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## Spectroscopic detection of bioaerosols with the wibs-4+: Anthropogenic and meteorological impacts

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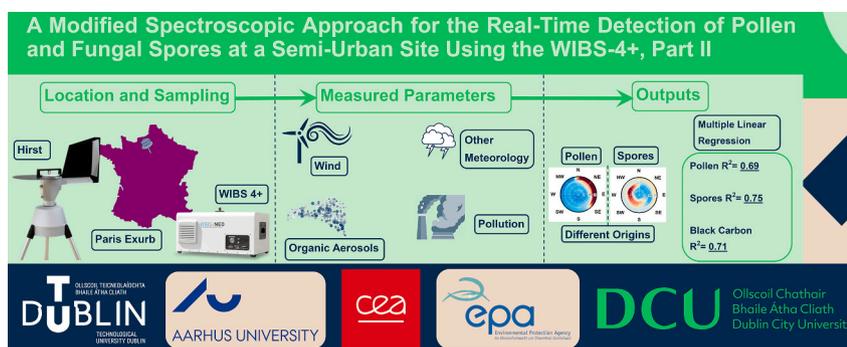
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## HIGHLIGHTS

- The WIBS-4+ study unveils the origins and environmental correlations of pollen and fungal spores in Île-de-France.
- Findings include positive correlation of some pollens with temperature and an inverse relationship with relative humidity.
- Challenges were encountered with the WIBS-4+ in capturing large fluorescent particles at high windspeeds.
- We traced the origins of spores from the west, while pollen came from the north-east.
- An interesting discovery was the lack of correlation between fungal spores and all measured pollutants.

## GRAPHICAL ABSTRACT



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## ABSTRACT

This research builds upon a previous study that explored the potential of the modified WIBS-4+ to selectively differentiate and detect different bioaerosol classes. The current work evaluates the influence of meteorological and air quality parameters on bioaerosol concentrations, specifically pollen and fungal spore dynamics.

Temperature was found to be the most influential parameter in terms of pollen production and release, showing a strong positive correlation. Wind data analysis provided insights into the potential geographic origins of pollen and fungal spore concentrations. Fungal spores were primarily shown to originate from a westerly direction, corresponding to agricultural land use, whereas pollen largely originated from a North-easterly direction, corresponding to several forests.

The influence of air quality was also analysed to understand its potential impact on the WIBS fluorescent parameters investigated. Most parameters had a negative association with fungal spore concentrations, whereas several anthropogenic influences showed notable positive correlations with daily pollen concentrations. This is attributed to similar driving forces (meteorological parameters) and geographical origins. In addition, the WIBS

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showed a significant correlation with anthropogenic pollutants originating from combustion sources, suggesting the potential for such modified spectroscopic instruments to be utilized as air quality monitors.

By combining all meteorological and pollution data along with WIBS-4+ channel data, a set of Multiple Linear Regression (MLR) analyses were completed. Successful results with  $R^2$  values ranging from 0.6 to 0.8 were recorded. The inclusion of meteorological parameters was dependent on the spore or pollen type being examined.

## 1. Introduction

### 1.1. Background

Primary biological aerosols (PBAP) originate from biological sources which include particles like bacteria, viruses, pollen and fungal spores. PBAP pose a potential risk to human, animal and plant health through the spread of allergenic and pathogenic materials (Brown and Hovmøll, 2002; Humbal et al., 2018). The release and transport of such biological matter can also pose certain environmental implications through the spread of invasive plant and fungal species (Card et al., 2007; Fetters et al., 2022; Madelin, 1994) as well as impacting radiative forcing by acting as cloud condensation/ice nuclei (Després et al., 2012; Haga et al., 2014; Pummer et al., 2012). In more recent decades, the allergenic potential of PBAP, originating primarily from exposure to pollen grains and fungal spores, has increased dramatically with ascending trends expected to continue and worsen with continuing climate change (Beggs, 2004; Paudel et al., 2021). As a result, there has been a drive towards the development and deployment of online/real-time instruments in recent years for the detection of PBAP, as opposed to traditional offline methods. Traditional instruments such as the Hirst volumetric sampler have been used extensively for monitoring ambient pollen and fungal spore concentrations since its development in the early 1950's (Hirst, 1952) and has seen very little technological development since (Adamov et al., 2021; Triviño et al., 2023). These methods typically involve the impaction of particulates onto a rotating collection tape that must then be examined microscopically, and any identifiable pollen/fungal spore manually counted. This process is extremely time-consuming and labour-intensive. As such, a series of alternative detection methods and principles have been trialled and developed in recent years for the detection of PBAP such as pollen and fungal spores (Tummon et al., 2021).

### 1.2. Modern PBAP detection

One popular approach used to detect PBAP is based on the principle of light induced fluorescence (LIF). This method utilises the natural fluorescent properties of PBAP when they are exposed to UV light and uses fluorescent aerosol particles (FAPs) as a proxy for PBAP. To date, a range of different LIF instruments (Addor et al., 2022; Huffman et al., 2020; Tummon et al., 2021) have been used to monitor different PBAP by selectively targeting the presence of specific bio-fluorophores (Buters et al., 2022). The WIBS is one such instrument, and has been deployed in its various iterations, to a myriad of outdoor (Crawford et al., 2014; Fernández-Rodríguez et al., 2018a; Li et al., 2022; O'Connor et al., 2014a, 2015a; Yue et al., 2017, 2022), indoor (Li et al., 2022; Zhou et al., 2017), and laboratory (Healy et al., 2012; Hernandez et al., 2016; Savage et al., 2017) environments to detect the presence of different PBAP. Originally, the WIBS was designed to detect fluorophores in 3 main detection channels (namely FL1, FL2 and FL3), selected to detect specific bio fluorophores such as tryptophan and NADH to name but a few (Kaye et al., 2005; Stanley et al., 2011). The WIBS has previously been shown to accurately identify fluorescent fractions representative of different PBAP such as pollen (O'Connor et al., 2014a; Tummon et al., 2021) and fungal spores (Fernández-Rodríguez et al., 2018b; O'Connor et al., 2015b). However, such studies have been restricted to relatively limited ambient environments generally devoid of complexity. In more diverse environments, the presence of anthropogenic pollution and

other interfering sources can hinder the ability of the WIBS to accurately and specifically identify PBAP. This can happen when large volumes of these particles outnumber the targeted PBAP particles, but can be mitigated through size filtering and sigma filtration (Hernandez et al., 2016; Pöhlker et al., 2012a; Savage et al., 2017).

In previous laboratory studies the presence of pollen specific fluorophores including chlorophyll has been shown to differentiate grass and herb pollen from other pollen sources and fungal spores (O'Connor et al., 2014b, 2011; Pöhlker et al., 2013). Thus, attempts have been made to incorporate this capability into real-time instruments such as the WIBS-4 (termed WIBS-4+). Indeed, previous work showed that the addition of 2 channels (FL4 and FL5) designed to detect such fluorescent components aided in the ability of the WIBS to distinguish between broad classes of PBAP in a more complex semi-urban environment (Markey et al., 2022). However, deviations between the WIBS-4+ and Hirst were noted which were suspected to be attributed to the differences in operating principles and influences of meteorological conditions and will be further examined in this study.

### 1.3. External parameters

Air quality parameters and meteorological conditions are important factors for instigating the release and detection of various PBAP. Various weather conditions such as temperature (Bruffaerts et al., 2018; Grinn-Gofroń et al., 2018; Matyasovszky et al., 2015; Ziska et al., 2019), humidity (Grinn-Gofroń et al., 2018; Ianovici, 2016) and wind speed (Dowding, 1987; Martínez-Bracero et al., 2022) are essential for the production and subsequent release of pollen and fungal spores. In the case of the traditional Hirst method, the sampling efficiency varies depending on wind conditions (Buters et al., 2022; Frenz, 2000). Wind parameters have also been shown to impact the sampling efficiency of the WIBS, especially when compared to traditional methods, owing to differences in working principles between both instruments, as the means of air intake differ slightly in their design philosophy, with the Hirst possessing a mono-directional air-inlet that uses a wind vane to sample in the direction of the prevailing wind, and the WIBS utilizing an omni-directional air inlet (Healy et al., 2012; O'Connor et al., 2014a). The sensing capability of the WIBS is also obstructed by the presence of other interfering aerosols present in urban environment which have been shown to fluoresce when sampled by the WIBS, particularly those that come from combustion sources such as black carbon from biomass or wood burning (Hernandez et al., 2016; Pöhlker et al., 2012b; Savage et al., 2017). Due to the proximity of the sampling site to a major urban city and the concomitant presence of non-biological pollutants and their additive effects on the allergenic potential of airborne pollens/fungal spores (Cariñanos et al., 2021; Grewling et al., 2019b, 2019a; Ørby et al., 2015; Sénéchal et al., 2015), the correlation between non-biological pollutants with the ambient concentrations of PBAP and FAPs were examined. Similarly, the effects of meteorological parameters on the sampling of the PBAP and FAP classes were also investigated for both monitoring methods.

## 2. Methodology

### 2.1. Sampling site

Both the traditional Hirst volumetric and WIBS-4+ were located at the same site at the CEA in Saclay 25 km to the South of Paris

(48.7247°N, 2.1488°E) from 21/04/17 to 15/06/17. The Hirst operated continuously, however the WIBS was not in operation for 5 days from the 05/05/17–10/05/17 due to power loss.

Additional details on the sampling site and instrumentation is provided in this study (Markey et al., 2022). A map of the sampling location is available within the supplemental materials, marked as Fig. S1.

## 2.2. Instrumentation

### 2.2.1. Hirst

Pollen and fungal spore data were acquired using the Hirst-Lanzoni 7-day volumetric which consists of a rotating drum onto which a silicone-coated tape is adhered. The trap was changed every 7 days and operated continuously using an air flow rate of 10 L/min and rotating 2 mm/h during which time the particulates sampled from the air impacted onto the tape and subjected to subsequent microscopic examination. Fungal spores and pollen grains were optically counted and identified using a 400× magnification. All above steps were carried out as recommended by the European Aeroallergen Network for pollen data reproducibility (EAS QC Working Group et al., 2014).

### 2.2.2. WIBS-4+

Ambient fluorescent aerosol particles (FAP) were monitored using the WIBS-4+ which operates on the basis of light induced fluorescence. The WIBS-4+ is a modified version of the WIBS-4 model that was developed by the University of Hertfordshire. Unlike traditional WIBS models which only detect FAPs in 3 fluorescent channels, the WIBS-4+ has the addition of 2 fluorescent channels designed to target different plant fluorophores including-but not limited to-chlorophyll (“WIBS sensors, n.d.”).

When a particle is detected by the WIBS, details on the particle shape, size and fluorescence intensity in each detection channel is recorded. If a particle possesses fluorescence intensity beyond a pre-determined threshold, it is classified as fluorescent. Depending on the fluorescence characteristics of the particle it can be categorised into 1 of 7 classes as previously defined by Perring et al., 2015 (Perring et al., 2015a). With the inclusion of the 2 additional fluorescent channels the WIBS-4+ can also classify particles into 3 additional classes, D, E, and DE as described previously (Markey et al., 2022).

## 2.3. Meteorological and pollution data

Meteorological and pollution data was also collected at the sampling site. Available meteorological parameters included: Temperature [°C] (Temp), Precipitation [mm] (Rain); Pressure [hpa] (Pres); Condensation particle counter [#/ $m^3$ ] (CPC); relative humidity [%] (RH); Wind Speed [m/s] (Wind\_s) and Wind direction [deg] (Wind\_d).

Available pollution data included: NO<sub>x</sub> [ $\mu\text{g}/m^3$ ]; SO<sub>4</sub> [ $\mu\text{g}/m^3$ ]; NH<sub>4</sub> [ $\mu\text{g}/m^3$ ]; NO<sub>3</sub> [ $\mu\text{g}/m^3$ ]; O<sub>3</sub> [ $\mu\text{g}/m^3$ ]; organic matter [ $\mu\text{g}/m^3$ ] (OM); black carbon from fossil fuels [ $\mu\text{g}/m^3$ ] (BC<sub>f</sub>); black carbon from wood burning [ $\mu\text{g}/m^3$ ] (BC<sub>wb</sub>); biomass burning organic aerosols [ $\mu\text{g}/m^3$ ] (BBOA); hydrocarbon-like organic aerosols [ $\mu\text{g}/m^3$ ] (HOA); less oxidized oxygenated organic aerosols [ $\mu\text{g}/m^3$ ] (LOOOA) and more oxidized oxygenated organic aerosols (MOOOA).

The normal distribution of the WIBS, Hirst and meteorological/pollution data was tested using the Shapiro-Wilk test with the results showing that the majority of daily data did not follow a normal distribution. A Spearman correlation test was selected to calculate the degree and the correlation between selected variables using the *nortest* (Gross and Ligges, 2015) and *corrplot* (Gross and Ligges, 2015) packages within R (R Development Core Team, 2020).

## 2.4. Multiple linear regression

A set of multiple linear regression (MLR) analyses were carried out

utilizing the obtained study datasets to investigate the possibility of using the WIBS fluorescent categories, pollution data and meteorological data to assist in the calculation of bioaerosol concentrations.

The collinearity of multiple independent variables was investigated using the variance inflation factor (VIF) in canonical correlation analysis (CCA). This tested for autocorrelating variables and shows those with the highest levels of collinearity (Sadyś et al., 2015). To ensure there would be no issues relating to collinearity, it was assumed that any VIF value above 2 was significant, and the corresponding should be removed (Johnston et al., 2018; Menard, 2002).

Detrended Correspondence Analysis (DCA) was then performed to confirm the previous assumption that all pollen or fungal parameters comprise a homogeneous dataset (Grinn-Gofroń et al., 2018). As a result of this, a Redundancy Analysis (RDA) was completed. DCA processing is used prior to RDA to assess the linearity of the data and visually inspect the ordination. This graphically displayed the degree of correlation between different fungal spore or pollen grain types, with all relevant variables. Longer arrows indicated stronger correlation and shorter arrows indicating weaker correlation. Positive correlation was determined by the proximity of a bioaerosol type to the end of an arrow. Negative correlation could be identified if a parameter is on the opposite side of the graphic to the arrow (Sadyś et al., 2015). In these calculations, the dependent variable was set as either all pollen or all fungal spore types. Before the above analysis, preliminary correlation plots calculated the Pearson correlation coefficient of every variable in the dataset which were then used for rapid visual guidance. A model was constructed for each dependent variable by including every available WIBS category, pollutant parameter, and meteorological parameter.

Wind direction was created by dividing the 360 Polar co-ordinates into 8 different parameters, each representing one 8th of the compass. Each parameter was input as a binary parameter. For example, every day would have a primary wind direction, with that direction having a value of “1” for the day, and the other 7 directions having a value of “0”.

For each analysis, a residual plot was created to test the quality of the data and the accuracy of each calculation. Following this, the MLR results could be treated as valid. Examples of non-quality data would be data that appeared non-linear, data that had outliers, or data with an unbalanced x or y axis. Subsequently, linear stepwise backwards regression analysis was performed to exclude non-significant parameters. Only once statistically significant parameters remained, were interaction plots created. These were created between all variable combinations, and a second regression analysis was completed. This was to check the impacts the interactions had each of the significant independent variables, and subsequent significant interactions were also included in the final correlative algorithm.

## 2.5. Geographical origins for airborne pollen and fungal spores

The geographical origin of ambient bioaerosols was examined using a source receptor approach available through the ZeFir-v3.7 package (Petit et al., 2017). A variant of two-dimension Non-parametric Wind Regression (NWR) known as Sustained Wind Incidence Method (SWIM) was used (Henry et al., 2009; Olson et al., 2012). This methodology determines spatial domains by highlighting their frequency of occurrence in 8 directional and 4 wind speed bins. This method has been applied in previous long-term monitoring studies from Saclay to determine the geographical origins of pollen, fungal spores and bacteria (Estève et al., 2018; Sarda-Estève et al., 2020, 2019), as well as in short term studies for various particulate types (Brilke et al., 2020; Petit et al., 2015).

## 2.6. HYSPLIT analysis of days with peak bioaerosol concentrations

Back trajectory analysis of peak pollen and fungal spores days were investigated using the HYSPLIT backward Lagrangian dispersion model (Stein et al., 2015). In all cases, the HYSPLIT maps generated have been

calculated for the peak date of each particle type as described in Table 1. The model was run from 23:00 on the peak day with a new trajectory calculated for each of the previous 24 h (Su et al., 2015; Zemmer et al., 2012). All trajectory points were grouped to show the accumulated frequency for all calculated trajectories at a selected height of 20 m above ground level.

### 3. Results & discussion

#### 3.1. Hirst and WIBS monitoring

In an effort to evaluate the potential of the WIBS-4+ instrument to reliably identify and differentiate between different bioaerosol classes, comparisons were made to the traditional volumetric Hirst instrument (O'Connor et al., 2013, 2014a, 2014b). The FAP fractions observed by the WIBS underwent a range of filtering and unsupervised learning approaches to isolate representative bioaerosol fractions. Detailed analysis of these methods and results are provided in a previous study, but in short, particles were filtered to minimum and maximum particle diameter, with minimum particle sizing of at least 2 µm removing the vast majority of possible interfering anthropogenic sources of particles (Markey et al., 2022). Utilizing the modified detection channels the WIBS-4+ was able to improve its differentiation proficiency. The additional FL4 and FL5 channels -unique to the WIBS-4+ model- were combined with the original WIBS detection bands to detect pollen specific fluorophores such as chlorophyll and azulenes in an effort to improve pollen class differentiation. For this reason, the WIBS-4+ was initially evaluated using pollen from grasses, herbs and trees & shrubs. A correlation plot of the results of the initial comparison between the Hirst and the WIBS-4+ is available within the supplementary materials as Fig. S3.

The WIBS provides information on the size, shape and fluorescent characteristics of particles sampled. Over the course of the campaign approximately 38 % of particles sampled possessed fluorescent intensity greater than the predetermined baseline value. These particles were then further classified based on their fluorescent characteristics in each WIBS detection channel as described by Perring et al., 2015 (Perring et al., 2015b). The inclusion of the FL4 and FL5 channels allowed the particles to be classified into 3 additional classes – D, E and DE as described previously (Markey et al., 2022). Presumptive association was seen through examination of the size distributions of the individual particle classes as shown in Fig. S2 in the supplementary materials, with reference to previous laboratory studies used to categories PBAP using other WIBS models. The contribution of larger particles in the ABC class is indicative of pollen grains as they regularly exceed the size ranges of other smaller aerosols with the same being seen for D and DE particles whereas, A and AB classes are dominated by particles smaller than 10 µm in size and have previously been shown to potentially represent fungal spore fractions (Hernandez et al., 2016; Markey et al., 2022; Savage et al., 2017). Initial correlation analysis further corroborated these findings. Following this, more in-depth filtering and unsupervised

learning techniques were applied to improve these associations, showing that the WIBS-4+ has the potential for the detection and differentiation between tree, herbaceous and grass pollen from each other and from fungal spores (Markey et al., 2022). The size distribution charts of the WIBS FAP classes can be seen in the supplementary materials.

Although aerobiological data was grouped into general classes for the purpose of assessing the differentiation ability of the added FL4 and FL5 channels, individual species of pollen and fungal spores were recorded by the Hirst/optical microscope method. Major contributing taxa are shown in Fig. 1. Overall, pollen concentrations were largely dominated by *Quercus* concentrations at the beginning of the campaign with Poaceae and Urticaceae then dominating for the remainder of the campaign as the spring season ended. With regards to fungal spores, ambient concentrations were largely dominated by Ascospores and *Cladosporium*. Major allergenic pollen and fungal spore taxa identified include Poaceae pollen and *Cladosporium* and *Alternaria* spores. A more detailed description of recorded trends and peak days etc. have been previously discussed (Markey et al., 2022).

#### 3.2. Influence of meteorological parameters on particle concentrations and geographical origin

Meteorological conditions are known to be a driving force for the production and subsequent release of both pollen grains and fungal spores, as was described previously. Spearman correlation analysis was carried out to determine any notable association between ambient daily pollen and fungal spore concentrations with weather (Table 2).

From the results depicted in Table 2, a notable positive correlation with temperature was observed for Poaceae and Urticaceae pollen and a negative correlation observed for *Quercus* pollen. The importance of temperature variables for the production and dispersal of grass and herb pollen has been well documented in literature (Emberlin, 1994; Frisk et al., 2023; Norris-Hill, 1997). Although the same trends are often depicted for tree pollen such as *Quercus*, studies have highlighted that temperatures exceeding 20 °C can have a negative effect on *Quercus* pollen production (Pérez-Badia et al., 2013). This negative association with temperature is likely due to the waning concentration of ambient *Quercus* pollen experienced following the peak in *Quercus* pollen on the 30th of April as temperatures continued to increase. A negative correlation was also observed for total pollen and relative humidity. Similar associations have been observed in multiple studies and are largely attributed to the reduced rate of anthesis as relative humidity increases (de La Guardia et al., 1998; Hart et al., 1994).

Conversely, the opposite was observed for the relationship between total fungal spores and Ascospores with relative humidity, both illustrating a positive correlation with rain. The relationship between airborne concentrations of Ascospores and relative humidity is well established (Elbert et al., 2007; Hernández Trejo et al., 2012; Li and Kendrick, 1995; Lim et al., 1998; Vélez-Pereira et al., 2023), and it is generally accepted that during periods of higher relative humidity and following periods of rainfall, the concentration of Ascospores increases. *Cladosporium* and Basidiospores saw a significant positive correlation with temperature which corroborates previous findings of these spore types favouring dry weather and higher temperatures (Flint and Thomson, 2000; Hollins et al., 2004; Spiers, 1985; Trout and Levetin, 2001).

Investigating the correlation between WIBS FAPs and weather further validates the initial link between A and AB particles with fungal spores discussed in the first part of this study, (Markey et al., 2022) achieving R<sup>2</sup> values of 0.8. The significant positive correlation seen for A/AB particles and rain/relative humidity mirrors that seen for fungal spores. This is largely driven by the high contribution of Ascospores to the total fungal spore concentrations – equating to over 50 % of spore concentrations seen during the campaign. Similarities between pollen (particularly for Poaceae and Urticaceae) and D type particles can also

**Table 1**  
HYSPLIT analysis of peak days.

PBAP type	Peak date	Concentration (pollen grain or spore/m <sup>3</sup> )
Grasses (Poaceae)	29/05/2017	354
Trees ( <i>Quercus</i> )	30/04/2017	579
Herbs (Urticaceae)	28/05/2017	275
<i>Alternaria</i>	29/05/2017    05/06/2017	412    540
Ascospores	03/06/2017	64,894
Basidiospores	22/05/2017    28/05/2017	2443    2417
<i>Cladosporium</i>	05/06/2017	19,995

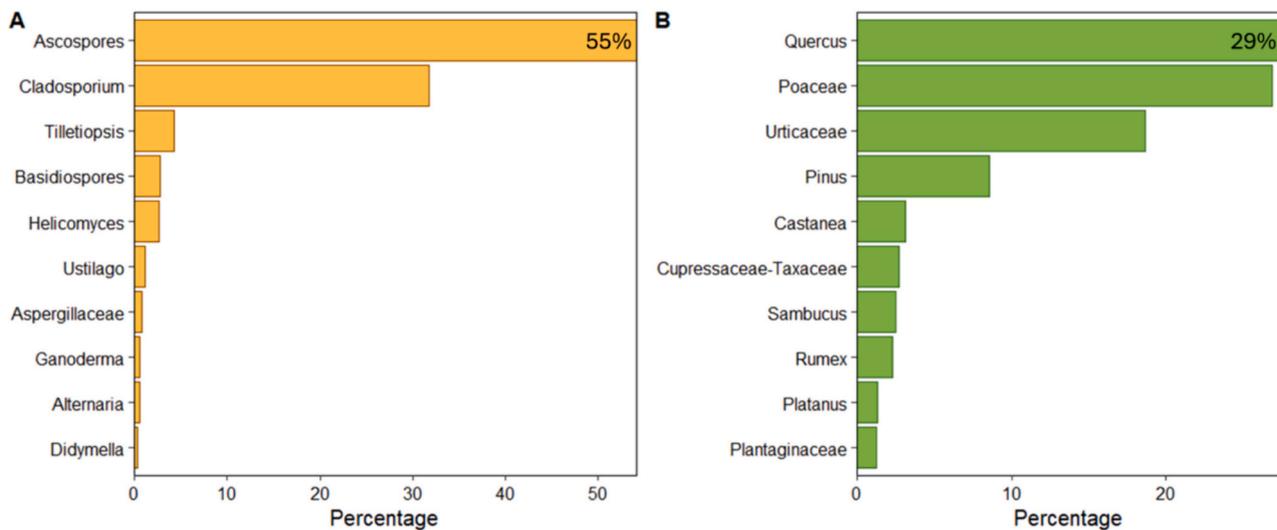


Fig. 1. Percentage distribution of top 10 (A) fungal spore and (B) pollen types.

Table 2

Spearman’s rank correlation between daily pollen/spore types and WIBS FAP concentrations with weather parameters.

	Temp	Wind_S	Wind_D	Pres	RH	Rain	CPC
Total Pollen	0.42**	-0.02	-0.3*	-0.14	-0.45*	-0.2	0.34
Quercus, L.	-0.51**	-0.12	0.14	-0.2	0.1	0.2	-0.19*
Urticaceae	0.79**	0.01	-0.3*	0.19	-0.33	-0.33	0.46**
Poaceae	0.89**	0.01	-0.34	0.06	-0.34*	-0.26	0.48*
Trees/Shrubs	-0.09*	-0.1	-0.07	-0.25	-0.25	0.01	0.12
Herbs	0.81**	0.01	-0.34*	0.15	-0.39	-0.37	0.49**
Total Fungal Spores	0.22	0.17	0.02	-0.29	0.47**	0.32	-0.29
Ascospores	-0.09	0.2	0.08	-0.3	0.70**	0.44*	-0.42
Cladosporium, Link	0.53**	0.12	-0.13	-0.22	0.1	0.09	0
Basidiospores	0.71**	-0.02	-0.13	0.03	-0.27	-0.24	0.44**
Alternaria, Nees	0.45	0.03	0.1	-0.32	-0.25	0.06	0.4
A	0.39	0.12	-0.02	-0.2	0.29**	0.17	-0.08
AB	0.03	0.04	0.06	-0.32*	0.58**	0.39**	-0.34*
ABC	-0.38**	-0.11	-0.1	-0.21	0.05	0.19	0.01
AC	-0.37**	0.16	0.24	-0.02	0.05	0.07	-0.26
B	0.04	-0.36*	-0.20*	0	-0.29*	-0.03	0.43**
BC	-0.23*	-0.18	-0.12*	0.03	-0.27*	-0.05	0.21
C	-0.26	0	-0.05	0.08**	-0.4**	-0.24	0.05
D	0.53*	-0.24	-0.46**	0.11	-0.25*	-0.2	0.64**
E	-0.34*	-0.18	-0.01	-0.11	0.04	0.09	0.09
DE	0.05	-0.14	-0.35**	0.02	-0.45**	-0.16	0.44**

\*significance at the 95 % level, \*\*significance at the 99 % level.

be observed based on their similar relationships with temperature and wind direction, again corroborating previous connections (Markey et al., 2022).

With regards to the WIBS particles, the influence of wind speed became increasingly significant and negatively correlated with increasing particle size >5 μm in size. This may be attributed to the stark differences in instrument flow rate and sampling efficiencies as previously discussed in the preceding study (Markey et al., 2022). This could further explain why the WIBS failed to account for several peaks in larger bioaerosol concentrations such as Poaceae pollen – which was under-sampled by the WIBS as a result of these conditions. The higher efficiency of the WIBS for sampling smaller particles could also explain why fungal spore detection seemed to marginally outperform pollen detection when comparing the WIBS FAPs to the Hirst concentrations.

Wind is an important release and transport mechanism for both fungal spores and pollen. In order to better understand the transport of airborne bioaerosols over Saclay the ZeFir source receptor model was used. The wind rose diagram, shown in Fig. 2, provides information on the statistical probability and distribution of the prevailing winds experienced during the sampling period. In this case, the prevailing

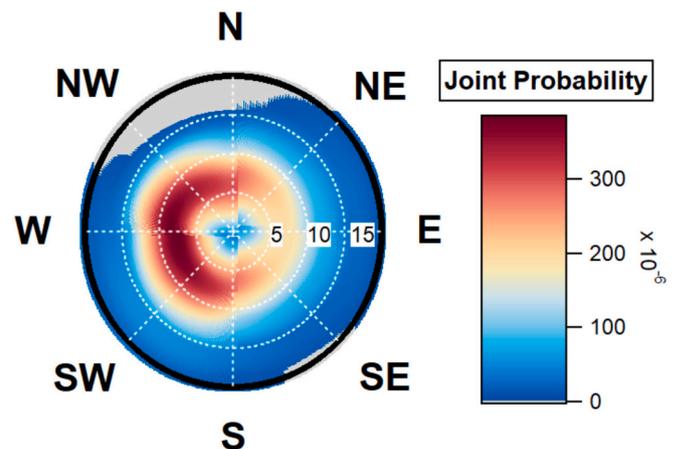


Fig. 2. Wind rose of prevailing winds during sampling period. White broken circles represent a wind speed scale in kilometer per hour (5 km/h, 10 km/h, 15 km/h).

wind predominantly originated from the western half of the windrows.

As shown in Fig. 3, ambient total pollen concentrations (pollen grains/m<sup>3</sup>) did not originate from the prevailing wind direction, instead coming from an easterly direction. Poaceae and Urticaceae also followed an easterly direction at strong wind speeds >15 km/h. This trend was also observed for the combined herbaceous pollen types which was largely dominated by Urticaceae pollen. However, although a similar easterly origin was observed from total tree and shrub pollen, the primary origin for *Quercus* (which represents approximately 30 % of all pollen samples) came from a south-easterly to the south-westerly direction at moderate wind speeds ranging from 10 to 20 km/h. The multi-directional origin may indicate several point sources. This explains why trees and shrub pollen have a combined weighted origin from the east due to the influence of summer tree and shrub taxa following *Quercus* pollination.

The geographical origin of ambient atmospheric fungal spores (particles/m<sup>3</sup>), highlighted in Fig. 4, shows a north-westerly direction at wind speed of >5 km/h but <15 km/h. Here we note that Ascospores also follows a north-westerly order, likely leading the directional origin of total spores due to their high concentrations. Conversely, *Cladosporium*, *Alternaria* and Basidiospores originated from a strong north-easterly direction, with *Cladosporium* and *Alternaria* also possessing a second source from a southern direction, at wind speed of >5 km/h.

Further land-cover analysis of potential fungal spore and pollen sources can allow for a more comprehensive understanding of their local specific geographical origins as is shown in Fig. S4 in the supplementary materials. The 30 km radius surrounding the sampling site is largely dominated by urban and industrial landscapes. However, several broad-leaved forest, coniferous forest and plenty of non-irrigated arable land are present mainly to the south/south-east of the sampling site.

Following the CORINE landcover classification and the site location, the broad-leaved forest contains: pure or mixed stands of beech (*Fagus*), oak (*Quercus*), hornbeam (*Carpinus*), lime (*Tilia*), maple (*Acer*), ash (*Fraxinus*), poplar (*Populus*), and birch (*Betula*) species among others.

Coniferous forest: pure or mixed stands of fir (*Abies*), pine (*Pinus*), spruce (*Picea*), cedar (*Cedrus*), cypress (*Cupressus*), juniper (*Juniperus*), yew (*Taxus*), and Douglas fir (*Pseudotsuga*) species, among others.

In the case of herb and grass pollen it is possible that Poaceae and Urticaceae pollen was transported along the air masses from natural grasslands and national forests situated close to Paris such as Forêt

domaniale de meudon and Verrières Forest, both of which are situated to the north-east of the sampling site. The same can be said for tree and shrub pollen which could have originated from similar sources as well as a nearby Arboretum (de la Vallée aux Loups), also positioned at a north-easterly direction from the sampling site. The source of *Quercus* pollen likely originated from the near-by forests located to the southeast of the sampling site such as Châtaigneraie du château de Corbeville. In comparison, the geographical origin of total and major fungal spore types likely originated from the non-irrigated arable land situated to the North-East and South-west of the Saclay site. Ascospores and *Cladosporium* spores represent the vast majority of the fungal spores sampled. These spores are associated with agricultural crop infections (Jesús Aira et al., 2012; Jordan, 1990; McCartney and Lacey, 1990; Shoute et al., 2018; Tournas, 2005; Trapero-Casas et al., 1996), and as such likely originated from areas containing crops such as the arable land surrounding the site.

To further investigate possible long-range transport of differing pollen and fungal spore types, HYSPLIT analysis was carried out to explore the origin of air masses on notable peak days during the campaign as summarised in Table 1. While the HYSPLIT back trajectory shows the primary wind direction on the 24 h preceding the peak concentration, the trajectory models shown herein do not include any bio-aerosol concentration data. These peak day trajectories should be observed in conjunction with the accompanying wind roses that describe the prevailing wind direction of higher bioaerosol concentrations. This is more reflective of the entire campaign.

As previously mentioned, most pollen originated from a north-easterly direction at high windspeeds (Fig. 3), but the peak day for most pollen types show wind coming from the south (Fig. S5). This shows that both the majority of the seasonal pollen, and the highest peak day, come from two separate sources that are not in the path of the prevailing wind, indicating two major sources of pollen, to the north-east, and to the south. In the case of herb and tree/shrub pollen, the peak days correspond to the peak days of Urticaceae and *Quercus* pollen respectively. On the peak day of grass pollen, the air masses of the previous 24 h originated largely from the west/south-west of the sampling location within France. Herbaceous pollen, on the other hand, originated from south-east region of France outside of Saclay and tree pollen originated from multiple directions over France, mainly coming from the south of France.

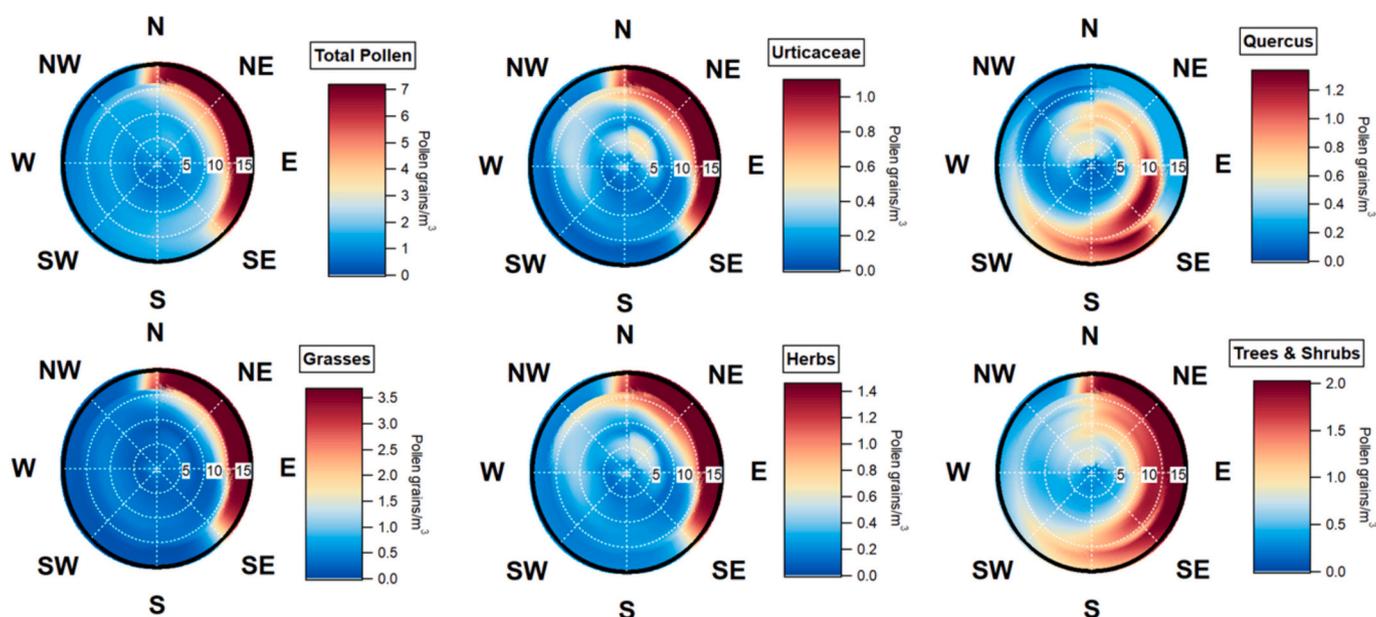


Fig. 3. Origin of Total pollen, Urticaceae, Quercus, Grass, Herb and Tree pollen as sampling site. The colour scale represents the estimated concentration (Pollen grains/m<sup>3</sup>) white gridlines represent a wind speed scale in kilometer per hour (5 km/h, 10 km/h, 15 km/h, 20 km/h).

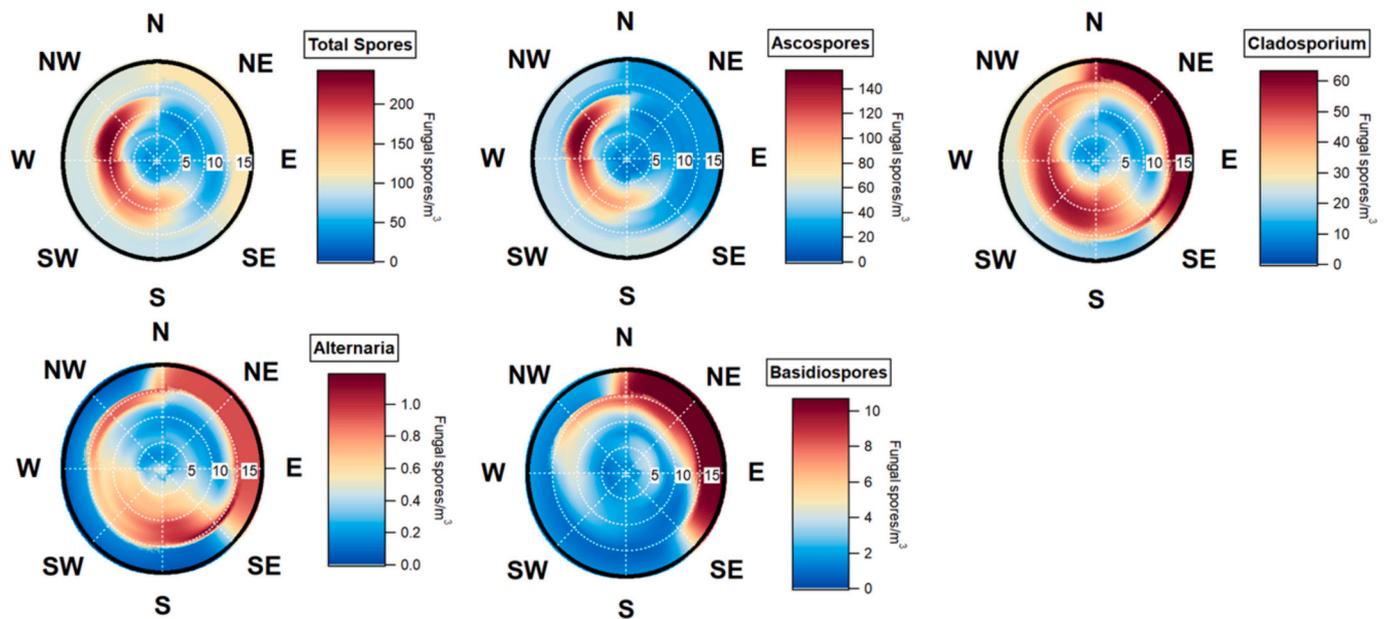


Fig. 4. Origin of Total fungal spores, Ascospores and Cladosporium, Alternaria and Basidiospores spores at sampling site. The colour scale represents the estimated concentration (Spores/m<sup>3</sup>) white gridlines represent a wind speed scale in kilometer per hour (5 km/h, 10 km/h, 15 km/h, 20 km/h).

The fungal spore HYSPLIT results combined with the wind roses also indicate two source directions, with one of these directions shared with pollen. There is a north-east source identifiable in the *Cladosporium* *Alternaria* and Basidiospore wind roses (Fig. 4), and a western source observed for *Cladosporium*, Ascospores, and *Alternaria* for both ZeFir and HYSPLIT analyses (Figs. 4 and S5). *Cladosporium* and *Alternaria* spores are often considered to be ubiquitous spore types found commonly in aerobiological studies and the presence of these two origins likely indicates the presence of multiple contributing sources.

### 3.3. Influence of air quality parameters

Due to the proximity of the sampling site to a major urban city and the concomitant presence of anthropogenic pollutants whose occurrence may strengthen the allergenic potential of airborne pollens/fungal spores (Carriñanos et al., 2021; Grewling et al., 2019a, 2019b; Ørby et al.,

2015; Sénéchal et al., 2015), the influence of pollutants on the concentrations of pollen grains, fungal spores and WIBS FAPs were also examined and are summarised in Table 3 (additional pollutants provided in supplemental Table S1). Notable positive correlation was observed for total pollen, Urticaceae and Poaceae pollen with O<sub>3</sub>, SO<sub>4</sub>, OM and LOOOA. Grass and herb pollen has been previously documented to illustrate positive correlations with certain pollutants such as O<sub>3</sub> (Adhikari et al., 2006; Goudarzi et al., 2022; Ouyang et al., 2019; Sousa et al., 2008). Positive correlations between pollen and pollutants can be attributed to comparable geographical origins and similar conditions needed for their release/production. From examination of their geographical origin (Fig. S7) these pollutants show a similar origin to that of Poaceae and Urticaceae pollen. Both these pollen types and pollutants appear to have originated from a north-easterly direction at wind speeds exceeding 15 km/h. In relation to the sampling site, this north-east origin corresponds to the city of Paris. It is, therefore, likely

Table 3  
Spearman's rank correlation between daily pollen/spore types and WIBS FAP concentrations with pollution parameters.

	NOx	SO <sub>4</sub>	NO <sub>3</sub>	OM	O <sub>3</sub>	BC <sub>ff</sub>	BC <sub>wb</sub>	LOO-OA
Total Pollen	0.11	0.32	0.15	0.51**	0.61**	0.34	0.34	0.55**
<i>Quercus</i>	0.31	-0.33*	0.43*	-0.03	0.03	0.14	0.29	-0.24
Urticaceae	-0.16	0.55**	-0.22	0.42**	0.35**	0.17*	0.02	0.65**
Poaceae	-0.28	0.53**	-0.33	0.45**	0.48**	0.06	-0.09	0.73*
Trees/shrubs	0.32	-0.1	0.39	0.28	0.43	0.31	0.39	0.13
Herbs	-0.2	0.47**	-0.28	0.4**	0.48**	0.13*	-0.04	0.61**
Total Spores	-0.57*	0.1	-0.39	-0.1	-0.15*	-0.44	-0.49*	-0.03
Ascospore	-0.44	-0.06	-0.2	-0.22	-0.26**	-0.41	-0.38	-0.26
Basidiospores	0.07	0.32	0.13	0.46**	0.47*	0.26	0.25	0.54**
<i>Alternaria</i>	-0.35*	0.22	-0.3	0.22	0.28	-0.16	-0.22	0.39*
<i>Cladosporium</i>	-0.6**	0.23	-0.5*	0.1	0.13	-0.37*	-0.49**	0.38
A	-0.55**	0.16	-0.39*	0.05	0.03	-0.38*	-0.48**	0.27
AB	-0.41	-0.01	-0.26	-0.1	-0.23**	-0.36	-0.41*	-0.05
ABC	0.5*	-0.1	0.53**	0.1	0.05	0.36**	0.46**	-0.1
AC	-0.11	-0.08	0.06	-0.22	-0.08	-0.21	-0.07	-0.42
B	0.73**	0.23	0.68**	0.55**	0.35*	0.74**	0.74**	0.38
BC	0.67**	0.06	0.64**	0.31	0.25	0.6**	0.69**	0.07
C	0.29**	-0.04	0.24**	-0.08	0.22	0.18*	0.3**	-0.05
D	0.29*	0.31*	0.16	0.5**	0.38**	0.46**	0.28*	0.72**
E	0.66*	0.14	0.72**	0.27	0.07	0.54**	0.62**	-0.05
DE	0.54*	0.07	0.42	0.34	0.42*	0.54**	0.53**	0.27

\*significance at the 95 % level, \*\*significance at the 99 % level.

that the origin of these pollutants came from Paris and were transported to the site in Saclay.

Temperature was found to be the dominant factor influencing the release of pollen, especially in the case of Urticaceae and Poaceae. In the case of O<sub>3</sub>, OM, secondary inorganic/organic aerosols such as SO<sub>4</sub> and LOOOA, several studies have highlighted increasing concentrations during summer months and higher temperatures (Bressi et al., 2014; Jiang et al., 2019; Ouyang et al., 2019; Zhang et al., 2019). In a study by Bressi et al. (2014), major sources of ambient SO<sub>4</sub> concentrations in Paris were found in the form of ammonium sulphate. The formation of ammonium sulphate was favoured during summer months due to higher temperatures and greater intensity/duration of sunshine promoting photochemical reactivity. In a similar study by Zhang et al. (2019), LOOOA in Paris were found to peak with increasing summer temperatures and were mainly associated with biogenic sources. Correlation analysis was also performed between pollutants and meteorological parameters to illustrate such trends (Table S2). The same association with summer weather conditions has also been found for photochemical oxidants such as O<sub>3</sub> (Ouyang et al., 2019). As a result of these comparable meteorological triggers, the production of these pollutants likely corresponded to concurrent release of pollen. Similarly, NO<sub>3</sub> correlated significantly well with *Quercus* pollen, again this connection is associated with comparable promoting conditions favouring the release of both aerosol types. *Quercus* pollen concentrations were highest at the beginning of the campaign when meteorological conditions were more representative of late spring and daytime temperatures had not exceeded 12 °C. The same conditions were found to also favour NO<sub>3</sub> concentrations. This corroborates previous studies which illustrated that NO<sub>3</sub> particulates in the form of ammonium nitrates show a clear seasonal pattern (Bressi et al., 2014) similar to pollen. In Paris, it is generally observed that higher concentrations of this particulate matter is seen during winter and spring followed by a sharp decrease during summer as the condensation of ammonium nitrate is favoured by lower temperature (Bressi et al., 2014), further supporting the trends observed during the present study.

In the case of fungal spores, concentrations were mainly negatively associated with pollutants or showed no significant correlation. In the case of total fungal spore and *Cladosporium* concentrations, a strong negative correlation was observed for NO<sub>x</sub> mirroring previous findings by (Grinn-Gofroń et al., 2011) for NO<sub>2</sub>. Total fungal spores and Ascospores also exhibited a negative association with O<sub>3</sub> which corroborates previous similar studies (Lin and Li, 2000; Roy and Gupta Bhattacharya, 2020; Sousa et al., 2008). However, the opposite was seen for basidiospores, as positive correlations with O<sub>3</sub> were seen, with similar trends having been documented with both (Adhikari et al., 2006) and (Grinn-Gofroń et al., 2011) illustrating positive correlations between O<sub>3</sub> and fungal spores. This relationship, as with many meteorological/anthropogenic variables, likely varies depending on location, time of year, climate etc.

Since the WIBS measures the intrinsic fluorescence of a particle, it can detect other particles that are not biological in nature but are fluorescent. In the previous part of this study (Markey et al., 2022), measures were taken to reduce the interfering impact of such particles by increasing the baseline fluorescent threshold from 3σ to 9σ, which has been shown to reduce the influence of interferents without affecting the representative biological fraction of FAPs (Savage et al., 2017). In order to investigate the impact of anthropogenic aerosols on the WIBS, FAP classes were also compared to the various pollutant parameters. Interestingly A and AB particles showed strong negative correlation with the majority of the investigated pollutants. Since A and AB particles have previously been classified as fungal spore in nature, these negative associations with air quality parameters indicate that these WIBS classes do not contain significant contamination from pollutant classes. These negative correlations seen for A/AB FAPs are comparable to the negative correlations seen for total fungal spore, *Cladosporium* and Ascospore concentrations with pollutant parameters. This adds further credence to

the classification of A/AB being as fungal spores (Fig. 5).

B, BC and E particles showed the strongest association with many of the pollutants investigated, with the strongest correlation seen for black carbon (BC<sub>ff</sub> and BC<sub>wb</sub>), NO<sub>x</sub> and NO<sub>3</sub> (Table S1) achieving *r* values >0.7. The production and formation of these pollutants are all associated with vehicle emissions or combustion processes. Several studies have previously noted the detection of anthropogenic aerosols using the WIBS. B type particles in particular have been linked with anthropogenic sources in past studies (Gabey et al., 2011; Toprak and Schnaiter, 2013; Twohy et al., 2016). However, only a limited number of investigations have assessed the WIBS characterisation of known anthropogenic aerosols. In one such study by Savage et al., 2017 (Savage et al., 2017) a number of non-biological samples were investigated. Soot type particles exhibit predominantly A-type fluorescence, whereas smoke type samples were classified predominantly as B-type particles (Savage et al., 2017). Due to the lack of correlation seen for A type particles and increased correlation seen for B and BC particles, it would appear that the pollutants investigated herein-especially black carbon concentrations-understandably show strong similarities to the smoke particles (produced following wood burning) classified by Savage et al., 2017 (Savage et al., 2017).

A similar study conducted by Yu et al. (2016) investigated the impact of combustion related particles on the real-time detection of fluorescent aerosols using the WIBS-NEO. It was found that when using LIF instruments like the WIBS near pollutant sources (such as Paris), fluorescent measurements experience heavy interference from anthropogenic aerosols such as black carbon originating from combustion processes. Notable positive correlation was seen for B particles >2 μm but <4 μm with black carbon (*r* = 0.58). Since the WIBS-4+ was operated in high gain mode for this investigation only particles >2 μm were collected. However, the correlation between these combustion aerosols and B and BC particles were highest for particles <3 μm in size showing similarities to previous findings (Yu et al., 2016). This also suggests that smaller modes of WIBS particles in these classes could represent possible anthropogenic fluorescent particles. The agreement between B type fluorescent particles and the temporal trends of NO<sub>x</sub> and black carbon could also be indicative of PAHs and/or their derivatives, even at larger particle size ranges (Miyakawa et al., 2015). Other interfering aerosols not compared to the WIBS in this study due the absence of co-located monitoring data such as mineral dust, HULIS like compounds and other secondary organic aerosols, have also been shown to possess fluorescence and could be contributing to fluorescent fractions of the sampled WIBS particles (Pöhlker et al., 2012a; Savage et al., 2017; Toprak and Schnaiter, 2013).

In the case of E type particles (showing fluorescence in the FL5 channel only), a notable correlation was observed for NO<sub>3</sub> ambient concentrations and similar geographical origins (Fig. S8). Typically, the fluorescent detection of ambient NO<sub>3</sub> is conducted at longer excitation wavelength greater than that of the traditional WIBS channels (excitation: 280 nm and 370 nm, emission: 310–400 nm and 420–650 nm) at an excitation wavelength of approximately 600 nm and emission detection in the region of 700–750 nm (Wood et al., 2003). The detection band of the FL5 channel covers this emission range. The correlation between E particles with NO<sub>3</sub> concentrations likely originates from detection of aerosols formed from reaction of ambient NO<sub>3</sub> emissions. In Paris and the surrounding area, NO<sub>3</sub> is mainly found in the form of ammonium nitrate, formed by the reaction of ammonia and nitric acid with major sources originating from agricultural release of ammonia and combustion sources for NO<sub>x</sub> (Bressi et al., 2014). This could also account for the strong correlation seen for NO<sub>3</sub> with NH<sub>4</sub> and NO<sub>x</sub> emissions (*r* = 0.85). Ammonium nitrate has been shown to fluoresce in the emission range of 650–700 nm when excited at similar wavelength to those used by the WIBS, such as 266 nm (Pan et al., 1999). Although ammonium nitrate is typically found in the fine mode of atmospheric aerosols, it can be present at coarser modes and under the right conditions observable by WIBS (Wall et al., 1988). In addition to this the correlation between NO<sub>3</sub>

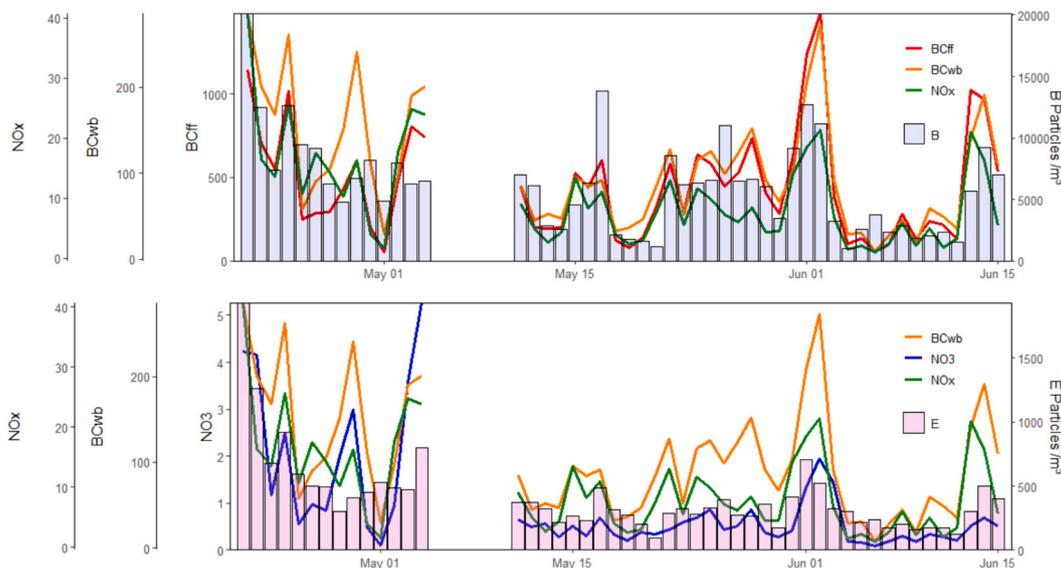


Fig. 5. Comparison between pollutants and (A) B WIBS particles and (B) E WIBS particles.

related particulates and E type fluorescent particles could be related to other sources such as combustion.

The potential of the WIBS to broadly classify bioaerosol classes in a diverse ambient environment has been shown to improve upon the addition of the FL4 and FL5 fluorescent channels. In this case, the implications of meteorological and air quality parameters on the operation of the WIBS has highlighted the possibility of deploying the modified WIBS or future iterations as an all-encompassing air quality monitor, capable of detecting and possibly classifying biological and anthropogenic particles. However, additional work is needed to further assess this potential application especially regarding the detection of pollutant particles at low gain operating modes in addition to addressing the limitations associated with sampling large particles at higher wind speeds.

### 3.4. Multiple Linear Regression analysis

In order to measure the combined effect of conditions on the potential selective detection of pollen and fungal spores by the WIBS-4+ a series of Multiple Linear Regression (MLR) algorithms were developed. The results of the MLR analysis can be seen below in Table 4. Six parameters were chosen to undergo MLR analysis. These included five categories from the ‘‘Pollen’’ and ‘‘Fungal Spores’’ groupings. For pollen, these were Poaceae, herb pollen, and tree pollen and for fungal spores, these were Ascospores and *Cladosporium*. Other fungal spore categories, namely Basidiospores and *Alternaria* were analysed but due to either the diversity of the spore groupings, or the overall concentrations (too low) results of note could not be obtained. After completing test analysis at various filter sizes, WIBS data was size filtered to >2 μm for fungal spores, and >8 μm for pollen grains. Furthermore, all analysis was performed with WIBS data fluorescent baseline thresholds of 3σ, 6σ, and 9σ, with the highest performing threshold being independently selected for each model.

After completion of the MLR analysis, the highest R<sup>2</sup> result was seen for Ascospores with an R<sup>2</sup> of 0.77. Next was tree pollen, with an R<sup>2</sup> of 0.73. The different pollen/fungal spore classes did not find their highest correlation values at the same fluorescent threshold level, with most parameters finding higher correlation at 9σ, while *Cladosporium* had higher correlation at the lower 3σ value. Pollen parameters had an identifiable correlation ‘‘ceiling’’ due to the peak period during the campaign, which no meteorological parameter, pollution measurement, or WIBS band, could reasonably overcome. This resulted in R<sup>2</sup> values of

Table 4

Multiple Linear Regression Analysis of selected Fungal Spores, Pollen Grains, and Black Carbon emission concentrations.

Dependent variable	WIBS particle size filtering	Regression	R <sup>2</sup>	Adjusted R <sup>2</sup>
Poaceae (9σ)	≥8 μm ≤30 μm	2396.47 + 0.44 × D + 85.09 × SO <sub>4</sub> - 176.28 × Cl - 69.40 × Wind North + 41.12 × Wind South-West - 2.84 × Relative Humidity - 2.27 × Pressure	0.63	0.57
Herbs (9σ)	≥8 μm ≤30 μm	- 13.99 + 0.75 × D - 0.08 × DE + 0.56 × Ozone - 39.92 × Wind South-East	0.68	0.65
Trees (9σ)	≥8 μm ≤30 μm	- 62.99 + 0.68 × DE + 84.66 × NH <sub>4</sub> - 168 × Wind North - 99.76 × Wind North-East + 68.45 × Wind East - 79.51 × Wind North-West	0.73	0.69
<i>Cladosporium</i> (3σ)	≥2 μm ≤8 μm	1467.45 - 4.68 × SUM (ABC + BC) + 3.94 × A + 2687.13 × Wind South + 317.09 × temperature - 0.39 × CPC	0.66	0.62
Ascospores (9σ)	≥2 μm ≤8 μm	1128.61 - 2.64 × B + 6.02 × A + 1539.35 × NO <sub>3</sub> - 6322.38 × Wind South - 7464.60 × Wind South-West	0.77	0.75
Black Carbon (3σ)	≤3 μm	737.43 + 6.03 × BC + 429.86 × Wind North + 608.46 × Wind North-East + 240.13 × Wind West - 197.88 × Wind Speed	0.74	0.71

0.63 and 0.68 for Poaceae and herb pollen, respectively. *Cladosporium* concentrations also peaked during this time period and had an R<sup>2</sup> of 0.66. Several parameters were significant in determining concentrations of more than one bioaerosol parameter. The FL4 and FL5 channels were the most important parameter for calculating concentrations of all three pollen classes, whereas the FL1 channel was used in determining ascospore and *Cladosporium* concentrations.

The FL1 channel had the strongest relationship with fungal spores out of all the WIBS fluorescence channels. Both *Cladosporium* and ascospores were related to A type WIBS particles, indicating that this particle class is composed largely of fungal spores. After many filtration levels and analyses, no WIBS band could be seen to reasonably correlate with basidiospores, but the overall length of this campaign means that individual days or short periods of unusual weather can have a disproportionate impact upon some results. For pollen grains, the FL4 and FL5 channels were of more relevance, with D type WIBS particle relating to both Poaceae and herb pollen concentrations and DE particles relating to tree pollen concentrations.

When constructing the MLRs, it was noted that the different pollution/weather parameters could not consistently account for the concentration variance seen for each pollen grain/spore type. This means while numerous parameters were significant, no single parameter was applicable across multiple different bioaerosol types. Between the 5 bioaerosol MLRs constructed, a total of 9 different pollution/weather parameters were seen as significant in calculating concentrations for one of the types, but no one parameter was significant for more than one bioaerosol type. This supports the idea that species specific algorithms and forecasts are necessary, rather than attempting to design one algorithm to obtain average concentration forecasts for all pollen or spore types.

While the FL4 and FL5 channels were important in calculating the pollen concentrations, and the FL1 channel was an appropriate match for fungal spore concentration predictions, further tests into other fluorescent aerosol types were carried out to see if either FL2 or FL 3 could be associated with any known aerosols. Upon completion of this analysis, the concentrations of Black Carbon measured at the sampling site were found to be reliably predicted using an MLR analysis involving the BC WIBS channel. This BC channels represents particles that fluoresce in FL2 and FL3 only and indicates to us that these fluorescence bands could be representative of black carbon and its primary emission sources, fossil fuels (BCff) and wood burning (BCwb). With this analysis, each of the fluorescence bands can be reliably associated with a different fluorescent particle type as follows; FL1 = Fungal Spores, FL2 and FL 3 = Black Carbon, FL4 and FL5 = Pollen Grains.

#### 4. Conclusion

This study focussed on the deployment and evaluation of a modified WIBS-4+ instrument for the detection and differentiation of multiple PBAP classes, the impact of various meteorological and air quality parameters was also investigated.

Correlation analysis between isolated WIBS FAPs and ambient bioaerosol concentrations with meteorological and air quality parameters showed comparable trends for suspected representative fractions – further corroborating associations between different WIBS particles and PBAP classes. Certain correlations between pollen concentrations and anthropogenic pollution concentrations were also attributed to similarities in preferential meteorological conditions for the release of such particulates as well as suggesting similar sources of origin as shown from the ZeFir analysis.

Differences in the sampling efficiency of the WIBS and Hirst were noted previously, however, it was found that the low flow rate of the WIBS-4+ in conjunction with higher wind speeds led to the under sampling of larger particles >5 µm in size – further explaining the discrepancies in the ability of the WIBS-4+ to record large pollen grains such as Poaceae and *Pinus* which were noted in the first part of this study.

Further analysis of wind data allowed for the apportionment of possible sources of PBAP over the entire campaign (ZeFir) as well as during the 24 h preceding notable peak days (HYSPLIT). In most cases, different directional origins of air were seen during peak days than for the entire campaign – highlighting the contribution of different local and long-range sources.

Due to the proximity of the sampling location to the mega city of Paris, various anthropogenic parameters were also investigated. It was noted that several pollen types illustrated positive correlation with several pollutants due to comparable conditions required for their production and similar sources. This co-occurrence could lead to increased respiratory reactions for allergy sufferers.

Interestingly, although precautions were taken to diminish the impact of interfering fluorescing anthropogenic aerosols, the modified WIBS-4+ showed good correlation between B and E type particles with several combustion related particle concentrations. These particulates could also potentially exhibit fluorescence in the FL2 and FL5 channels, as B and E type particles are defined as those which fluoresce in channels FL2 and FL5. The strong correlation seen for certain pollutants raises the argument that perhaps the WIBS-4+ could possess some merit as an overall air-quality monitor, including both anthropogenic and biological aerosol detection. Further work would be required to fully evaluate this potential in similar complex ambient environments, especially focussing on the detection of smaller particle sizes using lower gain WIBS modes.

The MLR analysis further brought into focus the benefits of the additional FL channels, as both the FL4 and FL5 channels were significant factors in the construction of all pollen-based regression equations. High multiple R<sup>2</sup> values were successfully obtained from all bioaerosols analysed. The best performing individual pollen regression was for tree pollen (R<sup>2</sup> = 0.73), followed by herb pollen (R<sup>2</sup> = 0.68). Individually, strong correlation was obtained for both *Cladosporium* (R<sup>2</sup> = 0.66) and ascospores (R<sup>2</sup> = 0.77) through use of the FL1 channel. Interactions between factors also assisted in increasing the MLR correlation values, with significant relationships observed between various WIBS channels, meteorological parameters and pollutant data. The channels that initially didn't correlate strongly with any bioaerosols, FL2 and FL3, were, upon subsequent analysis, found to strongly correlate with black carbon concentrations. This fluorescent particle was then added to the MLR analysis, with a high regression value of (R<sup>2</sup> = 0.74) obtained, resulting in all FL channels being reliably associated with a known fluorescent aerosol type.

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#### CRedit authorship contribution statement

**Emma Markey:** Conceptualization, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **Jerry Hourihane Clancy:** Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **Moisés Martínez-Bracero:** Formal analysis, Software, Visualization, Writing – original draft, Writing – review & editing. **Roland Sarda-Estève:** Data curation, Project administration, Writing – review & editing. **Dominique Baisnée:** Data curation, Project administration, Writing – review & editing. **Eoin J. McGillicuddy:** Resources, Writing – review & editing. **Gavin Sewell:** Validation, Writing – review & editing. **Carsten Ambelas Skjøth:** Conceptualization, Investigation, Software, Validation, Writing – review & editing. **David J. O'Connor:** Conceptualization, Data curation, Funding acquisition, Project administration, Supervision, Writing – review & editing.

#### Declaration of competing interest

The authors declare that they have no known competing financial

interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2024.173649>.

## References

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