Environment International 147 (2021) 106327



Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint





Size fractionation of bioaerosol emissions from green-waste composting

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ARTICLE INFO

Handling Editor: Guo-ping Sheng

Keywords:
Bioaerosols
Composting
Human health
Pathogens
Spectral Intensity Bioaerosol Sensor (SIBS)
Aspergillus fumigatus
Actinobacteria
Aspergillosis

ABSTRACT

Particle size is a significant factor in determining the dispersal and inhalation risk from bioaerosols. Green-waste composting is a significant source of bioaerosols (including pathogens), but little is known about the distribution of specific taxa across size fractions. To characterise size fractionated bioaerosol emissions from a compost facility, we used a Spectral Intensity Bioaerosol Sensor (SIBS) to quantify total bioaerosols and qPCR and metabarcoding to quantify microbial bioaerosols. Overall, sub-micron bioaerosols predominated, but molecular analysis showed that most (>75%) of the airborne microorganisms were associated with the larger size fractions (>3.3 μm d_a). The microbial taxa varied significantly by size, with Bacilli dominating the larger, and Actinobacteria the smaller, size fractions. The human pathogen *Aspergillus fumigatus* dominated the intermediate size fractions (>50% d_a 1.1–4.7 μm), indicating that it has the potential to disperse widely and once inhaled may penetrate deep into the respiratory system. The abundance of Actinobacteria (>60% at $d_a < 2.1$ μm) and other sub-micron bioaerosols suggest that the main health effects from composting bioaerosols may come from allergenic respiratory sensitisation rather than directly via infection. These results emphasise the need to better understand the size distributions of bioaerosols across all taxa in order to model their dispersal and to inform risk assessments of human health related to composting facilities.

1. Introduction

Bioaerosols (i.e. biogenic aerosols sized ~0.05–100 μm) represent a significant and growing health concern (Douwes et al., 2003; Douglas et al., 2017). Bioaerosol inhalation is associated with a range of negative health effects, including allergies, respiratory sensitisation, and infectious diseases (Douwes et al., 2003; Van Leuken et al., 2016; Bush and Portnoy, 2001; Gladding et al., 2003; Kim et al., 2017). Recycling of organic waste by composting is one of the biggest emitters of human health relevant bioaerosols (He et al., 2019; Veillette et al., 2018; Mbareche et al., 2017; Pankhurst et al., 2009, 2012; Pearson et al., 2015; Wéry, 2014; Conza et al., 2013; Robertson et al., 2019; Douglas et al., 2018). In particular, composting is associated with the release of significant amounts of sensitizers such as endotoxins and the causative agent of aspergillosis, *Aspergillus fumigatus* (Pankhurst et al., 2009; Taha et al., 2006; Rolph et al., 2018; Recer et al., 2001; Deacon et al., 2009). Predicting exposure to bioaerosols from composting has been performed using dispersion modelling to explore associations with health. Such approaches have been used as a key tool for regulators of the waste and recycling industry (Douglas et al., 2018a, 2017b, 2017c, 2016d; Williams et al., 2019). To support this, we need detailed characterisations of the emission sources of bioaerosols from compost sites in order to parameterise these models and improve exposure predictions (Douglas et al., 2016a, 2016b).

The ability of a particle to disperse, and the respiratory penetration once inhaled, are determined by particle size. Larger particles have faster deposition rates, and consequently smaller bioaerosols disperse much further (Bowers et al., 2009; Kanaani et al., 2008; Wilkinson et al., 2012; Reche et al., 2018). Once inhaled, smaller particles penetrate deeper into the respiratory system than larger ones (Douwes et al., 2003; Ivens et al., 1999) (Fig. 1). Therefore, size is an important factor in parameterising dispersion models and for evaluating risks to human health of different bioaerosols (Douglas et al., 2017; Douglas, 2013). Some studies have reported that the majority of bioaerosols at compost sites are in the range of ~0.5–2.1 μm and exist as single cells rather than aggregates (O'Connor et al., 2015; Gutarowska et al., 2015; Galès et al.,

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https://doi.org/10.1016/j.envint.2020.106327
Received 23 July 2020; Received in revised form 4 December 2020; Accepted 5 December 2020
Available online 31 December 2020
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2015; Tamer Vestlund et al., 2014; Feeney et al., 2018). In contrast, other studies have indicated larger sizes (>2.1 μm), with many of the microorganisms (particularly fungi) as aggregates, associated with the coarse fraction of bioaerosols (Gutarowska et al., 2015; Reinthaler et al., 1999; Byeon et al., 2008; Pahari et al., 2016). However, only one of these studies used culture independent methods (Galès et al., 2015), with all others using culture-based methods, fluorescence, and scanning electron microscopy. Culture-based methods, or qPCR of specific microbial groups (as used by Galès (Galès et al., 2015) do not account for the full diveristy of microorganisims present. As there is good evidence that the size distributions of microbial bioaerosols vary between taxa (Górny et al., 1999; Yamamoto et al., 2012), we need to use methods that consider the full diversity of the taxa present (e.g. high-throughput sequencing (Veillette et al., 2018; Pahari et al., 2016; Górny et al., 1999). Ultraviolet light-induced fluorescence (UV-LIF) based methods, such as the Wideband Integrated Bioaerosol Sensor (WIBS) (O'Connor et al., 2015; Feeney et al., 2018) and more recently, the Spectral Intensity Bioaerosol Sensor (SIBS, see (Nasir et al., 2019) can collect (optical based) size fractioned data in real-time, providing valuable data for modelling and surveillance of bioaerosols emissions at the broad scale. However, the SIBS cannot distinguish between taxa, or even between types of bioaerosols and needs to be used in conjunction with other methods to provide fine detail on bioaerosol composition. To improve our ability to assess risk and model exposure from compost bioaerosols, we need to develop a better understanding of the size distributions of all taxa present in the bioaerosols originating from compost.

Here we investigate size resolved bioaerosol emissions at an open windrow green waste composing site using qPCR and high throughput sequencing of the bacterial 16S rRNA gene and fungal ITS region. We compare molecular data to data collected simultaneously with SIBS, to determine what insights each method can deliver for characterising bioaerosols. We determine if bioaerosol emissions from compost sites vary in the abundance, diversity, or composition of bacteria and fungi between size fractions. We also investigate the prevalence of human pathogens in each size fraction to provide new insight into their dispersal, and the health risk associated with compost bioaerosol emissions.

2. Materials and methods

2.1. Compost site and sampling locations

Samples were collected at a green-waste compost site in the South East of the UK. The compost site, an open windrow site (i.e. composting takes place in open air and emissions are not contained, approximately 70% of UK facilities are of this type) processes approximately 40,000 metric tons per year of organic waste, which is predominantly household green waste collected at the road side. The site is 2.3 ha and is situated at the centre of an old airfield, now a rotational arable farm covering approximately 610 ha and predominantly producing wheat. There is a solar farm on the northern boundary of the compost site.

Sampling was performed on 28th July 2016 and consisted of six, two-hour air samplings on the leeward edge of the site, approximately 10 m from any turning and shredding activity (Figure S1). A second sampling was performed 24th November 2016 to determine the effect of bioaerosol emissions from the compost site in its local environment. Here we collected four, two-hour air samples upwind of the compost site (175 m), four, two-hour air samples on the compost site, and four, two-hour air samples downwind (250 m) of the compost site (Figure S1).

2.2. Bioaerosol sampling

2.2.1. Size fractionated bioaerosol sampling for molecular analysis.

Bioaerosol samples were collected using six-stage viable Andersen Cascade Impactors (Thermo Fisher, UK) which splits the sample into six size fractions as follows: stage one > 7 μm, stage two 4.7–7.1 μm, stage three 3.3–4.7 μm, stage four 2.1–3.3 μm, stage five 1.1–2.1 μm, and stage six 0.65–1.1 μm aerodynamic diameter (d_a) respectively (Fig. 1). The impactor was operated at 28.3 L min^{-1} according to manufacturer's instructions, except that the agar plates were filled with 40 mL 1.5% [w/v] agar (Sigma, UK) to achieve the correct sampling height, and two sterile polycarbonate filters (45 mm diameter) were placed onto the surface of the agar to collect bioaerosols. To prevent particle bounce, 150 μL of sterile glycerol 10% [v/v] (Sigma, UK) was applied to the filters (Xu et al., 2013; Blomquist et al., 1984). The filters were placed onto dry ice for transportation and then stored at −80 °C until analysis. DNA was isolated from filters following the optimised bioaerosol extraction method described in Ferguson *et al.*, (Ferguson et al., 2019) that uses sodium dodecyl sulphate buffer [10 mM Tris-HCl pH 8, 25 mM Na2EDTA pH 8, 100 mM NaCl] and Phenol/chloroform/isoamyl alcohol [25:24:1, 500 μL]. The 25 μL DNA extract from the duplicate filters (technical replicates) within each stage of the Impactor were pooled for analysis.

2.2.2. Fluorescence based measurements of bioaerosols with SIBS

Continuous real-time measurements of bioaerosol emissions were made with a SIBS (Droplet Measurement Technologies, Longmont, USA) on 28th July 2016. SIBS was co-located with six-stage Viable Andersen Cascade Impactors at the edge of the site (Figure S1). The measurements

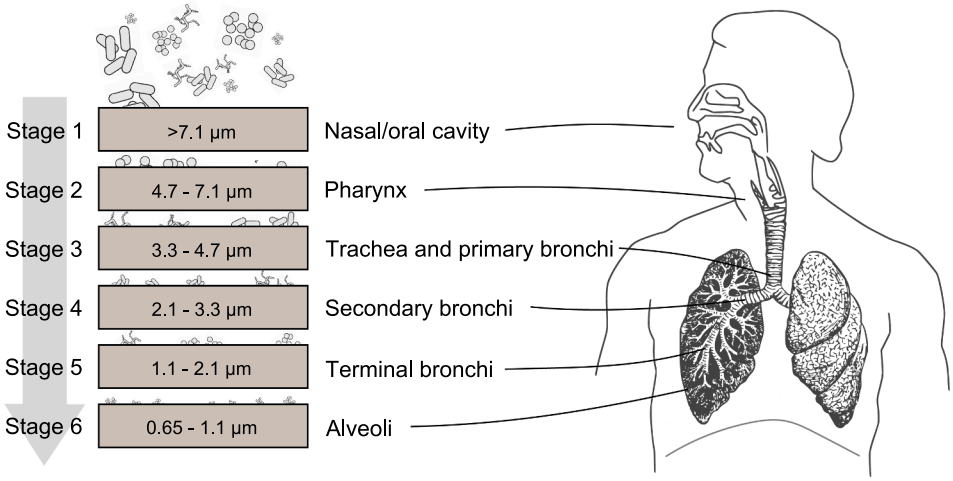


Fig. 1. Particle size fractions in relation to respiratory penetration for the size stages collected by the six-stage Andersen Cascade Impactor (ANDERSEN, 1958).

were made at a height of 1 m for a duration of four hours. The measurement principle of SIBS has been described by Nasir *et al.*, (Nasir et al., 2019, 2018). The SIBS used in this study had a sample flow rate of 0.3 L min^{-1} and derived the equivalent optical diameter (contrasting to aerodynamic diameter given by the Andersen Cascade Impactors) in the size range from 0.4 to 7 μm.

During the measurements, the SIBS recorded single particle data on the size along with fluorescence emission across 16 wavelength channels from 298 − 735 nm for two excitation wavelengths (285 nm and 370 nm). During continuous measurement mode, some particles had not been flashed (excited) due to the recharging of flash lamps and there were no fluorescent measurements for these particles. SIBS recorded excitation index (0 = Not excited, 1 = Excited) for each particle. The data on exited particles were processed further to generate histograms of total (excited), fluorescent (bioaerosols) and non-biological particle size distributions. Excited particles were segregated into fluorescent (bioaerosols) and non-biological particles based on a fluorescence threshold in each emission wavelength channel, determined by Forced Trigger (FT) measurement prior to the start of the sampling. The details on FT measurement has been described in Nasir et al., (Nasir et al., 2019, 2018). The mean plus 3 times the standard deviation (σ) of FT fluorescence intensity values in each channel was applied as fluorescence thresholds after Tian et al. (2020).

2.2.3. Meteorological and PM data collection.

Meteorological data (wind speed and direction, temperature, relative humidity, and atmospheric pressure) were collected with a Kestrel 4500 Weather Meter (Richard Paul Russell Ltd UK), during all sampling periods (Table S1). Atmospheric particulate matter (PM) mass fraction data, including total PM, PM_{10} (inhalable coarse fraction 2.5–10 μm d_a), $PM_{7.5}$ (2.5–7.5 μm d_a) and $PM_{2.5}$ particles (<2.5 μm d_a) were measured with an Aerocet 531S Handheld Particle Counter (Met One Instruments, Inc.) (Figure S2).

2.3. Identification and quantification of bacterial and fungal bioaerosols

For quantification and identification of bacterial and fungal bioaerosols we targeted the V3-4 region of the bacterial 16S rRNA gene (Klindworth et al., 2013) and the second fungal internal transcribed spacer (ITS2) region (White et al., 1990) (see Table S2 for primers), which have been widely used for quantitative analysis and metabarcoding analysis of environmental bacteria and fungi (Klindworth et al., 2013; White et al., 1990; Clark et al., 2018; Ferguson et al., 2017; Bani et al., 2019). qPCR was carried out using a Bio-Rad CFX96 Touch Real-Time PCR Detection System (Bio-Rad Laboratories) as described in Ferguson et al. (2019).

2.3.1. Metabarcoding of the microbial bioaerosol community

A two-step PCR was used to generate amplicon libraries (including 6 blank sequence libraries as controls of contamination) as described in Ferguson et al. (2019) and sequenced in equimolar amounts on one lane of a HiSeq 2500 System (Illumina) in rapid run mode (providing 2 × 300 bp sequences) at the Earlham Institute (formerly The Genome Analysis Centre), Norwich Research Park, Norwich, NR4 7UH, UK.

Sequence data were analysed following the guidelines for paired-read Illumina amplicon libraries in Dumbrell et al. (2017). Briefly, quality filtering was carried out with SICKLE (Joshi and Fass, 2011) reads were trimmed from the 3′ end when the PHRED score over a 30 bp window dropped to <30. Error correction was carried out on the trimmed reads with BayesHammer (Nikolenko et al., 2013) implemented with the default settings in SPAdes v3.7.1 (Bankevich et al., 2012). The paired forward and reverse reads were then paired-end aligned and primers removed using the PEAR algorithm (Zhang et al., 2014) implemented in PANDAseq (Masella et al., 2012). Further quality filtering was carried out in Mothur v1.35.1 (Schloss et al., 2009) to remove reads with ambiguous bases (N's), or that were overly short or long in comparison with the target amplicon length (<400 bp or >480 bp bacteria, or <250 bp or >500 bp for fungi). The quality filtered paired aligned reads were then clustered into operational taxonomic units (OTUs) at a 97% similarity threshold using VSEARCH v2.1.2 (Rognes et al., 2016). Chimeric sequences were removed in UCHIME (Wang et al., 2007) retaining 27271 bacterial and 5365 fungal OTUs. The representative reads from each OTU were then assigned a taxonomic group using the RDP classifier algorithm (Zhang et al., 2014) using the internal 16S rRNA training set 16 for bacterial OTUs and the UNITE for fungal OTUs (Kõljalg et al., 2005). OTUs assigned to chloroplasts were removed and the read depth in each sample was normalised by rarefaction to an even sampling depth of 20,097 reads for bacteria retaining all samples and 5320 for fungi, retaining all except two samples (one from stage four and six which had low numbers of reads), a random seed '1121983′ was sued to ensure repeatability of the analysis using the R package Phyloseq v3.10 (McMurdie and Holmes, 2013). Blank extractions were also sequenced in parallel, but none of the sequences from these samples passed our quality control filters or matched known bacterial taxa when the forward only reads were put though the bioinformatics pipeline described above.

To identify possible pathogenic bacteria we compared the bacterial OTUs to a list of known human pathogens compiled by Kembel *et al.,* (Kembel et al., 2012) (Table S3). We obtained representative 16S rRNA gene sequences for each pathogen from the NCBI reference sequence database (RefSeq) (Pruitt et al., 2007) and searched the representative sequence for each OTU against this database with the Basic Local Alignment Search Tool (BLAST) (Altschul et al., 1990). We defined a possible pathogen as any OTU that shared ≥99% sequence identity with a strain from the human pathogen reference database.

2.4. Statistical analysis

In order to quantify α-diversity we used species richness (the total number of OTUs) and community evenness (calculated as the reciprocal of Simpson's evenness (1/D)). To compare α-diversity across treatments we used generalised linear models (GLMs) with size fraction (a factor with six levels) as the predictor. For species richness (count data) we used a negative binomial error term and for community evenness we used Poisson error terms. To determine if there were differences in the community composition between the size fractions we carried out multivariate generalised linear modelling (MGLM) (Wang et al., 2012). The normalised OTU abundances were fitted to the predictor (size fraction) as described for α-diversity, with a negative binomial error term to account for overdispersion. Multivariate and univariate P-values were obtained by log-likelihood ratio tests (LRTs) and model-free bootstrapping with probability integral transform (PIT-trap) residuals (10,000 permutations). Univariate P-values were adjusted for multiple comparisons with the false discovery rate method (FDR) (Wang et al., 2012; Warton et al., 2017). To test if the bioaerosol size distributions upwind and downwind of the site were different to those onsite the distributions were compared with a Kolmogorov–Smirnov test (KS test). For all tests, an alpha value of $P < 0.05$ was used.

All statistical analyses were carried out in the R statistical language v3.6.2. and the cited associated packages (R Development Core Team, 2019). Preparation of the figures was carried out with the R packages '*ggplot2*′ v3.2.1 (Wickham, 2016) and '*phyloseq*' v3.10 (McMurdie and Holmes, 2013), box plots were constructed using the default settings in *ggplot2* after Krzywiniki *et ali.,* (Krzywinski and Altman, 2014). MGLM was carried out with '*mvabund*' v4.0.1 (Wang et al., 2012).

3. Results

3.1. Changes in bioaerosol abundance and α-diversity between size fractions

Bacterial and fungal abundance significantly decreased with

decreasing size fractions (Table 1, Fig. 2a and b). In both cases the decrease from the smallest to the largest size fraction (0.65–1.1 μm and >7.1 μm, respectively) was an order of magnitude; with bacterial bioaerosols ranging from 7.3 ± 0.3 to 6 ± 0.4 $\log_{10}$ 16S rRNA copies m^3, and fungal from 6.5 ± 0.4 to 5.1 ± 0.5 $\log_{10}$ ITS copies m^{-3}. This is consistent with the PM emitted from the compost site, where the highest concentration was in the $PM_{7.5}$ mass fraction (Figure S2). There was a parallel significant decrease in bacterial α-diversity (species richness, and diversity) as size decreased (Table 1, Fig. 2b and c). This was not observed for fungal bioaerosols, which showed consistent α-diversity across all size fractions (Table 1, Fig. 2e and f).

3.2. Size distribution of fluorescent particles (SIBS)

Bioaerosols made up about half (49%) of the total PM (excited particles) emitted from the compost site (Fig. 3a). In contrast to the molecular data (which considered only microbial bioaerosols), the SIBS showed that a vast majority of the total bioaerosols emitted from the compost site were in the smaller size fractions (Equivalent optical diameter < 0.6 μm, Fig. 3c).

3.3. Changes in bioaerosol community composition between size fractions

The composition of the bacterial and fungal bioaerosol community significantly changed with size fraction (Table 1, Figs. 4 and 5). In the larger size fractions (d_a > 3.3 μm) there was an even split between Actinobacteria and Firmicutes, which made up 15–20% of the bacterial community each (Figure 4 and S3). As size decreased, the community switched to one dominated by Actinobacteria; with a threefold increase in relative abundance of Actinobacteria to >60%. Most of the increase in Actinobacteria was due to increases in the relative abundance of the genera *Thermopolyspora*, *Thermomonospora*, *Thermobifida*, and *Saccharomospora* (Figure S4); notably the genera *Corynebacterium* bucked this trend and decreased in relative abundance with size fraction. Most of the decrease in the relative abundance of Firmicutes was due to decreases in the abundance of the Bacilli genera *Bacillus*, *Kurthia*, *Lactobacillus*, and *Ureibacillus* (Figure 4 and S3). Concurrent with this there was a decrease in the relative abundance of Bacteroidetes (Bacteroidia, Flavobacteriia, and Sphingobacteriia), Firmicutes (Bacilli and Clostridia), and Proteobacteria (Alphaproteobacteria, Betaproteobacteria, and Gammaproteobacteria) which decreased to <2% of the community in the smallest size fraction.

The dominant fungal phylum was Ascomycota, accounting for 40–80% of the fungal community across all size fractions (Fig. 5). The two most dominant Ascomycota orders were Eurotiales and Capnodiales (Fig. 5). Capnodiales were more abundant in the largest three size fractions d_a > 3.3 μm, where they comprised > 25% of the fungal community. In contrast Eurotiales (consisting of almost exclusively *Aspergillus fumigatus*) dominated in the intermediate size fractions (d_a 4.7–1.1 μm), where they made up >50% of the total fungal community. The Ascomycota classes Leotiomycetes and Sordariomycetes also followed this trend, with higher concentrations in the intermediate size fractions (>6% as d_a 3.3–4.7 μm). The next most abundant fungal phylum was the Basidiomycota, which made up > 6% of the fungal community (i.e. eight-fold less abundant than Ascomycota) (Figure S5). The dominant Basidiomycota class was Agaricomycetes, which in contrast to Ascomycota, showed a significant threefold increase with decreasing size (from ~1.5 to 5% of the community from the largest to smallest size fraction). The phylum Glomeromycota was almost exclusively present in the smallest size fraction, making up 6% of the fungal community (d_a 1.1–0.65 μm, Figure S5).

Table 1
Generalised linear models (GLM) describing changes microbial bioaerosol abundance, α-diversity, and community composition with respect to size (d_a).

	Bacteria		Fungi	
	χ^2 (df)	P-value	χ^2 (df)	P-value
Abundance	39 $_{5,35}$	< 0.001	41 $_{5,33}$	< 0.001
Species richness	37 $_{5,35}$	< 0.001	34 $_{5,33}$	0.038
Species diversity	156 $_{5,35}$	< 0.001	100 $_{5,33}$	0.13
Community composition	15,088 $_{5,35}$	< 0.001	5060 $_{5,33}$	< 0.001

X2 = test statistic, df = degrees freedom.

3.4. Identification of potential pathogens in size fractions

The most abundant human health relevant pathogen was the fungi *A. fumigatus*, which made up over 40% of the fungal community in the intermediate size fraction, corresponding to approximately 6 $\log_{10}$ *A. fumigatus* ITS copies m^{-3} (d_a 2.1–3.3 μm, Figure S6). Other potential fungal and bacterial pathogens were very low in abundance, making up less than1% of the community. For example, bacteria identified as the respiratory pathogen *Mycobacterium* comprised < 0.01% of the total bacterial community. In total, 210 (<0.001%) of bacterial OTUs had 99% (or more) identity to a pathogen, but none accounted for more than 1% of the total community. Although numbers of potential infectious bacterial pathogens were low, numbers of bacteria known to cause serious allergenic responses were exceptionally high. For example, Actinobacteria made up > 60% of all bacteria in the smallest size fraction (corresponding to approximately 5.1 $\log_{10}$ 16S rRNA copies m^3, Fig. 4).

Potential bacterial pathogens were more abundant in the larger size fractions, following the same general trends in abundance and diversity as the total bacterial community (Fig. 6). The only pathogen that increased in relative abundance with decreasing size was *Propionibacterium acnes*.

3.5. The influence of the compost facility on the bioaerosols upwind and downwind of the site

The size distribution bacterial bioaerosols downwind of the site was distinct to that on site (KS test, D = 0.3, P < 0.001) and upwind of the compost site (KS test, D = 0.53, P < 0.001, Fig. 7). Although elevated levels in the largest size fraction downwind of the site versus upwind suggest a possible influence of the compost site on the size distribution downwind of the site. The highest concentration of fungal bioaerosols was found 250 m downwind of the compost site (total 7.5 ± 0.4 $\log_{10}$ ITS rRNA copies m^{-3}), rather than on the edge of the site where they were over an order of magnitude lower (6.1 ± 0.5 $\log_{10}$ ITS rRNA copies m^{-3} Fig. 7. The fungal bioaerosol size distribution at the compost site was distinct to that upwind (KS test, D = 0.07, P < 0.001,) as well as downwind (KS test, D = 0.14, P < 0.001).

4. Discussion

4.1. The size profile of microbial bioaerosol emissions from compost sites

Molecular analysis showed that most microbial bioaerosols emitted from the compost site were larger than 7.1 μm. This is in broad agreement with culture-based studies, that also found that microbial compost bioaerosols are associated with the coarse fraction of PM (Gutarowska et al., 2015; Reinthaler et al., 1999; Pahari et al., 2016; Byeon et al., 2008). In contrast, the SIBS data showed that most bioaerosols (by number) were found in the < 0.6 μm size fraction. Taken together, this indicates that microorganisms emitted from the compost site were associated with the coarse fraction of PM (likely as aggregates comprising multiple microorganisms attached to inorganic and organic matter), but that overall, microbial bioaerosols formed a component of a total bioaerosol emission comprising a large number of submicron particles. The small bioaerosols detected by SIBS can include cellular fragments (e.g. endotoxins) or decomposition products of biological materials. These components pose a serious health risk in their own right

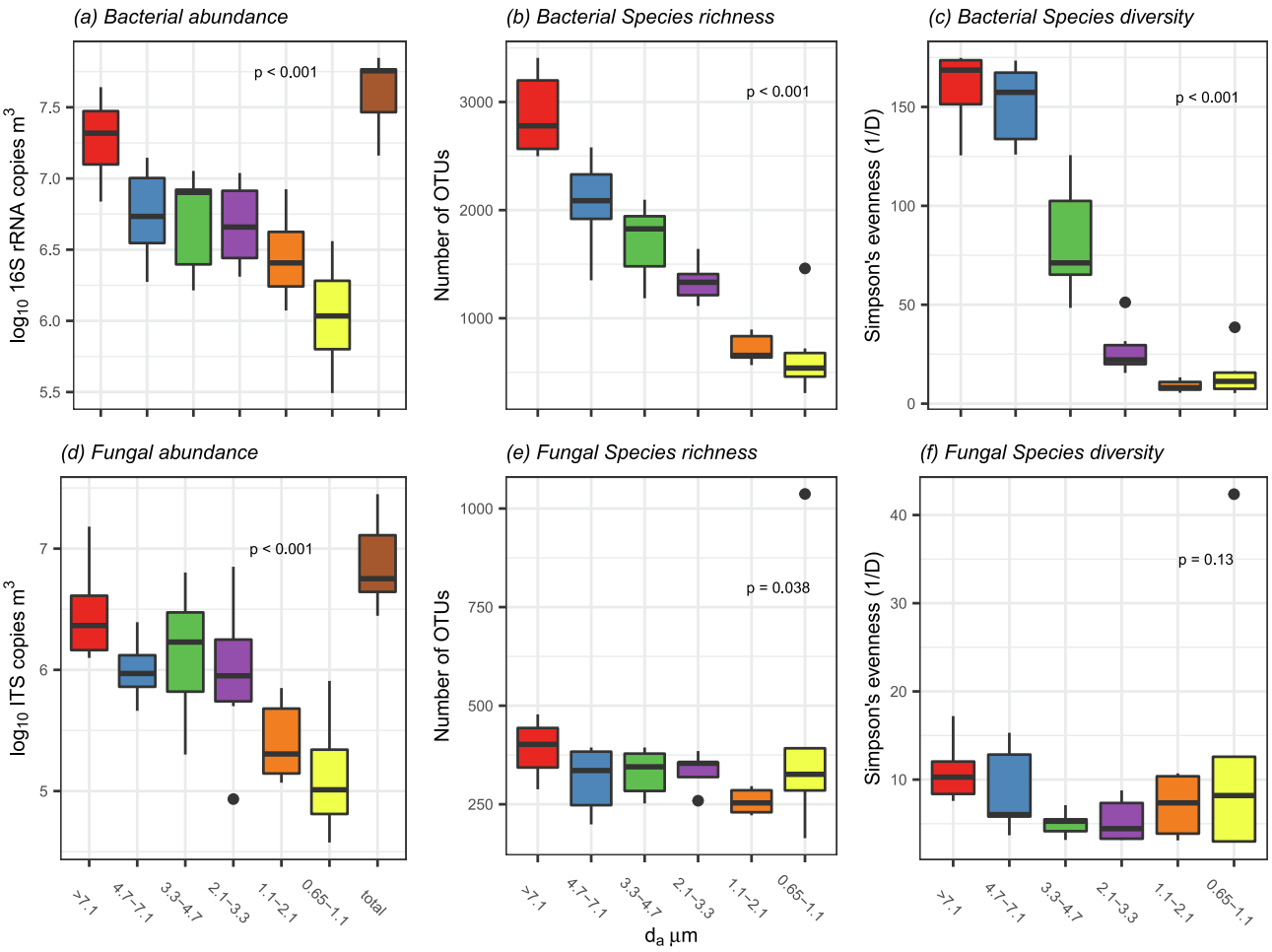


Fig. 2. Abundance and α-diversity of bacterial and fungal bioaerosols in each size fraction. *a* = bacterial abundance, *b* = bacterial species richness, *c* = bacterial species diversity, *d* = fungal abundance, *e* = fungal species richness, and *f* = fungal species diversity. ($n = 6$).

by acting as respiratory sensitizers, they also leave people more vulnerable to infection by live microbial bioaerosols (Pearson et al., 2015; Rolph et al., 2018). The extra context provided by the SIBS shows the value in considering the entire bioaerosol emission alongside the microbial component. Often the only context given to microbial bioaerosols is total PM (inorganic). As shown here, it is important to consider the other bioaerosol components, as they may enhance health risks from the microbial bioaerosols.

4.2. Changes in microbial community structure with bioaerosol size

Not only did microbial abundance vary with size, but there was clear differentiation in the bacterial and fungal community composition across size fractions. Specifically there was a decrease in relative abundance of Bacilli and increase in Actinobacteria as size decreased. For fungi there was increased relative abundance of Dothideomycetes, and Leotiomycetes in the larger size fractions, Sordariomycetes in the intermediate, and Eurotiomycetes in the intermediate to smaller size fractions. These size specific profiles are important when determining risk from bioaerosols, as it determines where they will deposit if inhaled (Fig. 1). But also as size is a key parameter and prerequisite for inclusion of deposition into dispersion modelling of compost bioaerosols (Douglas et al., 2016). As our results show that the microbial community composition varies across size ranges, accurate dispersal models of specific taxa from compost sites requires information about size ranges.

We propose that compost age and site activiy may be key drivers in determining the size profiles for the different taxa we observed. During composting, organic matter is broken down into smaller particles by a succession of mesophilic and thermophilic microorganisms. The dominant taxa involved depends on the composition of the starting material, but in general, the early stages are characterised by high concentrations of Bacillales, Corynebacteriales, and Clostridales and the latter stages by Actinomycetales. Entrobacteriales, Saccharomycetales, and *Aspergillus* (Watanabe et al., 2009; Danon et al., 2008; Antunes et al., 2016; Huhe et al., 2017). Our data showed that taxa associated with the early stages of composting, such as Bacillales and Corynebacteriales, are associated with larger bioaerosols; and late composting stage taxa, such as Actinobacteria and *Aspergillus*, are associated with smaller bioaerosols. The age of compost could be used as a predictor of size profiles and taxonomic composition of microbial bioaerosols. If this is found to be the case, the composition and size profiles of microorganisims emmited from composting (including those associated with various activities e.g. shredding, turning, screening, etc.) could be generalised and predicted. This would allow for more nuanced regulation of composting based on specific risk profiles accounting for composting activity and age.

Although the overall size distribution in this study contrasted with Galès *et al.*, (Galès et al., 2015), who found that most bacteria aerosolized at a compost site were emitted as single cells. Both studies agree on the size ranges for specific taxa (e.g. Actinobacteria and *A. fumigatus*), as do other studies using visual methods (Tamer Vestlund et al., 2014) and filtration combined with culture (Deacon et al., 2009). Therefore, we do not think that the contrasting overall size profiles contradict each other, but reflect some diferences in the site charactertristics, for example, compost age or site activity. The site in this study involved

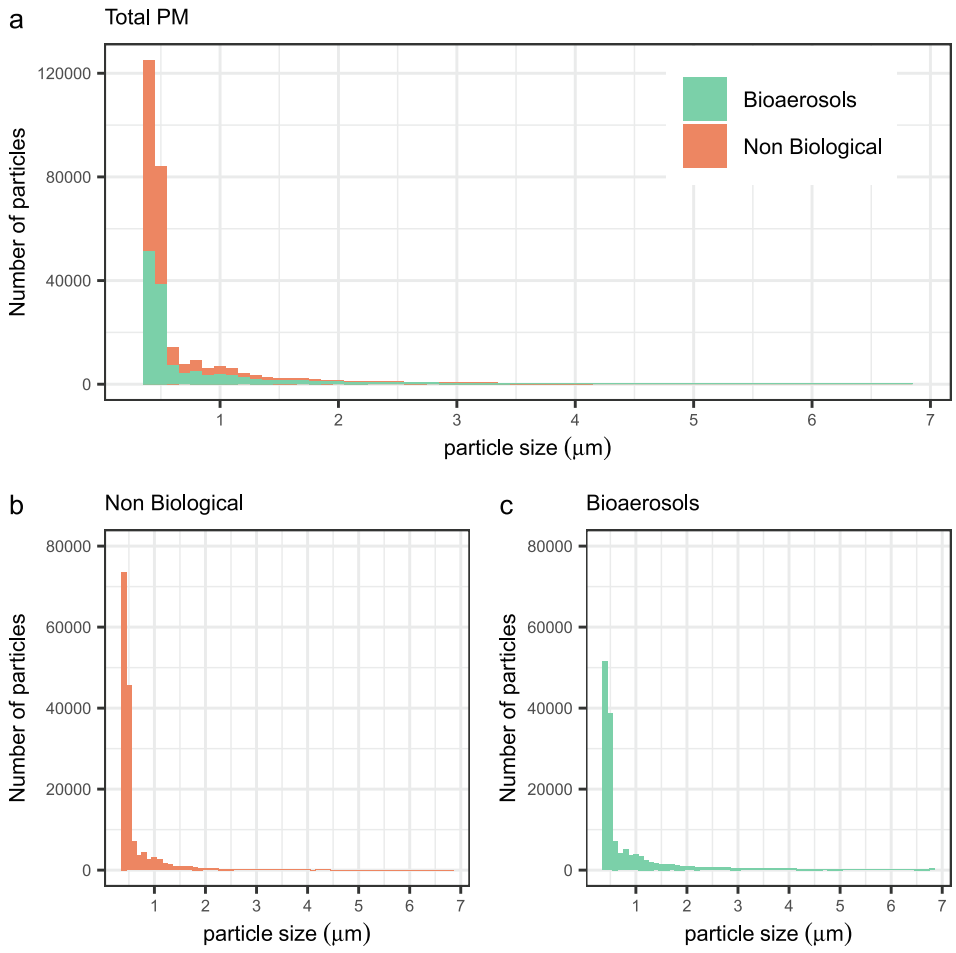


Fig. 3. Size distribution of particles measured by SIBS concurrently with molecular sampling. a = Total PM, b = non-biological particles, and c = bioaerosols (fluorescent particles).

composting activities from all stages of the process and therefore emmited microbial taxa from all stages of the process. Some micro-oganisms (partucualy Actinobacteria) are preferentially emmited from composting, but this may vary depending on compost age, activity, or material composistion (Veillette et al., 2018). It is therfore possible that the site in Galès *et al.*, (Galès et al., 2015) was preferentially emitting bioaerosols from the latter stages, resulting in a different microbial bioaerosol profile.

We observed a peak in the concentration of *A. fumigatus* in the 2.1–3.1 μm size fraction. This is consistent with other studies which have put the size range of *A. fumigatus* bioaerosols between 1 and 3 μm (Deacon et al., 2009; Galès et al., 2015; Reponen et al., 1996). It is generally assumed that $PM_{2.5}$ is the best proxy for microbial bioaresols (Williams et al., 2019). Our results confirm the assumption that $PM_{2.5}$ is a suitable proxy for dispersion modelling of *A. fumigatus* (Williams et al., 2019). But other taxa did not fall into the $PM_{2.5}$ size range. Therefore, taxa specific profiles are needed for dispersion modelling. Further the prodominance of microbes in the larger size fractions reported here and by others (Gutarowska et al., 2015; Reinthaler et al., 1999; Byeon et al., 2008; Pahari et al., 2016) indicates that a larger sizes (i.e. > $PM_{7.5}$) may be a better proxy for modeling the overall microbial bioaresol emission.

4.3. Pathogens in compost bioaerosol emissions.

None of the bacterial genera containing potential human pathogens we identified made up more that 1% of the total bacterial community, but this is not to say that there was not a significant health risk posed. For example, in absolute terms our data still suggests 1000 pathogenic *Mycobacterium* 16S rRNA copies m^3 of air. The pathology of *Mycobacterium* is complex and comprises both host immune response as well as the dose. Even low doses of *Mycobacterium* can elicit infection (1 CFU), and prior exposure to *Mycobacterium* (including non-pathogenic strains) can result in worse outcomes from subsequent tuberculosis infection (Hernandez-Pando et al., 1997; Johnson et al., 2007; Saini et al., 2012). Our results therefore indicate a health risk, especially for workers who daily spend hours at a time at a time exposed to bioaerosol emissions.

Although we did not detect high numbers of specific bacterial pathogens, a large proportion of the bacterial community was comprised of Actinobacteria (~20–70% depending on size fraction) which are widely reported as dominant in compost site bioaerosols (Pankhurst et al., 2009, 2012). Their small size (as reported here) means that they would be deposited in the bronchi and alveoli (Fig. 1) where they can cause severe respiratory sensitisation. Actinobacteria can cause serious respiratory disease such as hypersensitivity pneumonitis, commonly known as mushroom worker's lung (also allergic alveolitis) (Paściak et al., 2014; Kagen et al., 1981; Lacey and Dutkiewicz, 1994; Moore et al., 2004; Skóra et al., 2013; Barka et al., 2016; Rintala, 2011). In addition to this, respiratory sensitisation caused by non-infectious Actinobacteria may also leave people more vulnerable to secondary infection from other pathogens present (e.g. *Mycobacterium* and *A. fumigatus*) (Lacey and Dutkiewicz, 1994). In this instance, it is the sheer numbers of Actinobacteria, combined with the small size, which presents a significant health risk as allergens and sensitizers, rather than as infectious agents.

By far the most dominant pathogen was the fungal pathogen *A. fumigatus*, making up more than 40% of all fungal sequences (1×10^6

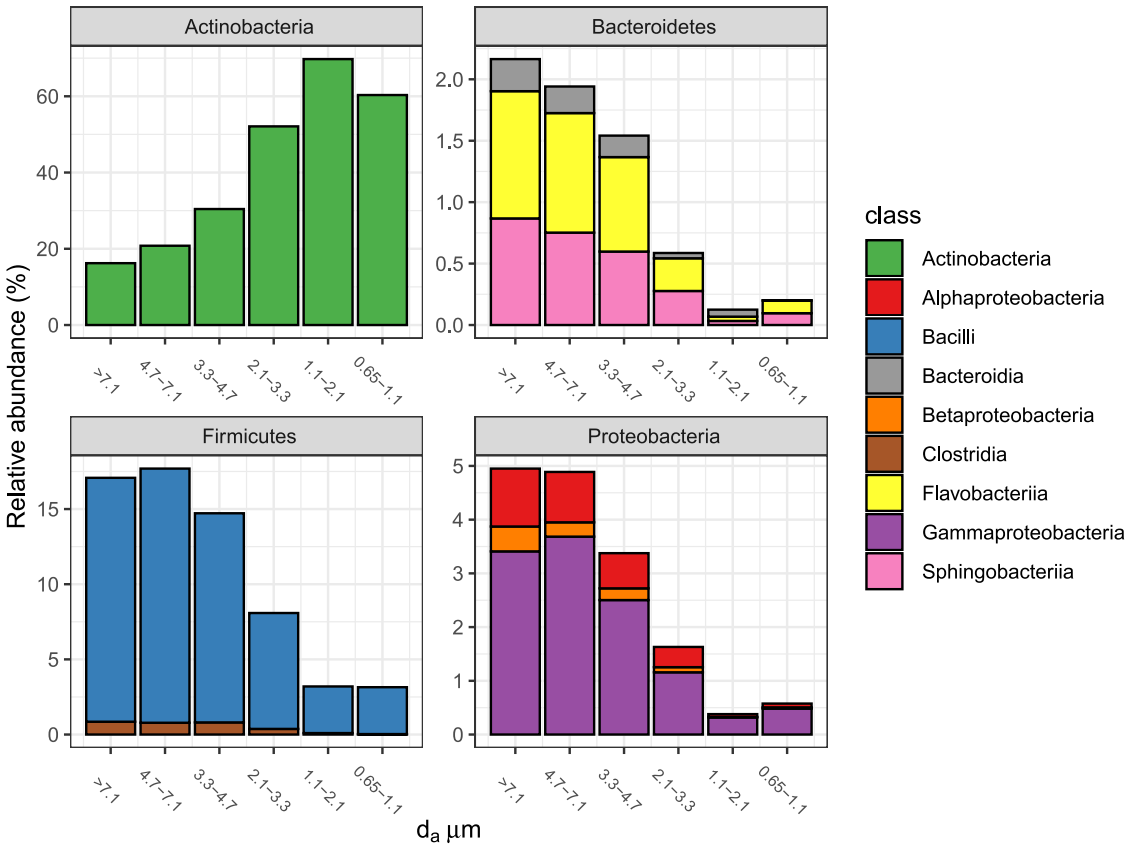


Fig. 4. Relative abundance of dominant bacterial taxa that showed significant changes in relative abundance between size fractions (adjusted univariate $P < 0.05$, Table S4).

m^3 air). The size range of *A. fumigatus* bioaerosols (2.1–3.1 µm) makes them a significant risk factor, a) because it enables them to disperse over large distances, and b) because it enables them to settle in the distal airways once inhaled. Globally *Aspergillus* spp. are estimated to be responsible for over 200,000 cases of invasive aspergillosis, 1.2 million cases of pulmonary aspergillosis, and 4.8 million cases of allergic bronchopulmonary aspergillosis annually; with most cases caused by *A. fumigatus* (Verweij et al., 2015; Denning et al., 2013, 2011). It is not remarkable to find high concentrations of *A. fumigatus* in compost, as it plays a vital role in breaking down complex organic matter during the later stages of the process (Clark et al., 1983; Anastasi et al., 2005; Kwon-Chung and Sugui, 2013; Tekaia and Latgé, 2005). Our results, however, confirm that vast numbers of *A. fumigatus* are aerosolised during the process, prosing a significant risk to health.

Currently, *A. fumigatus* is the only specific taxa that UK legislation requires compost sites to test for (Agency, 2018). Our data confirms that although it is vital to monitor this pathogen, there are high numbers of other bacterial respiratory sensitizers (such as Actinobacteria) which are only monitored non-specifically as 'total mesophilic bacteria'. The current monitoring methods may entirely overlook or undercount Actinobacteria, which are hard to grow in culture (Jiang et al., 2016; Janssen et al., 2002). Further to this, the SIBS showed that most of the bioaerosols emitted were not microbial and small in size. As discussed, these non-microbial bioaerosols represent a sensitizing health risk that could cause respiratory diseases or enhance the infectivity of the microbial agents present. Current legislation does not consider the size of microbial bioaerosols, which is a key factor in determining the risk they pose (Fig. 1). The use of culture-based methods in the current monitoring protocols also reduces specificity in monitoring. In future we should move towards molecular methods (Ferguson et al., 2019) (including size fractionation) as well UV-LIF based measurements, such as the SIBS, to capture a comprehensive understanding of the full diversity of taxa present and the relative health risks posed based on size. This is important, not just in compost sites as shown here, but also in the wider environment, as other studies have shown that human pathogens such as *Legionella* also show specific size associations (Tanaka et al., 2020).

4.4. Influence of the compost facility on the bioaerosol community at the landscape level

For both bacterial and fungal bioaerosols the highest concentrations downwind were for larger particles ($d_a > 2.1$ µm). Especially for bacteria, where it is only in the largest size fraction ($d_a > 7.1$ µm) that concentrations remain elevated after 250 m. Other studies have also reported that the most PM emitted from composting are large particles, and that smaller particles are from the wider environment (Gutarowska et al., 2015; Reinthaler et al., 1999; Byeon et al., 2008; Pahari et al., 2016). There are a couple of possible explanations for the elevation in concentrations of large bioaerosols at 250 m, but not other size fractions, downwind of the compost site (Fig. 7). One is simply that the vast majority of PM and microbial bioaerosols emitted from the site were large (Figure 2 and S2). The second is that smaller particles from other sources diluted the signal from the compost site. Therefore, the main impact over the bioaerosol community at short distances is from large bioaerosols from nearby sources. This is potentially significant as pathogens and respiratory sensitizers were more abundant in the larger size fractions.

4.5. Can we integrate real time UV-LIF based measurements (SIBS) with molecular data for biomonitoring?

The real time measurements with SIBS gives valuable context to the molecular microbial data; showing that most of the bioaerosols emitted

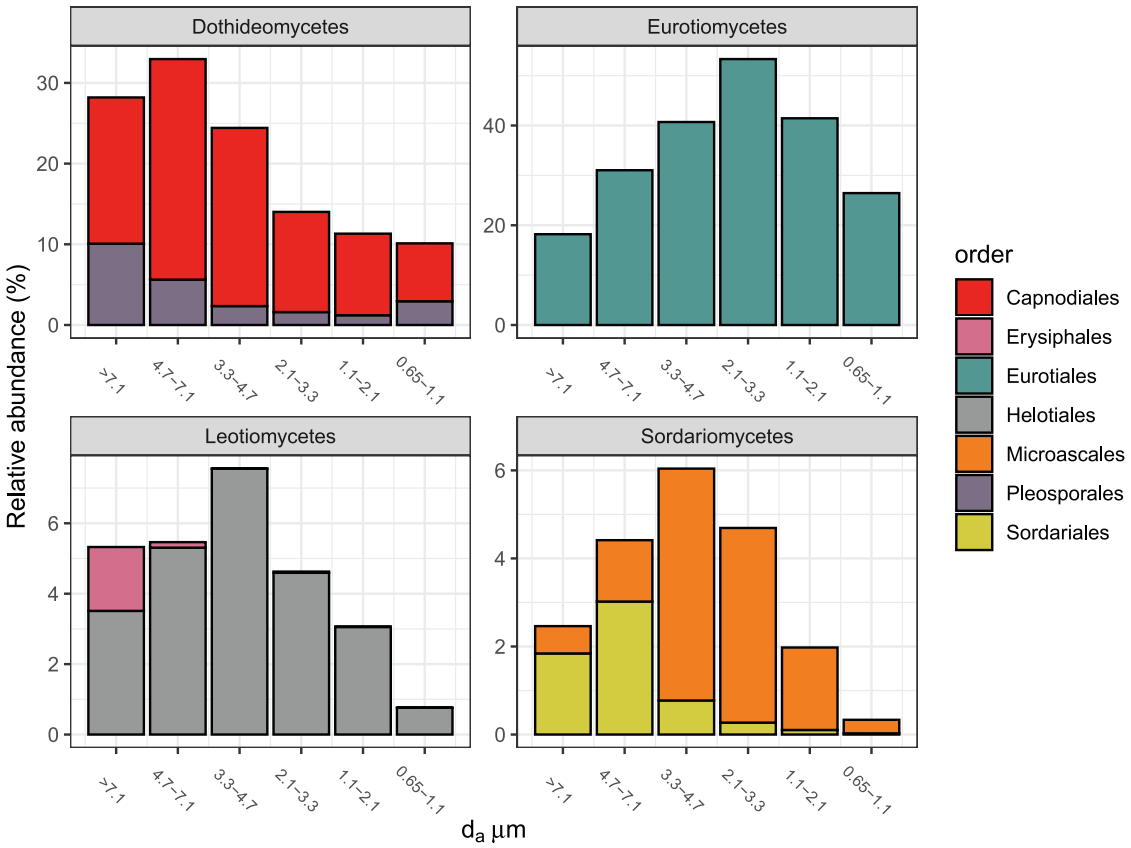


Fig. 5. Relative abundance of dominant Ascomycota taxa that showed significant correlations with size fraction (adjusted univariate $P < 0.05$, Table S4).

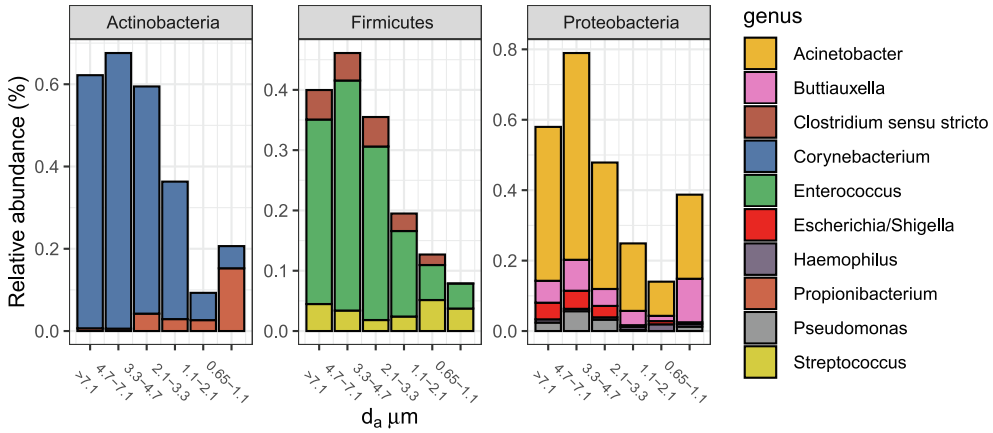


Fig. 6. Relative abundance of potential bacterial pathogen containing genera that showed significant correlations with size fraction.

at the site were not whole microorganisms, but other bioaerosols with intrinsic biofluorophores less than 0.6 µm in diameter. This is in broad agreement with other studies using SIBS (Nasir et al., 2019) and the WIBS (another UV-LIF based detection system) (O'Connor et al., 2015; Feeney et al., 2018) and arises from the fact that measurement principle of these methods is fundamentally different to the molecular qPCR data. This SIBS measures optical diameter of the particle, whereas the Andersen Cascade Impactor measures aerodynamic diameter. This, however, does not account for the differences in size distribution observed, indeed the difference arises because the molecular methods are not measuring individual particles, rather the size of the particle microorganisms where found on. The single particle UV-LIF based real time bioaerosols detection systems (SIBS/WIBS) interrogates the characteristic intrinsic fluorescence emission in each individual particle irrespective of size; whereas the qPCR counts the number of genes, which could be many thousands of genes for a single particle. In short, the SIBS counts each bioaerosol separately but the qPCR may count many genes for each bioaerosol. This is analogous to the difference between number size distribution and mass size distribution of airborne particles such that the former gives more emphasis to small particles than larger while the latter is more influenced by larger size particles.

Both methods are complementary, and as we have shown both methods are required to characterise the entire bioaerosol emissions and health risks from a given source. The aim of the study must be considered when selecting which method to use. The SIBS and similar methods can offer fine scale temporal profiles of the entire bioaerosol fingerprint. Offering the capability to comprehend the contribution of bioaerosols to total PM and the impact of various process and duration of high emission

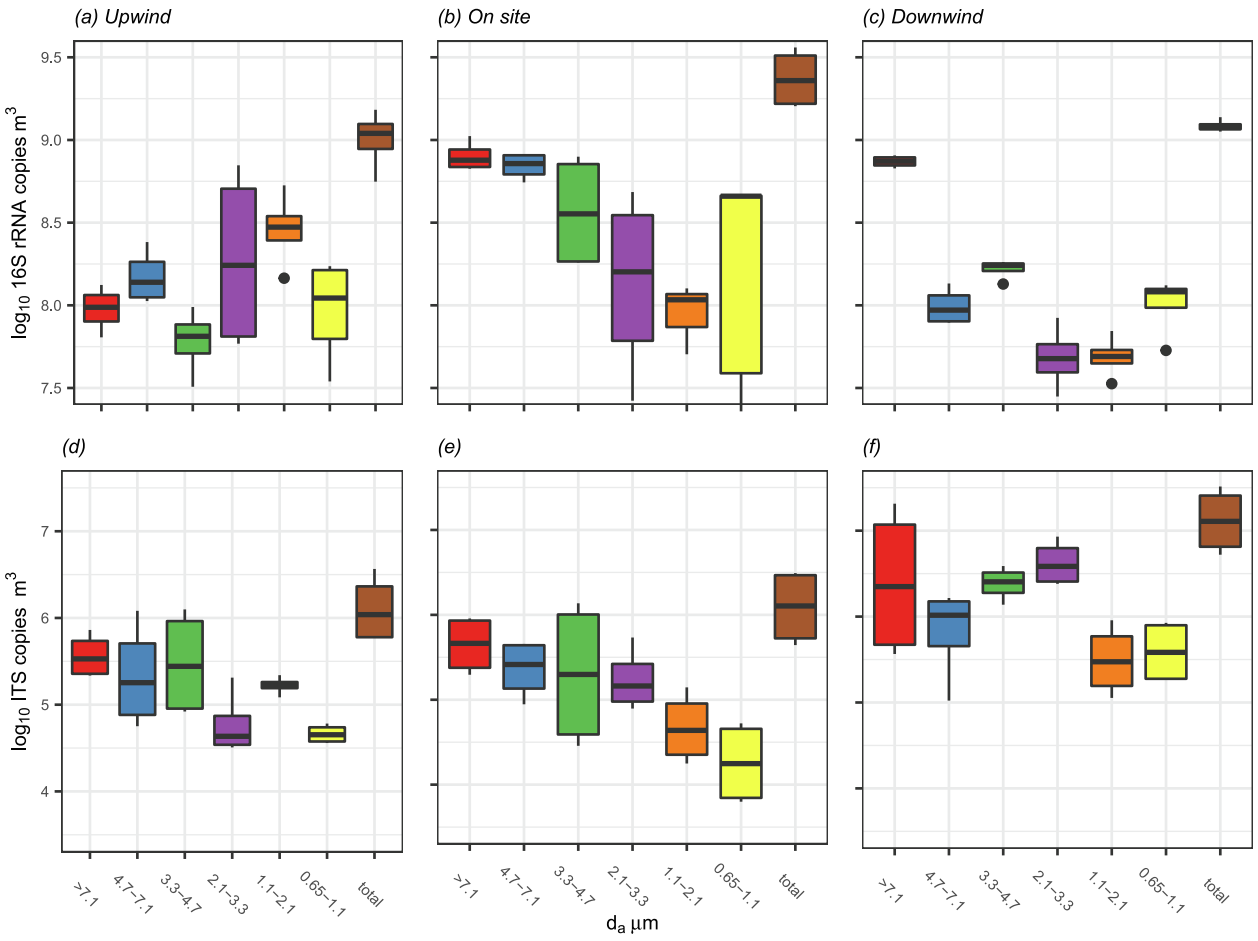


Fig. 7. Abundance of bacterial and fungal bioaerosols upwind, downwind, and at the compost site in each size fraction. *a* = upwind bacterial abundance, *b* = on site bacterial abundance, *c* = downwind bacterial abundance, *d* = upwind fungal abundance, *e* = on site fungal abundance, *f* = downwind fungal abundance.

events. In contrast, molecular methods cannot give fine temporal data as they can only be integrated over minutes (often hours) due to the requirement to sample for long periods to collect sufficient DNA for analysis (Ferguson et al., 2019). However, the SIBS has a limited ability to distinguish between different types of microbial bioaerosols in a broad sense, not between specific taxa or within a particle aggregate (Nasir et al., 2019). What remains to be determined is if studies where both types of data are available can be used to determine spectral fingerprints from the SIBS that can be utilised to detect specific microbial taxa, to enable real-time monitoring of microbial bioaerosol release.

4.6. Limitations of the study

A potential limitation of the study is primer selection, particularly so for fungal metabarcoding as there is no consensus on the best target region, let alone the best primers. A recent study found higher fungal diversity when sequencing the ITS1 region rather than the ITS2 region (as used in this paper) (Mbareche et al., 2020). Optimal primer choice is context dependant, other studies have found no difference between ITS1 and ITS2 (Blaalid et al., 2013), indeed the ITS region may not be the best choice for studying fungal diversity at all (Lücking et al., 2020). The fungal diversity recovered in this study is similar to that reported in other research into compost bioaerosols (Mbareche et al., 2020). But it should be noted that the variety of primers used across the literature (particularly for fungi) currently limits the ability for comparison between studies (particularly for measures of microbial diversity).

Metabarcoding gives limited taxonomic resolution, 16S rRNA amplicons can only give identification to genus level, whereas ITS amplicons can in some cases give species resolution. As pathogen identification requires strain level resolution, this only allows us to identify potential pathogen containing genera. Alternative approaches to overcome this would be to follow up on potential pathogens detected here with specific PCR based identification on a pathogen by pathogen basis; or to use a metagenomic approach in the first instance. These methods both have their limitations, using PCR to detect individual pathogens would be time consuming and unless a study (such as this one is first carried out to identify target pathogens) could miss significant pathogens present. Metagenomics does not require prior knowledge of the potential pathogens, but it does require high concentrations of DNA, which are challenging to achieve for bioaerosols sampling (Ferguson et al., 2019). A further limitation of metagenomics is that abundant taxa get most of the coverage, so rare pathogens can be missed or not sequenced with enough depth for robust identification. A combination of all three methods is required; metabarcoding for broad identification of taxa in all environments, metagenomics for identification of strains and pathogen virulence genes, followed up with PCR based methods for specific conformation of the presence of pathogens.

5. Conclusions

In this study, we observed shifts in microbial abundance, diversity and community composition with size (Fig. 8). This confirms that when assessing the risk and modelling the dispersion of composting bioaerosols, size does matter. We found that most of the microbial bioaerosols emitted from compost sites are associated with larger size fractions (>3.3 µm) and would end up in the nasal/oral cavity if inhaled.

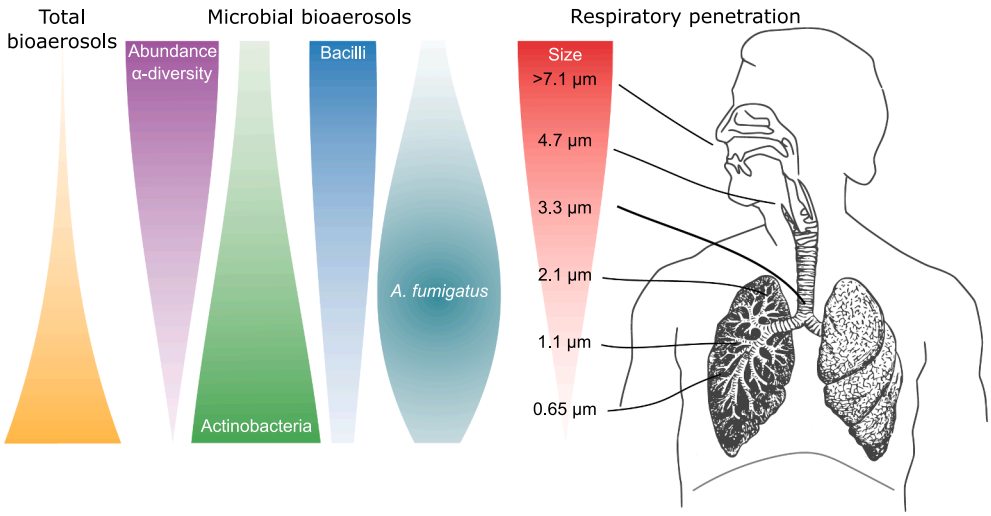


Fig. 8. Summary of key findings.

However, UV-LIF based measurements with SIBS showed that these microbial bioaerosols formed a component of a total bioaerosol emission comprising high numbers of sub-micron particles.

We found high numbers of the human pathogen *A. fumigatus* in intermediate size fractions, enabling it to deposit in the secondary and terminal bronchi. Although relative abundances of infectious bacteria were low, Actinobacteria were abundant in compost bioaerosols. Combined with their small size (<3.3 μm) this presents a high risk of respiratory sensitisation due to deep inhalation to the bronchi and alveoli sacs. This could lead to serious respiratory complications such as hypersensitivity pneumonitis. This is critical information as it confirms that size profiles, including those for specific taxa need to be accounted for when parameterising dispersion models and determining risks of bioaerosols.

Author contributions

R.F., C.W., A.J.D., F.C., Z.N., S.T., and I.C. conceived and planned the experiments. R.F., C.N., S.G-A., and Z.N. performed the field sampling. R.F. and C.N. performed the molecular work. Z.N. performed all the SIBS sampling and associated data analysis. R.F performed the formal analysis, visualisation, data curation, and prepared the original draft. All authors contributed to writing, review & editing and approved the final manuscript. Funding Acquisition for NE/M010813/1 by I.C., A.J.D., and C.W., for NE/M010961/1 by F.C., and S.T., for NE/M01163/1 by S.T. and Z.N., and for the Aerosol Society undergraduate research bursary by R.F.

Funding

The Natural Environment Research Council (NERC) supported this work (NE/M010813/1, NE/M010961/1 and NE/M01163/1). The Aerosol Society funded C.N. with an undergraduate research bursary.

8. Data availability

Sequences from this study are available through the European Nucleotide Archive under Project accession number PRJNA646699. All other data generated or analysed during this study are included in the supporting data files 1 (qPCR) and 2 (SIBS).

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.

Acknowledgements

We thank the site operators for providing site access. We thank Dr Toni Gladding and Dr Catherine Rolfe, Faculty of Science, Technology, Engineering & Mathematics, School of Engineering & Innovation at the Open University, UK for the lone of the Six-Stage Viable Andersen Cascade Impactors.

Declaration of Competing Interest

The authors declare no conflict of interest.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2020.106327.

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Ferguson, Robert M. W.; Neath, Charlotte E. E.; Nasir, Zaheer A.; Garcia Alcega, Sonia; Tyrrel, Sean F. ...

2020-12-31
Elsevier
https://doi.org/10.1016/j.envint.2020.106327
Ferguson RM, Neath CE, Nasir ZA, et al., (2021) Size fractionation of bioaerosol emissions from green waste composting. Environment International, Volume 147, February 2021, Article number 106327

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