Abundance and Diurnal Trends of Fluorescent Bioaerosols in the Troposphere over Mt. Tai, China, in Spring

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Abstract

Primary biological aerosol particles are ubiquitous in the global atmosphere and can affect cloud formation, deteriorate air quality, and cause human infections. Mt. Tai (1,534 m a.s.l.) is an elevated site in the North China Plain where atmospheric aerosols reflect both regional advection and long-range transport. In this study, we deployed a Wideband Integrated Bioaerosol Sensor (WIBS-4A) and collected total suspended particles and eight-stage size-segregated aerosol samples at the summit of Mt. Tai in spring from 21 March to 8 April 2017 to quantify the abundance, size distributions, and diurnal variations of fluorescent bioaerosols and to investigate the effect of different fluorescence thresholds of WIBS for ambient bioaerosol recognition. During the whole sampling period, the number concentration of fluorescent particles (>0.8 μm) was 647 ± 533 L⁻¹, accounting for 26.9% ± 10.0% by number of the total particles in that size range. Three-dimensional excitation-emission matrix fluorescence of water-soluble organic matter in size-segregated aerosols shows that humic-like substances (HULIS) are mainly in the fine mode (<2.1 μm) while protein-like substances are mainly in the coarse mode (>2.1 μm). From the diurnal variation, it is shown that bioaerosols can undergo transformation during long-range transport and contribute to HULIS. For bioaerosol recognition, we find that 6σ-threshold can lead to better classification of fluorescent aerosol particles for fungal spores. Overall, our results constrain the abundance of primary bioaerosols in the troposphere over East Asia and elucidate the processes for their evolution via mountain/valley breezes and long-range transport.

1 Introduction

Primary biological aerosol particles (PBAP) that are emitted directly from the biosphere contribute a large fraction of the atmospheric particulate mass (Després et al., 2012; Pöschl, 2005; Pöschl et al., 2010). PBAP comprise animal and plant debris, pollen grains, fungal spores, bacteria, virus, proteins, and other materials of biological origin (Després et al., 2012; Xie et al., 2017). Estimation of terrestrial PBAP emission is still highly uncertain, ranging from 56 to 1,000 Tg/year (Boucher et al., 2013; Jaenicke, 2005). The physical dimensions of PBAP range from nanometers (e.g., viruses) to hundreds of micrometers (e.g., pollen grains; Fröhlich-Nowotny et al., 2016). The abundance and physical states of bioaerosols not only depend on their source emissions, but also are related to their atmospheric history, through processes such as condensation of organic coatings (Mikhailov et al., 2015; Pöhlker, Wiedemann, et al., 2012; Pöschl et al., 2010), photooxidation by oxidants (Shiraiwa et al., 2012), clustering with dust particles (Barberán et al., 2015; Yamaguchi et al., 2016), and transformation during long-range transport and contribute to HULIS. Airborne measurements suggest that PBAP can be lofted to high altitudes and transported over regional and continental distances (Fu et al., 2014; Hu et al., 2017; J. Lee et al., 2017; Pratt et al., 2009). Mountainous sites are ideal for the studies of aerosols in the free troposphere and its interaction with the planetary boundary layer (PBL; Choularton et al., 2008; Fu et al., 2008, 2014; Matthias-Maser...
et al., 2000; Wang et al., 2012). Mt. Tai is the most elevated location on the North China Plain, and has become a focus for pollution research due to the complicated sources and evolution processes of pollutants (Fu et al., 2008; Gao et al., 2018; W. Li et al., 2011). The mountain is located at the east of the East Asian Continent (Kawamura et al., 2013). When subtropical westerlies prevail, air masses transporting aerosols over the North China Plain can affect the downwind areas of the Korean Peninsula, Japanese Islands, and the North Pacific Ocean (Fu et al., 2012; Xu, Wei, Chen, Zhu, et al., 2017). According to a year-round study at Mt. Tai (Zhang et al., 2014), the summit of Mt. Tai is within the PBL in the daytime of spring season. Freshly emitted PBAP and other nonbiological aerosols can be transported upward in daytime by valley breezes together with the large-scale convective air mass. During this transport, aerosols will be involved in processes such as photooxidation and multiphase reactions in-cloud droplets. While at night, especially after midnight, the PBL lowers to about half the altitude of the mountain (Zhang et al., 2014). The air mass over the summit station is therefore representative of the lower free troposphere, which is most often derived from a regional scale or long-range transport (Fu et al., 2012; Zhang et al., 2014). Mountain breezes transport airborne aerosols and gases downward, thereby influencing the local regions under the boundary layer.

Comparing with land surface environments, field characterization of bioaerosols at high altitudes is still very limited (Després et al., 2012; Fu et al., 2014). The bioaerosols at this site have been discussed mainly based on bulk filter analysis for molecular tracers and genetic materials (Fu et al., 2008; Wang et al., 2009; M. Wei et al., 2017; Xu, Wei, Chen, Zhu, et al., 2017). Genetic sequencing of the fungal species and bacteria in aerosols and cloud water has been reported for this site (M. Wei et al., 2017; Xu, Wei, Chen, Sui, et al., 2017; Xu, Wei, Chen, Zhu, et al., 2017). These studies presented fungal sequencing data showing variations in PM2.5.

The measurement of PBAP based on UV light/laser-induced fluorescence (UV-LIF) method is fast and non-destructive by using online instruments such as Ultraviolet Aerodynamic Particle Sizer (HAirston et al., 1997), Wideband Integrated Bioaerosol Sensors (WIBS; Kaye et al., 2005) and Single-Particle Fluorescence Sensor (Pan et al., 2007). This technique has been applied for online PBAP characterization in forest, rural, urban, and suburban environments (Gabey et al., 2011; Huffman et al., 2013; Pöschl et al., 2010; Yue et al., 2017). Autofluorescence of PBAP is caused by natural bio-fluorophores. Typical biological fluorophores include amino acids (e.g., tryptophan, tyrosine); structural chemicals such as chitin, cellulose, lignin, and sporopollenin; coenzymes such as Nicotinamide Adenine Dinucleotide (NADH) and Nicotinamide Adenine Dinucleotide(P)hosphate (NAD(P)H), flavins and vitamins, pigments (e.g. chlorophyll-a, chlorophyll-b), nucleic acids (e.g. DNA and RNA), and secondary metabolites (Fennelly et al., 2017; Pöhlker, Huffman, et al., 2012). Nonbiological fluorescent compounds including HULIS, mineral dust containing transition metals and rare-earth cations, PAHs, soot, and SOA are potential interferences for fluorescent biological aerosol particles (FBAP) measured by UV-LIF method (H. Lee et al., 2013; Pöhlker, Huffman, et al., 2012; Savage et al., 2017). While the excitation-emission (Ex-Em) spectral bands of WIBS-4A are tuned to excite at the maximum fluorescence of tryptophan and NAD(P)H, it should be noted that many other biofluorophores, such as riboflavin, ergosterol, and chlorophylls, and the nonbiological fluorophores listed above can also fluoresce in the sensing spectral regions of WIBS-4A albeit often with lower quantum yields (Pöhlker, Huffman, et al., 2012; Savage et al., 2017).

Attribution of the fluorescent particles to specific types of bioaerosols is an active field of research. The majority of the atmospherically relevant biological fluorophores has been summarized in Pöhlker, Huffman, et al. (2012). A simple way of categorizing fluorescent bioaerosols is by using the particle’s spectral property. For WIBS, seven types of fluorescent particles can be classified (for definitions please refer to the method section, Table S1 and Perring et al., 2015). According to the spectral features of a vast number of biological compounds in literature (Savage et al., 2017; Pöhlker, Huffman, et al., 2012; and references therein), bio-fluorophores of high or medium relevance in the atmosphere for the fluorescence-resolved fluorescent aerosol particle (FAP) measurement are tryptophan and tyrosine (FL A, FL AB); NADH and NADPH (FL C, FL BC); cellulose and lignin (FL AB); riboflavin (FL B, FL BC); vitamin B6 compounds (FL A); lipofuscin and ceroid (FL BC); phenolics (FL BC, FL C); terpenoids and dipicolinic acid (FL BC); ergosterol (FL ABC);
sporopollenin, flavonoids, chitin, and alkaloids (FL C). It should be bear in mind, however, that the actual fluorescence of these bio-fluorophores are rather complex presenting fluorescence characteristics beside the main features shown above, due to effects such as physical quenching (Savage et al., 2017). With respect to biological species (Hernandez et al., 2016), more than 90% of 13 types of bacteria were classified as FL A particles or FL AB particles for Bacillus subtilis; nearly 80% of the fungal spores (27 types) were categorized as FL A particles, following by FL AB and FL ABC type; pollens were partitioned into FL ABC and FL BC particles, collectively representing approximately 80%, with FL C and FL AB also representing around 10% for species like Eucalyptus. Another study by Savage et al. (2017) shows that as the size of pollen materials and fungal spores increase, they have the tendency to be classified as particles with multiple Ex-Em pairs (e.g., FL AB and FL ABC).

Currently, it is still difficult to robustly determine the representativeness of single fluorescent particles for PBAP characterization by fluorescence technique (Kaye et al., 2005; Perring et al., 2015; Pöhlker, Huffman, et al., 2012). To eliminate nonbiological fluorescent compounds or assess their influence on FBAP measurements, one method is to determine an appropriate fluorescence intensity threshold above which particles are defined as fluorescent particles (Savage et al., 2017). While increasing the thresholds can eliminate weakly fluoresced nonbiological particles, small bioaerosols with weak fluorescence intensity are also prone to be excluded. As shown in the previous experiments on dust, PAHs, soot, HULIS and brown carbon, 6σ- and 9σ-thresholds (refer to section 2 for definitions) are not capable of eliminating PAHs (e.g., pyrene, phenanthrene, and naphthalene), fullerene, and diesel soot (Savage et al., 2017). In the fine mode, the 9σ-threshold helps to decrease the interference from wood smoke soot and brown carbon (e.g., methylglyoxal + glycyne, glycolaldehyde + methylamine, and glyoxal + ammonium sulfate). In the coarse mode, HULIS (Pony Lake fulvic acid) was largely eliminated. For dust particles, although increasing the fluorescence thresholds were effective to classify them to be non-FAP, the mixing state of dust particles with biological particles was still not clear, hindering to reach a conclusive method to eliminate these particles. Moreover, it has also been revealed that, for some fungi species, like Aspergillus brasiliensis, Rhizopus stolonifer and Aspergillus versicolor, more than 80% of their fine mode particles were misclassified into non-FAP by applying both 3σ- and 9σ-threshold baselines, which indicates that even using 3σ-threshold, biological aerosol abundance can be underestimated for some species (Savage et al., 2017). Currently, discussions of the effects of varying fluorescence intensity thresholds on FBAP characterization have not been applied for ambient measurements.

The objective of this study is to investigate the abundances, size distributions, and diurnal variations of biological aerosol particles in the troposphere over Mt. Tai (1534 m a.s.l.) in East China. WIBS-4A was first utilized at this site to provide real-time monitoring of fluorescent bioaerosols. Offline fluorescence measurements and chemical characterization of molecular markers for airborne fungal spores were combined with online fluorescent bioaerosol characterization. Taking advantage of this mountain site, the effect of long-range transport of bioaerosols is also discussed. In addition, we discuss the effects of different fluorescence threshold criteria on ambient biological aerosol categorization, in order to optimize the analytical strategies using UV-LIF techniques to characterize bioaerosols in atmospheric samples.

2. Materials and Methods

2.1. Sampling

Sampling was conducted at the summit of Mt. Tai, China (1534 m a.s.l.; 36.26°N, 117.10°E), from 21 March to 8 April 2017. The location is the same as for the previous study by W. Li et al. (2011). Total suspended particle (TSP) samples (n = 34) were collected onto quartz fiber filters (TISSUQUARTZ-2500QAT-UP, Pallflex) on a day/night basis using a high-volume air sampler (Tisch 4010126, United States). Size-segregated aerosol samples (n = 6) were collected onto Munktell microglass fiber papers using an eight-stage nonviable Andersen cascade impactor (Anderson, United States). The 50% cutoff sizes of the impactor are: >9.0, 9.0–5.8, 5.8–4.7, 4.7–3.3, 3.3–2.1, 2.1–1.1, 1.1–0.7, 0.7–0.4, and <0.4 μm. The filters were first enveloped with aluminum foils and baked at 450 °C for 6 hours. Sampled filters were stored at −20 °C before the analysis. Details of the sampling record of the date and time are provided in Tables S2 and S3. Hourly meteorological data were obtained from the meteorological station at the summit of Mt. Tai (Station ID: 54826).
2.2. WIBS Measurement and Analyses

The principles of the operation and measurement of WIBS have been described in previous studies (Gabey et al., 2011; Kaye et al., 2005; Yue et al., 2017). Briefly, individual airborne particles were introduced into a measuring chamber through stainless-steel and silicone tube. The tube was set vertically into the wind. The inlet was around 2 m above ground. The tubing length was approximately 60 cm with bending less than 30°. For particle measurement, a continuous-wave 635 nm diode laser was used for the initial determination of the optical diameters of particles and to count the number of particles. For particles larger than 0.5 μm, fluorescent emissions of particles were detected in two wavelength ranges, 310–400 and 420–650 nm, excited by xenon wavelengths centered at 280 and 370 nm successively. The size threshold for total particle analysis was set to 0.8 μm (Gabey et al., 2011; Healy et al., 2012; Yue et al., 2016). Assuming a density of 1 g/cm³, the mass concentrations of FAPs were calculated (Heald & Spracklen, 2009).

2.3. WIBS Fluorescence Thresholds

Fluorescence thresholds were determined as the average fluorescence intensities plus multifold standard deviations by using forced trigger mode data for the three fluorescence channels FL1 (Ex/Em: 280 nm/310–400 nm), FL2 (280 nm/420–650 nm) and FL3 (370 nm/420–650 nm; Gabey et al., 2010; Gabey et al., 2011; Mason et al., 2015; Perring et al., 2015; Twohy et al., 2016; Ziemba et al., 2016). The elastically scattered UV light at 370 nm is much higher than the fluorescence signal and therefore saturates the detector of the 310–400 nm channel (Kaye et al., 2005). From the histogram of fluorescence intensity in background mode (Figure S1), 3σ-threshold (average + threefold standard deviations) criteria located at the right bottom side of the Gaussian-like distributions of the fluorescence intensities. In this study, 3σ-thresholds were applied to be consistent with many previous studies (Toprak & Schnaiter, 2013; Twohy et al., 2016; Yu et al., 2016; Yue et al., 2016, 2017), while the effect of applying more rigorous standards will be discussed at the end of the discussion session. FAPs were classified using the fluorescence-based scheme and they were categorized as FL A, FL AB, FL ABC, FL AC, FL B, FL BC, and FL C fluorescent particles following literature convention (Perring et al., 2015; Yue et al., 2017; Table S1).

2.4. Offline Fluorescence and Factor Analyses of Excitation-Emission Matrices

Offline fluorescence was measured for size-segregated aerosol and TSP samples. Three eightths (3/8) of each stage of the size-segregated aerosol samples and an aliquot (30 mm, diameter) of TSP samples, respectively, were cut for the extraction of the water-soluble compositions by adding 20 ml Milli-Q (18.2 MΩ cm) water and ultrasonating the mixture for 20 min. The extracts were filtered via syringe-driven filters (Millex-GP, 0.22 μm). A fluorometer (Fluoromax-4, Horiba) and a UV-Vis spectrophotometer (U3900H, Hitachi) were used to analyze the excitation-emission matrix (EEM) and absorbance of water-soluble contents of aerosols, respectively (Fu et al., 2015; Yue et al., 2016). Excitation and emission wavelength ranges were 240–455 (5 nm step) and 290–550 nm (2 nm step), respectively. Fluorescence spectra were obtained in a Signal/Reference mode with instrumental bias correction. The inner filter effect was corrected by absorbance spectrum measured with the spectrophotometer (McKnight et al., 2001). The EEM spectra of all samples were corrected by subtracting the blank sample, especially for each size range of size-segregated aerosol samples. First- and second-order Rayleigh scattering were removed by interpolation. Fluorescence was converted into Raman units (RU L m⁻³).

A parallel factor analysis (PARAFAC) model with nonnegativity constraint was applied to extract independent components of EEMs treating TSP and size-segregated aerosol samples as a whole (drEEM 0.2.0 MATLAB toolbox; Murphy et al., 2013). Analyses were performed according to the tutorial (Murphy et al., 2013) and previous studies (Chen, Ikemori, et al., 2016; Chen, Miyazaki, et al., 2016; Yue et al., 2017). EEM spectra data were normalized to unit variance in sample mode. The number of components was determined from the 2- to 7-component models by inspecting the residual errors (Figure S2), spectral loadings (Figure S3), and split-half analysis (S4C6T3, Figure S4; Murphy et al., 2013). The core consistency was 41.9%, which is acceptable for real-world data sets (Chen, Ikemori, et al., 2016; Murphy et al., 2013). A 10⁻⁸ convergence criterion was used to check the model convergence. The three-component model explains >98.9% of the variations within the data set. The three components were designated as PLOM (protein-like organic matter), HULIS-1, and HULIS-2 by visual inspection of the peak location (Figure 3; Chen, Miyazaki,
et al., 2016; Coble, 2007). The data set was reverse-normalized at the end of the analysis. The maximum intensities of the components in the original measurement scale (RU L m⁻³) are reported. Besides, fluorescence indices were calculated from the excitation/emission pairs for the humification index (HIX, ratio of the integrated fluorescence intensity in the range of 436–480 to the range of 300–344 nm both excited at 255 nm) and biological index (BIX, ratio of emission intensity of 380 nm to 430 nm both excited at 310 nm; Fu et al., 2015).

2.5. Organic Marker Analyses

Detailed procedures for the analysis of organic markers are well documented in previous studies (Fu et al., 2008; Schauer et al., 1996; Simoneit et al., 2004). Briefly, for each TSP sample, a filter aliquot was extracted three times with dichloromethane/methanol (2:1, v/v) under ultrasonication for 10 min. Solvent extracts were filtered through quartz wool, concentrated by a rotary evaporator and nitrogen-stripped to dryness. Derivatization was then carried out by reaction with 30 μl of N,O-bis(trimethylsilyl)trifluoroacetamide containing 1% trimethylsilyl chloride and 10 μl of pyridine at 70 °C for 3 hr. Prior to gas chromatography/mass spectroscopy (GC/MS) analysis, the derivatives were mixed with 40 μl of n-hexane containing 1.43 ng/μl of the internal standard (C₁₃ n-alkane). GC/MS measurements were performed on an Agilent GC (model 7890) coupled to Agilent Mass Selective Detector (model 5975C). The mass spectrometer was operated on Electron Ionization mode at 70 eV and scanned between 50 and 650 Da. GC/MS spectra for one real sample and the blank sample are presented in Figure S5. The response factors of target compounds were determined with authentic standards. Recoveries of the tracers were better than 80%. Field blanks were subtracted from real samples. Target compounds were identified by comparison with authentic standards and literature data (Fu et al., 2008; Fu et al., 2009).

3. Results and Discussion

Figure 1 shows the time series of meteorological parameters, number concentrations, and fractions of FAPs and non-FAP. During the sampling period, intermittent cloud events occurred with sporadic rain and snow precipitation. The average temperature was 3.1 °C (−4.8 to 12.6 °C) and the mean relative humidity was 67.5% (21.0% to 99.0%; Table S4). For most time of the period, the winds were mainly from north, west, and south directions, indicating that the air masses coming from the surrounding North China Plain dominated.

3.1. Abundance of Bioaerosols

The abundance of total FAP (>0.8 μm) in the ambient atmosphere by volume was 647 ± 533 L⁻¹ (26.9% ± 10.0%, Figures 1 and S6 and Tables S5 and S6), which is at the scale of the concentration for clean period in winter in Beijing (Yue et al., 2017), but one order of magnitude lower than the concentration in autumn in Nanjing (Yu et al., 2016). The most abundant type of FAP was FL B particles, which is the same as the observations in Beijing and Nanjing (Yu et al., 2016; Yue et al., 2017). Laboratory experiments of bacteria, fungal spores, and pollen grains have very rarely measured these PBAF as FL B particles (Hernandez et al., 2016; Savage et al., 2017), although Perring et al. (2015) showed that some mold spores are detected as this type. The dominance of FL B particles may suggest nonbiological interferences, although particles containing riboflavin can also contribute to this type of aerosols (Savage et al., 2017). For mass concentrations, FAP represented more than half of total particles by average (61.4%, Figure S6).

The FAP concentration at Mt. Tai was much higher than the measurements in other high-altitude sites. For example, the FAP concentration was two orders of magnitude higher than that above the High-Altitude Research Station Jungfraujoch in winter (6.3 ± 5.7 L⁻¹, 3,580 m a.s.l.; Crawford et al., 2016). The lower FAP level at the Jungfraujoch site is a combination of the winter biological inactivity, the high altitude of this site which causes weak interactions of PBL, and the dominant marine air masses at this site (Crawford et al., 2016; Z. Li et al., 2017). The FL1 particle concentration in this study was 190 ± 226 L⁻¹ which is much higher than the concentration at Puy de Dôme mountain in France (12 ± 6 L⁻¹, 1,465 m a.s.l) in summer using WIBS-3 (Gabey et al., 2013). The average abundance of FL3 (281 L⁻¹) fluorescent aerosol at this site was also higher than the Puy de Dôme mountain (95 L⁻¹). Regional pollution can also lead to higher FAP concentration since nonbiological fluorophores may be included, which can be seen from the WIBS measurements in Beijing, another city frequently influenced by pollution of North China Plain (K. Wei et al., 2016; Yue et al.,
Alternatively, varying climatic zones, WIBS prototypes and FAP definitions may also contribute to these differences (Pöhlker, Huffman, et al., 2012; Savage et al., 2017). Arabitol, mannitol, and trehalose are produced in large amounts by a variety of fungi. Arabitol and mannitol can also be emitted by lichen (Lewis & Smith, 1967). Algae and higher plants can also contribute to mannitol (Lewis & Smith, 1967). Trehalose is also present in bacteria, yeast, and a few higher plants (Medeiros et al., 2006). In this study, the mass concentrations of arabitol, mannitol, and trehalose of TSP samples were 3.3 ± 2.4, 4.6 ± 3.5, and 3.9 ± 3.6 ng/m³, respectively. Their abundance are lower than those in previous reports during nondust periods in spring (23, 15, 13 ng/m³; Wang et al., 2012). The concentrations of these sugar polyols were an order of magnitude lower than those in early June (90, 90, and 23 ng/m³, respectively), which was influenced by biomass burning pollution (Xu, Wei, Chen, Zhu, et al., 2017). Sugar polyol concentrations were also lower than those in late June (11, 24, 9.0 ng/m³; Fu et al., 2008, 2012), when the higher concentrations were attributable to the increased biological activity in the warmer seasons. A strong positive linear correlation ($R^2 = 0.85$, $p < 0.001$) was found between arabitol and mannitol, which supports the idea that they are both tracers for airborne fungal spores since significantly multiple sources may lower the correlation level (Fu et al., 2008; Gosselin et al., 2016). Mannitol and trehalose were positively correlated ($R^2 > 0.88$, Table S7). The Pearson correlation coefficients ($R^2$) of biomass burning tracers with fungal tracers were less than 0.08 and did not pass the two-tailed $t$ test, which indicates that biomass burning was not a significant source of fungal materials during this sampling period (Table S7). This result contrasts with biomass burning is one of the main sources of fluorescent interferences of bioaerosol detection by UV-LIF method. Levoglucosan, mannosan, and galactosan are three main tracers for biomass burning. The average mass concentration of levoglucosan was 38.4 ± 29.8 ng/m³, which is much lower than those (mean 426 ng/m³) reported in Mt. Tai aerosols collected in early summer when wheat-burning was active in the North China Plain (Fu et al., 2008). The three biomass burning tracers strongly correlate with each other ($R^2 > 0.88$, Table S7). The Pearson correlation coefficients ($R^2$) of biomass burning tracers with fungal tracers were less than 0.08 and did not pass the two-tailed $t$ test, which indicates that biomass burning was not a significant source of fungal materials during this sampling period (Table S7). This result contrasts with

Figure 1. Time series of meteorological parameters and WIBS results. (a) Temperature and RH; (b) wind speed and wind direction; for simplicity, wind directions are defined as north wind (N): 315°–359° and 0°–45°, east wind (E): 45°–135°, south wind (S): 135°–225°, west wind (W): 225°–315°; (c) number fractions of fluorescent particles and non-FAP to total particles; (d) number concentrations of fluorescent particles and non-FAP. According to their spectral properties, fluorescent particles are classified as FL A, FL AB, FL ABC, FL AC, FL B, FL BC, FL C (Table S1 for full description of definitions). In-cloud, snow, and rain periods are indicated by color shading. RH = relative humidity; FAP = fluorescent aerosol particle.
the biomass-burning active period in early June, during which biomass contributed to fungal particles significantly (Fu et al., 2012).

PAHs are major interferences of the detection of FBAP (Pöhlker, Huffman, et al., 2012; Savage et al., 2017). The total concentration of PAHs was 4.76 ng/m³ (0.11–14.4), which is at the same scale of the concentration in summer (14.6; Fu et al., 2008). The correlation coefficients ($R^2$) between PAHs and the three biological tracers were lower than 0.01 (Table S7). All the coefficients between FL-size-resolved FAPs and PAHs were also found to be <0.29 (Tables S8 and S9), which indicates that they were not important interferences of the measurement of FBAP in this study.

3.2. Size Distribution

As shown in Figure 2, all types of FAPs showed a predominant mode. Except for the coarse peak (~2.5 μm) of FL ABC particles, other FAPs were dominated by the fine mode (~1 μm). Compared to single Ex-Em pair

Figure 2. Size distributions of FL-resolved FAPs by applying different fluorescence thresholds (3σ-, 4σ-, 5σ-, 6σ-, and 9σ-threshold). Dotted lines show the size distributions of the eliminated FAPs from the 3σ- to 6σ-threshold (black) and from the 6σ- to 9σ-threshold (white). Solid lines show the fraction of FAPs excluded from the 3σ- to 6σ-threshold relative to 3σ-threshold. The black and gray parts indicate the decrease and increase of particle number concentrations, respectively. (a) FL A, (b) FL AB, (c) FL ABC, (d) FL B, (e) FL BC, and (f) FL C. FAP = fluorescent aerosol particle.
FAPs, fluorescent particles with multiple Ex-Em pairs (FL AB, AC, BC, and FL ABC) had greater contribution of larger aerosols. This agrees with the findings of laboratory experiments which reveal a change pattern of the fluorescent particle type (A-AB-ABC, B-BC-ABC, and C-BC-ABC) for individual fungi, intact and fragmented pollen, and bio-fluorophores (Savage et al., 2017). This can be explained that as particle size increases, the increasing quantity of constituent biomolecules leads to fluorescence intensity exceeding the baseline of other spectral band. This change pattern has also been observed in the atmosphere in winter in Beijing (Yue et al., 2017) and in laboratory experiments with nonbiological interferences (Savage et al., 2017). Collectively, as to number concentration, the fluorescent bioaerosols resided mainly in the fine mode (<~3 μm), while for mass concentration, they were dominated by coarse (2.5–10 μm) and Lcoarse particles (>10 μm; Figure S6).

To identify and quantify independent chromophore components of water-soluble organic matter, PARAFAC model was applied to the size-segregated aerosol samples. PARAFAC model can decompose the excitation and emission matrix of fluorescent mixtures to individual chemical components (Murphy et al., 2013). Three components were found and identified as PLOM, HULIS-1, and HULIS-2 according to their spectral location on the EEM maps (Figure 3a; Chen, Miyazaki, et al., 2016; Yue et al., 2017). PLOM showed a bimodal distribution of a fine mode peak (around 0.6 μm) and a coarse mode peak (around 5.0 μm; Figures 3b and S7). The fine mode peak may include protein molecules, decomposed peptides, amino acids and smaller bacteria, and so forth, while fungal spores, pollen fragments may constitute the coarse mode peaks (Després et al., 2012; Pöschl, 2005). By comparison, the size distributions of the number and surface area concentrations of FL1-FAP are also shown here (Figure 3b). They showed a fine (~1 μm) and coarse (~6 μm) predominant mode, respectively. Tryptophan and tyrosine are two widely contained...
amino acids which have relatively high fluorescence quantum yields (Pöhlker, Huffman, et al., 2012). Materials containing these amino acids contribute to both FL1-FAP and PLOM (Catalá et al., 2016; Pöhlker, Huffman, et al., 2012). It has been shown that the fluorescence intensity of single particles scale as the particle size to the power between two and three (Sivaprakasam et al., 2011; Taketani et al., 2013). This is because the inner fluorescent molecules may not contribute to the total fluorescence intensity, especially for large particles (Sivaprakasam et al., 2011; Taketani et al., 2013). However, our results contrast with this feature. The resolved PLOM component are water-soluble organic matter. Additional nonnitrogen-containing compounds (e.g., phenol compounds and naphthalene) can also be included (Chen, Ikemori, et al., 2016). The discrepancy may be also caused by the different quantum yields of biological fluorophores in single particle state and in water solution (Pöhlker, Huffman, et al., 2012). Different sizing methods (aerodynamic vs optical) for nonspherical aerosol particles may also be a potential reason for the shift of the size distribution (Ramachandran & Cooper, 2011).

Figure 4. Diurnal variations of the fluorescent aerosol particles for nighttime (00:00–06:00) and daytime (08:00–16:00). (a) The number concentrations and fractions of FAP, relative number concentration of FL-resolved FAPs, and FL-size-resolved FAPs in nighttime and daytime. The areas of the pie charts represent the relative abundance of total particles (>0.8 μm). (b) Diurnal variations of the number concentrations of FAP, non-FAP, FL-resolved FAPs, and FAP fraction. FAP = fluorescent aerosol particle.

Figure 5. Correlations between fine (0.8–2.5 μm) and coarse (2.5–10 μm) mode FL BC and HULIS-1. Eight samples collected in entire in-cloud periods are excluded from the calculation.
Fluorescent aerosol particles are categorized by aerosol particles and the fungal spore tracer mannitol of TSP samples. Not passing the two shown in Tables S8 and S9. (* indicates that the correlation coefficient does not pass the two-tailed t test with significance level p < 0.001). TSP = total suspended particle. Figure 6. Pearson correlation coefficients \( R^2, n = 26 \) between fluorescent aerosol particles and the fungal spore tracer mannitol of TSP samples. Fluorescent aerosol particles are categorized by fluorescence property and size ranges: fine (0.8–2.5 \( \mu \)m), coarse (2.5–10 \( \mu \)m), and Lcoarse (>10 \( \mu \)m). Eight samples collected in entire in-cloud periods are excluded from the calculation. Details of correlation coefficients with arabitol, trehalose, and biomass burning tracers levoglucosan, mannosan, and galactosan are shown in Tables S8 and S9. (* indicates that the correlation coefficient does not pass the two-tailed t test with significance level p < 0.001). TSP = total suspended particle. HULIS-2 and HULIS-1 also exhibited two size modes, however, the coarse mode peak of HULIS-1 was much weaker than the fine peak. According to the spectral positions (Chen, Ikemori, et al., 2016; Chen, Miyazaki, et al., 2016), HULIS-2 has been designated as a less-oxygenated species of terrestrial origin, while HULIS-1 has been appointed to a highly oxygenated species which may be due to the photodegradation of organic chromophores, such as PLOM and HULIS-2. The dominant fine mode of HULIS-1 can be explained by the fragmentation of chromophores through the oxygenation reaction pathways (Chen, Miyazaki, et al., 2016). Fluorescence indices can provide insights into the origins of fluorophores. The BIX is used to estimate the contribution of autochthonous biological activity. High values (>1) have been shown to correspond to a predominately biological origin of organic matter, whereas low values (<0.6) indicated few biological materials (Fu et al., 2015; Huguet et al., 2009). The HIX was introduced to estimate the maturation level of dissolved organic matter in soil (Zsoltay et al., 1999). Through the humification process, the microbial availability of organic matter decreases and its aromaticity increases. Low values (<4) correspond to autochthonous or microbial origin, while high values (>10) are indicative of strongly humified organics, mainly of terrestrial origin (Birdwell & Engel, 2010; Huguet et al., 2009). In this study, BIX was smaller for the fine mode particles (<2.1 \( \mu \)m, 0.8–1.1, average: 0.9) than for the coarse mode particles (>2.1 \( \mu \)m, 0.9–1.5, 1.2). Similarly, HIX decreased from fine (1.7–3.4, 2.4) to coarse mode (0.6–3.0, 1.1). BIX of aerosols between 3.3 and 5.8 \( \mu \)m were in the very lower-right side of Figure 3c. BIX values of TSP aerosol samples in Beijing in spring were between 0.8 and 1.4, similar to the range in this study (Yue et al., 2016). Comparison with previous data for dissolved organic matter in river and oceanic waters, aquatic and soil humic substances, as well as rain and fog-water samples, the scatter plot of HIX and BIX values shows that coarse aerosols were contributed by more biological materials, while fine mode aerosols contained more humified matter of terrestrial source (Birdwell & Engel, 2010; Fu et al., 2015). This is apparent in Figure 3b, where the ratio of PLOM to HULIS is much higher in the coarse size range than the fine size range. 3.3. Diurnal Variations of Bioaerosols and the Transformation of Bioaerosols to HULIS As illustrated in Figure 4, the concentrations of total FAP (747 vs 458 L\(^{-1}\), >0.8 \( \mu \)m), non-FAP and all FL-resolved FAPs were higher during the day (08:00–16:00) than at night (00:00–06:00) and showed a diurnal peak in the early afternoon and minimum shortly before sunrise. The fungal spore tracers also showed higher daytime abundance of biological matter (Figure S8). This feature was similar to most of the primary and secondary organic tracers during the nonbiomass burning periods in late June at the same site (Fu et al., 2012). However, the fraction of FAP was similar at night (23.2%) and during the day (20.8%). The relative abundance of FL-resolved FAPs was also similar between day and night with the exception of FL A and FL BC (Figure 4a). The inverse day/night contrast of these two kinds of fluorescent particles suggests the transformation of protein-like bioaerosols of FL A type to HULIS detected as FL B particles. During long range transport, protein-like substances FL A particles can be photo-oxidized, contributing to HULIS which was part of the FL BC particles. The fluorescence fingerprint of the highly oxygenated HULIS-1 (Figure 3a) shows that it can also contribute to FL BC particles, which is further supported by the moderate correlation between fine \( R^2 = 0.60 \)/coarse \( R^2 = 0.50 \) mode FL BC and HULIS-1 (Figure 5). One formation pathway of
Physically, higher thresholds will eliminate weakly fluorescent particles in each channel, either nonbiological particles or fluorescent bioaerosols (Savage et al., 2017). This can specifically cause a change for the categorization of fluorescent particle and of their size distributions.

First, in this field measurement, increasing fluorescence threshold from 3σ to 6σ removed a large fraction of FAP. In general, by applying the 6σ-threshold, the average number fraction of FAP in total particles larger than 0.8 μm reduced by 15% (from 26.9% to 11.9%, Table S6). In particular, more than half of the fine (0.8–2.5 μm) and coarse (2.5–10 μm) mode FAP were recategorized as nonfluorescent particles (Figures 2 and S6). As an example, the concentration of FL A decreased by one order of magnitude, from 107 to 8.40 L−1 μm. As to mass concentration, FAP contribution to total particulate reduced by 18.8% (from 61.4% to 42.6%, Figure S6). The decrease of FAP mass with 6σ-threshold criteria was significant in fine mode aerosols while Lcoarse particles reduced only by a slight degree (Figure S6).

Second, applying more strict fluorescence thresholds causes the shift of FAP categorization. Namely, biological species classified into multiple Ex/Em band FAPs were reduced to be single Ex/Em band FAPs. For instance, from 3σ- to 6σ-threshold, the number concentration of FL B (>~7 μm), Lcoarse mode FL AB and FL BC particles increased (Figure 2). This feature has also been observed in the laboratory measurements of bioaerosols (Savage et al., 2017). Savage et al. (2017) found that the fraction of fungi Saccharomyces cerevisiae to be classified as FL ABC and FL AB particles decreased, while the FL A fraction increased across the whole size range when increasing the baseline from 3σ- to 9σ-threshold.

Third, another effect is the shift of the size distribution of FLI FAPs and FL BC to the coarse mode. This means that the fine mode particles will be more prone to being influenced than coarse mode particles. One reason should be due to the smaller quantity of fluorophores they contain. Another reason can be that nonbiological interferences such as SOA, HULIS mainly reside in the fine mode.

Furthermore, for bioaerosol characterization, here we show the evidence of the removal of both nonbiological matter and bioaerosols by increasing fluorescence threshold. On the one hand, as illustrated in Figure S9, compared to the correlation coefficients when using 3σ-threshold (0.59 < R² < 0.63), the coefficients of fine mode FL A with non-FAP decreased to insignificant values by applying the 6σ-threshold (R² < 0.18), indicating a removal of nonbiological particles from this particle type. FL AB, AC showed a similar trend. On the other hand, the correlation of FL ABC with non-FAP in fine mode became stronger, from 0.12 to 0.31 (R²). This feature suggests that by applying the 6σ-threshold, a proportion of bioaerosols was misclassified into non-FAPs. In addition, FL B and FL C particles in fine mode still strongly correlated with the fine mode non-FAPs (For FL C, 3σ: R² = 0.72–0.90; 6σ: R² = 0.69–0.90; 9σ: R² = 0.67–0.88), which indicates that they might have similar source with non-FAPs. This is similar to the findings in winter in Beijing (Yue et al., 2017) and is also indicated by the moderate correlations with biomass burning tracers (Tables S8 and S9).

### 3.4. Generic Effect of Fluorescence Threshold on Bioaerosol Characterization

The purpose of adjusting fluorescence threshold is to reduce the interference of nonbiological matter and maintain the biological aerosols. Here we use the method through comparing FAP with biological tracers and with non-FAP to assess the effects of different fluorescence thresholds for bioaerosol characterization.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Avg.</th>
<th>Sdev.</th>
<th>Min.</th>
<th>Max.</th>
</tr>
</thead>
<tbody>
<tr>
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<td>23</td>
<td>6.7</td>
<td>86</td>
</tr>
<tr>
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<td>7.4</td>
<td>6.2</td>
<td>34</td>
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<tr>
<td>N₃mannitol (L⁻¹)</td>
<td>8.7</td>
<td>6.6</td>
<td>0.40</td>
<td>29</td>
</tr>
<tr>
<td>N₆mannitol (L⁻¹)</td>
<td>8.0</td>
<td>5.8</td>
<td>0.54</td>
<td>26</td>
</tr>
</tbody>
</table>

Note. Same to Figure 7, the Wideband Integrated Bioaerosol Sensor results are calculated as the sum of FL A (2.5–10 μm) + FL AB (2.5–10 μm) + FL ABC (>2.5 μm) + FL BC (>10 μm) under 3σ- and 6σ-threshold. Eight samples collected in entire in-cloud periods are excluded from the calculation.

HULIS-1 is the photooxidation of PLOM substances, such as tryptophan, tyrosine, phenol, and 4-phenoxyp phenol (Bianco et al., 2014; Laurentiis et al., 2015). The phenolic compounds can be produced by microorganisms, plants, and pyrolysis of lignin (Hättenschwiler et al., 2000; Yee et al., 2013).

### 3.5. Improvements of Fungal Spore Classification

Specially, we explore the effect of fluorescence threshold on characterization of fungal spores. Eight in-cloud samples (TSP) were excluded from the following discussions for the sake of characterizing bioaerosols under general background condition since precipitation and high RH can induce the release of bioaerosols (Huffman et al., 2013; Yue et al., 2016) and rupture of bioaerosols (China et al., 2016) by a large amount,
respectively. This can also be seen from the spikes of FAPs in Figure 1. The mass concentrations of FAP particles were calculated (Heald & Spracklen, 2009) and averaged over the high-volume TSP sampling periods. Correlation analysis was performed between the mass concentrations of fluorescent aerosols and molecular tracers. Overall, positive correlations were found for fungal polyols with FAPs (3σ-threshold), $R^2 = 0.72$ between FL1-FAP and mannitol, and $R^2 = 0.68$ between FL1-FAP and arabitol. Compared with the 3σ-threshold, the coarse mode FL1-FAP mass concentration resulting from the 6σ-threshold showed similar correlation coefficients with fungal polyols mannitol ($R^2 = 0.70$) and arabitol ($R^2 = 0.68$).

Size-resolved correlation coefficients between mass concentrations of FAPs and biological tracers (TSP) showed that the 6σ-threshold could lead to better classification of FAPs for fungal spores (Figure 6 and Tables S8 and S9). As an example, the best correlation with mannitol was found for Lcoarse FL ABC ($R^2 = 0.75$), FL BC ($R^2 = 0.59$), and coarse mode FL AB ($R^2 = 0.57$). Generally, the fine mode FAPs showed no clear linear correlation with mannitol, which is reasonable as fungal spores often reside in the coarse mode (Fröhlich-Nowoisky et al., 2016). By applying a 6σ-threshold, all the correlation coefficients increased except for FL ABC and fine FL AB particles. The coefficients increased significantly for coarse FL A (from 0.29 to 0.54) and Lcoarse FL B (from 0.35 to 0.55). These enhancements could be partly due to the exclusion of weakly fluorescent bioaerosols such as bacteria or bacteria clusters and nonbiological interferences (Savage et al., 2017), whereas the decreased correlation coefficients can be explained by the change of fluorescence particle type. Namely, for instance, when applying higher fluorescence intensity thresholds, previously categorized FL ABC particles would fluoresce weakly in any of the three excitation/emission bands and be demoted to FL AB, FL BC, or FL AC particles, simultaneously contributing to the increased correlation relationships of other FAPs with mannitol.

Furthermore, we found evidence of better quantification of fungal spores from the comparison of the estimated concentration based on molecular tracers and WIBS. The number concentration of fungal spores was calculated by assuming 0.49 and 0.38 pg per spore for mannitol and arabitol, respectively (Liang et al., 2017).

Figure 7. Correlations among the estimated number concentrations of fungal spores from mannitol, arabitol, and WIBS. WIBS results are calculated as the sum of FL A (2.5–10 μm) + FL AB (2.5–10 μm) + FL ABC (>2.5 μm) + FL BC (>10 μm) under 3σ- and 6σ-threshold. Eight samples collected in entire in-cloud periods are excluded from the calculation. WIBS = Wideband Integrated Bioaerosol Sensor.

Figure 8. Schematic diagram showing the effect of mountain-valley breezes and regional wind transport on the biological matter of tropospheric aerosols over Mt. Tai (1534 m a.s.l.). PBL = planetary boundary layer.
As to WIBS, the criteria for the selection of fluorescence category and size range is that at least one of the $R^2$ under $3\sigma$- and $6\sigma$-threshold is larger than 0.50 (Figure 6). Thus, the sum of FL A (2.5–10 μm) + FL AB (2.5–10 μm) + FL ABC (>2.5 μm) + FL BC (>10 μm) for $3\sigma$- and $6\sigma$-threshold was used as an estimate. The estimated concentrations of fungal spores from mannitol ($N_{\text{mannitol}}$ = 8.7 ± 6.6 L$^{-1}$) and arabitol ($N_{\text{arabitol}}$ = 8.0 ± 5.8 L$^{-1}$) were equivalent and well correlated ($R^2 = 0.94, p < 0.001$; Table 1 and Figure 7). The estimation of fungal spores from WIBS were 45 ± 23 (N$_5$) and 18 ± 7.4 L$^{-1}$ (N$_6$), both of which were higher than the values from molecular tracers (Table 1). The positive correlation was found more significant between $N_{6\sigma}$ and $N_{\text{mannitol}}$ than that between $N_{5\sigma}$ and $N_{\text{mannitol}}$ ($R^2$: 0.62 vs 0.46, $p < 0.001$; Figure 7). Besides, the fitting slope also revealed a better representation of fungal spores under $6\sigma$-threshold (0.78) than $3\sigma$-threshold (2.2).

4. Conclusions

In this article, we first deployed the online bioaerosol instrument (WIBS) on the top site of Mt. Tai, North China Plain, together with filter sampling of TSP and size-segregated particles. The number concentrations, mass abundance, size distributions, and diurnal variations of fluorescent bioaerosols were characterized. The abundance of total fluorescent aerosols was 647 ± 533 L$^{-1}$, accounting for approximately one fourth of total particles (>0.8 μm). FAP represented 61.4% (mean) of total aerosol mass for the size range. All types of FAPs showed a predominant mode, ~2.5 μm for FL ABC particles and ~1 μm for the other six types of FAPs. The PARAFAC model resolved three factors, PLOM, HULIS-1, and HULIS-2 for the water-soluble contents of aerosols. Their size distributions showed that the fluorescence of coarse aerosols was contributed mainly by biological materials, while humified matter contributed largely to the fluorescence of fine particles.

The effect of applying different fluorescence thresholds for WIBS to characterize ambient bioaerosols are also discussed. The shift of the size distributions and recategorization of FAPs have been revealed as the laboratory experiments (Savage et al., 2017). Besides, we find that while applying stricter criteria ($6\sigma$-threshold) can improve the characterization of biological materials, despite the risk to eliminate small FBAP with weak fluorescence. Furthermore, here we recommend constraining the sum of FL A (2.5–10 μm) + FL AB (2.5–10 μm) + FL ABC (>2.5 μm) + FL BC (>10 μm) under $6\sigma$-threshold as an estimate of fungal spores, although cautious are still needed for other studies to quantify this type of bioaerosol.

As illustrated in Figure 8, this mountain, in addition to large-scale atmospheric convection, can serve as an enhancer for transporting regional aerosols to free troposphere by valley breezes. During the long-range transport, PBAP can be degraded by photooxidation and contribute to HULIS, which can influence cloud formation and oxidation potentials in the troposphere, thus potentially affecting the ocean-atmosphere interactions and marine biogeochemical cycles when the westerlies prevail (Ariya et al., 2009; Estilllore et al., 2016; Sun & Ariya, 2006). Mountain breezes at night can also bring long-range transported aerosols down to the local areas of upwind side of the mountain. Our results provide useful information to better understand the abundance and evolution of tropospheric aerosols with respect to the interactions of the PBL and atmospheric chemistry over East Asia.
nents and their relationship to chemical structure. Environmental Science & Technology, 50(20), 10,859–10,888. https://doi.org/10.1021/acs.est.6b02541