Climate change impact on fungi in the atmospheric microbiome

Running title: Atmospheric microbiome composition

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Abstract

The atmospheric microbiome is one of the least studied microbiomes of our planet. One of the most abundant, diverse and impactful parts of this microbiome is arguably fungal spores. They can be very potent outdoor aeroallergens and pathogens, causing an enormous socio-economic burden on health services and annual damages to crops costing billions of Euros. We find through hypothesis testing that an expected warmer and drier climate has a dramatic impact on the atmospheric microbiome, conceivably through alteration of the hydrological cycle impacting agricultural systems, with significant differences in leaf wetness between years (p-value <0.05). The data were measured via high-throughput sequencing analysis using the DNA barcode marker, ITS2. This was complemented by remote sensing analysis of land cover and dry matter productivity based on the Sentinel satellites, on-site detection of atmospheric and vegetation variables, GIS analysis, harvesting analysis and footprint modelling on

trajectory clusters using the atmospheric transport model HYSPLIT. We find the seasonal spore composition varies between rural and urban zones reflecting both human activities (e.g. harvest), type and status of the vegetation and the prevailing climate rather than mesoscale atmospheric transport. We find that crop harvesting governs the composition of the atmospheric microbiome through a clear distinction between harvest and post-harvest beta-diversity by PERMANOVA on Bray-Curtis dissimilarity (p-value <0.05). Land cover impacted significantly by two-way ANOVA (p-value <0.05), while there was minimal impact from air mass transport over the three years. The hypothesis suggests that the fungal spore composition will change dramatically due to climate change, an until now unforeseen effect affecting both food security, human health and the atmospheric hydrological cycle. Consequently the management of crop diseases and impact on human health through aeroallergen exposure need to consider the timing of crop treatments and land management, including post harvest, to minimize exposure of aeroallergens and pathogens

Introduction

Biological particles within the atmosphere have several constituent fractions, such as microorganisms, pollen and other small plant matter (Fröhlich-Nowoisky *et al.*, 2016). These form the atmospheric microbiome and this has received relatively little scientific attention compared to e.g. soil or water (Bissett *et al.*, 2017; Aalismail *et al.*, 2019; Holman *et al.*, 2019). The atmospheric microbiome originates from marine (Wilson *et al.*, 2015) and terrestrial surfaces (Cáliz *et al.*, 2018) and can have profound effects on the climate through aerosol-cloud processes (Andreae and Rosenfeld, 2008). Conversely, climate itself can have

an effect on general bioaerosol production, including microorganisms, over terrestrial and marine surfaces (Fröhlich-Nowoisky *et al.*, 2016). This thereby closes a feedback mechanism where the atmospheric microbiome depends on complex interactions between climate, vegetation and anthropogenic factors (Grinn-Gofroń *et al.*, 2019).

About 70% of the global ice-free land cover is directly affected by human use (IPCC, 2019) and substantial fractions of the terrestrial surface are managed forests or cropland. The type of land cover can influence the abundance and diversity of species present in the atmosphere as the development and decay of plant material can contribute to short term bursts of spore emissions, e.g. related to changes in osmotic pressures (Després *et al.*, 2012). Anthropogenic influences on vegetation which can affect the atmospheric microbiome include forestry, agriculture in rural regions and landscaping in the urban zone. The atmospheric microbiome largely follows the pattern of crop distributions (Bebber, Holmes and Gurr, 2014) and as climate change is moving the agricultural margins towards the polar regions so the microbiome is responding (Bebber, Ramotowski and Gurr, 2013). Initial steps have been taken to elucidate the effects of land on the atmospheric microbiome (Makiola *et al.*, 2019), but the impact of these is not well studied (Baldrian, 2017; Cavicchioli *et al.*, 2019). Reviews of the anticipated responses of fungal pathogens to climate change (e.g. Bebber, Ramorowski and Gurr (2013); Magyar *et al.* (2021); Cavicchioli *et al.* (2019)), highlight the possible outcomes of altered temperatures, UV radiation and CO_2 levels on fungal growth but do not address how altered emission of fungal spores would affect pathogen-crop dynamics pre- and post-harvest, for example by altering the overwintering capability of fungi through

increased/decreased fitness and adaptative mechanisms. Climate change has also been found to advance the harvest date within the last three decades (Ren *et al.*, 2019), but how this change in timing, warmer climate and change in water availability affect the atmospheric microbiome has not been determined.

Human exposure to allergenic fungi is dependent on spore emission into the atmosphere which occurs over several months each year (Banchi et al, 2020). Climate change is known to impact the duration of spore seasons for the allergenic fungi, *Cladosporium* and *Alternaria*, resulting in longer allergen exposure in some regions, as discussed in Anees-Hill *et al.* (2021). Furthermore, the authors also highlight several knowledge gaps in understanding of fungal spore seasonality, including the need for studies on the contribution of land use to atmospheric fungal populations. These knowledge gaps are not restricted to fungal spores. Many species within the atmospheric microbiome are impacting human health either directly or by acting in concert and exacerbating severe conditions in the respiratory system, recently exemplified by allergenic pollen correlating with the infection rate of SARS-CoV-2 infection rates (Damialis et al, 2020). Covid-19 particles are small and are not expected to airborne for extended amounts of time (Jarvis 2020, Stadnytskyi, V., et al 2020), recently supported by studies from the urban background in Leipzig, Germany (Dunker et al, 2021). However, this general statement is challenged in an editorial (The Lancet Respiratory Medicine, 2020) and with the detection of Covid-19 particles in outdoor air in Italy (Setti et al, 2020). These conflicting views covered in a Covid-19 article concerning expert views (Lewis, 2020) illustrate the knowledge gaps around bioaerosols, partly driven by difficulties in capturing and analyzing the atmospheric microbiome. A thorough understanding of the atmospheric

microbiome is clearly missing. For fungal spores the picture is particular complex as their presence and abundance is related to land use and the use of mitigation techniques (e.g. pesticide application), which impacts on human health and directly links to climate and climate change including feedback processes (Andreae and Rosenfeld, 2008; Fröhlich-Nowoisky *et al.*, 2016; Cáliz *et al.*, 2018).

As such, the microbiome is important to climate (Andreae and Rosenfeld, 2008), human health (Pulimood *et al.*, 2007; Arikoglu *et al.*, 2016) and food security (Dixon, 2012; Kettles and Luna, 2019; Rodriguez *et al.*, 2019) underlining the necessity for an understanding of how the microbiome is affected by this triangle of climate, vegetation and anthropogenic factors. Emission and deposition rates in relation to the atmospheric microbiome are poorly constrained (Randall *et al.*, 2013) and studies often limited to a single year (e.g. (Abrego *et al.*, 2018; Brennan *et al.*, 2019; Ovaskainen *et al.*, 2020). Recent developments in sampling techniques and eDNA approaches are expected to reduce this important knowledge gap (Kettles and Luna, 2019). Likewise, novel databases in the UK with sufficient information about crop land and harvesting have become available for vegetation-atmosphere studies. In particular when such databases are combined with newest remote sensing products covering large areas (Ovaskainen *et al.*, 2020). Next generation sequencing methods, such as metabarcoding, reveal entire microbial populations in any given sample (Abrego *et al.*, 2018). This was not possible with previous DNA sequencing techniques and metabarcoding may also identify species within genera which are otherwise impossible to distinguish morphologically (Brennan *et al.*, 2019). By combining

these new methods, an understanding of how the species-specific fraction of the atmospheric microbiome is connected with surface processes can fill an important knowledge gap that benefits agriculture, forestry, healthcare services, and contributes towards better predictions of climate change. We present our methodology for metabarcoding, meteorological assessments, atmospheric footprint modelling and remote sensing analysis of vegetation status, prior to evaluating the impact of climate change on the fungal component of the atmospheric microbiome through local environmental variables, atmospheric transport and land management.

Methods

Study design

The study was designed to examine the effect of climate change on fungi within the atmospheric microbiome. Climate change was defined through three areas; 1) local environmental variables, 2) atmospheric transport and 3) land management. Firstly, atmospheric DNA concentrations, dry matter productivity and fungal community compositions were examined in the study of the impact of local environmental variables on atmospheric fungi. Then atmospheric footprints were examined to study the effect of atmospheric transport on fungal community structure and finally, fungal community structure was examined in detail against

147 meteorological parameters and harvest timing to study the effect of land
148 management.

149 We collected airborne material from two sites in Worcestershire, UK, during the
150 main harvesting season over the years 2016-18. One site (Fig. 1) is classified as
151 rural (52.2544°, -2.2537°) and another as urban (52.1969°, -2.2422°). The
152 sampling period was defined as starting from the week in July when 5% of
153 cereals have been harvested according Agriculture and Horticulture
154 Development Board (AHDB)/ADAS harvesting data (AHDB, 2020) and ending
155 when 95% of cereals have been harvested (an overall period of 10 weeks).

156 Interactions between atmospheric fungi and environmental variables were
157 examined at two temporal resolutions; weekly and annually. Sample sizes were
158 determined to be suitable sampling sizes based on knowledge of the
159 representative fungal spore genera (*Alternaria* and *Cladosporium*) distributions
160 determined by microscopy as part of the UK national bioaerosol monitoring and
161 previous experience with eDNA analysis (e.g. Brennan *et al* (2019)). Daily air
162 samples were pooled into weekly samples prior to DNA extraction. Weekly
163 samples were defined as the weekly DNA samples from one year (2017), while
164 annual samples consisted of weekly DNA samples which were pooled across the
165 sampling period, therefore giving one annual DNA sample per year per site (six
166 samples total). Finally, for the analysis of weekly atmospheric fungi during crop
167 growth and harvest, the weekly samples were also analysed individually covering
168 an extended period in the year 2017 (between 29th June and 1st November) for
169 the rural site, hence 18 weekly samples.

170

Air sampling

At the rural site we used a Burkard multi-vial cyclone sampler placed on a flat surface at 3.8m above ground level, giving an inlet height of 4.4m. This sampler collects air at 16.5L min^{-1} and uses a mini-cyclone to deposit airborne material into 1.5mL Eppendorf tubes as detailed in Brennan *et al.*, (2019). Each day at 9 am a carousel replaces the existing tube with a new one, allowing for downstream analysis of biological material. At the urban site we used a Burkard single-vial cyclone sampler placed at 10m above ground according to recommendations for placements in urban areas (Rojo *et al.*, 2019). The flow rate and tube deposition are the same for both samplers. The single cyclone requires manual changing of tubes, here done daily at 9 am, except over weekends when three days samples were collected into one tube. Both samplers rotate, each has a fin that ensures the inlet is directed towards the wind. The samplers are placed in an elevated position, which provides representative sampling of the area for large bioaerosols, such as pathogens from agricultural areas, ensuring more biological material in the trap compared with higher elevations found above 10m (Rojo *et al.*, 2019).

DNA extraction

Pooling of samples was performed by re-suspending collected materials in 100µL FastDNA Spin Kit lysis buffer (MP Biomedicals, CA, USA) and vortexing at full speed on a Vortex-Genie 2 for 5 min. Samples were brought to a final volume of 1mL then DNA was extracted using the FastDNA Spin kit (MP Biomedicals, CA, USA) and following the manufacturers' instructions. After extraction pooled

weekly DNA for each year and site was quantified using a Nanodrop™ 2000 (Thermo Scientific, UK).

Fungal metabarcoding

Samples were profiled using the internal transcribed spacer region (ITS2), a widely studied, non-coding and highly conserved DNA region frequently used for fungal metabarcoding. Primers used for amplification were; forward – 5'-GCATCGATGAAGAACGCAGC-3' and reverse – 5'-TCCTCCGCTTATTGATATGC-3' (White *et al.*, 1990). Sequencing was performed by a commercial provider (Eurofins Genomics, Freiberg, Germany) on the Illumina MiSeq. Samples were demultiplexed based on their index sequence before primer sequences were trimmed with no mismatched pairs progressed for analysis. Bioinformatic analysis was performed in R using the Dada2 ITS workflow which, in brief, consists of quality filtering, pair merging, denoising and chimera removal followed by taxonomic assignment (Callahan *et al.*, 2016; Callahan, McMurdie and Holmes, 2017). Negative controls were included in DNA extraction and PCR sequencing. A positive control comprising *Cladosporium, Alternaria*, grass pollen and tree pollen was amplified and sequenced. These genera are known to be abundant, with *Cladosporium* spp. often considered to be the most abundant spore type in the air (Sadyś *et al.*, 2016). Subsequent phylogenetic analysis was performed using the R packages 'phyloseq' (McMurdie and Holmes, 2013) and 'vegan' (Oksanen *et al.*, 2015).

Meteorology

Hourly meteorological data from Pershore (52.1001°, -2.0600°) were extracted from the Met Office Integrated Data Archive System (MIDAS) (Office, 2012) and averaged to match the corresponding period of atmospheric sampling. Pershore was the nearest climate station to the observations site; 16.4km from the urban site at Worcester and 21.5km from the rural site. Seasonal differences were also examined for each of the years 2016, 2017 and 2018. To this end, data from a 22-week period (Table S7) was used as it covers the entire harvest season in 2016, 2017, and 2018 (AHDB, 2020) as well as the extended sampling period in 2017 and the four weeks prior to sample collection in all years. This additional four-week pre-period was selected to capture any potential droughts as fungal spore production often depends on environmental conditions during the latent period in the days and weeks prior to emission (Pariaud *et al.*, 2013; Newlands, 2018). Drought can be a complex matter to explore and there is, according to UK Met Office, no investigation of climate change on droughts in the UK. Therefore, droughts are also loosely defined as an extended period of dry weather with much less rain than usual and an aspect that is generally explored in higher detail in the Annual State of the UK climate reports (Kendon *et al.*, 2019). This data set was complemented with data from two advanced weather stations installed in 2017, with additional sensors generally not available from the MIDAS network (e.g. radiation, flux data, soil data and leaf wetness). The weather stations are co-located with the multi-vial cyclones and here we complement the MIDAS data with leaf wetness for the years 2017 and 2018 available at 30min resolution, which we use to calculate both the seasonal and daily sum of wet leaf periods (Fig. 4 and Table S6) for the 2017 and 2018.

Footprint modelling

Backwards modelling or so-called atmospheric footprint modelling with an atmospheric transport model is commonly done in order to identify potential source areas for the airborne catch of pollen (Skjøth *et al.*, 2015) and spores (Fernández-Rodríguez *et al.*, 2015). We use the, arguably most commonly applied, model HYSPLIT (Stein *et al.*, 2015) using a cluster of trajectories with a starting height of 500 m at the observational sites, similar to other aerobiological studies (e.g. Fernández-Rodríguez et al, (2015); Bilińska *et al.*, (2017)). Twelve trajectories are calculated each day, one for each two-hour period covering the entire observational period similar to Fernández-Rodríguez et al, (2015). This will cover the large daily variations in air mass transport. Trajectories are calculated 48 h back in time to represent the path of the air masses towards the observations site. Atmospheric transport models like HYSPLIT are sensitive to the temporal and spatial resolution of the input data (Bilińska *et al.*, 2017) and it is therefore recommended to use available data with the highest detail possible (Hernández-Ceballos *et al.*, 2014). We have therefore used HYSPLIT-ready global data with the highest possible resolution: A 0.5° x 0.5° data set available for the period 1st Sep 2009 – 11th June 2019 from the NOAA ftp servers: ftp://arlftp.arlhq.noaa.gov/pub/archives/gdas0p5. The location of the air mass in space and time, here represented by the trajectory, is given by a 3D geographical coordinate (lon,lat,height) every hour. These coordinates are grouped into observational years and processed in ArcGIS ver 10.3 using the point density found within the spatial analyst extension. This provides the geographical density of the coordinates as a raster data set with 0.1° x 0.1°

resolution as a representation of the atmospheric footprint area for each campaign year.

Remote sensing

Vegetation type, amount and status have previously been identified as important variables responsible for geographical variations in fungal spore concentrations (O'Connor *et al.*, 2014; Skjøth *et al.*, 2016). We have therefore combined the 10-day remote sensing product with 300 m resolution with a detailed land cover map for the UK within the nearest 30km. The remote sensing product is the Dry Matter Productivity (DMP), which is a global product available from the Copernicus Global Land Service: https://land.copernicus.eu/global/products/dmp. The land cover map cover specific crops with an area of at least 2ha using the Sentinel 1 and 2 satellites and has been shown to be suitable for air-vegetation studies in relation to fungal spores (Apangu *et al.*, 2020). Here the land cover data is used to extract the pixel values from the DMP product into four different groups covering the land cover classes urban, forest, grassland and cropland. We have here extracted data within the nearest 30 km of the observation sites following previous studies (e.g. (O'Connor *et al.*, 2014; Apangu *et al.*, 2020)) as studies suggest that the spore load within a region is mainly due to local emission sources complemented by occasional long range transport episodes (e.g. Apangu *et al.*, 2020).

Data analysis

In analysis of local environmental variables on atmospheric fungi, the atmospheric DNA concentrations, dry matter productivity and fungal community compositions were examined. The Pearson product moment correlation coefficient was calculated for atmospheric DNA levels each week between June and September and the statistical significance of any differences between weeks and sites across the three years was tested with t-tests and one-way ANOVA. To examine the influence of sampling site (urban and rural) we then calculate the average and standard deviation of Dry Matter Productivity for every 10 days for each campaign year for each land cover class, here available as time series (Fig S3) with significance tested by two-way ANOVA. The most prevalent fungi at each site were determined by examining the top 10 taxa that agglomerated at genus level after proportional transformation using the phyloseq 'transform_sample_counts(physeq, function(x) x/sum(x))' function. Phylum, family and species level agglomeration was also considered.

In the study of the effect of atmospheric transport on fungal community structure, the raster data generated from footprint modelling was normalized to contain values from 0 to 100, which enabled an easy comparison of the footprint areas between the different years.

To assess the effect of land management on atmospheric fungi, the fungal community structure was examined in detail against harvest timing and meteorological parameters. The relative abundance of atmospheric fungi in 2017 was determined by examining the top 10 taxa that agglomerated at genus level

315 as previously described. To examine weekly variations in alpha-diversity
316 Shannon and Simpson alpha-diversity indices were calculated from the Amplicon
317 Sequence Variant (ASV) data, according to Nearing *et al.*, (2018); normality was
318 tested using Shapiro-Wilks and either Kruskal-Wallis/Wilcoxon-ranked pairs or t-
319 tests/ANOVA were used to test alpha diversity significance depending on
320 normality. Nonmetric multidimensional scaling (NMDS) based on Bray Curtis
321 dissimilarity (Bray and Curtis, 1957) was used to visualise differences between
322 harvest and post-harvest atmospheric communities and was tested for
323 significance using PERMANOVA. Beta-diversity measures were performed on
324 ASV data which had been filtered to taxa occurring at least twice in 10% of
325 samples in order to exclude low abundance taxa, prior to proportional
326 transformation using the phyloseq 'transform_sample_counts(physeq, function(x)
327 x/sum(x))' function. It has been shown that filtering low abundance taxa does not
328 significantly affect beta-diversity measures but may remove ASVs that result from
329 rare species, false positives, sequencing errors or chimeras. Bray-Curtis
330 dissimilarity was used as a beta-diversity measure and was visualised by
331 nonmetric multidimensional scaling (NMDS). This was tested for significance
332 through PERMANOVA using the adonis function in vegan.

333 The top ten most abundant species during the extended sampling period in 2017
334 were correlated using Pearson's product moment correlation coefficient against
335 weekly percent completed harvest data for the following cereals; winter wheat,
336 spring wheat, winter oilseed, spring oilseed, winter barley, spring barley and oats
337 (AHDB, 2020).

338 To examine seasonal differences the meteorological parameters of temperature,
339 relative humidity and precipitation were analysed using Pearson's product

moment correlation coefficient over the three years of sampling. In 2017 these parameters were also examined against the abundance of the top ten genera and total DNA concentrations at the rural site. Daily leaf wetness data gathered from the advanced weather stations in 2017 and 2018 were analysed by t-test.

Results and Discussion

Local environmental variables

Seasonality in environmental variables has previously been demonstrated to influence community structure of the atmospheric microbiome (Cáliz *et al.*, 2018; Fan *et al.*, 2019) and, in 2017, this was confirmed in our data as a decrease in atmospheric DNA concentration from June to September (S1) (r=-0.646, p=0.043 (to 3dp)). The atmospheric microbiome largely comprises of pollen, fungi and bacteria, and during June to September it is possible that grass and other pollens will be contributing to the total DNA concentration and these will show a decline towards the later months of the year (Brennan *et al* 2019). However it has been shown that fungi and their mycelial components can contribute 11% of the atmospheric microbiome (Tordoni *et al.* 2020) and the time period covered here includes the well documented spore seasons for fungi such as *Alternaria* and *Cladosporium* (Anees-Hill *et al.* 2021). Some variation in DNA concentrations over the sampling period was also expected as a result of changing meteorological parameters. Fungal spores are known to be affected by meteorological factors such as relative humidity, dew point, wind levels and solar radiation, which have previously been found to impact airborne spore levels in

positive and negative directions (Jiřík *et al.*, 2016; Li *et al.*, 2017; Kowalski and Pastuszka, 2018). It has been shown that the highest atmospheric spore levels are generally recorded in the summer and the lowest in winter (De Linares *et al.*, 2010; Fan *et al.*, 2019) reflecting a species-specific relationship with temperature, supported by the findings of Pyrri and Kapsanaki-Gotsi (2017). The exception to this is that dry and warm climates may see a local decrease in mid-summer (De Linares *et al.*, 2010).

Here, we also find a change in local climate and environment may also change the local atmospheric microbiome with respect to abundance and composition. Over all three years combined, the urban site had a higher amount of atmospheric DNA compared to the rural site ($t = -2.979$, $df = 56.448$, p-value = 0.004 (to 3dp)) and the week-to-week variation at both sites varied by a factor of two to four. This difference is remarkable as the remote sensing shows a statistically significant difference in land use types within 30km ($p < 0.005$, $F = 2.691$ to 3dp) with a lower amount of dry matter productivity in the urban area, compared to any of the analysed land cover types found in the nearby rural region (Fig. 2; S2). This does not correspond with previous findings of increasing fungal abundance in more vegetative areas than urban (Lin *et al.* 2018), however this could be explained by a larger proportion of other bioaerosol components, such as bacteria, in urban environments.

In 2016 and 2017 abundances of the top ten genera at both the rural and urban sites were comparable in both years (Fig. 1; S3) and reflected the difference in DNA levels between sites. However, in 2018 a shift in community structure was observed which accompanied increased variation between the urban and rural

389 sites. There was decreasing abundance of *Alternaria* and increases in
390 *Cladosporium* and *Mycosphaerella* (Fig. 1) which corresponded with 20% lower
391 DNA concentrations at both sites in 2018, although not statistically significant.

392 When broken down at lower taxonomies (e.g. Fig. 1) our observations
393 considerably extend results of other studies using optical methods for recognition
394 of *Alternaria* and *Cladosporium* spp. (Damialis *et al.*, 2015; Kasprzyk *et al.*, 2015)
395 as the atmospheric concentrations of both these genera have been shown to
396 respond to harvest (Irga and Torpy, 2016; Skjøth *et al.*, 2016; Olsen *et al.*, 2019),
397 climate-driven increases in temperature and drier conditions (Sindt, Besancenot
398 and Thibaudon, 2016). Many *Alternaria* species are morphologically
399 indistinguishable and conventional airborne counts are generally performed to
400 genus level, leaving the proportion of allergenic species, such as *A. alternata,*
401 undefined (Kasprzyk *et al.*, 2015), despite the focus in many monitoring
402 programmes to identify airborne allergens (Grinn-Gofroń *et al.*, 2019). In our
403 study *Alternaria* often dominated the atmospheric microbiome, but mainly
404 consisting of *A. metachromatica* while *A. alternata,* known for causing allergenic
405 asthma (Pulimood *et al.*, 2007), was a smaller fraction. These two species are
406 morphologically very similar. The phytopathogenicity of *A. metachromatica* is
407 undisputed (Bashir, Mushtaq and Akhtar, 2014; Al-Lami, You and Barbetti, 2019),
408 but its allergenic potential remains undetermined. The greater taxonomic
409 resolution of *Alternaria* provided by metabarcoding demonstrates the potential to
410 re-examine the abundance of genus and species which are difficult to distinguish
411 morphologically and a considerable opportunity for further study (Woudenberg *et*
412 *al.*, 2013).

413

Atmospheric transport

The atmospheric footprint calculations show that that there was very little difference between the three years and that the air masses mainly passed over Wales and West Midlands and parts of Ireland before arrival in Worcester. Areas like south East England, France and Scotland had from very small to no contribution to the spore catch (Fig. 2). This suggests that the major differences in the airborne DNA observed between 2016-18 were not due to mesoscale atmospheric transport, an often-applied hypothesis (Skjøth *et al.*, 2012). Here the alternative hypothesis must be accepted that major differences in annual spore concentrations are either due to micro scale atmospheric processes or processes related to production of spores in the vegetation. This supports other findings of local contributions to the atmospheric microbiome (Liu *et al.,* 2019; Apangu *et al*., 2020), and it is likely that local emission sources contribute the majority of crop pathogens and fungal allergens within a region, but this should be considered in context with the potential for long distance transport influencing the atmospheric microbiome (Grewling *et al.* 2020; Triadó-Margarit, Cáliz and Casamayor, 2022).

Land management

In 2017, most variation in abundance occurred within the top few taxa. The top ten taxa at genus level showed a decline in relative abundance along with a more even distribution from week 11 onwards (Fig. 3; S4). This was reflected in weekly variation in alpha-diversity measures (Shannon's and Simpson's Diversity Indices) for species richness and evenness (S5). Shannon's diversity index displayed a trend for greater species diversity and evenness in later weeks and

Simpsons diversity index also showed a general increase over time except most notably in weeks 13 and 14. As Shannon's index provides a balanced consideration of species richness and evenness while Simpson's has a greater weight on evenness it may be postulated that these two weeks display reduced sample evenness with less impact on diversity. Shapiro-Wilk tests showed the Simpson's diversity index was normally distributed (p=0.270) but Shannon's was not (p=0.021) therefore Kruskal-Wallis tests showed the significance of status (pre- or post- harvest) on Shannon's diversity index (Chi-squared = 5.366, df = 1, p=0.02), but ANOVA on Simpsons diversity index was not found to be significant (F = 0.805, df=1, p=0.383). While the general trend of increasing diversity would be expected as a seasonal response to changing abiotic factors, such as temperature and rainfall, which lead to peaks of airborne fungi in other European countries (Sarda-Estève *et al.*, 2019), this did not fully explain the observed pattern. The autumn period, with less DNA in the air, also corresponds with a reduction in dry matter productivity (e.g. Fig. 2) and the end of the cereal harvest season. It is known that levels of fungal spores may be higher during harvest in some crops, such as cotton, citrus, grapes and cereals (Lee *et al.*, 2004; Skjøth *et al.*, 2012) but there is little known about the impact of agricultural practices on atmospheric fungal spore behaviour (Anees-Hill *et al.*, 2021) despite the importance of crop disease forecasting, which often involves airborne spore dispersal, to the agricultural industry. Where recent studies have looked at similar topics, they have focused on bacteria (Mhuireach *et al*. 2021) or the broader atmospheric microbiome (Finn *et al.* 2021) but still demonstrate the significance of agricultural practices to atmospheric community structure. Here, harvest timing and conditions were key parameters for the airborne spore community through a clear distinction between harvest and post-harvest beta-diversity by

PERMANOVA on Bray-Curtis dissimilarity (p-value = 0.005, F statistic = 4.966, R^2 = 0.237, df = 1). Furthermore, numerous species varied significantly between harvest and post-harvest periods and we find the emergence and abundance of crop pathogens in the air matches well with crop harvests (S6). During the 18-week sampling period in 2017 air temperature and relative humidity were moderately or strongly associated ($p<0.05$) with five of the top ten most abundant genera, four of which contain important pathogenic species; *Mycosphaerella, Alternaria, Puccinia and Botrytis*; *Mycosphaerella* showed a strong positive correlation with air temperature (r=0.759, p=0.00026) and a strong negative correlation with relative humidity (r=-0.858, $p=5.31x10^{-6}$). This was also the case for *Alternaria* but at a moderate level (r=0.677, p=0.002031 and r=-0.620, p=0.006039 respectively). *Puccinia* was positively correlated for both variables (r=0.521, p=0.02651; r=0.519, p=0.02741). *Botrytis* showed a moderately negative association with relative humidity (r= -0.521, p=0.02651). Between the three years there were no statistically significant differences in mean temperature, soil temperature, relative humidity or precipitation over the entire the study period (S7), however leaf wetness duration and frequency was significantly lower in 2018 than 2017 in rural and urban regions ($p = <0.05$) (Fig 4; S8). Leaf wetness is known to impact spore productivity (Crandall and Gilbert, 2017). This corresponded with a notable dry spell of six weeks throughout July and into August in 2018, when some of the warmest days were observed and provided good harvest conditions causing earlier harvest completion but also widespread drought (Kendon *et al.*, 2019). In 2018, harvest was completed earlier for cereals than 2016 and 2017 (Fig. 4; S9). Many pathogens we observed can infect a broad range of cereal crops but others, e.g. *Pyrenophora tritici-repentis* and *Puccinia graminis* correlated strongest with harvesting of

winter cereals (S6), suggesting that this has been the main emission source. In the 2017 time series, by the 27th Sept (start of week 13) winter oilseed rape (OSR), winter barley, spring wheat, spring barley and oats harvests had reached 100% while winter wheat was 96% complete and spring OSR was 70% complete. Abundance of *Mycosphaerella tassiana* correlated with the harvest of all cereals, while *Alternaria metachromatica*, *Puccinia coronata* and *Puccinia coronati-agrotidis* correlated with harvest of winter and spring wheat, spring OSR, spring barley and oats. *Blumeria graminis* correlated similarly, except not with winter wheat. *Pyrenophora tritici-repentis* and *Puccinia graminis* correlated with winter barley and winter OSR but none of the others (Table 2). Previously, data concerning which crop is harvested when, (also termed activity data), has been notoriously difficult to obtain for environmental studies (Kendon *et al.*, 2019) despite previous recommendations to enable the access to data for environmental studies (Flechard *et al.*, 2013). Here, the unique and easily accessible UK data base with weekly progression of the harvesting (AHDB, 2020) has been applied to explain observed changes in atmospheric fungal spore communities. The consequences of altered atmospheric fungal communities on crop pathogen persistence in the environment is not well-determined. Usually, fungi overwinter as spores or sclerotia, and the effect of climate change on the overwintering capability of these survival structures is not clearly established. It is known that climate change is altering the overwintering capability of fungal spores (Prank *et al.*, 2019) and, given sufficient time, can potentially alter host ranges (Gange *et al.* 2011). This aspect, as well as the extended growing period, will affect emission dynamics. As we observed in 2018, our results indicate that pre-season harvest conditions with significantly longer periods of dry vegetation, can have a very large impact on the structure of

516 the spore community, in particular in crop pathogens like *Alternaria.* It is known
517 that fungi require a period of infection (vegetative growth) on host plants prior to
518 lifecycle completion during sporulation (Pariaud *et al.*, 2013; Cao *et al.*, 2014)
519 and during this period are vulnerable to the environment. These spores may be
520 released naturally by suitable weather conditions or anthropogenically by
521 harvesting machinery (Skjøth *et al.*, 2012). The long dry period in 2018, with
522 significantly reduced leaf wetness, is therefore expected to have reduced the
523 period of vegetative growth and hence the emission potential for fungi within the
524 study region. This change in vegetative growth is expected the be the main
525 reason for the observed reduction in key species and an overall change in
526 species composition. It should here be noted that overall amount of precipitation
527 did not differ that much between the years. Prolonged dry spells/droughts are
528 expected to be more frequent under climate change (IPCC, 2019) and while
529 there is debate whether climate change trends are associated with increases or
530 decreases of the hydrological cycle, theory suggests that there will be more
531 extremes of wet and dry periods without a change in overall precipitation Feistel
532 and Hellmuth (2021). Studies of predicted patterns show that the hydrological
533 cycle will be affected by climate change at important stages, with greater water
534 volumes during periods of snow melt (Javadinejad, Dara and Jafary, 2020) and
535 increased flooding during wet seasons combined with increased drought during
536 dry periods (Oo, Zun and Kyi, 2020). Our findings fit with these responses to
537 changing climate conditions and associated with this will inevitably be a reduction
538 in leaf wetness during increased dry spells. It is therefore likely that climate
539 change will impose a shift in the airborne microbiome community, supported by
540 our results contrasting the years 2016 and 2017 with 2018.

Our taxonomic assessment of airborne spores using molecular methods, conducted over three years at contrasting sites, clearly shows the atmospheric microbiome is governed by three factors: vegetation type, climatic conditions and anthropogenic factors, particularly the management of the agricultural landscape. Local harvesting has previously been shown to be the main contributor of *Alternaria spp*. in the city of Copenhagen (Skjøth *et al.*, 2012). Our combined use of remote sensing, detection of weather variables and bioinformatics show substantial local variations in the atmospheric microbiome that has previously not been observed and that areas with less vegetation (e.g. urban zones) surprisingly can be very productive with respect to spores. Many studies on the atmospheric microbiome have separated spores into dry and wet spores, and often attribute temperature and variables connected to the hydrological cycle such as precipitation, relative humidity or vapour pressure as explanatory variables (Grinn-Gofroń *et al.*, 2019). This study takes these findings much further and directly relates to the so-called “One Health Approach”, jointly supported by World Health Organisation and Food and Agricultural Organisation. A key to address this overarching topic is to use and further develop molecular approaches in the analysis of the atmospheric microbiome such as eRNA methods (Yates et al, 2021) and machine learning (Ahmad et al, 2021).

Conclusion

The aim of our study was to examine the impact of climate change on the fungal component of the atmospheric microbiome through local environmental variables, atmospheric transport and land management. We find that the abundance and composition of the atmospheric microbiome changes in response

to local climate and environment. Seasonal spore composition varies between rural and urban zones and that urban zones with less vegetation can be very productive with respect to spores. We find that mesoscale atmospheric transport is less important for spore composition and concentrations. Instead, we find that human activities, type and status of the vegetation, combined with leaf wetness and the prevailing climate are more important. Our findings confirm the connection to the hydrological cycle, but instead suggest a much stronger connection with vegetation type and status, particularly leaf wetness; a key ecosystem variable during spore development. Atmosphere-vegetation processes directly affect leaf wetness with a known feedback mechanism from both climate change and anthropogenic management. We therefore predict that prolonged spells of dry weather create reduced leaf wetness, leading to decreased abundance and change the distribution in the atmospheric microbiome due to unfavorable growing conditions for many fungi. One remaining question is whether climate-induced changes to atmospheric microbiome will result in increased or decreased disease outbreaks as climate change may affect both viability and transport pattern of the atmospheric microbiome. Future work should address how climate driven change in harvest timing affects pathogen interactions with host plants.

Overall, our findings suggest that a move towards a warmer and drier climate will have a dramatic impact on the atmospheric microbiome: on amount, composition and seasonal variation which may affect both food security and human health, through alteration of crop pathogen dynamics and allergenic fungi seasonality.

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Competing interests

The authors declare no competing interests

References

Aalismail, N. A. *et al.* (2019) 'Functional metagenomic analysis of dust-associated microbiomes above the Red Sea', *Scientific Reports*, 9(1), pp. 1–12. doi: 10.1038/s41598-019-50194-0.

Abrego, N. *et al.* (2018) 'Give me a sample of air and I will tell which species are found from your region: Molecular identification of fungi from airborne spore samples', *Molecular Ecology Resources*, 18(3), pp. 511–524. doi: 10.1111/1755-0998.12755.

AHDB (2020) *No Title*, *Harvest progress in Great Britain.* Available at: https://ahdb.org.uk/cereals-oilseeds/gb-harvest-progress.

Ahmad, F., Mahmood, A., Muhmood, T., 2021. Machine learning-integrated omics for the risk and safety assessment of nanomaterials. Biomaterials Science 9, 1598–1608. https://doi.org/10.1039/D0BM01672A

Al-Lami, H. F. D., You, M. P. and Barbetti, M. J. (2019) 'Incidence, pathogenicity and diversity of Alternaria spp. associated with alternaria leaf spot of canola (Brassica napus) in Australia', *Plant Pathology*. Blackwell Publishing Ltd, 68(3),

pp. 492–503. doi: 10.1111/ppa.12955.

Anees-Hill, S., Douglas, P., Pashley, C.H., Hansell, A., Marczylo, E.L., 2021. A systematic review of outdoor airborne fungal spore seasonality across Europe and the implications for health. Science of The Total Environment 151716. https://doi.org/10.1016/J.SCITOTENV.2021.151716

Andreae, M. O. and Rosenfeld, D. (2008) 'Aerosol-cloud-precipitation interactions. Part 1. The nature and sources of cloud-active aerosols', *Earth-Science Reviews*, 89(1–2), pp. 13–41. doi: 10.1016/j.earscirev.2008.03.001.

Apangu, G. P. *et al.* (2020) 'Air mass trajectories and land cover map reveal cereals and oilseed rape as major local sources of Alternaria spores in the Midlands, UK', *Atmospheric Pollution Research*. doi: 10.1016/j.apr.2020.06.026.

Arikoglu, T. *et al.* (2016) 'The characteristics of indoor and outdoor fungi and their relation with allergic respiratory diseases in the southern region of Turkey.', *Environmental monitoring and assessment*. Netherlands, 188(6), p. 380. doi: 10.1007/s10661-016-5371-7.

Baldrian, P. (2017) 'Forest microbiome: Diversity, complexity and dynamics', *FEMS Microbiology Reviews*. Oxford University Press, 41(2), pp. 109–130. doi: 10.1093/femsre/fuw040.

Banchi, E., Ametrano, C.G., Tordoni, E., Stanković, D., Ongaro, S., Tretiach, M., Pallavicini, A., Muggia, L., Verardo, P., Tassan, F., Trobiani, N., Moretti, O., Borney, M.F., Lazzarin, S., 2020. Environmental DNA assessment of airborne plant and fungal seasonal diversity. Science of The Total Environment 738, 140249. https://doi.org/10.1016/J.SCITOTENV.2020.140249

636 Bashir, U., Mushtaq, S. and Akhtar, N. (2014) ‘First report of Alternaria
637 metachromatica from Pakistan causing leaf spot of tomato’, *Pakistan Journal of*
638 *Agricultural Sciences*, 51(2), pp. 315–318.

639 Bebber, D. P., Holmes, T. and Gurr, S. J. (2014) ‘The global spread of crop pests
640 and pathogens’, *Global Ecology and Biogeography*, 23(12), pp. 1398–1407. doi:
641 10.1111/geb.12214.

642 Bebber, D. P., Ramotowski, M. A. T. and Gurr, S. J. (2013) ‘Crop pests and
643 pathogens move polewards in a warming world’, *Nature Climate Change*, 3(11),
644 pp. 985–988. doi: 10.1038/nclimate1990.

645 Bilińska, D. *et al.* (2017) ‘Source regions of ragweed pollen arriving in south-
646 western Poland and the influence of meteorological data on the HYSPLIT model
647 results’, *Aerobiologia*. Springer Netherlands, 33(3), pp. 315–326. doi:
648 10.1007/s10453-017-9471-9.

649 Bissett, A. *et al.* (2017) ‘Erratum: Introducing BASE: The Biomes of Australian
650 Soil Environments soil microbial diversity database [GigaScience. 5, 1, (2016) (1-
651 11)] DOI: 10.1186/s13742-016-0126-5’, *GigaScience*. GigaScience, 6(5), p. 1.
652 doi: 10.1093/gigascience/gix021.

653 Bray, J. R. and Curtis, J. T. (1957) ‘An Ordination of the Upland Forest
654 Communities of Southern Wisconsin’, *Ecological Monographs*, 27(4), pp. 325–
655 349. doi: 10.2307/1942268.

656 Brennan, G. L. *et al.* (2019) ‘Temperate airborne grass pollen defined by spatio-
657 temporal shifts in community composition’, *Nature Ecology and Evolution*, 3(5),
658 pp. 750–754. doi: 10.1038/s41559-019-0849-7.

Cáliz, J. *et al.* (2018) ‘A long-term survey unveils strong seasonal patterns in the airborne microbiome coupled to general and regional atmospheric circulations’, *Proceedings of the National Academy of Sciences*. Proceedings of the National Academy of Sciences, 115(48), pp. 12229–12234. doi: 10.1073/pnas.1812826115.

Callahan, B. J. *et al.* (2016) ‘DADA2: High-resolution sample inference from Illumina amplicon data’, *Nature Methods*. Nature Publishing Group, 13(7), pp. 581–583. doi: 10.1038/nmeth.3869.

Callahan, B. J., McMurdie, P. J. and Holmes, S. P. (2017) ‘Exact sequence variants should replace operational taxonomic units in marker-gene data analysis’, *ISME Journal*. Nature Publishing Group, 11(12), pp. 2639–2643. doi: 10.1038/ismej.2017.119.

Cao, X. *et al.* (2014) ‘Development of Weather- and Airborne Inoculum-Based Models to Describe Disease Severity of Wheat Powdery Mildew’, *Plant Disease*. Scientific Societies, 99(3), pp. 395–400. doi: 10.1094/pdis-02-14-0201-re.

Cavicchioli, R. *et al.* (2019) ‘Scientists’ warning to humanity: microorganisms and climate change’, *Nature Reviews Microbiology*. Springer US, 17(September). doi: 10.1038/s41579-019-0222-5.

Crandall, S. G. and Gilbert, G. S. (2017) ‘Meteorological factors associated with abundance of airborne fungal spores over natural vegetation’, *Atmospheric Environment*. Elsevier Ltd, 162, pp. 87–99. doi: 10.1016/j.atmosenv.2017.05.018.

Damialis, A. *et al.* (2015) ‘Fungi in a changing world: growth rates will be elevated, but spore production may decrease in future climates’, *International Journal of Biometeorology*, 59(9), pp. 1157–1167. doi: 10.1007/s00484-014-

0927-0.

Damialis, A., Gilles, S., Sofiev, M., Sofieva, V., Kolek, F., Bayr, D., Plaza, M.P., Leier-Wirtz, V., Kaschuba, S., Ziska, L.H., Bielory, L., Makra, L., del Mar Trigo, M., Traidl-Hoffmann, C., 2021. Higher airborne pollen concentrations correlated with increased SARS-CoV-2 infection rates, as evidenced from 31 countries across the globe. Proceedings of the National Academy of Sciences of the United States of America 118. https://doi.org/10.1073/PNAS.2019034118/-/DCSUPPLEMENTAL

Després, V. R. *et al.* (2012) 'Primary biological aerosol particles in the atmosphere: A review', *Tellus, Series B: Chemical and Physical Meteorology*. doi: 10.3402/tellusb.v64i0.15598.

Dixon, G. R. (2012) 'Climate change-impact on crop growth and food production, and plant pathogens', in *Canadian Journal of Plant Pathology*, pp. 362–379. doi: 10.1080/07060661.2012.701233.

Dunker, S., Hornick, T., Szczepankiewicz, G., Maier, M., Bastl, M., Bumberger, J., Treudler, R., Liebert, U.G., Simon, J.C., 2021. No SARS-CoV-2 detected in air samples (pollen and particulate matter) in Leipzig during the first spread. Science of The Total Environment 755, 142881. https://doi.org/10.1016/J.SCITOTENV.2020.142881

Fan, X.-Y. *et al.* (2019) 'More obvious air pollution impacts on variations in bacteria than fungi and their co-occurrences with ammonia-oxidizing microorganisms in PM2.5', *Environmental Pollution*. doi: 10.1016/j.envpol.2019.05.004.

Feistel, R., Hellmuth, O., 2021. Relative Humidity: A Control Valve of the Steam

Engine Climate. Journal of Human, Earth, and Future 2, 140–182. https://doi.org/10.28991/HEF-2021-02-02-06

Fernández-Rodríguez, S. *et al.* (2015) 'Potential sources of airborne Alternaria spp. spores in South-west Spain', *Science of the Total Environment*. Elsevier, 533, pp. 165–176. doi: 10.1016/j.scitotenv.2015.06.031.

Finn, D. R., Maldonado, J., de Martini, F., Yu, J., Penton, C. R., Fontenele, R. S., Schmidlin, K., Kraberger, S., Varsani, A., Gile, G. H., Barker, B., Kollath, D. R., Muenich, R. L., Herckes, P., Fraser, M., & Garcia-Pichel, F. (2021). Agricultural practices drive biological loads, seasonal patterns and potential pathogens in the aerobiome of a mixed-land-use dryland. Science of The Total Environment, 798, 149239. https://doi.org/10.1016/J.SCITOTENV.2021.149239

Flechard, C. R. *et al.* (2013) 'Advances in understanding, models and parameterizations of biosphere-atmosphere ammonia exchange', *Biogeosciences*. Copernicus GmbH, 10(7), pp. 5183–5225. doi: 10.5194/bg-10-5183-2013.

Fröhlich-Nowoisky, J. *et al.* (2016) 'Bioaerosols in the Earth system: Climate, health, and ecosystem interactions', *Atmospheric Research*. Elsevier Ltd, pp. 346–376. doi: 10.1016/j.atmosres.2016.07.018.

Gange, A.C., Gange, E.G., Mohammad, A.B., Boddy, L., 2011. Host shifts in fungi caused by climate change? Fungal Ecology 4, 184–190. https://doi.org/10.1016/J.FUNECO.2010.09.004

Grewling, Ł., Magyar, D., Chłopek, K., Grinn-Gofroń, A., Gwiazdowska, J., Siddiquee, A., Ianovici, N., Kasprzyk, I., Wójcik, M., Laffársová, J., Majkowska-Wojciechowska, B., Myszkowska, D., Rodinkova, V., Bortnyk, M., Malkiewicz, M.,

731 Piotrowska-Weryszko, K., Sulborska-Różycka, A., Rybniček, O., Ščevková, J.,
732 Šikoparija, B., Skjøth, C.A., Smith, M., Bogawski, P., 2022. Bioaerosols on the
733 atmospheric super highway: An example of long distance transport of Alternaria
734 spores from the Pannonian Plain to Poland. Science of The Total Environment
735 819, 153148. https://doi.org/10.1016/J.SCITOTENV.2022.153148

736 Grinn-Gofroń, A. *et al.* (2019) 'Airborne Alternaria and Cladosporium fungal
737 spores in Europe: Forecasting possibilities and relationships with meteorological
738 parameters', *Science of the Total Environment*. Elsevier B.V., 653, pp. 938–946.
739 doi: 10.1016/j.scitotenv.2018.10.419.

740 Hernández-Ceballos, M. A. *et al.* (2014) 'Improvement in the accuracy of back
741 trajectories using WRF to identify pollen sources in southern Iberian Peninsula',
742 *International Journal of Biometeorology*. Springer New York LLC, 58(10), pp.
743 2031–2043. doi: 10.1007/s00484-014-0804-x.

744 Holman, L. E. *et al.* (2019) 'Detection of introduced and resident marine species
745 using environmental DNA metabarcoding of sediment and water', *Scientific*
746 *Reports*. Nature Publishing Group, 9(1). doi: 10.1038/s41598-019-47899-7.

747 IPCC, 2019 (2019) *Summary for Policymakers. In: Climate Change and Land: an*
748 *IPCC special report on climate change, desertification, land degradation,*
749 *sustainable land management, food security, and greenhouse gas fluxes in*
750 *terrestrial ecosystems*.

751 Irga, P. J. and Torpy, F. R. (2016) 'A survey of the aeromycota of Sydney and its
752 correspondence with environmental conditions: grass as a component of urban
753 forestry could be a major determinant', *Aerobiologia*. Springer Netherlands,
754 32(2), pp. 171–185. doi: 10.1007/s10453-015-9388-0.

Jarvis, M.C., 2020. Aerosol Transmission of SARS-CoV-2: Physical Principles and Implications. Frontiers in Public Health 8, 813. https://doi.org/10.3389/FPUBH.2020.590041/BIBTEX

Javadinejad, S., Dara, R., Jafary, F., 2020. Climate Change Scenarios and Effects on Snow-Melt Runoff. Civil Engineering Journal 6, 1715–1725. https://doi.org/10.28991/CEJ-2020-03091577

Jiřík, V. *et al.* (2016) 'Bioaerosols in the suburbs of ostrava during a one year period', *Central European Journal of Public Health*. Czech National Institute of Public Health, 24, pp. S55–S60. doi: 10.21101/cejph.a4534.

Kasprzyk, I. *et al.* (2015) 'Air pollution by allergenic spores of the genus Alternaria in the air of central and eastern Europe', *Environmental Science and Pollution Research*, 22(12), pp. 9260–9274. doi: 10.1007/s11356-014-4070-6.

Kendon, M. *et al.* (2019) 'State of the UK climate 2018', *International Journal of Climatology*, 39(S1), pp. 1–55. doi: 10.1002/joc.6213.

Kettles, G. J. and Luna, E. (2019) 'Food security in 2044: How do we control the fungal threat?', *Fungal Biology*. Elsevier B.V. doi: 10.1016/j.funbio.2019.04.006.

Kowalski, M. and Pastuszka, J. S. (2018) 'Effect of ambient air temperature and solar radiation on changes in bacterial and fungal aerosols concentrationin the urban environment', *Annals of Agricultural and Environmental Medicine*. Institute of Agricultural Medicine, 25(2), pp. 259–261. doi: 10.26444/aaem/75877.

Lee, K. *et al.* (2004) 'Personal exposures to inorganic and organic dust in manual harvest of California citrus and table grapes', *Journal of Occupational and Environmental Hygiene*, 1(8), pp. 505–514. doi: 10.1080/15459620490471616.

778 Lewis, D., 2020. Is the coronavirus airborne? Experts can't agree. Nature 580,
779 175. https://doi.org/10.1038/D41586-020-00974-W

780 Li, Y. *et al.* (2017) 'Concentrations and size distributions of viable bioaerosols
781 under various weather conditions in a typical semi-arid city of Northwest China',
782 *Journal of Aerosol Science*. Elsevier Ltd, 106, pp. 83–92. doi:
783 10.1016/j.jaerosci.2017.01.007.

784 Lin, W.R., Wang, P.H., Tien, C.J., Chen, W.Y., Yu, Y.A., Hsu, L.Y., 2018.
785 Changes in airborne fungal flora along an urban to rural gradient. Journal of
786 Aerosol Science 116, 116–123.
787 https://doi.org/10.1016/J.JAEROSCI.2017.11.010

788 Liu, H., Hu, Z., Zhou, M., Hu, J., Yao, X., Zhang, H., Li, Z., Lou, L., Xi, C., Qian,
789 H., Li, C., Xu, X., Zheng, P., Hu, B., 2019. The distribution variance of airborne
790 microorganisms in urban and rural environments. Environmental Pollution 247,
791 898–906. https://doi.org/10.1016/J.ENVPOL.2019.01.090

792 De Linares, C. *et al.* (2010) 'Dispersal patterns of Alternaria conidia in Spain',
793 *Agricultural and Forest Meteorology*. Elsevier B.V., pp. 1491–1500. doi:
794 10.1016/j.agrformet.2010.07.004.

795 Magyar, D., Tischner, Z., Páldy, A., Kocsubé, S., Dancsházy, Z., Halász, Á.,
796 Kredics, L., 2021. Impact of global megatrends on the spread of microscopic
797 fungi in the Pannonian Biogeographical Region. Fungal Biology Reviews 37, 71–
798 88. https://doi.org/10.1016/J.FBR.2021.03.006

799 Mhuireach, G.Á., Betancourt-Román, C.M., Green, J.L., Johnson, B.R., 2019.
800 Spatiotemporal Controls on the Urban Aerobiome. Frontiers in Ecology and
801 Evolution | www.frontiersin.org 1, 43. https://doi.org/10.3389/fevo.2019.00043

802 Makiola, A. *et al.* (2019) ‘Land use is a determinant of plant pathogen alpha- but
803 not beta-diversity’, *Molecular Ecology*, 28(16), pp. 3786–3798. doi:
804 10.1111/mec.15177.

805 McMurdie, P. J. and Holmes, S. (2013) ‘Phyloseq: An R Package for
806 Reproducible Interactive Analysis and Graphics of Microbiome Census Data’,
807 *PLoS ONE*, 8(4). doi: 10.1371/journal.pone.0061217.

808 Nearing, J. T. *et al.* (2018) ‘Denoising the Denoisers: an independent evaluation
809 of microbiome sequence error-correction approaches’, *PeerJ*. PeerJ, 6, p. e5364.
810 doi: 10.7717/peerj.5364.

811 Newlands, N. K. (2018) ‘Model-Based Forecasting of Agricultural Crop Disease
812 Risk at the Regional Scale, Integrating Airborne Inoculum, Environmental, and
813 Satellite-Based Monitoring Data’, *Frontiers in Environmental Science*. Frontiers
814 Media SA, 6. doi: 10.3389/fenvs.2018.00063.

815 O’Connor, D. J. *et al.* (2014) ‘Atmospheric concentrations of Alternaria,
816 Cladosporium, Ganoderma and Didymella spores monitored in Cork (Ireland)
817 and Worcester (England) during the summer of 2010’, *Aerobiologia*. Kluwer
818 Academic Publishers, 30(4), pp. 397–411. doi: 10.1007/s10453-014-9337-3.

819 Office, M. (2012) *No Title*, *Met Office Integrated Data Archive System (MIDAS)*
820 *Land and Marine Surface Stations Data (1853-current). NCAS British*
821 *Atmospheric Data Centre. 2020*. Available at:
822 https://catalogue.ceda.ac.uk/uuid/220a65615218d5c9cc9e4785a3234bd0.

823 Oksanen, J. *et al.* (2015) ‘Package “vegan”’, *R package version 2.3-4*.

824 Olsen, Y. *et al.* (2019) ‘Grain harvesting as a local source of Cladosporium spp.

825 in Denmark', *Aerobiologia*. Springer Netherlands, 35(2), pp. 373–378. doi:
826 10.1007/s10453-018-09556-w.

827 Oo, H.T., Zin, W.W., Thin Kyi, C.C., 2020. Analysis of Streamflow Response to
828 Changing Climate Conditions Using SWAT Model. Civil Engineering Journal 6,
829 194–209. https://doi.org/10.28991/CEJ-2020-03091464

830 Ovaskainen, O. *et al.* (2020) 'Monitoring Fungal Communities With the Global
831 Spore Sampling Project', *Frontiers in Ecology and Evolution*. Frontiers Media
832 S.A., 7. doi: 10.3389/fevo.2019.00511.

833 Pariaud, B. *et al.* (2013) 'Shared influence of pathogen and host genetics on a
834 trade-off between latent period and spore production capacity in the wheat
835 pathogen, Puccinia triticina', *Evolutionary Applications*, 6(2), pp. 303–312. doi:
836 10.1111/eva.12000.

837 Prank, M. *et al.* (2019) 'Climate change impacts the spread potential of wheat
838 stem rust, a significant crop disease', *Environmental Research Letters*. Institute
839 of Physics Publishing, 14(12). doi: 10.1088/1748-9326/ab57de.

840 Pulimood, T. B. *et al.* (2007) 'Epidemic asthma and the role of the fungal mold
841 Alternaria alternata', *Journal of Allergy and Clinical Immunology*, 120(3), pp.
842 610–617. doi: 10.1016/j.jaci.2007.04.045.

843 Pyrri, I., Kapsanaki-Gotsi, E., 2017. Functional relations of airborne fungi to
844 meteorological and pollution factors in a Mediterranean urban environment.
845 Fungal Ecology 30, 48–54. https://doi.org/10.1016/J.FUNECO.2017.08.007

846 Randall, D *et al.* (2013) *Clouds and Aerosols. In: Climate Change 2013: The*
847 *Physical Science Basis. Contribution of Working Group I to the Fifth Assessment*

Report of the Intergovernmental Panel on Climate Change Coordinating Lead Authors: Lead Authors.

Ren, S. *et al.* (2019) 'Heat and drought stress advanced global wheat harvest timing from 1981-2014', *Remote Sensing*, 11(8). doi: 10.3390/rs11080912.

Rodriguez, P. A. *et al.* (2019) 'Systems Biology of Plant-Microbiome Interactions', *Molecular Plant*. Cell Press, pp. 804–821. doi: 10.1016/j.molp.2019.05.006.

Rojo, J. *et al.* (2019) 'Near-ground effect of height on pollen exposure', *Environmental Research*. Elsevier Inc., 174(April), pp. 160–169. doi: 10.1016/j.envres.2019.04.027.

Sadyś, M. *et al.* (2016) 'Comparisons of fungal spore distributions using air sampling at Worcester, England (2006–2010)', *Aerobiologia*, 32(4), pp. 619–634. doi: 10.1007/s10453-016-9436-4.

Sarda-Estève, R. *et al.* (2019) 'Variability and geographical origin of five years airborne fungal spore concentrations measured at Saclay, France from 2014 to 2018', *Remote Sensing*. MDPI AG, 11(14). doi: 10.3390/rs11141671.

Setti, L., Passarini, F., de Gennaro, G., Barbieri, P., Perrone, M.G., Borelli, M., Palmisani, J., di Gilio, A., Torboli, V., Fontana, F., Clemente, L., Pallavicini, A., Ruscio, M., Piscitelli, P., Miani, A., 2020. SARS-Cov-2RNA found on particulate matter of Bergamo in Northern Italy: First evidence. Environmental Research 188, 109754. https://doi.org/10.1016/J.ENVRES.2020.109754

Sindt, C., Besancenot, J. P. and Thibaudon, M. (2016) 'Airborne Cladosporium fungal spores and climate change in France', *Aerobiologia*. Springer Netherlands, 32(1), pp. 53–68. doi: 10.1007/s10453-016-9422-x.

871 Skjøth, C. A. *et al.* (2012) 'Crop harvest in Denmark and Central Europe
872 contributes to the local load of airborne Alternaria spore concentrations in
873 Copenhagen', *Atmospheric Chemistry and Physics*, 12(22), pp. 11107–11123.
874 doi: 10.5194/acp-12-11107-2012.

875 Skjøth, C. A. *et al.* (2015) 'Pollen from alder (Alnus sp.), birch (Betula sp.) and
876 oak (Quercus sp.) in the UK originate from small woodlands', *Urban Climate*.
877 Elsevier, 14, pp. 414–428. doi: 10.1016/j.uclim.2014.09.007.

878 Skjøth, C. A. *et al.* (2016) 'Alternaria spores in the air across Europe: abundance,
879 seasonality and relationships with climate, meteorology and local environment',
880 *Aerobiologia*, 32(1), pp. 3–22. doi: 10.1007/s10453-016-9426-6.

881 Stadnytskyi, V., Bax, C.E., Bax, A., Anfinrud, P., 2020. The airborne lifetime of
882 small speech droplets and their potential importance in SARS-CoV-2
883 transmission. Proceedings of the National Academy of Sciences of the United
884 States of America 117, 11875–11877. https://doi.org/10.1073/pnas.2006874117

885 Stein, A. F. *et al.* (2015) 'Noaa's hysplit atmospheric transport and dispersion
886 modeling system', *Bulletin of the American Meteorological Society*. American
887 Meteorological Society, pp. 2059–2077. doi: 10.1175/BAMS-D-14-00110.1.

888 The Lancet Respiratory Medicine, 2020. COVID-19 transmission—up in the air.
889 The Lancet Respiratory Medicine 8, 1159. https://doi.org/10.1016/S2213-
890 2600(20)30514-2

891 Tordoni, E., Ametrano, C.G., Banchi, E., Ongaro, S., Pallavicini, A., Bacaro, G.,
892 Muggia, L., 2021. Integrated eDNA metabarcoding and morphological analyses
893 assess spatio-temporal patterns of airborne fungal spores. Ecological Indicators
894 121, 107032. https://doi.org/10.1016/j.ecolind.2020.107032

895 Triadó-Margarit, X., Cáliz, J., Casamayor, E.O., 2022. A long-term atmospheric
896 baseline for intercontinental exchange of airborne pathogens. Environment
897 International 158, 106916. https://doi.org/10.1016/J.ENVINT.2021.106916

898 White, T. J. *et al.* (1990) 'Amplification and Direct Sequencing of Fungal
899 Ribosomal Rna Genes for Phylogenetics', *PCR Protocols*, (January), pp. 315–
900 322. doi: 10.1016/b978-0-12-372180-8.50042-1.

901 Wilson, T. W. *et al.* (2015) 'A marine biogenic source of atmospheric ice-
902 nucleating particles', *Nature*, 525(7568), pp. 234–238. doi: 10.1038/nature14986.

903 Woudenberg, J. H. C. *et al.* (2013) 'Alternaria redefined', *Studies in Mycology*.
904 Centraalbureau voor Schimmelculturen, 75, pp. 171–212. doi: 10.3114/sim0015.

905 Yates, M.C., Derry, A.M., Cristescu, M.E., 2021. Environmental RNA: A
906 Revolution in Ecological Resolution? Trends in Ecology & Evolution 36, 601–609.
907 https://doi.org/10.1016/J.TREE.2021.03.001

908

Figures

909 Figure 1. Relative abundance (%) of top 10 atmospheric fungal genera at urban
910 and rural locations over three years

Figure 2. A. Decreasing dry matter productivity in the UK in 2017 (top-bottom = 1st July, 1st Aug, 1st Sept); B. Dry matter productivity over 2016, 2017 and 2018 at urban and rural locations and C. The atmospheric footprint showing the source of air masses during the 2017 sampling period.

Figure 3. Relative abundance of top 10 atmospheric fungal genera over 18 weeks during summer and autumn in 2017. Week 2 corresponds to cereal crop harvesting exceeding 5% and week 11 corresponds with when 95% of cereal crops have been harvested based on AHDB/ADAS data (indicated by arrow).

Figure 4. Leaf wetness duration (minutes/day) from July to September in two years at urban and rural locations and the proportion of harvest completed over the corresponding 12-week harvest periods (July-September).