	0.18	-0.12	0.12	-0.08			-0.18	
<i>Epicoccum</i>	0.60	0.56	0.63	0.45	0.77	-0.43	-0.17	0.19	0.16	0.12	0.20	0.09	0.09	-0.14	-0.13	-0.16	0.09	-0.08	-0.17	
<i>Fusarium</i>	0.53	0.53	0.51	0.25	0.68	-0.37	-0.20	0.19	0.16	0.16	0.24	0.18	0.18	-0.14	0.10	-0.10	0.09	-0.08	-0.17	
<i>Ganoderma</i>	0.33	0.20	0.39	0.33	0.46	-0.21	-0.20	0.08	0.16	0.16	0.09	0.19	0.19	-0.09	0.11	-0.08	0.11	0.13	-0.16	
<i>Leptosphaeria type</i>	0.53	0.64	0.57	0.43	0.65	-0.27	-0.12	0.18	0.15	0.13	0.23	0.11	0.11	-0.13	0.11	0.13			-0.12	
<i>Nigrospora</i>	0.41	0.35	0.54	0.32	0.65	-0.24	-0.10	0.18	0.09	0.14	0.15	0.11	0.11	-0.12	0.12	-0.12			-0.11	
<i>Paraphaeosphaeria</i>	0.50	0.39	0.43	0.36	0.62	-0.28	-0.11	0.18	0.17	0.13	0.17	0.14	0.14	-0.12	0.13	-0.15			-0.18	
<i>Periconia</i>	0.68	0.53	0.68	0.39	0.79	-0.42	-0.16	0.18	0.17	0.10	0.18	0.18	0.18	-0.12	0.11	-0.15	0.08	-0.10	-0.15	
<i>Peronospora</i>	0.45	0.40	0.42	0.45	0.67	-0.29	-0.13	0.11	0.17	0.10	0.14	0.14	0.14	-0.12	0.14	-0.11			-0.15	
<i>Pithomyces</i>	0.50	0.30	0.57	0.58	0.74	-0.36	-0.13	0.11	0.17	0.10	0.11	0.18	0.18	-0.12	0.09	-0.11			-0.15	
<i>Pleospora</i>	0.44	0.48	0.38	0.28	0.62	-0.23	-0.10	0.19	0.17	0.15	0.13	0.09	0.09	-0.10	0.14	-0.10	0.08	-0.10	-0.14	
<i>Puccinia</i>	0.71	0.59	0.54	0.43	0.78	-0.48	-0.13	0.19	0.18	0.15	0.28	0.09	0.09	-0.14	-0.08	0.09			-0.15	
<i>Spegazzinia</i>	0.16	0.24		0.14	0.11			0.09	0.11	0.14	0.10	0.11	0.11	-0.10	-0.10	-0.11			-0.19	
<i>Sporormiella</i>	0.22	0.21	0.34	0.13	0.40	-0.15	-0.10	0.09	0.09	0.15	0.21	0.11	0.11	-0.12	-0.12	-0.11			-0.08	
<i>Stemphylium</i>	0.65	0.48	0.55	0.45	0.78	-0.44	-0.13	0.10	0.09	0.15	0.21	0.16	0.16	-0.16	0.09	-0.10			-0.18	
<i>Tilletia</i>			0.37	0.37	0.27			0.09											0.09	
<i>Torula</i>	0.59	0.48	0.56	0.53	0.77	-0.43	-0.10	0.15	0.11	0.16	0.16	0.11	0.11	-0.08	-0.08	-0.10			-0.20	
<i>Ustilago</i>	0.76	0.69	0.68	0.50	0.80	-0.46	-0.10	0.13	0.11	0.13	0.33	0.16	0.16	-0.14	-0.08	-0.10			-0.18	
<i>Venturia</i>	0.40	0.23	0.43	0.31	0.58	-0.29	-0.12	0.13	0.18	0.16	0.10	0.10	0.10	-0.10	0.09	-0.10			-0.12	
<i>Xylaria</i>	0.33	0.34	0.72	0.32	0.69	-0.20	-0.15	0.13	0.09	0.13	0.18	0.19	0.19	-0.11	-0.11	-0.16			-0.16	

TME daily mean air temperature; RH daily mean relative humidity; WINDME daily mean wind velocity; PRECIP daily amount of precipitation; only statistically significant values are included in the table ($p < 0.05$).

Table 5
RDA summary for samples collected in the provinces of the Central and Eastern Black Sea region of Turkey.

	Axes	Province				
		Çankırı	Gümüşhane	Artvin	Giresun	Sinop
Eigenvalues	I	0.306	0.488	0.254	0.103	0.137
	II	0.001	0.000	0.002	0.005	0.000
	III	0.000	0.000	0.000	0.000	0.000
	IV	0.000	0.000	0.000	0.000	0.000
Fungal spores-environment correlation	I	0.563	0.710	0.560	0.449	0.443
	II	0.172	0.175	0.190	0.106	0.077
	III	0.043	0.084	0.060	0.052	0.044
	IV	0.030	0.126	0.074	0.050	0.076
Cumulative percentage variance of fungal spore data	I	30.6	48.8	25.4	10.3	13.7
	II	30.7	48.8	25.6	10.8	13.7
	III	30.7	48.9	25.6	10.8	13.8
	IV	30.7	48.9	25.7	10.8	13.8
Cumulative percentage variance of fungal spores-environment relationship	I	99.8	99.9	99.2	95.0	99.4
	II	100.0	100.0	99.9	100.0	99.7
	III	100.0	100.0	100.0	100.0	100.0
	IV	100.0	100.0	100.0	100.0	100.0
Sum of all eigenvalues/total inertia	1.000	1.000	1.000	1.000	1.000	
Sum of all canonical eigenvalues	0.307	0.489	0.257	0.108	0.138	
Explained fungal spore data variance [%]	30.7	48.9	25.7	10.8	13.8	

Southern Europe (Herrero et al., 2006; Reyes et al., 2016). The latter study was performed at a similar latitude as the present study. Nevertheless, certain clear differences in the airborne spore season timing were observed. First of all, Reyes et al. (2016) reported that *Periconia* spores were abundant mainly in winter months, but in our study, *Periconia* spores peaked in summer. In our study, *Epicoccum* also peaked in early summer, but Reyes et al. (2016) recorded an *Epicoccum* peak in autumn. These discrepancies indicate the importance of conducting new local spore season timing studies in different regions.

4.2. Impact of meteorological parameters varies from one fungal species to another

The abundance of all spore types identified throughout the study area was positively and significantly correlated with the daily mean air temperature. The highest correlations with TME were documented for *Cladosporium* (up to $r_s = 0.84$ in Gümüşhane), *Alternaria* (up to $r_s = 0.81$ in Sinop) and *Ustilago* (up to $r_s = 0.80$ in Sinop), whereas the remaining taxa showed correlations mostly in a range of 0.4–0.7. As shown by numerous aerobiological studies, most airborne fungal spores are positively temperature-dependent (Oliveira et al., 2009; De Linares et al. 2010; Almaguer et al., 2014; Sadyś et al., 2015, 2016; Akgül et al., 2016; Grinn-Gofroń and Bosiacka, 2015; Grinn-Gofroń et al., 2018). There are, however, a few reports on a significant negative relationship between air temperature and the spore count or concentration. Sadyś et al. (2015) recorded a moderate reverse effect of air temperature on the *Leptosphaeria* spore concentration, while another their study reported a weak negative impact on *Didymella* spore counts (Sadyś et al., 2016). Grinn-Gofroń et al. (2018) detected a weak negative correlation

between the mean daily and hourly air temperature and the number of *Stachybotrys* and *Stemphylium* spores. Antón et al. (2019) reported a weak negative correlation of daily average air temperature with *Coprinus* and *Leptosphaeria* spore concentrations. This negative relationship between temperature and spore count may be due to rain. Indeed, precipitation correlates negatively with temperature in many regions of the world, as rain reduces the temperature (Trenberth and Shea 2005). Wet-weather fungi, e.g. most ascomycetes and coelomycetes actively release their spores in rain, reaching the highest concentrations during continuous rain (Gottwald et al., 1997; Arseniuk et al., 1998). Some authors suggest that dry spores can also be released during rain by mechanical shock or fast air flow when rain drops spread (Hirst and Stedman 1963; Allitt 2000). Consequently, they may negatively correlate with temperature.

Daily, weekly or monthly correlations between temperature and spore counts are commonly used to depict a meteorological background for the presence of spores in the air, but frequently this simple analysis generalises the real effect of temperature on the spore count. Therefore, in this study, we clustered spore counts into three large classes – below the median (low spore count), between the median and the 90th percentile (moderate) and above the 90th percentile (high) and calculated TME for each class. Although in the case of several spore types, the spore count clearly increased with temperature, there are some spore types that do not respond to temperature, which is very similar for each class of the spore count (from 5 per 30 taxa in Sinop to 17/30 taxa in Gümüşhane; for more details see Fig. 7 and Table S3). Moreover, we also detected that the highest temperature was recorded with a moderate spore count (e.g. *Didymella*, *Venturia*, *Fusarium*, *Nigrospora* at selected stations) or even with low spore counts (e.g. *Sporormiella*,

Table 6

Forward selection of explanatory variables with the significance test of meteorological parameters explaining variance in the fungal spore composition in the air in the provinces of the Central and Eastern Black Sea region of Turkey.

Variables	Province		Gümüşhane		Artvin		Giresun		Sinop	
	Çankırı	<i>p</i>	Explained data variance [%]	<i>p</i>	Explained data variance [%]	<i>p</i>	Explained data variance [%]	<i>p</i>	Explained data variance [%]	<i>p</i>
TME	30	0.002*	45	0.002*	22	0.002*	9	0.002*	12	0.002*
RH	1	0.020*	3	0.002*	3	0.002*	2	0.002*	2	0.002*
WINDME	0	0.240	1	0.002*	1	0.020*	0	0.136	0	0.514
PRECIP	0	0.836	0	0.524	0	0.162	0	0.896	0	0.798

TME, daily mean air temperature; RH, daily mean relative humidity; WINDME, daily mean wind velocity; PRECIP, daily amount of precipitation.

* $p < 0.05$ (significance level).

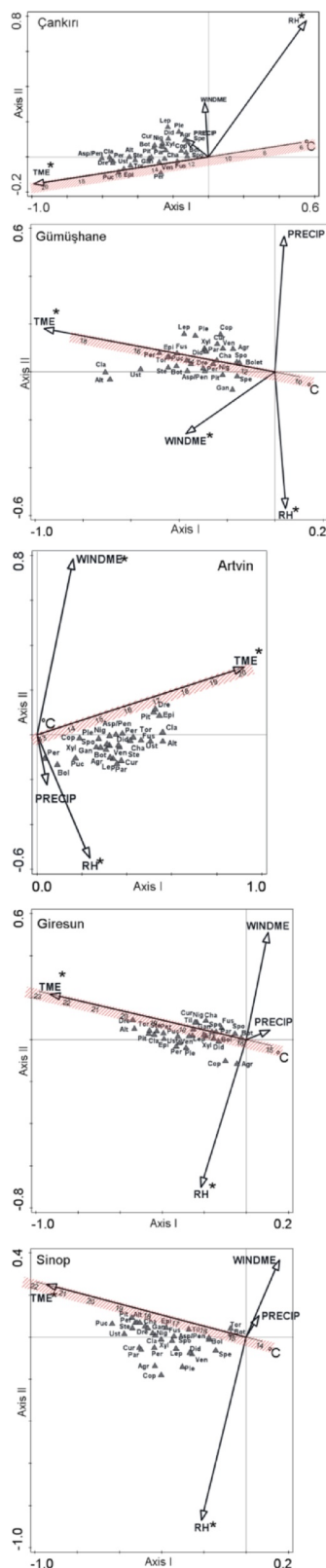


Fig. 6. Diagrams with the ordination of fungal spores and meteorological variables along the first two RDA axes for five sites in northern Turkey.

Coprinus, *Chaetomium*, *Peronospora*, *Botrytis*). This reversed relationship between the spore count and temperature occurred in Çankırı (12 taxa) and Artvin (7 taxa). Potentially, this phenomenon may be connected with low tolerance of the above-mentioned airborne fungi to high

temperatures (Ingold, 1971; Cosentino et al., 1990). For example, the development of *Peronospora* mold is enhanced in shady, wet and cold conditions (Aylor and Taylor, 1982), which agrees with our results from Artvin and Çankırı, where the highest *Peronospora* counts were recorded at the lowest temperatures. It is interesting that only in the subtropical climate province of Giresun the *Peronospora* spore count increases with the temperature.

Apart from temperature, many spore types were significantly negatively (in colder climates: Çankırı, Gümüşhane) or positively (in warmer climates: Artvin, Giresun, Sinop) correlated with relative humidity. As an increase in temperature is required for better dispersion and transport of spores, relative humidity is also important during the growth of fungi and the release of some spore types (Herrero et al., 2006). Especially during long, light rains and high humidity, release mechanisms largely counteract the run-off of spores by precipitation (Dey et al., 2019). This may be confirmed by high positive correlations between precipitation and *Leptosphaeria* and *Didymella* spores detected in the UK, Poland and Spain (Sadyś et al., 2015; Grinn-Gofroń et al., 2018; Antón et al., 2019). However, negative correlations between the spore count and humidity or precipitation are also possible as in Çankırı and Gümüşhane in this study, or in Worcester, West England (for *Epicoccum*, Sadyś et al., 2015, 2016). This may be an effect of spore removal with water run-off when heavy rains dominate at a site and this is probably the case of Gümüşhane in this study. The daily spore counts were also weakly positively correlated with the daily mean wind speed in provinces with the continental climate (Çankırı, Gümüşhane), and weakly negatively correlated in provinces with the subtropical climate at the coastal stations (Giresun, Sinop). This negative correlation may be explained by the fact that winds blowing from the sea usually have the highest speed due to the low surface roughness (Barthelmie et al., 1996), but they rarely transport airborne spores. Nevertheless, it should be mentioned that meteorological variables such as temperature, humidity, rainfall or wind can differently affect different types of spores: airborne conidia are positively correlated mainly with temperature, whereas basidiospores and ascospores with humidity and rainfall, and teliospores are negatively correlated with humidity (Herrero et al., 2006).

Considering that air temperature is the driving factor for daily spore counts it is worth noting that it increases in many places in the world. Similarly, air temperature in the study period (2010–2012) was, on average, slightly lower than in 2013–2019 (e.g. from 0.2 °C in Artvin, 0.5 °C in Sinop, 0.8 °C in Giresun, 1.1 °C in Çankırı to 2.1 °C in Gümüşhane; Figure S7). It seems that warming in Çankırı, Gümüşhane (stations with continental climate) has proceeded more intensively than in the coastal area. Moreover, spore counts in Çankırı and Gümüşhane are highly positively correlated with temperature. Consequently, an increase in temperature may cause an increase in spore counts of strongly temperature-dependent spore types such as *Cladosporium*, *Aspergillus/Penicillium*, *Alternaria*, especially in the continental climate. These taxa have allergenic properties, so we may expect (taking into account meteorological factors only) that the exposure to allergenic spores will increase if climate warming continues. However, Olsen et al. (2020) documented that *Alternaria* and *Cladosporium* counts have significantly decreased in recent years along with the minimum temperature increase. They attributed this decrease to changes in land use – primarily the growth of Copenhagen urban areas. In contrast, urban areas cover a small portion of our study area (< 1%, Table S1), potentially having little impact on spore counts.

The results of the redundancy analysis identified the air temperature as the main meteorological variable affecting the abundance of each fungal spore type in the air and the total airspora composition, similarly to other studies involving RDA (Li and Kendrick, 1995; Grinn-Gofroń et al., 2018), but contrary to the results of the study by Sadyś et al. (2015), who reported the importance of various factors that change from year to year. Li and Kendrick (1995) and Sadyś et al. (2015) assessed the importance of individual meteorological variables only

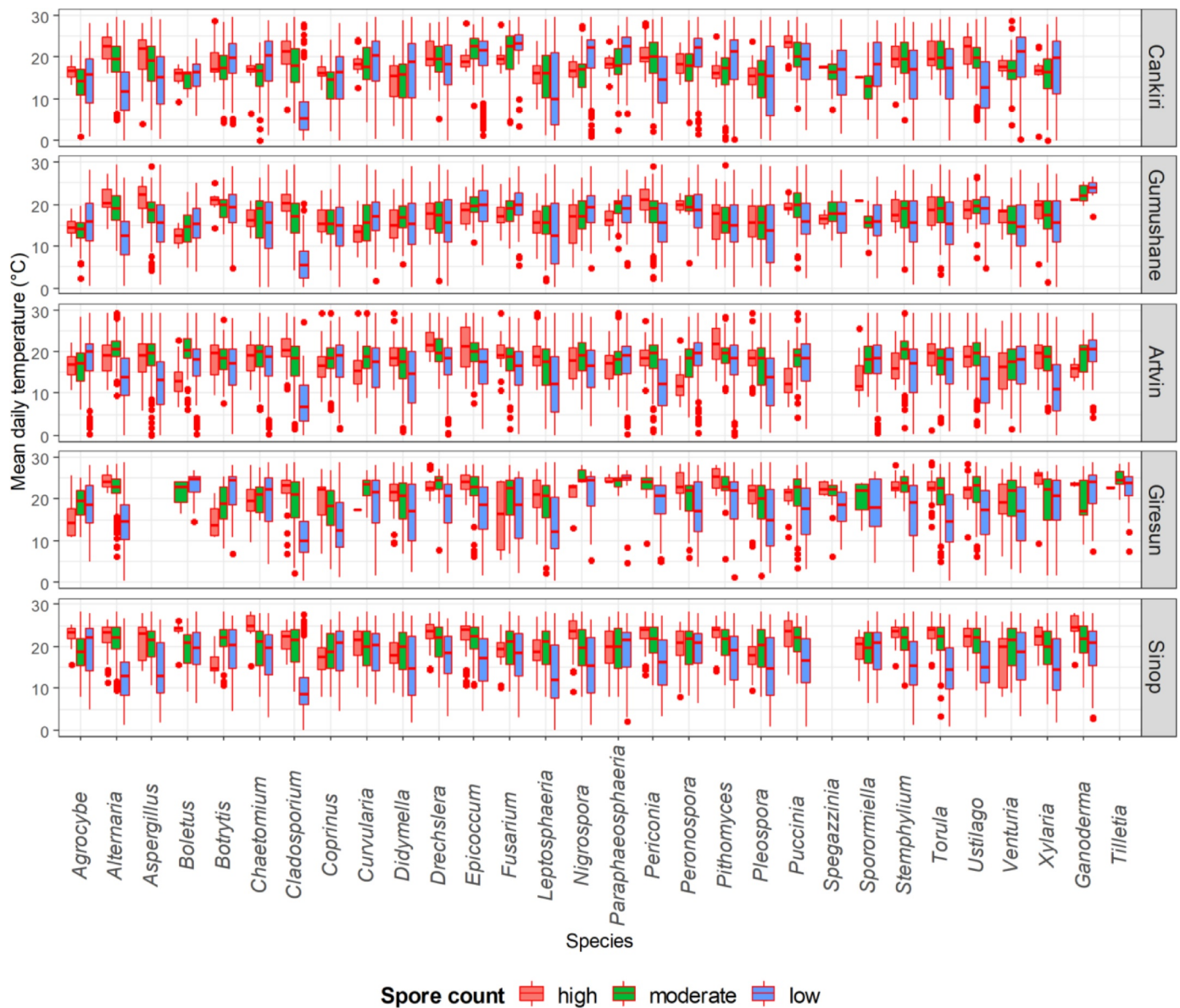


Fig. 7. Temperature range for spore count classes distinguished on the basis of percentiles: spore count (SC) > 90th percentile (high count), SC ≤ 90th percentile and SC > median (moderate count), SC ≤ median (low count). For test statistics, see Table S2 (Supplementary material).

tentatively, on the basis of the length of vectors in the ordination plot, without the stepwise forward selection of explanatory variables.

Percentage of variance in the fungal spore data explained by primary meteorological factors was clearly higher in two provinces with continental climate: Çankırı and Gümüşhane. This climate type is characterised by, inter alia, significant annual temperature differences (hot summers and cold winters). Long gradients of environmental factors (wider ranges of factor values) can increase the amount of explained variance in biotic variables (Grinn-Gofroń and Bosiacka, 2015). Nevertheless, the main meteorological factors explained a maximum of 49% of the airborne spore data variance (in Gümüşhane), while at coastal stations this value dropped to 11 and 14% for Giresun and Sinop, respectively. It is clear that there was another important factor affecting the composition of airborne spores.

4.3. Importance of wind direction and land use for spore source detection

We assumed that part of the variance in the spore count may be explained by land use and we collected some evidence to support this

assumption. We obtained significant positive correlations between the wind-direction-aggregated spore count and the area covered by grasslands (Çankırı, Gümüşhane, Giresun, Sinop), coniferous forests (Giresun, Sinop, Gümüşhane), mixed forests (Çankırı, Giresun), woodland-shrub (Çankırı, Gümüşhane) and arable land (Çankırı). To date, such analyses have been performed mainly in pollen-oriented studies, documenting the impact of land use on pollen concentrations (Oteros et al., 2015; Maya-Manzano et al., 2017; Ciani et al., 2020; Rojo et al., 2020), but rarely to determine sources of fungal spores (Sadyś et al., 2014; Fernandez-Rodriguez et al., 2015). According to our results, grasslands constitute the main possible source areas for fungal spores in the Central and Eastern Black Sea region of Turkey. Even if grasslands cover a small area around a trap (as in Sinop and Giresun, see Fig. 2 and Table S1), this correlates well with airborne spore counts. This corroborates the results for *Alternaria* from Badajoz, Spain, where grassland and cropland were potential sources of spores (Fernandez-Rodriguez et al., 2015). In our study, it is interesting to note that the highest positive correlation for grassland in Çankırı was found in the case of *Puccinia* spores. *Puccinia* species are phytopathogenic fungi

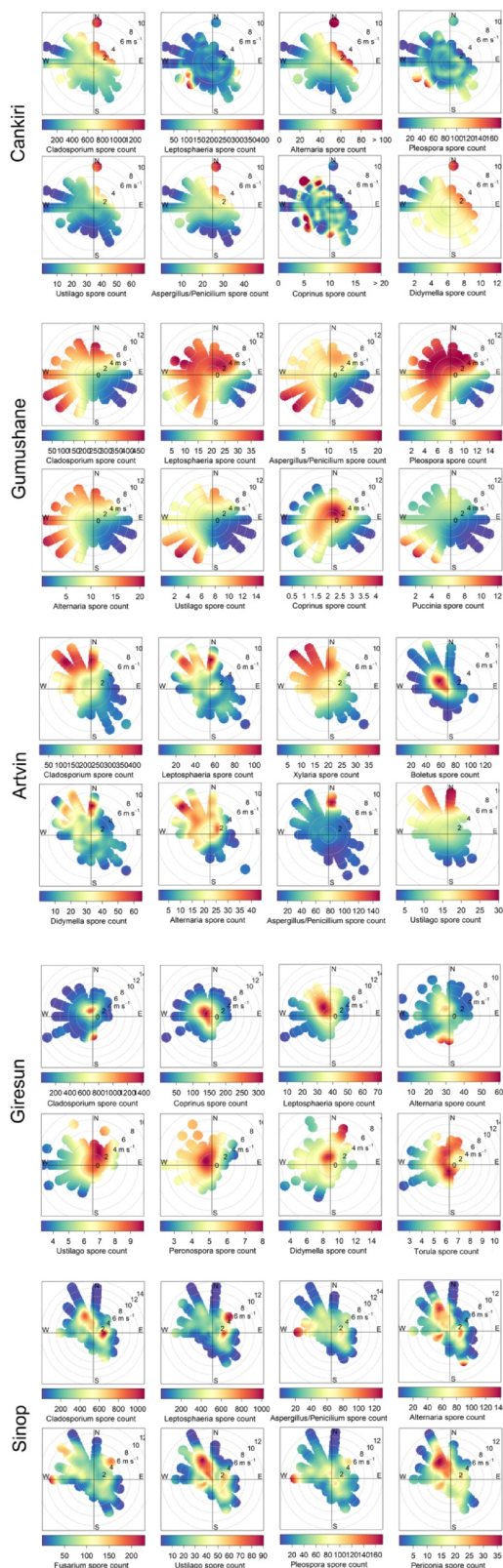


Fig. 8. Polar plots showing the relationship between the spore count and wind conditions (direction and speed).

affecting grasses, weeds, ornamental and horticultural plants (Camacho et al., 2018). Erdoğan et al. (2010) found that *Puccinia acarnae*, *P. annularis*, *P. eryngii*, *P. heterophyllae*, *P. hieracii*, *P. jasmimi*, *P. menthae*, *P. nigrescens*, *P. pulverulenta*, *P. punctata* occur in northern

Turkey. These species are oligophagous and affect different weed plants such as those belonging to the Lamiaceae family, *Epilobium* sp., *Hieracium* sp., *Eryngium* sp., *Picnoman acarna* – all these species grow in grasslands (Schaminée et al., 2016). Cropland was also indicated as a possible source of *Alternaria* and *Cladosporium* spores in Denmark (Skjøth et al., 2012; Olsen et al., 2019). In this study, we identified cropland within a 5 km distance from the trap as a potential source of airborne fungal spores for Çankırı (site with the largest cropland in this study, see Table S1).

We suppose that apart from grassland and cropland, also orchards and forests are significant sources of airborne spores in the Central and Eastern Black Sea region of Turkey. Specifically, hazelnut and cherry orchards cover large areas in Giresun (Ünal et al., 2010; İkinci and Bolat, 2015), where airborne *Coprinus* spores account for more than 25% of the total spore pool, whereas at sites without orchards it was no more than 1%. *Coprinus* is a genus of saprotrophic fungi that are typical for orchards (Russo et al. 2015), therefore our results confirm that large quantities of airborne *Coprinus* spores recorded in Giresun may be considered as a good indicator for orchard occurrence in the surrounding of the study site. Also forests seem to be major contributors of spores. Although no significant correlations with the forest area in Artvin was found, this site features the largest area covered by forests or shrub-woodland cover classes (up to 80%). It is clear that fungal taxa preferring forests and humid environments, such as *Boletus* (Salerni and Perini, 2004), *Xylaria* (Osono and Takeda, 2001) and *Didymella* (Van Steekelenburg, 1985), reached the highest spore counts in Artvin. It seems that coniferous forests are larger contributors of fungal spores than broadleaved forests as the area of the latter did not correlate positively with the spore count. The area of coniferous forests, on the other hand, was significantly positively correlated with the wind-direction-aggregated spore count in three out of the five sites in this study. This finding seems to be confirmed by the high incidence of fungal species in coniferous forests (compared to broadleaved woodlands), regardless of whether they are semi-natural forests, pine plantations or are composed of exotic conifer species (Humphrey et al., 2000). The area of coniferous forests in our study was significantly correlated with the spore count at a distance of 5–15 km from the site (Sinop) or even 25–30 km from the trap (Gümüşhane and Giresun). A relatively large distance may result from a larger spore production, assuming that it depends on larger plant biomass in forests than in grasslands (Li et al., 2015).

The airborne spore count significantly correlated with grassland, cropland and forest area at different distances from the trap. Grassland and cropland located close to the trap correlated with airborne spores. On the other hand, forest areas at larger distances (from 5 to 30 km) correlated with airborne spores. This may indicate that grasslands and croplands are sources of fungal spores deposited at shorter distances (local), whereas fungal spores originating from forests may be dispersed further. This difference is probably due to the larger plant biomass and the higher spore release level in forests, which implies greater potential for dispersion (Kuparinen et al., 2007; Li et al., 2015). Another phenomenon is that the correlation between land use types and spore counts was similar for different fungal taxa. This was evident in Çankırı and less evident in Gümüşhane and suggests that different fungal spores have a common source area. This finding was also confirmed on polar plots, where several different species showed a similar pattern of transport to the traps (Fig. 8).

The role of wind direction should be emphasized when studying the land use effect on the airborne spore count. By combining the wind direction data with spore counts, we were able to obtain information on the dominant spore transport pathways and also potential transport routes that did not bring spores to the study sites. Integration of spore transport pathways with land use provides information on potential sources of airborne spores. However, the accuracy of the estimates largely depends on the quality of wind direction data. The wind data in our study originated from closely located stations, which ensures a good

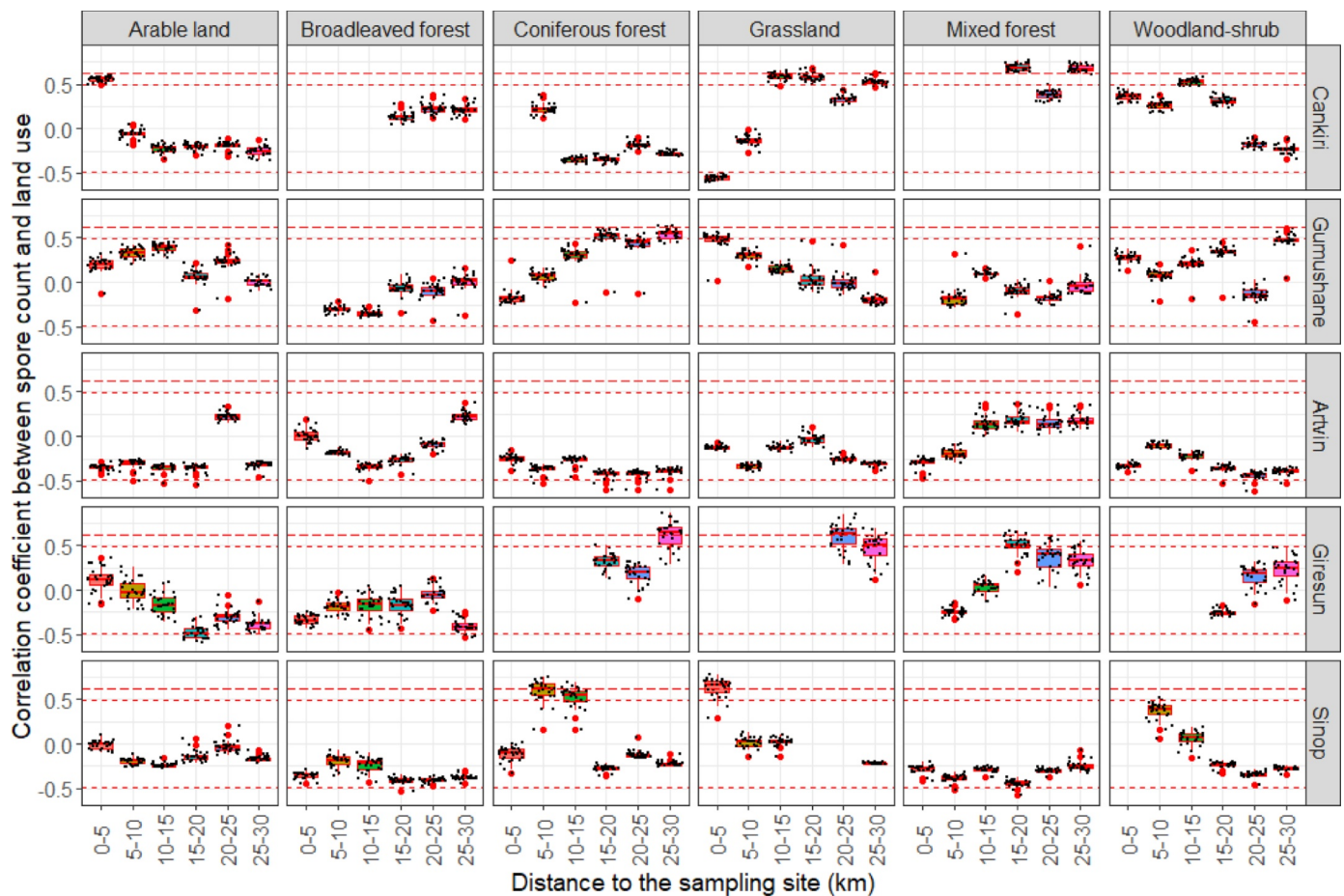


Fig. 9. Spearman correlations between the wind-direction-aggregated spore count and the land use type proportion in respective sectors at different distances from the spore trap. Dashed and long-dash red lines show the statistical significance threshold of the correlation coefficient ($p = 0.05$ and $p = 0.01$, respectively). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

quality of data. However, as meteorological stations in Artvin and Sinop were not located in the immediate vicinity of the spore trap, wind directions at the trap location may be slightly different due to the complexity of the terrain (especially in Artvin). This may be the reason for not detecting significant correlations between the forest area and the spore count in Artvin. Future work will focus on measuring the airborne spore count directly at potential sources, grassland, cropland and coniferous forest, and adding high resolution back-trajectories together with remote sensing phenological data (Bogawski et al., 2019b) to better identify sources of fungal spores.

5. Conclusions

This is the first study describing the airborne spore composition in the Central and Eastern Black Sea region of Turkey against the background of meteorological variables and land use. It should be emphasized that the dataset collected in this study is a unique collection of spore samples from the area that has never been sampled aerobiologically before. It was shown that the airborne spore composition and the sporulation season timing could vary significantly within just 100 km (distance between Gümüşhane and Giresun), and therefore we emphasize the importance of local studies on airborne spores. We have proposed a new methodology for investigating changes in airborne spore counts and identifying potential spore sources. It can be used anywhere, provided that airborne spore data, as well as meteorological and land use data are available. First, we assessed the combined effect of primary meteorological variables by multivariate statistics and followed with detailed analysis of the most important factor –

temperature. The longer temperature gradients, characteristic of two continental climate provinces: Çankırı and Gümüşhane, resulted in the increased amount of the explained variance in the spore occurrence, compared to other provinces. Then, we defined classes of the airborne spore count (based on percentiles) and calculated the temperature range for these classes, obtaining information on how a particular spore type responds to a temperature increase. It turned out that between 17% (Sinop) and 56% (Gümüşhane) of the taxa did not respond positively to temperature when clustered based on the spore count. In these cases, no significant differences between spore count classes were found or the highest spore count was recorded at a lower temperature. The main novelty of this study was to combine wind direction with airborne spore data, integrating them into 16 main world directions and overlapping with land use data. This method allowed us to identify potential sources of fungal spores in the studied area of Turkey, which significantly varied from site to site. Nevertheless, grasslands and croplands, especially within a 5–10 km distance from a trap, proved to be the main potential sources of fungal spores. We also suggested that coniferous forests may also be the large source of airborne spores and spores from these forests can be transported over longer distances than those from grasslands and cropland.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.agrformet.2020.108191.

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