Fungal Spore Seasons Advanced Across the US over Two-Decade Climate Change

by

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Abstract

Phenological shifts due to climate change have been extensively studied in macroorganisms, yet the responses of fungal spores—crucial microorganisms that act as airborne allergens and play important roles in ecosystems—remain understudied. This hinders our understanding of global change impacts and its implications for both ecology and public health. To bridge this gap, we leveraged a long-term, continental-scale dataset of airborne fungal spores collected by the US National Allergy Bureau. We extracted ten metrics describing the timing (e.g., start and end of season) and intensity (e.g., peak concentration and integral) of fungal spore seasons from both ecological and public health perspectives, defined with percentiles of total spore concentration and allergenic thresholds of spore concentration, respectively. Using linear mixed effects models, we quantified temporal shifts in these metrics across the continental US. We revealed that the onset of the spore season has significantly advanced from both ecological (11 days, 95% confidence interval: 0.4 ~ 22 days) and public health (31 days, 14 ~ 48 days) viewpoints in two decades. Accordingly, the ecological spore season and allergy season tended to be longer. Nevertheless, the total spore concentration in an ecological spore year and in a spore allergy season tended to decrease. The start of the spore season was significantly correlated with climate change variables, such as temperature and precipitation. Overall, our findings suggest possible climate-driven advanced and prolonged fungal spore seasons, highlighting the importance of climate change mitigation and adaptation in public health decision-making.

Table of Content

1. Introduction

The shifts of phenology—the timing of seasonal activities of living organisms—in response to climate change are evident and extensively documented across diverse ecosystems, from polar lands to tropical oceans (Bradley et al., 1999; Menzel, 2000; Parmesan, 2007; Walther et al., 2002). Current phenological knowledge has been focusing on prominent macroorganisms, which has gained significant public attention. For instance, North American and European studies show a consistent trend towards earlier biological spring events, such as bird migrations, bird singing, butterfly appearance, amphibian spawning and plant leafing and flowering, in response to a warming climate (Beebee, 1995; Kusano & Inoue, 2008; Menzel et al., 2020; Piao et al., 2019; Roy & Sparks, 2000; Sparks, 1999). However, corresponding shifts in microorganism phenology, such as the timing and intensity of fungal spore production, have received limited focus. Given the importance of fungal spores as airborne allergens affecting public health and the importance of this life stage to fungi, which are of fundamental importance in ecosystems, understanding the phenological shifts in fungal spore productions is crucial for assessing the impact of climate change on ecosystems and informing decision-making in public health.

Fungal spores are important for both ecological communities and public health. From an ecological perspective, fungal spores are engaged in multiple important processes. Fungal spores are reproductive propagules that enable fungi to reproduce and spread (Wyatt et al., 2013). Spores are often dispersed by air, water, or other organisms, facilitating the colonization of new environments (Wyatt et al., 2013). Variation in the quantity, composition, and arrival time of fungal spores has been demonstrated to change a number of important ecological processes, such as fungal community assembly, decomposition rate, nutrient cycling, and plant productivity and competition (Dighton, 2018; Dighton & White, 2017; Leopold & Fukami, 2021; Levetin, 2016;

Orwin et al., 2011; Peay, 2018; Peay et al., 2012; Read & Perez-Moreno, 2003; Smith & Peay, 2021; Van Der Heijden et al., 2008). From a public health perspective, exposure to airborne fungal spores may trigger allergic reactions resulting in asthma exacerbations or aggravating allergic rhinitis. The allergic symptoms could range from mild respiratory discomfort to serious respiratory distress upon inhalation, sometimes inducing life-threatening reactions (Horner et al., 1995; Hughes et al., 2022; Jenkins et al., 1981; Kurup et al., 2000). In asthmatic patients, especially children, dominant allergenic fungi, such as *Alternaria*, *Cladosporium*, *Aspergillus*, and *Penicillium*, can precipitate acute symptoms (Batra et al., 2022; Chen et al., 2014; Tham et al., 2017). A serum-specific lgE test in the US suggests that 22% of allergic patients have allergic responses to at least one fungal allergen (Kwong et al., 2023). Furthermore, hospital admissions and mortalities related to asthma increased on days marked by high fungal spore concentrations (R. W. Atkinson et al., 2006; Dales et al., 2000; Newson et al., 2000). Fungal spores represent an important life stage of fungi, which are of fundamental importance in ecosystems; they are also major airborne allergens affecting public health. Understanding the phenological shifts of fungal spores is crucial for assessing the impact of climate change on ecosystems and informing decision-making in public health.

Fungal physiological studies show that climate change, including warmer temperatures and altered precipitation, has the potential to alter the times of year in which airborne fungal spore concentrations are elevated (herein referred to as fungal spore seasons). Previous studies reported that warmer temperatures and altered rainfall patterns have been shown to affect the life cycle of fungi and subsequently the presence of their spores in the atmosphere (Diez et al., 2013; Gregory, 1967; Webster et al., 1989). In the initial stages, warm and wet conditions can synergistically accelerate fungal growth and spore formation. Specifically, elevated temperatures

can stimulate the rapid growth of fungi and induce spore production (Jesús Aira et al., 2012); the post-rainfall moisture retained in the soil and leaves provides the necessary conditions for fungal growth and spore development (Ganthaler & Mayr, 2015). A previous study on mushroom fruiting phenology suggested a widening fruiting season in Europe (Kauserud et al., 2012). Subsequently, the process of spore release into the air is also enhanced by such warm and humid conditions, which facilitate the detachment of spores from their parent structures and promote their initial entry into the air (Gabey et al., 2010; Katial et al., 1997; Tilak, 2009), potentially leading to an upsurge in airborne spore density (Ganthaler & Mayr, 2015). Contrastingly, the process of aerosolization—the wide dispersion of spores through the air—tends to be more efficient under drier conditions. Dryness can reduce the agglutination of spores, thereby increasing their potential to become airborne and travel over longer distances (Sache, 2000). Ultimately, the extensive spread and viability of spores as they move through different climates depend on various factors, with some spore types resilient across wide environmental ranges (Wyatt et al., 2013), while others strictly require certain temperatures and humidity levels to survive (Golan et al., 2023). This multistage perspective underscores the nuanced impacts of weather patterns on fungal spore dynamics, thus impacting airborne fungal spore phenology and intensity, which in return, could have implications for ecosystems and public health. Therefore, it is critical to closely monitor and analyze the temporal patterns of fungal spores as they respond to ongoing climatic shifts.

Despite the recognized importance of fungal spores and potential shifts in their phenology under a changing climate, comprehensive evaluations are limited. Only a few studies in Europe attempted to analyze long-term trends of fungal spore phenology and intensity, but many limitations persisted among them. First, they were usually constrained to one or two

locations, yielding inconclusive results. Second, the results of currently available studies are inconsistent even under similar rising temperatures, reflected by some studies reporting an earlier spore season, while others suggesting a delayed season (Damialis et al., 2015; Grinn-Gofroń et al., 2016; Ščevková et al., 2016). Likewise, the trends in peak concentration were also heterogeneous over space in Europe (Damialis et al., 2015; Millington & Corden, 2005). Differences between sites and uncertainty in characterizing fungal spore phenology could have posed challenges to obtaining generalizable insights. Third, existing studies usually defined the spore seasons from a purely ecological perspective, with the start and end of the spore season identified when the cumulative integral of spore concentrations exceeded a certain percentage of the annual integral (Anees-Hill et al., 2022). Although this method is reasonable, it has less relevance for addressing health concerns associated with allergenic fungal spores. Compared to this, extensive studies on pollen, another airborne allergen, usually took an approach more relevant to public health, using specific allergy thresholds to determine the start of an allergic allergen season (Jato et al., 2006)—a methodology that could be explored for spores as well. Using both approaches—the ecological cumulative integral method and the public health threshold method—could offer a more comprehensive view of fungal spore seasons, encompassing ecological patterns and health implications.

In this study, we aim to quantify the long-term trends in the phenology and intensity of fungal spore seasons and their correlation with climate change. To achieve this, we analyzed data from aerial samplings collected over a 20-year period (2003 ~ 2022) from 55 monitoring stations across the continental United States. Our methodology integrates two approaches to define the spore seasons: the first uses cumulative integrals to define ecological spore seasons, consistent with prior ecological studies (herein: the ecological method); and the second applies the National

Allergy Bureau (NAB) allergy thresholds to identify spore allergy seasons (Portnoy et al., 2004), an approach consistent with current public health messaging and recommendations (herein: the public health method). We subsequently employ statistical methods to quantify the trends in fungal spore season metrics and their relationships with climate change, including changes in temperature and precipitation. We hypothesize that the fungal spore phenology has shifted from 2003 to 2022, identified by an earlier start, and longer length of the spore season. We also anticipate increases in the intensity of fungal spore seasons, such as peak and annual concentration, mirroring the shifts in allergenic pollen phenology in North America (Anderegg et al., 2021). Additionally, we hypothesize that the fungal spore season metrics correlate with climatic variables, including temperature and precipitation during the same period. Our study deepens the understanding of fungal spore ecology and could inform health-related policies and actions in response to the impact of climate change on allergenic fungal spores.

2. Methods

2.1. Data source for fungal spore concentrations and climate data

We acquired a unique fungal spore concentration dataset from the National Allergy Bureau (NAB) counting stations, part of the American Academy of Allergy, Asthma and Immunology (AAAAI) Aeroallergen Network. NAB has a long history of monitoring aeroallergens in North America, thus providing long-term spore data over a wide spatial scale (Levetin et al., 2022). To our knowledge, the NAB spore data is the most accurate spore data available because of their stringent sampling protocol, including the sampler location selection and spore identification (Portnoy et al., 2004). At each NAB station, airborne fungal spores are sampled over a 24-h period, typically using a Rotorod Sampler or Burkard spore trap (Portnoy et al., 2004). According to the guidelines for setting up an NAB station, the sampler is positioned on a rooftop

free of obstructions, with no unusual local sources of fungal spores surrounding (Portnoy et al., 2004). Finally, the collected spores are identified and counted by certified experts, whose certifications need to be maintained (Portnoy et al., 2004). Daily average fungal spore concentrations (spores m^{-3}) are calculated by measuring the number of spores measured divided by the volume of air sampled over 24 hrs. Fungal spores are classified to the genus level when possible, resulting in 23 genera, as well as categories for "Other Fungi," and "Unidentified Fungi." Despite the certifications of the experts, there is high uncertainty in morphological identification (Fig. S2). We focused on the total concentration of all fungal spore taxa in our study, with unidentified and Cladosporiaceae spores contributing to 46% and 44% of the total concentration, respectively (Fig. S2). Additionally, given the lack of studies on North American fungal spore phenology, our study of the total spore concentration fills an important gap. Furthermore, our focus on total spore concentration aligns with the public health definition of the allergy season based on NAB guidelines, which sets allergy thresholds based on total spore concentration.

From the NAB dataset, we acquired a time series of total fungal spore concentration from 2003 to 2022 from 75 counting stations across the continental US. In order to have enough data to analyze temporal trends, stations were eligible for inclusion in the study if they contained at least ten days with non-zero concentrations in each station-year and at least three years of data. Overall, 638 station-year combinations across 55 NAB counting stations met our inclusion criteria. These stations are located in eight ecoregions in the continental US: 28 stations in the Eastern Temperate Forests ecoregion, 13 stations in the Great Plains ecoregion, six stations in the Mediterranean California ecoregion, and four stations in the North American Deserts ecoregion (US EPA, 2015; Fig. S1). The stations span a wide climatic gradient, with mean annual

temperature ranging from −11.7 °C to 41.6 °C, and total annual precipitation ranging from 0 mm to 2,115.9 mm.

We obtained the climate data at each station from the National Aeronautics and Space Administration (NASA)-Daymet project (Thornton et al., 2022). The Daymet project provides 1-km gridded daily surface climate data, covering the whole duration and spatial coverage of the NAB spore data. Previous studies have recommended the Daymet dataset for analyzing NAB data (Katz et al., 2023; Wozniak & Steiner, 2017). We calculated mean annual temperature (MAT, °C) and total annual precipitation (TAP, mm) at each station for the years with spore sampling.

2.2. Pre-processing of spore data

We filled in gaps in daily spore concentration using linear interpolation for each station. Most of the station-year combinations contained missing data. Large gaps existed occasionally in winter seasons. This occurs because NAB stations prioritize monitoring airborne pollen, leading them to occasionally suspend monitoring during winter when pollen is not present. Some smaller gaps also existed due to some stations not operating during weekends. The missing data inevitably caused information loss and introduced potential bias in calculating spore season metrics, such as the start and end dates of the spore allergy season. For instance, if the actual start date of the spore allergy season corresponds to a date with missing data, this information was lost, potentially skewing the results. To mitigate such issues and improve the accuracy of subsequent analyses, smaller gaps in each station, which were fewer than 14 consecutive days, were linearly interpolated by taking the first data previous and subsequent to the gap. Linear interpolation has been commonly used to deal with missing data in aerobiological studies (Anderegg et al., 2021; Gabarra et al., 2002; Picornell et al., 2021). Additionally, station-year combinations with large

gaps were excluded from our analysis, and the detailed criterion will be stated for each fungal spore season metric subsequently.

To mitigate the impact of observational errors and day-to-day variability on the extraction of fungal spore season metrics, we employed the Whittaker-Henderson smoothing method for each station (Wehrens et al., 2015; Whittaker, 1922). Smoothing enhances the quality of the data by providing more stable and representative signals, enabling a clearer analysis of underlying trends and recurring patterns in spore season metrics. The Whittaker-Henderson smoother is particularly advantageous for its capabilities in auto-interpolating missing observations, adapting to data boundaries, and allowing for precise adjustment of smoothness levels (Eilers, 2003). It has been widely adopted for use in prior phenological research (P. M. Atkinson et al., 2012; Li et al., 2020). We used a smoothness parameter of 100 for the Whittaker-Henderson smoothing. To ensure the robustness of our results, we conducted a sensitivity analysis on this parameter (Fig. S3). The analysis, illustrated in Figure S2, confirmed that the observed trends in spore season were similar across the range of smoothing parameters tested (Fig. S2). The pre-processed data were subsequently used to extract fungal spore metrics.

2.3. Characterizing fungal spore season

2.3.1. Detecting seasonal cycles

To characterize the fungal spore season in each station, we visualized fungal spore calendars by averaging daily raw concentrations from 2003 to 2022 (Fig. S5). Because the seasonal cycles of spore concentration did not necessarily coincide with calendar years (i.e., January 1st ~ December 31st) in many stations, we defined spore years in order to calculate ecologically summary metrics (e.g., start and end of season). Specifically, we computed the median fungal spore concentration of each day for years with available data in each station; the beginning day

of a spore year was then defined as the day when the trough value appeared in each station's spore median concentration curve (Fig. 1A). After pre-processing raw data and defining the spore year for each station, we assessed the data completeness for each station and spore year combination by dividing the number of days with available data by 365. The data completeness subsequently served as a threshold criterion for the inclusion of station-spore year combinations in our future analysis.

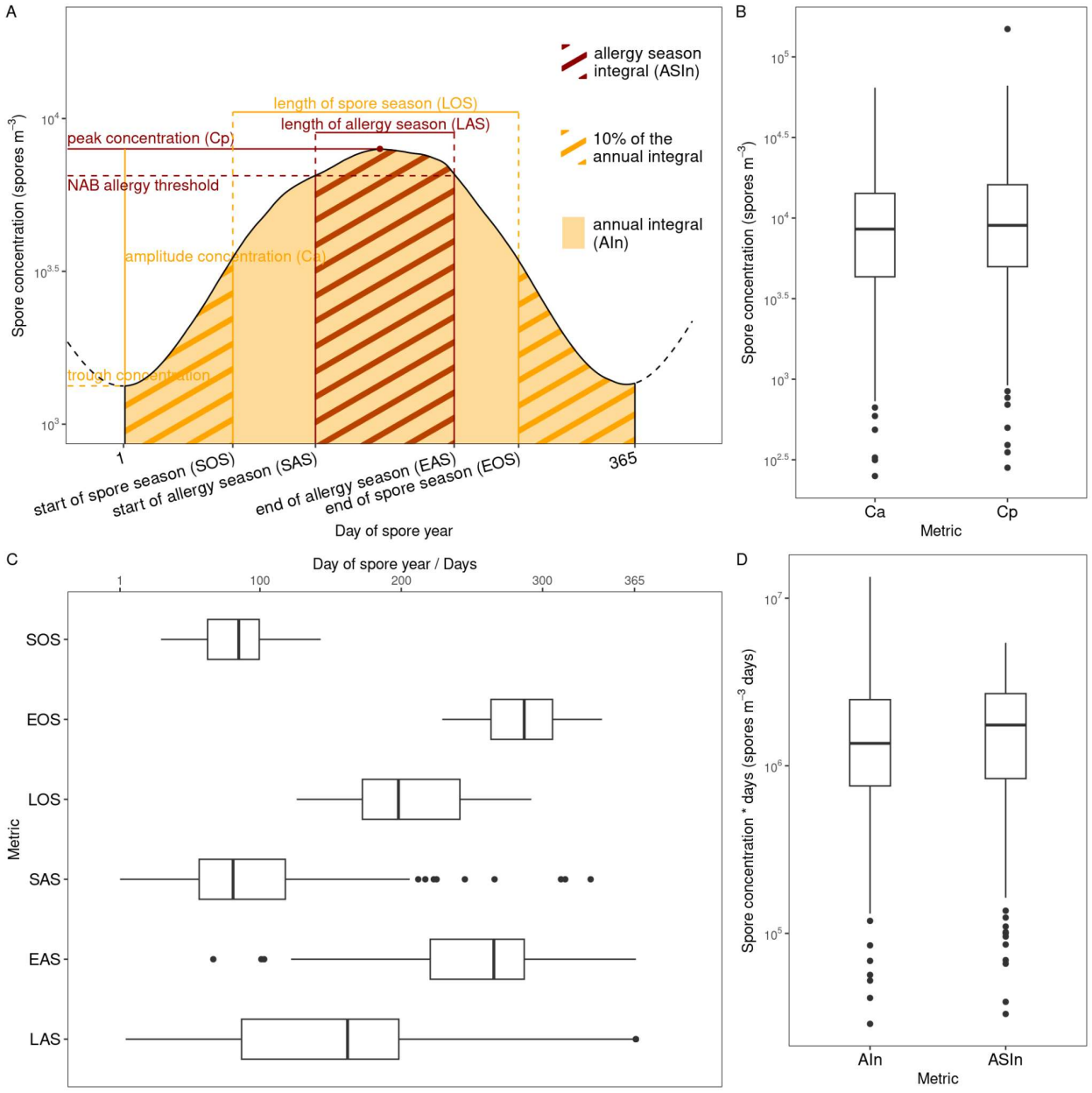

Fig. 1 Definition and numerical summary of ten fungal spore metrics. The annotation of ten fungal spore metrics (A). Orange indicates metrics defined in the ecological approach, while dark red indicates metrics defined in the public health approach. Each box represents each metric's interquartile range (IQR), with the median value indicated by the line inside. Whiskers extend to the minimum and maximum values within 1.5 times the IQR, while points beyond the whiskers are considered outliers. Phenology metrics are in the unit of day of spore year (DOY) or days (for season lengths LOS, LAS) (C), while intensity

metrics are in the unit of spores m^{-3} (B) or spores m^{-3} days (D), displayed on a natural logarithm-transformed scale. The abbreviations of metrics used in (B), (C), and (D) are defined in (A).

2.3.2. Defining spore season phenology

Within each spore year, we extracted ten fungal spore season metrics, encompassing the phenology and intensity of fungal spore season, in both ecological and public health methodologies (Fig. 1A). For the ecological method, we characterized the start of spore season (SOS) and the end of spore season (EOS) as the day of the spore year (DOY) when the cumulative sum of smoothed daily spore concentrations exceeded the 10th and 90th percentile of the annual spore concentration integral, respectively (Fig. 1A). This definition allowed us to characterize the timings of seasonal increase and decrease of fungal spore concentration. We then calculated the length of spore season (LOS, days) by subtracting SOS from EOS (Fig. 1A). Since the annual spore integral was a principal reference in defining the spore season, we required the data completeness after interpolation of each station-spore year to be 100% for metric SOS, EOS, and LOS to ensure the accuracy of the values.

For the public health method, we identified spore season phenology based on the four allergy thresholds as defined by NAB: “low,” “moderate,” “high,” and “very high.” According to the NAB definitions, the moderate-level risk, set at 6500 spores m^{-3}, indicates that many individuals could be sensitive to the spores, potentially leading to allergy symptoms (Portnoy et al., 2004). We thus adopted 6500 spores m^{-3} as the allergy threshold in our definition of allergy season. Therefore, the start of allergy season (SAS) and the end of allergy season (EAS) is the first and last day of the spore year (DOY) of the first and last ten consecutive days with the smoothed spore concentration above the threshold of 6500 spores m^{-3} (Fig. 1A). Since sampling may not cover a whole spore year, to remove concerns that sampling started after the true SAS or

ended before the true EAS, or such station-spore year with incomplete sampling, we excluded detected SAS if ten consecutive days before this SAS contained missing data. Likewise, the detected EAS was removed if any of the ten consecutive days following it were incomplete. The length of allergy season (LAS, days) was the interval between SAS and EAS when both SAS and EAS were available (Fig. 1A).

2.3.3. Defining spore season intensity

We also extracted metrics related to the intensity of fungal spore season from both ecological and public health perspectives. For each station-spore year, we calculated the peak and trough concentrations by taking the maximum and minimum spore concentrations in a station-spore year, respectively (Fig. 1A). Therefore, the amplitude concentration (Ca, spores m^{-3}) was defined by the difference between peak concentration and trough concentration (Fig. 1A). We calculated the total spore concentration in an ecological spore year as the annual integral (AIn, spores m^{-3} days) by multiplying the average daily concentration by 365 days (Fig. 1A). From a public health perspective, the peak concentration (Cp, spores m^{-3}) was defined as the maximum spore concentration in a station-spore year (Fig. 1A). We also defined total spore concentration in a spore allergy season as the allergy season integral (ASIn, spores m^{-3} days) by multiplying the average daily concentration during the allergy season by the LAS (Fig. 1A). We adopted the following filtering criteria on data completeness for each station-spore year to balance the quality of metric extraction and sample size (Table S2). We kept station-spore year combinations with more than 80% available data after interpolation for metrics Ca, Cp and AIn; We kept station-spore year combinations with more than 80% available data after interpolation during the allergy season for metric ASIn. After extracting all the metrics, we characterized the overall spore

season phenology and intensity across all the stations by taking the median value of each metric across all years in the study period.

2.4. Detection of temporal trends

We quantified the temporal trends of the ten fungal spore metrics at the continental US level. In order to focus on the long-term trends of the ten metrics, we included stations with five or more years of extracted metrics. To satisfy the normality assumption in subsequent statistical models, we applied a natural log transformation to all the intensity metrics in our analysis, including Ca, Cp, AIn, and ASIn. To explore the trends in metrics at each station, we used Theil-Sen linear regression of each metric against year at each station (Sen, 1968). This nonparametric method is known for its robustness to outliers and suitability for exploratory data analysis with noisy metric values. These station-level trends are presented in Fig. S6.

To capture the average temporal trends in spore metrics across all stations, we fitted the following linear mixed-effects model of each fungal spore metric against year, with random intercepts and slopes on stations:

$$y_{it} = (\beta_0 + b_{0i}) + (\beta_1 + b_{1i})x_{it} + \varepsilon_{it} \quad (1)$$

where the response y_{it} is each fungal spore metric (intensity metrics Ca, Cp, AIn, and ASIn were log-transformed) in each station i, year t, while the independent variable x_{it} here is year t. A fixed intercept β_0 indicates the average spore metric across all stations when x_{it} is zero, b_{0i} is the random intercept for station i, capturing the station-specific deviation from the overall average when x_{it} is zero, the fixed slope, β_1, quantifies the average temporal trend in the fungal spore season metric per year across all stations, b_{1i} is the random slope for station i, accounting for the variability in the rate of change specific to that station, random error ε_{it} is assumed to be independent and normally distributed with mean 0 and variance σ^2.

We then used the model estimated parameters and predicted random effects to predict both overall trends and station-specific trends:

$$\hat{y}_{it} = (\hat{\beta}_0 + \hat{b}_{0i}) + (\hat{\beta}_1 + \hat{b}_{1i})x_{it} \quad (2)$$

where $\hat{y}_{it}$ is the predicted fungal spore season metric at station i in year t, the term ($\hat{\beta}_0$ + $\hat{b}_{0i}$) predicts the fungal spore season metric at station i when x_{it} is zero, the term ($\hat{\beta}_1$ + $\hat{b}_{1i}$) predicts the temporal trend in the spore metric at station i per year, x_{it} is the year. These predicted continental and station levels trends will be presented in Fig. 4.

2.5. Assessment of climate change impact

The varying rates of climate change across the continental US, including divergent patterns of warming and drying (Portmann et al., 2009), may alter fungal spore season phenology and intensity in different areas. To investigate this, we analyzed the relationship between climate and observed metrics at each station. For each station-spore year, we averaged daily mean temperature (°C) to calculate the mean annual temperature (MAT, °C). Total annual precipitation (TAP, mm) was derived by integrating daily precipitation (mm) within each spore year. To analyze the effect of climate change on metric trends, we fitted linear mixed-effects models sharing the same structure with model (1), except using MAT_{it} (°C) or TAP_{it} (mm) at station i in year t as the independent variable x_{it}, respectively. The fixed slope, β_1, represents the average sensitivity of the spore metric to changes in climatic variables across all stations, b_{1i} is the random slope for station i, accounting for the variability in the sensitivity of the fungal spore season metric specific to that station. Using equation (2) above, we predicted the fungal spore season metric under the climatic condition x_{it} for each station and time point. The prediction can be found in Fig. S7. All computations were carried out using R 4.2.0 (Bates et al., 2014; Pinheiro & Bates, 1995).

3. Results

3.1. Fungal spore season characteristics

Spatial variations in spore seasonality, encompassing both timing and magnitude, were evident among different stations, as presented in Fig. 2. Stations characterized by warmer, humid summers and cold, dry winters, such as Dayton, OH, Berkeley, MO, and Oklahoma City, OK, experienced an increase in airborne spore concentrations typically at the onset of the calendar year (Fig. 2A). Conversely, stations situated within the Mediterranean California ecological region, known for its hot, dry summers and mild winters, displayed a different pattern, where winter and spring spore concentrations surpassed those observed in the summer. At these stations, a notable increase in spore concentrations began around the middle of the calendar year (Fig. 2A). The observed mismatch in seasonal patterns underscore the necessity for station-specific definitions of the "spore year" to account for the unique cycles at each location. Stations with distinct precipitations showed variability in spore season intensity. Stations such as Tampa, FL, Houston, TX, and Sarasota, FL, with higher annual precipitations, experienced higher spore concentrations throughout the year (Fig. 2B). Alternatively, stations receiving less precipitation, such as La Jolla, CA and Las Vegas, NV, tended to record lower spore concentrations (Fig. 2B). The calendars of all the stations are presented in Fig. S5.

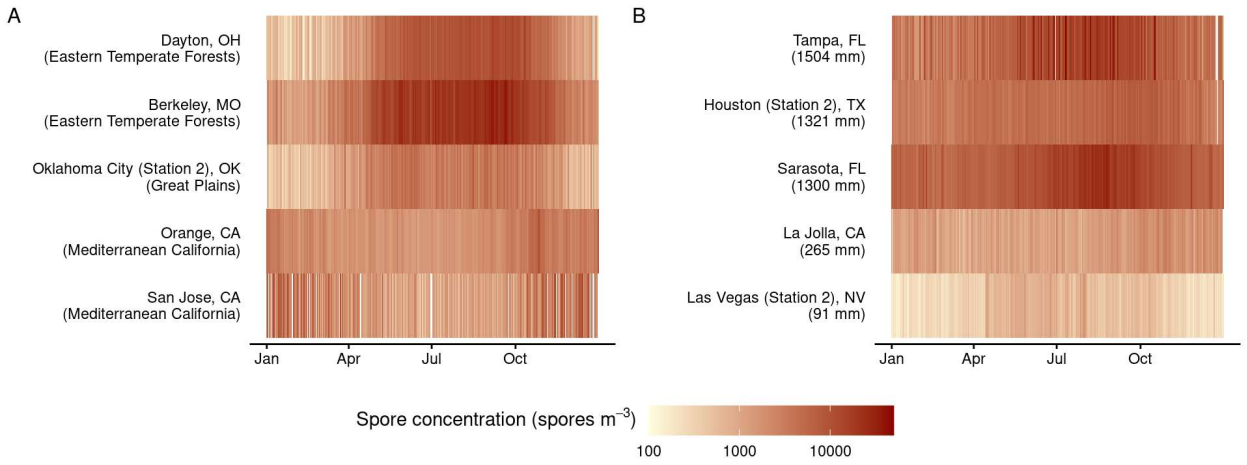

Fig. 2 Fungal spore calendar for ten sampling stations. The daily long-term mean of fungal spore concentration is displayed, 2003-2022. Darker colors indicate higher concentrations, while missing data are represented in white. (A) Stations show variable seasonality across ecological regions. (B) Stations with higher long-term mean of total annual precipitation (mm) show higher intensity of fungal spore seasons.

After defining the spore year for each station, we observed the overall spore season characteristics across the continental US. From an ecological perspective, the spore season lasted a median of 198 days, with a median SOS of 85 day of spore year (DOY) and a median EOS of 287 DOY (Fig. 1C). From a public health perspective, the allergy season started at 81 DOY and ended at 266 DOY, resulting in a median LAS of 162 days (Fig. 1C). The spore season intensity had a median amplitude concentration of 8,538 spores m^{-3} and a median peak of 9,009 spores m^{-3} (Fig. 1B). The median AIn and ASIn were 1.36 million spores m^{-3} days and 1.75 million spores m^{-3} days, respectively (Fig. 1D). The ecological and public health approaches lead to similar estimates of timing and quantities of spore seasons.

3.2. Temporal trends of spore metrics

Thirteen to 28 stations for each metric were included in our analysis (Table S1). We observed spatial variations in station-level trends of metrics. For the metric SAS, eighteen out of 28 stations, including San Jose, CA, Twin Falls, ID, and Oklahoma City, OK, experienced, on average, anadvanced allergy season over time (Fig. 3). Conversely, the SAS in ten stations, such as Vancouver, WA, and Olean, NY, was delayed, on average, over time (Fig. 3). We also identified spatial variabilities in the trends of other metrics across different stations (Fig. S6). For instance, the annual spore integral in fifteen out of 22 stations decreased, on average, over time, while seven stations were exposed to higher annual spore integral over time (Fig. S6).

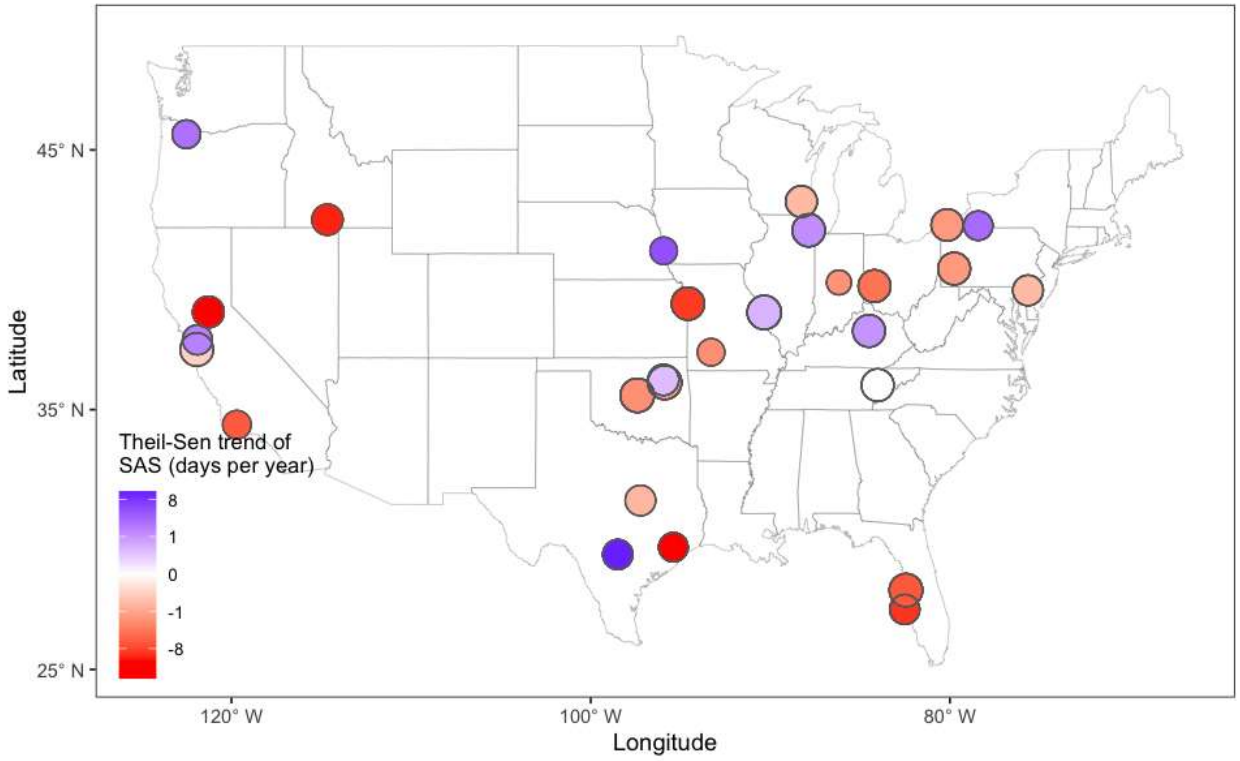

Fig. 3 Station-level temporal trends of the start of allergy season (SAS). The trends were estimated by Theil-Sen linear regression. Warm colors indicate earlier seasons, and circle sizes are proportional to the years of data at each station.

Across the continental US, we observed an overall significant advancing trend in the onset of spore seasons, using both the ecological and the public health approach for calculating season start (SOS and SAS, respectively; Fig. 4A, Fig. 4F). Between 2003 and 2022, we detected an advance in the SOS of 11 days, on average, across the continental US, corresponding to a rate of 0.59 (95% confidence interval: 0.02 ~ 1.16) days year^{-1} ($p < 0.05$; Fig. 4A, Fig. 5). The SAS in the continental US advanced significantly at the rate of 1.64 (0.76 ~ 2.53) days year^{-1} ($p < 0.05$), equivalent to an advancement of 31 days from 2003 to 2022 (Fig. 4F, Fig. 5). In contrast, no significant shifts were found in the end of the spore season (EOS) or the end of the allergy season (EAS). The EOS and EAS only moved earlier by a statistically insignificant 0.012 days and ten days, respectively, over the two decadal period ($p = 1$ for EOS, $p = 0.32$ for EAS; Fig. 4B, Fig. 4G, Fig. 5). Length of the season changes were less pronounced, with LOS and LAS both showing non-significant extensions of 11.6 days and 16 days over the two decadal period, respectively, occurring at a rate of 0.61 (−0.13 ~ 1.35) days per year for LOS ($p = 0.1$; Fig. 4C, Fig. 5) and 0.84 (−0.74 ~ 2.42) days per year for LAS ($p = 0.3$; Fig. 4H, Fig. 5). Regarding the intensity of the spore seasons, Ca exhibited significant decline across the continental US over the study period (Fig. 4D), while Cp, AIn, and ASIn all declined insignificantly (Fig. 4I, Fig. 4E, Fig. 4J).

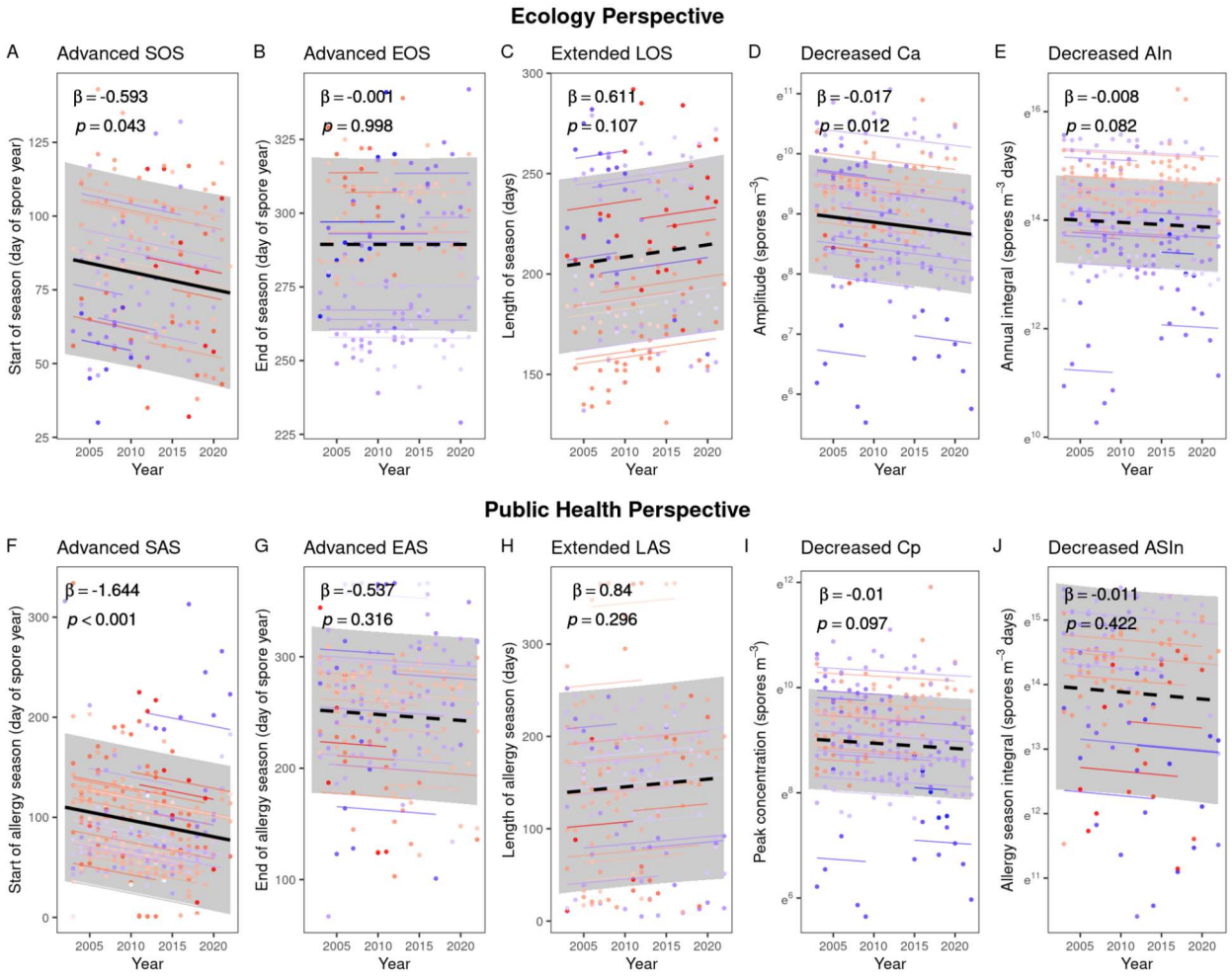

Fig. 4 Temporal trends of ten fungal spore metrics across all the stations. The estimated trends from linear mixed effects models across stations of year against the start of season (SOS; A), start of allergy season (SAS; F), end of season (EOS; B), end of allergy season (EAS; G), length of season (LOS; C), length of allergy season (LAS; H), amplitude (Ca; D), peak concentration (Cp; I), annual integral (AIn; E), and allergy season integral (ASIn; J). Solid black lines indicate significant ($p < 0.05$, *t*-test) slopes. Shaded areas indicate the 95% confidence intervals of the fixed effect. Points are individual years at individual stations. Colors are station-level Theil-Sen linear regression slopes, with warmer colors indicating earlier day of year, longer days, or higher intensity. The slope of colorful lines are model predicted station-level trends. The top row presents metrics defined in the ecological method, while the bottom row shows metrics defined in the public health method. Intensity metrics are transformed using the natural logarithm.

3.3. The relationship between spore season shifts and climate change

We identified significant correlations between several pairs of metrics and climate variables, as shown in Fig. 5. Our findings indicate a significant negative correlation between temperature and SOS, implying that higher temperatures may be associated with an earlier onset of the spore season ($p < 0.05$; Fig. 5). Specifically, a one-degree Celsius increase in MAT is projected to advance the SOS by 2.2 days (95% confidence interval: 0.98 ~ 3.43; Fig. 5). Later EOS was significantly related to warmer conditions: a one-degree Celsius increase in MAT caused a delay of 1.97 (0.86 ~ 3.08) days in EOS ($p < 0.05$; Fig. 5). Extended LOS was also significantly correlated with warmer temperatures, with an extension of 4.27 (2.3 ~ 6.25) days per degree Celsius increase in MAT ($p < 0.05$; Fig. 5). However, rainfall had opposite impacts on the phenology of spore season and allergy season. Wetter years were correlated with later SOS and shorter LOS, with a delay of 0.02 (0.01 ~ 0.03) days in SOS and a shortening of 0.02 (0.002 ~ 0.03) days in LOS per mm increase in TAP ($p < 0.05$; Fig. 5). Conversely, more precipitation was characterized by earlier SAS and longer LAS ($p < 0.05$; Fig. 5). Numerically, a one-mm increase in TAP was associated with 0.04 (0.02 ~ 0.06) days of advancement in SAS and 0.06 (0.03 ~ 0.09) days of extension of LAS (Fig. 5). Both the annual spore integral and the allergy season integral were positively correlated with total annual precipitation ($p < 0.05$; Fig. 5).

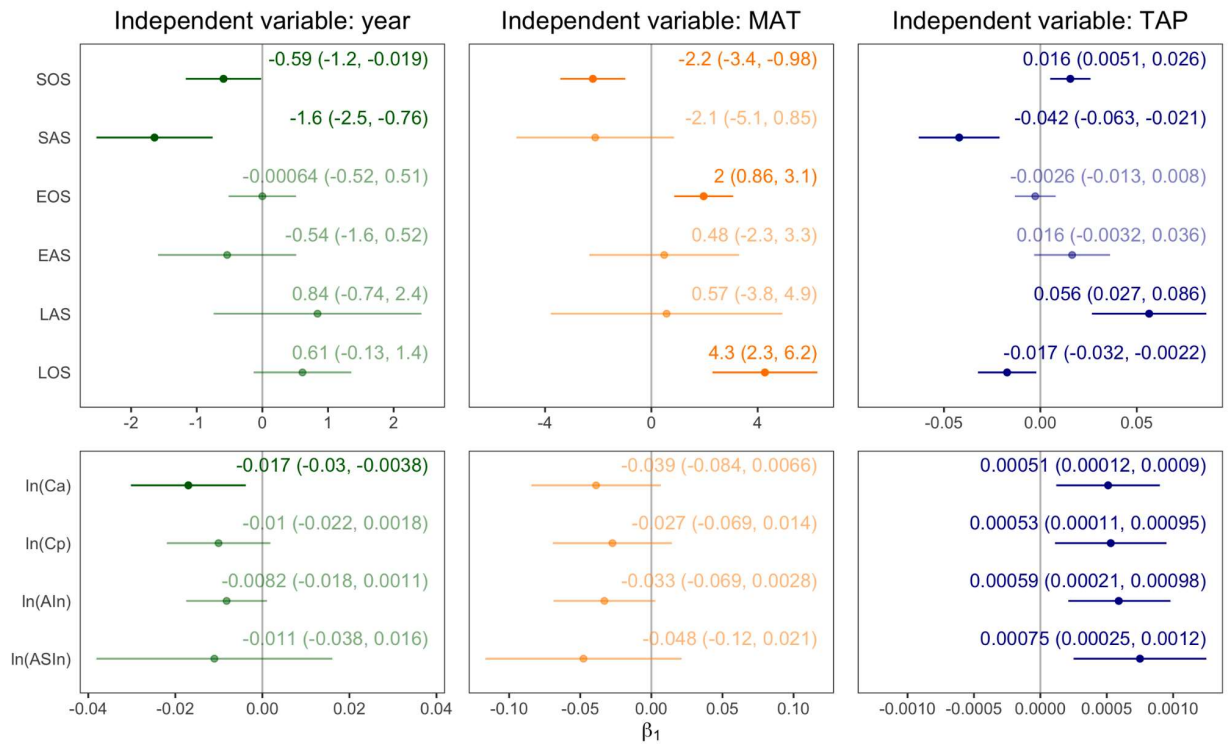

Fig. 5 Summary of coefficients β_1 in three mixed-effects models for each metric. Points are estimated values of β_1. Error bars indicate the 95% confidence intervals of the fixed slope. Specific values are annotated. Colors are different independent variables in the model. Non-transparent colors present significant coefficients ($p < 0.05$, *t*-test).

4. Discussion

Our analysis supports the hypothesis of a multi-decadal, continental-scale shift in fungal spore phenology and intensity under climate change. Specifically, over the period from 2003 to 2022, across the continental United States, we identified a significantly advancing start of fungal spore seasons—defined both by when fungal spore concentrations exceeded some percentile value of total annual concentrations and by when fungal spore concentrations exceeded the NAB-defined allergy threshold—and a non-significant extending length of seasons. Additionally, our analysis shows a significant decrease in the amplitude, and non-significant decreases in the peak concentration, annual integral, and allergy season integral. Notably, these observed trends were

found to be strongly correlated with climate change, particularly warming temperatures and altered precipitation. These results contribute to a growing body of evidence suggesting the impacts of climate change on phenological shifts of organisms, especially microorganisms, with implications for both ecology and public health.

4.1. Shifts in fungal spore seasonality

Our findings revealed notable shifts in fungal spore phenology across the continental US. Using two different, yet complementary, methods for determining seasons of elevated airborne spore concentrations, we documented a significant earlier onset of both the ecological spore season and the spore allergy season, along with a non-significant trend towards longer durations, although the extension of the seasons was not statistically significant (Fig. 4A, Fig. 4F, Fig. 4C, Fig. 4H). We did not observe significant changes in peak concentration, annual spore integral, and allergy season integral over time (Fig. 4). Our observations aligned with published research from other regions. Notably, a study conducted over 19 years in Northern Italy highlighted a shift towards earlier onset of the spore season by 0.7 days annually and an expansion of season length by 1~2 days each year—findings that closely mirror our own (Marchesi, 2020). Studies from Poland and Slovakia reported more pronounced shifts, finding that the start of the spore season advanced four and 2.5 days per year, respectively, with the spore season lengthening at a rate of five or 8.2 days annually for *Alternatia* and *Stemphylium*, respectively, in Slovakia (Grinn-Gofroń et al., 2016; Ščevková et al., 2016). Moreover, the decreasing trends in peak spore concentrations and annual integrals have received support from multiple studies (Damialis et al., 2015; Grinn-Gofroń et al., 2016; Marchesi, 2020; Olsen et al., 2020). On the other hand, contrasting patterns have been observed in other studies. A study in Greece noted a delayed spore season onset (Damialis et al., 2015), while research in Denmark recorded no clear trends in the timing or

duration of the spore season (Olsen et al., 2020). Opposite trends in spore season intensity, such as increases in spore counts over time, have also been documented in long-term studies spanning 13 to 34 years in locations like Slovakia, the UK, and France (Millington & Corden, 2005; Ščevková et al., 2016; Sindt et al., 2016). The inconsistencies across studies may arise from methodological variations. Many previous studies have centered their analysis on data from a single monitoring station, which could introduce biases and limit the generalizability of their conclusions. Our study of start and length of season expanded on this by incorporating data from 16 different stations throughout the continental United States, offering a broader perspective on the phenomenon. These differences highlight the sensitivity of fungal life cycles to climate conditions and the regional phenological differences this creates. Our research emphasizes the need for comprehensive and methodologically consistent data collection across extensive geographic areas to fully understand fungal spore phenology and its implications for ecosystems, human health, and climate change.

The analysis of airborne fungal spores in our study contributes to the broader field of aerobiology, which also encompasses significant airborne allergens such as pollen. We can further contextualize our findings by comparing them with a recent study examining pollen trends across the continental U.S., which, like this study, also utilized the data from the National Allergy Bureau (NAB). This research documented a shift towards earlier pollen season onset, with start dates advancing by ~20 days and a season duration increase of ~8 days over the 28 year period spanning 1990 to 2018 (Anderegg et al., 2021). These changes mirror the phenological shifts in fungal spore seasons observed in our research qualitatively and quantitatively, highlighting parallel trends in pollen and spore phenologies. However, in contrast to our results, an increase in the pollen annual integral was reported (Anderegg et al., 2021).

Further contextualization in global change biology comes from comparing our research with phenological studies on macroorganisms like plants and insects. Prior research has identified an overall trend towards earlier spring events across various taxonomic groups, with an average rate of 0.28 days per year (Parmesan, 2007). More specifically, tree phenology has been advancing by 0.33 days per year, while butterfly spring phenology has shown an advancement of 0.37 days per year (Parmesan, 2007). The rate at which these macroorganisms are experiencing spring advancements is in line with the advancement rate we have found in fungal spores, which are produced by microorganisms. This similarity illustrates that fungal spore phenology can serve as a bioindicator of global environmental change, paralleling that of more conspicuous macroorganism phenology. Such findings underscore the potential of fungal spores to be sensitive proxies for monitoring and understanding the effects of climatic shifts on ecological systems.

4.2. Climate change impacts on spore season shifts

Our investigation has demonstrated significant correlations between the seasonality of fungal spores and climatic variables. We found a marked trend of earlier spore season onset with increasing temperatures (Fig. 5), consistent with similar studies in Europe (Anees-Hill et al., 2022). There is established evidence of a strong positive correlation between spore concentrations and mean temperature (Burch & Levetin, 2002; Troutt & Levetin, 2001), suggesting a more rapid accumulation of spores in the atmosphere under global warming, possibly reaching predetermined thresholds or percentages sooner than before. This correlation aligns with mycological research indicating that key fungal life cycle events occur earlier as a result of global warming, such as the advancement and extension of the green season which provides more favorable conditions for fungal growth. For example, related studies have

observed earlier fungal fruiting in response to warmer temperatures (Kauserud et al., 2012; Klironomos et al., 1997; Troutt & Levetin, 2001).

Moreover, we found that increased rainfall correlates with an earlier start and an extended duration of the allergy season, as well as higher spore concentrations (Fig. 5). This aligns with the findings of a century-long North American mycological study that suggests heightened moisture accelerates fungal fruiting (Diez et al., 2013). Although some research argues that rainfall might reduce airborne spore levels by causing a washout effect (Katial et al., 1997; Sindt et al., 2016), this perspective primarily considers the direct impact of daily precipitation on daily spore counts. In contrast, the inherent moisture from rainfall can encourage fungal growth and sporulation (Kendrick, 2017), potentially leading to elevated spore levels in the air (Ganthaler & Mayr, 2015). Thus, the net effect of increased precipitation can promote higher overall spore concentrations, despite a possible short-term negative correlation between rainfall and spore density. Our analysis extends beyond the daily impact, analyzing the relationship between total annual precipitation and annual spore metrics, which helped us highlight the impact of precipitation regime on an interannual scale rather than the impact of rainfall on a daily scale. However, higher moisture could result in a delayed ecological spore season onset and a contraction of the season. Such conditions suggest a compression of airborne spore presence around the peak period. Therefore, we propose that moist environments may lead to a concentration of spore activity within a narrower timeframe, emphasizing the complex interplay between rainfall patterns and spore dynamics.

4.3. Implications for ecology, public health, and climate change

The observed shifts in fungal spore phenology and seasonal intensity bear important implications for ecological systems, public health, and climate change mitigation strategies. Ecologically,

fungi are crucial in processes such as decomposition, nutrient cycling, and shaping plant diseases. Alterations in the timing and volume of spore distribution may lead to disruptions in these processes, with possible consequences for ecosystem function and biodiversity. An asynchrony between the phenology of fungi and the life cycles of their host plants could profoundly affect plant-fungal interactions (Song et al., 2023). Additionally, with many insects relying on fungi for nutrition (Douglas, 2009), a shift in spore availability could create cascading effects through the food web, affecting not only these insects but also the broader array of species that interact with them, including predators, parasites, and their plant hosts. Our findings, therefore, provide far-reaching implications of fungal spore season changes for ecosystems.

From a public health standpoint, these alterations are meaningful. Despite fungal spores being major air allergens, their study has been overshadowed by pollen research. Evidence suggests that health impacts associated with fungal spore exposure, such as hospitalization risks, lung function decline, and enhanced morbidity, may outweigh those linked to pollen in asthmatic individuals (Hughes et al., 2022). With fungal spore seasons potentially lasting twice as long as pollen seasons and prolonged exposure spanning several months (Hughes et al., 2022), the extended window of susceptibility is notable (Hughes et al., 2022). It is worth mentioning that atmospheric fungal spore concentrations can exceed pollen levels by orders of magnitude (Hughes et al., 2022). Notably, our research introduces a unique method by assessing allergy seasons through specific spore allergy thresholds, thereby bridging the gap between ecological conditions and public health imperatives.

We have observed trends indicating the onset of earlier and longer allergy seasons that could affect respiratory health, particularly in vulnerable populations (Fig. 4F, Fig. 4H). Such changes can weaken current allergy management practices, as individuals may unknowingly

encounter allergens outside the traditionally expected time window, leading to unforeseen, severe allergic reactions. Public health entities must therefore integrate these temporal shifts when developing awareness campaigns and advising on allergy prevention measures. Extended exposure to fungal spores tends to exacerbate allergic symptoms over longer periods, potentially heightening the incidence of allergic rhinitis, aggravating asthma symptoms, and increasing hospital admissions. Our study can serve as an alert to both patients and clinicians about the crucial role of fungal spores in allergy pathogenesis, thereby reinforcing its critical contribution for public health advocacy.

Beyond the importance of fungal spores for allergies, certain species of environmentally dwelling fungi produce infectious arthroconidia that can cause severe disease in humans when inhaled. Invasive fungal infections are on the rise globally and are of particular public health concern given high mortality rates, limited therapeutics and no vaccines, and rising antifungal resistance (Fisher & Denning, 2023). Geographic expansion of many human-pathogenic fungal species has been documented - including *Coccidioides*, *Blastomyces, Histoplasma,* and *Crypotoccoccus* spp., potentially associated with climate change (Gorris et al., 2019; Mazi et al., 2023; Nnadi & Carter, 2021). Warming temperatures may cause such pathogenic fungi to produce more spores and/or spend more time in these more thermotolerant latent structures (Frías-De-León et al., 2022). For instance, lengthened duration of spore production by *Histoplasma capsulatum* as well as *Microsporum* and *Trichophyton* genera, which cause histoplasmosis and dermatophytosis, respectively, have been observed (Frías-De-León et al., 2022; Jr, 2018; Nnadi & Carter, 2021). While we were unable to examine spore concentration for specific pathogenic species in this study, the findings here nonetheless may help inform our understanding of the transmission of invasive fungal infections.

Ultimately, our study stands out by addressing an important gap in allergen and phenological research, specifically regarding microorganism—fungal spores. While the focus has traditionally been on macroorganisms to assess the impact of climate change on biological systems, our findings underscore the sensitivity of microorganisms to climate change. This is a significant insight, as fungal spores, much like larger organisms, respond to the climate variability. By paralleling the shifts in allergenic fungal spores with those seen in macroorganisms, we contribute to the rising evidence that supports the use of a broad spectrum of biological indicators – from the macroscopic to the microscopic – to monitor and understand the complexities of climate change. This study heightens the urgent need for cross-disciplinary initiatives in climate change mitigation and adaptation, informed by a more inclusive set of biological markers and health risk assessments. This stresses the shared responsibility of the biological and public health communities to respond to these environmental challenges, fostering resilient biological systems and safeguarded public health in an era of climate change.

4.4. Limitations

While our investigation provides valuable contributions to understanding fungal spore trends amid climatic impacts, it is necessary to acknowledge several limitations and identify directions for future work. One limitation is our focus on total daily spore concentrations, which aggregates data across all fungi genera. By not examining the community structure of the fungal spores, we overlook the distinct responses that different fungal taxa, such as dry or wet adapted fungi, may have to varying climate conditions. Future research should aim to disaggregate the spore concentration data, where possible, assessing trends at the genus level or even species level when identification is feasible. This approach could provide a more detailed understanding of how different fungal communities are responding to climate change, enabling targeted ecological and

public health interventions. Secondly, although this study is the most comprehensive analysis of its kind in the continental United States, the relatively small number of monitoring stations, coupled with missing data at some stations, limits our capacity to attribute spore phenological shifts to climate change. Expanding the network of spore monitoring sites will enhance the geographic representation, thereby enhancing the statistical strength of future analyses. Lastly, the varied time spans represented by the stations in our dataset can present another limitation. Stations with shorter durations of data collection may not capture the full impact of climate changes and could reduce the analytical power for detecting long-term phenological shifts. To address this, future efforts should intensify in maintaining existing stations and establishing new ones with the goal of achieving long-term, consistent data collection that can robustly track climate trends and impacts.

4.5. Conclusions

In summary, we identify advancing fungal spore season onset and extending season length, as well as decreased concentrations over the past two decades across the continental United States. These trends in fungal spore metrics significantly correlate with climate change, particularly altered temperature and precipitation. These trends have the potential to disrupt ecological systems and intensify public health issues related to allergies and respiratory illnesses. Therefore, we call for greater attention towards fungal spore research in climate change studies. Furthermore, given the potential health impacts, it also emphasizes the importance of enhancing public awareness and preparing the health sector for these shifts under climate change.

Appendices

Table S1: Summary of trend and observations included analysis for each metric. Displayed is the number of stations and observations included in the model, the slope of spore metrics with log-transformation for intensity metrics, change over 2003-2022 in day for timing metrics and % for intensity metrics(back-transformed), and the p-value of the mixed effects model for each metric, including start of spore season (SOS), start of allergy season (SAS), end of spore season (EOS), end of allergy season (EAS), length of spore season (LOS), length of allergy season (LAS), amplitude concentration (Ca), peak concentration (Cp), annual integral (AIn), and allergy season integral (ASIn).

Metric	Number of stations	Number of observations	Slope	Change	p-value
SOS	16	163	–0.5925	–11.26d	0.0431
SAS	28	321	–1.6437	–31.23d	0.0003
EOS	16	163	–0.0006	–0.01d	0.998
EAS	21	208	–0.537	–10.2d	0.316
LOS	16	163	0.6108	+11.61d	0.1067
LAS	20	196	0.8404	+15.97d	0.2964
ln(Ca)	20	221	–0.017	–27.62%	0.0118
ln(Cp)	21	246	–0.0101	–17.42%	0.097
ln(AIn)	22	266	–0.0082	–14.46%	0.0822
ln(ASIn)	13	144	–0.011	–18.91%	0.4224

Table S2: Sensitivity test on data completeness for four fungal spore season intensity metrics. Displayed is the number of observations included in the model, the slope of spore metrics with log-transformation, change over 2003-2022 in % for concentrations (back-transformed), and the *p*-value of the mixed effects model for each intensity metric, including amplitude concentration (Ca), peak concentration (Cp), annual integral (AIn), and allergy season integral (ASIn).

Metric	Data completeness	Number of observations	Slope	Change	p-value
ln(Ca)	100%	161	–0.0118	–20.12%	0.137
	90%	194	–0.0155	–25.54%	0.0257
	80%	221	–0.017	–27.62%	0.0118
	70%	233	–0.0145	–24.13%	0.0256
	60%	254	–0.0115	–19.63%	0.0705
ln(Cp)	100%	162	–0.0103	–17.73%	0.1548
	90%	213	–0.0104	–17.90%	0.1007
	80%	246	–0.0101	–17.42%	0.097
	70%	265	–0.0083	–14.54%	0.1546
	60%	372	–0.0083	–14.57%	0.1047
ln(AIn)	100%	163	–0.0067	–11.95%	0.2307
	90%	222	–0.0075	–13.33%	0.1277
	80%	266	–0.0082	–14.46%	0.0822
	70%	292	–0.0122	–20.69%	0.0198
	60%	403	–0.0069	–12.32%	0.1182
ln(ASIn)	100%	105	–0.0101	–17.54%	0.5008
	90%	128	–0.0095	–16.45%	0.5075
	80%	144	–0.011	–18.91%	0.4224
	70%	152	–0.0066	–11.80%	0.6163
	60%	175	0.0083	+17.13%	0.5068

Table S3: Temporal trends in the relative abundance of two main taxa across all the stations.

Taxa	**Random effects**	**SD**			
Unidentified	Intercept	0.16			
	Year	0.000076			
	Residual	0.16			
	Fixed effects	**Estimate**	**SE**	**t-value**	**p-value**
	Intercept	4	2.7	1.5	
	Year	–0.0018	0.0013	-1.3	0.18
Cladosporiaceae	**Random effects**	**SD**			
	Intercept	0.23			
	Year	0.000022			
	Residual	0.16			
	Fixed effects	**Estimate**	**SE**	**t-value**	**p-value**
	Intercept	0.098	2.7	0.036	
	Year	0.00021	0.0013	0.15	0.88

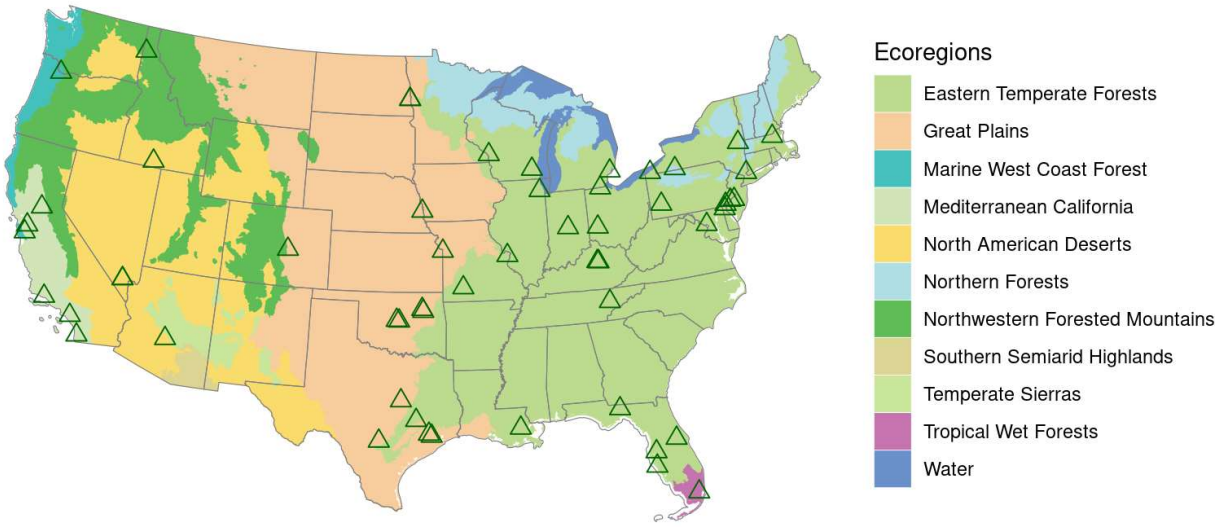

Figure S1: Map of 55 fungal spore counting stations associated with the National Allergy Bureau (NAB) distributed in different ecoregions within the continental United States that were included in the analysis of this study.

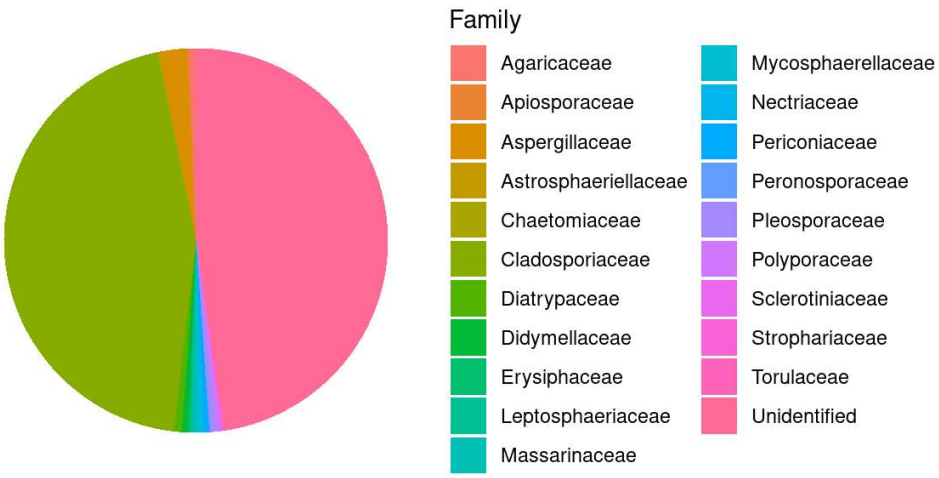

Figure S2: Relative abundance of each spore family during the study period across all the stations.

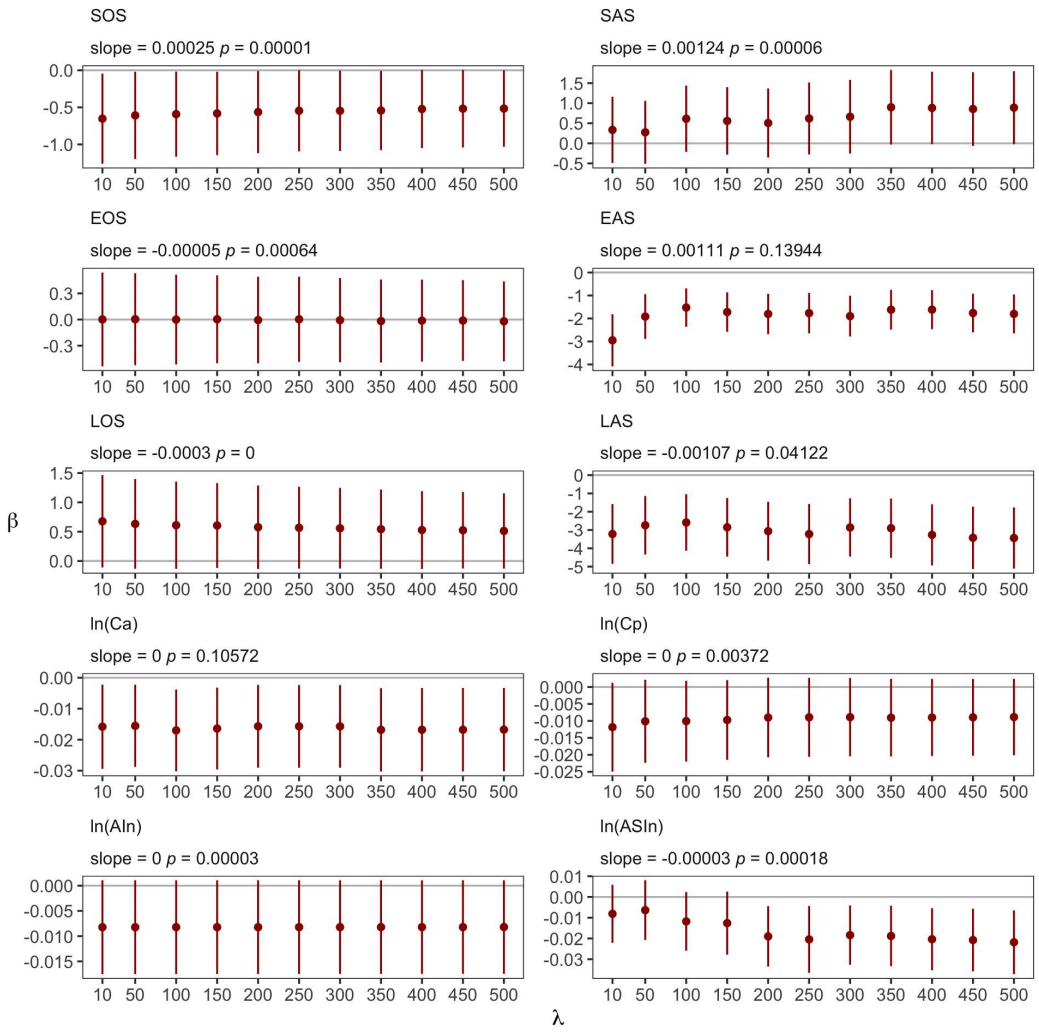

Figure S3: Sensitivity test on the smoothness parameter, lambda, applied in Whittaker-Henderson smoothing for ten fungal spore season metrics. The lambda was varied from ten to 500. This analysis confirmed that the observed trends in spore season were similar across the range of smoothing parameters tested. Points are estimated values of β_1 in the linear mixed-effects models for trend detection. Error bars indicate the 95% confidence intervals of the fixed slope. Annotated slope and *p*-value describe the linear regression result of fixed slopes against lambda (*t*-test). Metrics defined in ecological approach are in the left panel, while metrics defined in the public health approach are in the right panel. Colors are different independent variables in the model.

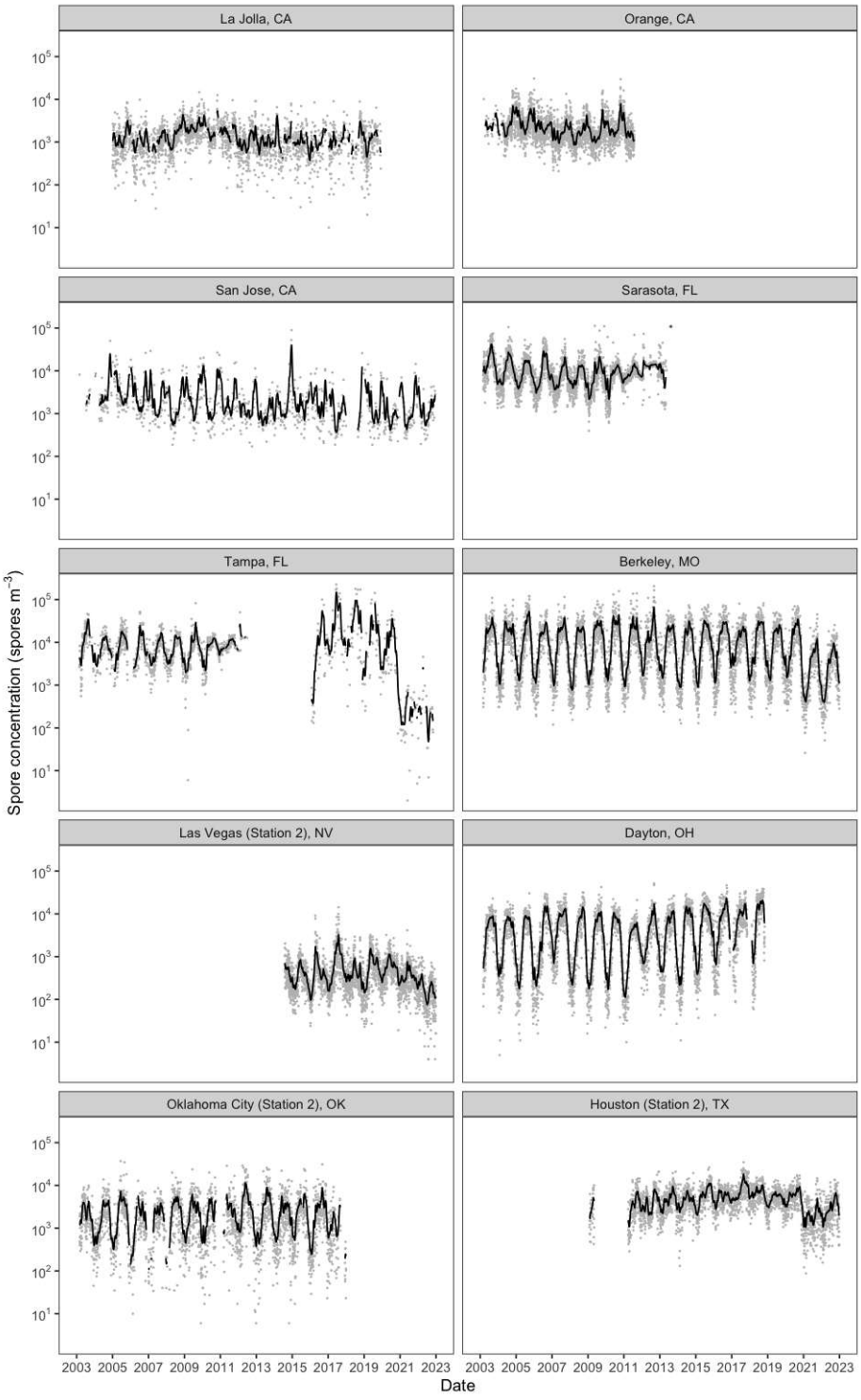

Figure S4: Spore curves of pre-processed data.

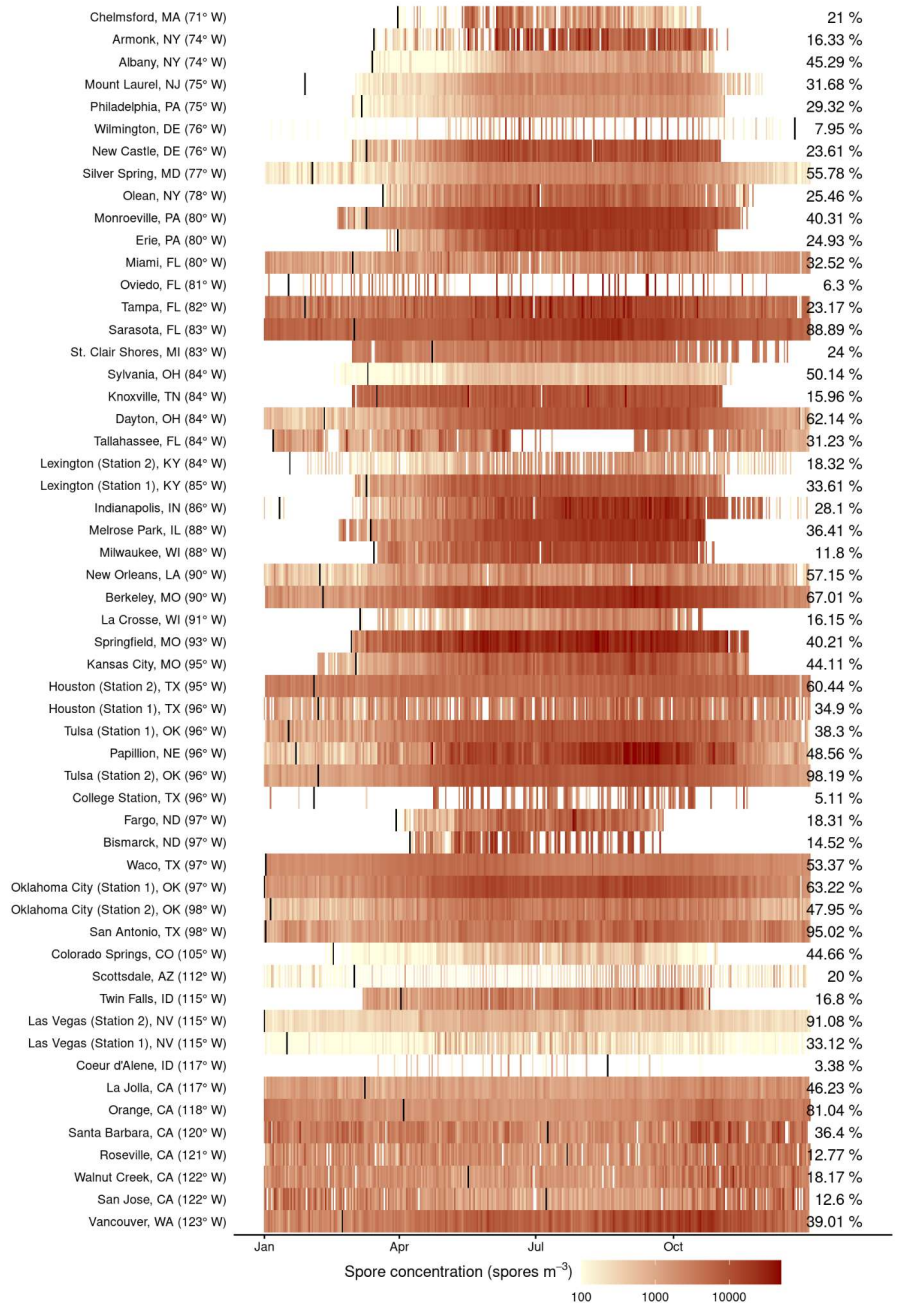

Figure S5: Fungal spore calendar for all the 55 stations meeting our inclusion criteria. Displayed is the daily long-term mean of fungal spore concentration, 2003-2022. Darker colors indicate higher concentrations, while missing data are represented in white. Vertical black lines are the start of the spore year. Annotated numbers are the average data availability across available years for each station. Stations are ranked by longitude.

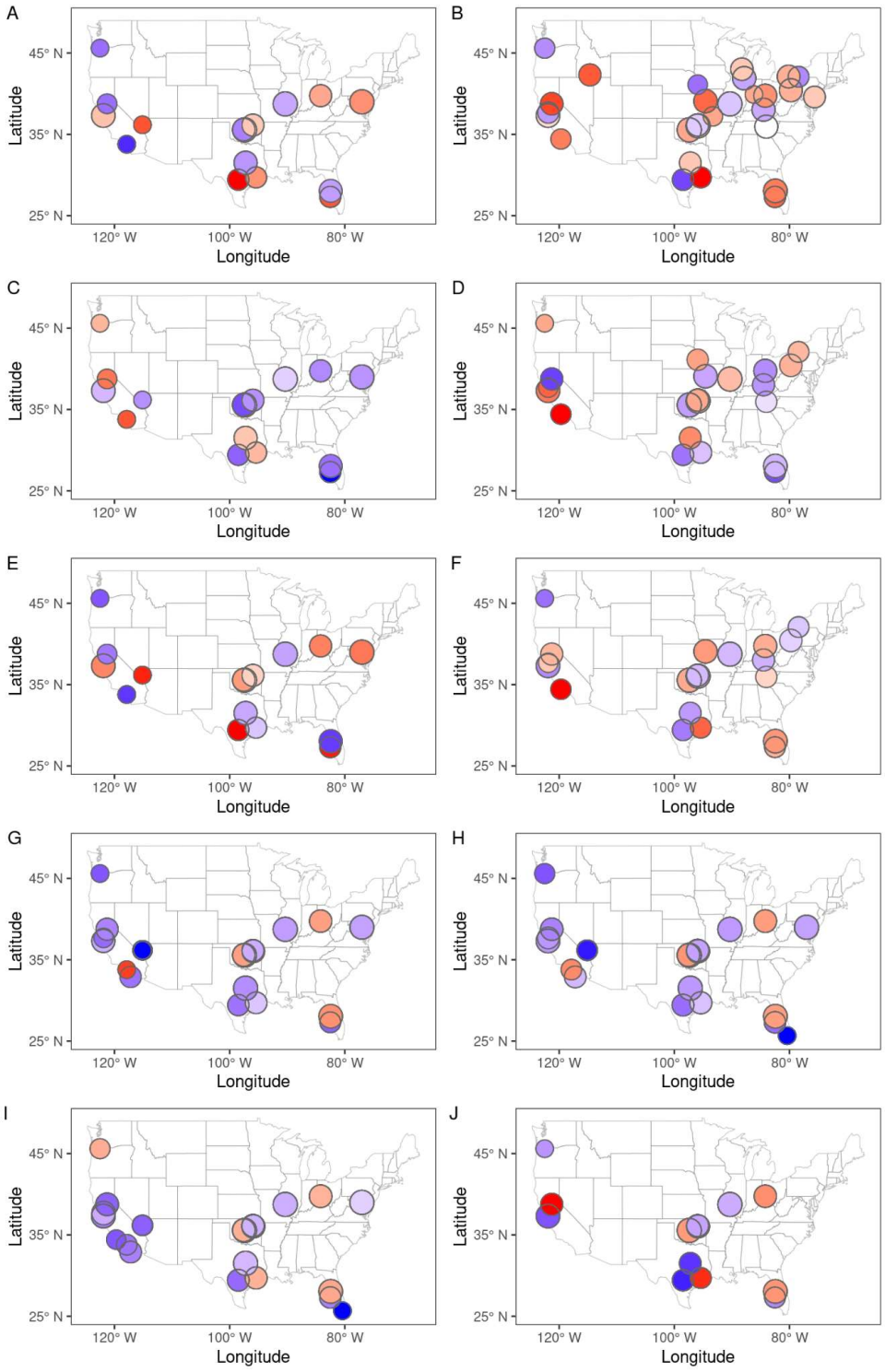

Figure S6: Station-level temporal trends of the start of spore season metrics. The trends were estimated by Theil-Sen linear regression. Redder colors indicate earlier DOY, longer days, or higher concentration, and circle sizes are proportional to the years of data at each station.

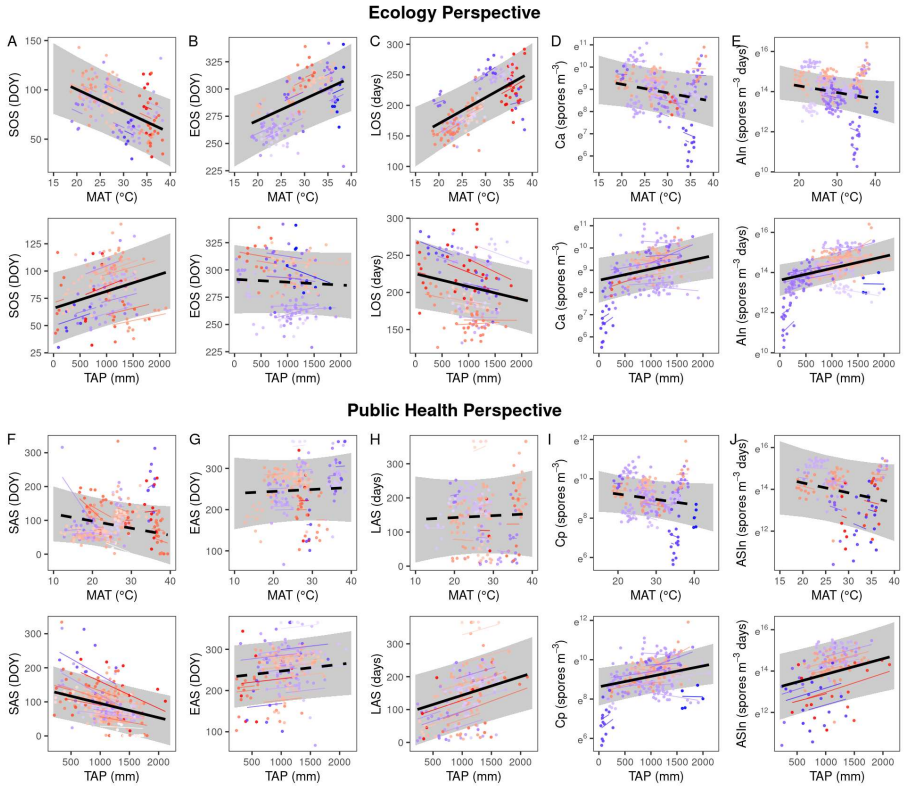

Figure S7: Correlation between fungal spore metrics and climate variables. Black lines are predicted slopes from linear mixed effects models across all stations. Warming correlated with the advanced start of ecological season (SOS; A) and the extended length of ecological season (LOS; C). Wetting correlated with the delayed start of ecological season (SOS; A), the advanced start of allergy season (SAS; F), the shortened length of ecological season (LOS; C), the extended length of allergy season (LAS; H), and the increased annual integral (AIn; E) and allergy season integral (ASIn; J). Solid black lines indicate significant ($p < 0.05$, *t*-test) slopes. Shaded areas indicate the 95% confidence intervals of the fixed effect. Points are individual years at individual stations. Point/line colors are stations. Colors are station-level Theil-Sen linear regression slopes, with warmer colors indicating earlier day of year, longer days, or higher intensity. The slope of colorful lines are model predicted station-level correlations. Intensity metrics are transformed using the natural logarithm.

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