

Car cabin filters as sampling devices to study bioaerosols using eDNA and microbiological methods

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Abstract The aim of this study was to examine whether bioaerosols could be isolated and quantified from used car cabin filters. Car cabin filters are widely available and can provide a vast untapped resource for sampling of bioaerosols in areas with enhanced air pollution. We developed a test system where we exposed car cabin filters to birch pollen under compressed air to represent airflow onto the filter. The flow of pollen within the test system was confirmed by microscopy and real-time PCR. Testing of extraction methods was performed on the most prevalent types of filters in UK cars and confirmed it was possible to extract and quantify viable fungi, birch pollen or proteins from car filters. The main challenge of their use is envisaged to be the lack of temporal resolution as car cabin filters are not routinely changed at intervals greater than 1 year; however, the systematic recording of the different routes driven during the sampling interval has been enabled through the common use of GPS, smartphones or similar technologies. Car filters therefore provide substantial possibilities to monitor exposure of harmful bioaerosols in the polluted traffic regions defined by the road network. This method could also be applied to studying allergen exposure associated with

bioaerosols and their delivery into the human respiratory system. These findings demonstrate that car cabin filters have the potential to be used to isolate and quantify a range of bioaerosols including pollen and fungi, as well as fractions of bioaerosols, such as proteins.

Keywords Bioaerosols · Pollen · Fungi · Filters · Environment · Allergy

1 Introduction

Any biological component capable of being transported in the air is considered a bioaerosol, and these include plant materials, such as pollen, or microbial material, such as fungal spores (Jones and Harrison 2004; Douglas et al. 2017). Bioaerosols are significant because of their ability to affect human and crop health (e.g. see Kim et al. 2017; Brown and Hovmøller 2002).

Human health concerns related to bioaerosols include respiratory problems, such as asthma, chronic obstructive pulmonary disease (COPD) and allergic rhinitis (e.g. Kim et al. 2017; D'amato et al. 2007). These are thought to be triggered by a range of factors, including chemical particulates and biological particles, which induce an immune response in predisposed individuals; however, the evidence for the involvement of bioaerosols such as pollen is mixed. It has

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been shown that pollen is among the environmental factors causing increases in incidents of asthma requiring emergency treatment (Gleason et al. 2014; Gonzalez-Barcala et al. 2013; Ghosh et al. 2012). Contrary to this, Marchetti et al. (2017) found no association between pollen levels and asthma; indeed they reported a lower incidence of allergic rhinitis in areas with higher pollen levels, which was hypothesized to be related to increased rates of desensitization. A study on allergenic bioaerosols shows several orders of magnitude in the sensitization to *Alternaria* within major cities (Heinzerling et al. 2009). This pattern is not reflected in the overall exposure of *Alternaria* in major cities of Europe (Skjøth et al. 2016).

Given the variation within findings, further research is necessary to ascertain why the evidence is conflicting. It is known that the immune response triggered by allergens, such as pollen, is modulated by molecules, such as pollen-associated lipid mediators (PALMs) (Gilles et al. 2009). There are several sources of lipids which may be associated with allergens; the pollen coat may contain lipids as part of its protective mechanism, lipids may also be bound to the allergens, and they may also originate from the pollen microbiome (Bublin et al. 2014). The lipid profiles of the primary species of allergenic pollen have been documented and key lipid molecules associated with immune responses identified (Bashir et al. 2013); however, data on the effects of the environment on pollen lipid profiles are lacking. One possibility is that there may be environmental factors that modulate lipid production resulting in altered allergenicity. Aside from the potential effect of PALMs on the immune response, it has also been shown that allergen levels are not directly correlated with pollen levels (Buters et al. 2010, 2015). This suggests that current methods of counting pollen may not be the best indicator of allergenicity and associated symptoms. One possible reason is that current sampling methods do not provide the required temporal and spatial resolution to produce data to match the scale of the health events being studied. In the majority of studies, bioaerosol data are collected from static pollen monitoring stations which sample a given volume of air per unit of time. These may be sited in a variety of locations and the roofs of university or hospital buildings are the most common locations (e.g. Sikoparija et al. 2017; Smith et al. 2014).

Current methods of air sampling use deposition or impaction to gather particles. Deposition onto a surface is a passive method of sampling, while impaction relies on drawing in a given volume of air and subsequent binding on to a surface. The collection of particles in both types of sampling relies on collection media (coated microscope slides or agar plates usually) or collection vessels (such as tubes or ELISA wells). Methods commonly used for subsequent analysis include microscopy, DNA-based techniques such as PCR and immunological techniques such as ELISA. Examples of samplers include Burkard 7-day cyclone and multi-well samplers, Andersen samplers and ChemVol samplers. These types of samplers are frequently used in static locations, whereas mobile sampling is less prevalent in the design of studies. Recently, unmanned aerial vehicles (UAVs) have provided an improvement to mobile bioaerosol sampling and the use of samplers fixed to vehicles has been briefly investigated (Brown 1991), with West and Kimber (2015) providing a comprehensive review of the latest air sampling techniques used in plant pathology, all of which are largely translatable to health research. Over recent decades, the incorporation of filters into vehicle ventilation and air-conditioning systems has become a common method of improving cabin air quality. These are generally pleated fibre or activated carbon (charcoal) filters which reduce the exposure of vehicle occupants to particulate air pollutants and bioaerosols such as pollen. The effectiveness of these filters has been shown both through the capture of residues in the filters (He et al. 2016) and through the biochemical and symptomatic markers in human participants (Muala et al. 2014). The ability of activated carbon filters to capture micro-organisms has been noted, this can cause microbial growth on the filter which reduces its ability to capture gases and volatile organic compounds and due to this, several antimicrobial compounds have been tested for incorporation into filters (Sim et al. 2014; Hwang et al. 2015). Filter design is constantly being refined to provide additional benefits and it is likely that future approaches will incorporate several different membranes and compounds, such as those detailed in Saikin et al. (2017) that allow capture of many different pollutants, particulates and bioaerosols.

While static samplers are a valuable means of getting data, they are limited by collecting from a local

environment with little or no spatial resolution. Repeatedly, studies have shown substantial spatial variation of aeroallergens in the urban environment (Werchan et al. 2017; Skj  th et al. 2013). Greater spatial resolution or sampling over larger areas instead of point-based measurements would therefore be of benefit and is well aligned with recent findings on pollen and asthma (Pollock et al. 2017). Using vehicular filters would provide a means for mobile sampling without needing the modification of vehicles or samplers and also provide access to an untapped data resource with the potential to be used for studies of the environmental effects on asthma epidemiology. Gathering samples in this manner would be limited to analysing cumulative bioaerosol content along any given route or within defined regions driven, but offers potential for mapping bioaerosol content at a finer resolution than currently achieved by conventional sampling methods. Current methods rely on sampling over a set period of time (generally 24 h or greater), at a static location where the bioaerosols collected depend on local sources of emission and atmospheric transport. Using vehicle filters would still rely on these factors but with a mobility element that would broaden the local sources of emission covered and at a greater air sampling rate which would reduce the amount of sampling time required to achieve comparable data. An additional benefit is that with the increased interest in citizen science, there is the scope for public involvement in large-scale health studies. The aim of this study was to examine whether bioaerosols and molecules associated with allergenicity, such as proteins, could be isolated and quantified from used car cabin filters using standard methods.

2 Materials and methods

2.1 Test system

In order to approximate bioaerosol transport through a car ventilation system within a laboratory, a test system was designed that included a source of compressed air, pollen and a car cabin air filter (CAF). The mechanism of cabin air ventilation is dependent on the car model, but generally has a heating function, which may or may not be associated with air conditioning. As car cabin filters only receive air flow when turned on, this system is representative

of a functioning cabin ventilation system. The test system comprised of a PVC tube of 1000 mm length by 110 mm diameter. The leading end of the tube was sealed with a push-fit cap with an entry port in the centre for compressed air. The car CAF was cut to 100 mm and installed at the open end of the tube and held in place using an open-centred rubber seal. Birch pollen grains were weighed to 0.025 g and placed on a platform inside the tube, by the compressed air entry port. The number of pollen grains present in 0.025 g pollen was calculated by suspension in 1 mL sterile distilled H₂O, thorough vortexing and counting using a haemocytometer at 100 × magnification. Counts were repeated six times.

Activation of the compressed air distributed the pollen throughout the test tube and resulted in pollen impaction onto the CAF. The air flow was maintained for 10 s, after which the compressor was stopped prior to removal of the filter from the test system for genomic DNA extraction. The test system was operated at 20 °C and 50% humidity, and air velocity was measured with an airflow anemometer (AirflowTM, Buckinghamshire, UK) to be 0.39 m s⁻¹, thereby providing an airflow rate of 184 L min⁻¹.

Initial experiments determined the proportion of pollen impacting on the filter in the test system. For this, one 1.5-mL tube was located in the centre of an expanded CAF sample. Expanding the CAF to remove the concertina structure provided a flat surface through which air could flow, while supporting the 1.5-mL tube in the centre to collect pollen. Birch pollen was then run through the system as described, and the pollen grains collected in the 1.5-mL tube were suspended in 100 µL water and, following a vigorous vortex to remove pollen grains adhered to the sides of the tubes, a 20 µL aliquot counted on a haemocytometer at 100 × magnification. This was repeated in triplicate for each particulate and carbon filters, and the mean level of pollen hitting the filter was calculated from the amount of pollen collected in each tube with each tube opening representing 1/100th of the surface area of the filter.

2.2 DNA extraction

Using the test system, the potential to extract pollen DNA from two of the three major types of car filter in the UK (activated charcoal/carbon and particulate) was examined. The third major type of CAF relies on

electrostatic charge and this was not replicated here. The CAFs used in this test system were for a Ford Focus. In this instance, the filters used were manufactured by Crosland (Euro Car Parts Ltd. Middlesex, UK).

A comparison of DNA extraction from the two filter types exposed to pollen in the test system was performed. The first method of DNA extraction tested followed the method of Radosevich et al. (2002) with the following amendments; $\frac{1}{4}$ of the 100 mm CAF sample was cut into approximate 1-cm strips and placed in a 50-mL centrifuge tube with 43 mL PBS-T (0.01 M phosphate buffer, 0.0027 M KCl, 0.137 M NaCl₂, 0.05% Tween 20). Tubes were vortexed vertically for 90 s in a Vortex Genie 2 (Scientific Industries, Inc., New York, USA), and a Q Series ultrasonic water bath (Ultrawave, Cardiff, UK) was used to sonicate the samples for 10 min, maximum power at 22 °C, prior to a 5 s vortex. As per Radosevich et al. (2002), the suspension was poured into clean 50-mL centrifuge tubes and the process repeated for a second wash of the filter strips using 25 mL PBS-T. Centrifugation was performed on both collected sample washes at room temperature for 30 min at $1400 \times g$. Pellets for each sample were combined in 1.5 mL PBS-T and centrifuged at $16,000 \times g$, 8 min. Pellets were suspended in 200 μ L PBS-T to provide a 'filter concentrate'. DNA extraction was performed on 100 μ L of the filter concentrate using a DNeasy PowerSoil DNA isolation kit following the manufacturer's instructions (Qiagen, Hilden, Germany) with the following modification of the homogenization step to 60 s at speed 6.5 in a FastPrep (MP Biomedicals, California, USA).

The second method of DNA extraction used the DNeasy PowerMax Soil DNA isolation kit (Qiagen, Hilden, Germany). CAF samples were cut into 1-cm strips and then placed in the 50-mL PowerMax[®] Soil PowerBead tubes for DNA extraction following the manufacturer's instructions.

2.3 Real-time PCR on laboratory test samples

Genomic DNA extracted and cleaned-up from the CAFs by the different methods detailed were amplified by real-time PCR using primers for birch BP8 for 5'-ACGATCGAGTTTTCATCAACAAA-3' and BP8 rev 5'-GACCTTATTGTCTTCACGGTCCTT-3' (Müller-Germann et al. 2015). Reactions consisted of

1 $\times$ qPCRBIO SyGreen Blue Mix Separate-ROX (PCR Biosystems, London, UK), 0.15 μ M each primer, 8 μ L DNA and molecular-grade H₂O to 20 μ L final volume. Cycling was performed in a Lightcycler 480 (Roche Diagnostics, Burgess Hill, UK) using Axygen 96-well plates (Corning, New York, USA). Parameters were 95 °C for 5 min followed by 40 cycles of 95 °C 5 s, 60 °C 30 s followed by a melt curve of 95 °C 1 min, 40 °C 1 min, 60 °C 1 s, continuous to 95 °C with fluorescence measured five times for every degree increase. Standard curves were generated from the DNA extracted from 0.25 g (2.5×10^8) birch pollen grains serially diluted (tenfold) to produce a series of standards covering the equivalent of 10^8 – 10^3 pollen grains.

2.4 Car filters

Six car cabin filters (four particulate filters and two activated carbon filters) were collected during routine annual (12 months $\pm$ 1 month) services by a qualified mechanic and stored at -20 °C prior to analysis. All filters were received from the garage within 2 months of each other. A 5×5 cm subsample was cut from each filter into 1-cm strips and placed in a 50-mL centrifuge tube and processed via the adapted method of Radosevich et al. (2002). Filtrate was examined for the presence of pollen and micro-organisms at $\times 400$ magnification, and DNA extraction using the DNeasy PowerSoil DNA extraction kit was performed as described previously.

To examine the effects of any inhibitors present in the resultant DNA extracts, two approaches to cleaning up the DNA prior to PCR were made, either diluting the DNA or using a commercial DNA clean-up method; the DNeasy PowerClean[®] clean-up kit (Qiagen, Hilden, Germany). This gave a total of four DNA treatments: undiluted (neat), 1 in 10 dilution, 1 in 100 dilution and the clean-up kit. All dilutions were performed in molecular-grade H₂O, and the DNeasy PowerClean[®] clean-up kit purification was performed according to the manufacturer's instructions. For each clean-up method, 50 μ L of the DNA extracts was used from both types of filters and DNA extraction methods, and all experiments were repeated in triplicate. The amount of DNA present ($\text{ng } \mu\text{L}^{-1}$) in the filtrate was quantified using a NanoDrop 2000 (Thermo Fisher Scientific, Waltham, USA).

After DNA extraction the quantity of birch pollen on the car cabin filters was determined by qPCR as described previously using the method of Müller-Germann et al. (2015) with a standard curve generated from a known quantity of birch pollen grains (Allergon, Thermo Fisher Scientific, Waltham, USA) determined by haemocytometry. Total eukaryotic DNA was also amplified, using primers ITS86F/ITS4 (ITS86F; 5'-GTGAATCATCGAATCTTTGAA-3', ITS4; 5'-TCCTCCGCTTATTGATATGC-3') and qPCR BIO SyGreen Blue Mix Separate-ROX (PCR Biosystems, London, UK) following the method and cycling parameters detailed in Op De Beeck et al. (2014). Instead of quantification against a standard curve, quantification cycles (Cq) were used to compare the effects of inhibitors on the amplification of DNA.

The quantity of birch pollen collected on CAF was assessed against microscopic counts from 04/2016 to 03/2017 inclusive, to represent the mean 12-month period during which the cars were driven. As the CAF were all from cars residing in the Worcester area, the qPCR was compared against the total amount of birch pollen sampled at the University of Worcester by a standard method of volumetric sampling (using a Burkard 7-day volumetric spore sampler) onto adhesive tape and subsequent microscopy, following the method described in Skjødth et al. (2015).

Two filters were selected at random from the six and 100 µL of the extracted filtrate was diluted to 1 in 10, 1 in 100 and 1 in 1000 in maximum recovery diluent (Oxoid Ltd. Cheshire, UK). From each concentration, 100 µL aliquot was spread onto nutrient agar and Rose Bengal agar (with chloramphenicol 100 mg L⁻¹) (Oxoid Ltd. Cheshire, UK) in triplicate to measure total viable count and fungi, respectively. Nutrient agar plates were incubated for 4 days at 30 °C and Rose Bengal plates for 6 days at 20 °C before the number of colony forming units (CFU) were counted.

2.5 Protein concentration

A 100 µL aliquot from each filtrate was centrifuged at 1400 g for 10 min to pellet debris. The supernatant was then centrifuged at 17,000 × g for 10 min to pellet proteins. Total protein concentration in the supernatant was measured by bicinchoninic acid assay (BCA) using a PierceTM BCA protein assay kit (Thermo Fisher Scientific) following the

manufacturers' 96-well microplate instructions. Bovine serum albumin (BSA) was used as a standard from 2 mg mL⁻¹, and protein concentration was measured at 562 nm absorbance.

2.6 Statistics

To compare the abundance of bacteria and fungi on car filters, CFU from each type of filter were log-transformed and compared by *t* test. The difference between DNA extraction methods and type of car filters was examined by Mann–Whitney *U* tests on untransformed data. Two-way ANOVA tested the effect of PCR inhibition on fungal and pollen amplification. Pollen amounts were calculated using the fit point method of quantitative analysis, with the threshold of background fluorescence set at three times the standard deviation. Protein concentration was interpolated from the BSA standard curve generated by four-parameter logistic regression. All data were analysed in GraphPad Prism version 7.0 (GraphPad Software, Inc. California, USA) except for qPCR analysis which was conducted using the proprietary LightCycler 480 software (Roche Diagnostics, Burgess Hill, UK).

3 Results

3.1 Test system validation

Haemocytometer counts showed a mean of 2.5×10^7 birch pollen grains in 0.025 g. In the test system, the approximate mean number of pollen grains impacting on the filter was 2.7×10^5 ($\pm 1.37 \times 10^5$), thus representing a 100-fold loss within the test system, likely due to electrostatic adherence of pollen grains to the tube walls, imperfect filtration of the air allowing birch pollen to pass through the filter, turbulence in the tube or clumping of pollen causing uneven distribution of pollen grains onto the filter (27). Knowing the amount of pollen impacted on the filter allows the efficiency of subsequent DNA extraction and quantification by real-time PCR to be determined.

3.2 DNA extraction

Quantitative PCR for birch pollen on filters from the test system had an efficiency of 1.89 and slope of

– 3.63. The amount of birch pollen detected on carbon and particulate filters extracted using the DNeasy PowerMax DNA isolation kit (Qiagen, Hilden, Germany) was lower than on both types of filters extracted with a sonication step, and a Mann–Whitney U test showed the difference was significant ($U = 0$, $P < 0.01$). There was no difference in the median amount of birch DNA detected on either type of filter ($U = 2.5$, $P = 0.5$).

3.3 Car filters

Bacteria were cultured from the two filters tested in similar quantities (Fig. 1). The mean total viable count and fungal count were both 3.3×10^3 CFU on a 5×5 cm segment of filter; however, fungi were found in greater quantities on filter B than filter A. Overall these results confirm the existence of viable micro-organisms on the car cabin filters. Pollen grains and micro-organisms were visible in filtrates at $\times 400$ magnification. Overall there was no significant difference between the total viable count and fungal counts ($t = 0.532$, $df\ 2$, $P = 0.65$), suggesting that fungi comprise the majority of micro-organisms present.

The amount of DNA in the filtrates ranged from 48.8 to 342.3 ng μL^{-1} . Quantitative PCR for Birch pollen on car filters had an efficiency of 1.999 and slope of -3.323. Birch pollen was present on all except one of the car filters in undiluted DNA extracts. Diluting the DNA tenfold subsequently resulted in the detection of a small quantity of birch pollen on the

previously negative filter (Fig. 2). When the dilution factors were taken into account, the maximum amount of birch pollen detected on 5 cm² of filter was 2.83×10^5 grains and the minimum was 2.38×10^3 grains. Greater counts were calculated on five out of the six filters tested once dilution was considered; however, when counts were transformed by $\log_{10}(-Y + 1)$, this was shown to be insignificant by one-way ANOVA ($F = 1.645$, $P = 0.23$). The pollen and spore integrals for the Worcester area of 18 pollen species one fungal species were 27,833 pollen grains/fungal spores quantified in total over the 12-month sampling period using a Burkard volumetric spore sampler. Of this, 16% were identified as birch pollen. The mean total DNA extracted from the filters was 1.9 ng μL^{-1} , < 1% of which was birch. These values are not directly comparable because the DNA concentration will contain all species over the entire 12-month sampling period, while the microscopic counts are restricted to the pollen species manually counted during the active pollen season (approximately 9 months) and only spore, *Alternaria*, where a previous study has shown that *Alternaria* account for less than 1% or the total spore count (Grinn-Gofron et al. 2019).

The filters from cars driven on roads all generally followed the same trend of Cq results when amplified using eukaryotic ITS primers. Undiluted samples showed the lowest Cq values and therefore the greatest DNA quantity, while the 1 in 100 dilutions showed the highest, with the 1/10 dilution and cleaned-up DNA falling in between (Fig. 3). The mean Cq of undiluted

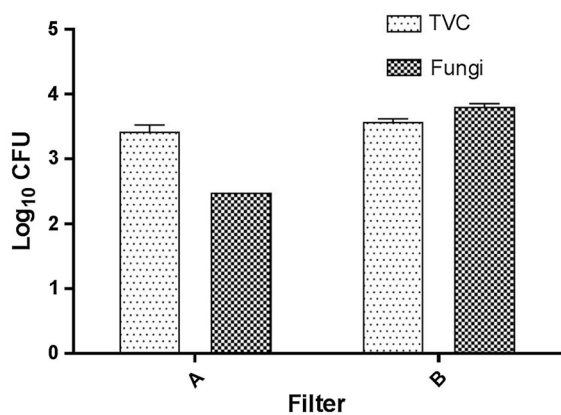


Fig. 1 Number of colony forming units from total viable counts (TVC) and fungal colonies on two road driven car filters. Error bars represent SEM across both filters ($n = 3$)

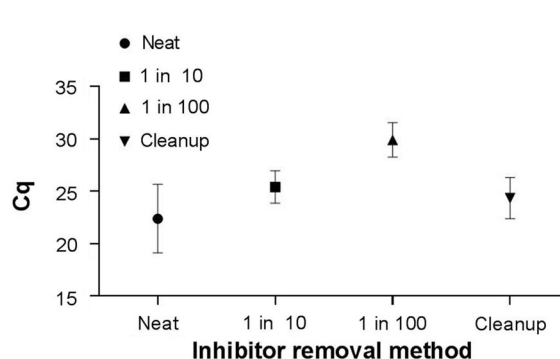


Fig. 2 Inhibitor removal for qPCR of birch pollen isolated from 'on-road' car cabin filters. The quantification cycle (Cq) represents the cycle at which the fluorescence exceeds background, and error bars represent SEM across all six filters ($n = 18$)

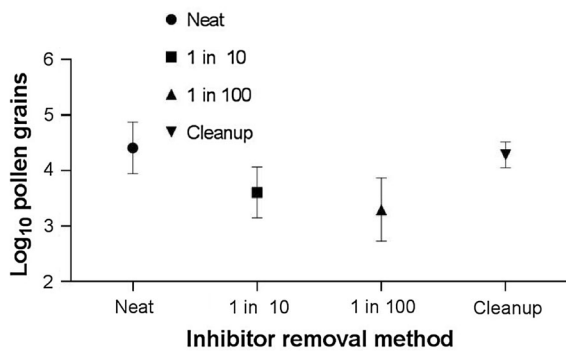


Fig. 3 Inhibitor removal for qPCR of eukaryotic DNA isolated from ‘on-road’ car cabin filters. Error bars represent SEM across all six filters ($n = 18$)

samples was 22.40 (SD 3.272) and of cleaned-up DNA was 24.35 (SD 1.953), while the 1/10 and 1/100 samples had Cq values of 25.42 (SD 1.535) and 29.88 (SD 1.640), respectively, and the differences between these were significant ($F = 186.9$, $df\ 3$, $P < 0.0001$). There was no uniform increase between the undiluted, 1/10 and 1/100 in any of the filters tested, which may indicate inhibitory effects. Given that two-way ANOVA showed significant differences in Cq values from the filters from different vehicles ($F = 57.16$, $df\ 5$, $P < 0.0001$), it is likely that both eukaryotic DNA quantity and inhibitory molecules are unique to each filter tested.

Overall there was no effect of dilution on the quantification of birch pollen; however, when eukaryotic DNA was amplified it appeared that there may be some effect of inhibitor molecules on the quantification cycle.

Table 1 Total protein (mg mL⁻¹) extracted from used car filters

Filter	Total protein mg mL ⁻¹ (2dp) (± SD)
A	0.10 (± 0.06)
B	0.67 (± 0.13)
C	1.47 (± 0.01)
D	1.37 (± 0.01)
E	0.42 (± 0.01)
F	0.91 (± 0.11)

3.4 Protein concentration

Total protein concentration was extrapolated from the BSA standard curve and ranged from 0.10 to 1.47 mg mL⁻¹ across the six car filters (Table 1). The difference in protein concentration was significant between the filters ($F = 58.62$, $P < 0.0001$).

4 Discussion

Using filters that extract bioaerosols from car cabins it was possible to isolate pollen, fungi and bacteria by culturing and through extraction of genomic DNA. The amount of DNA extracted from filters was comparable to amounts collected by Radosevich et al. (2002) from air filters. Using car cabin filters provides a new, mobile approach to air sampling which will be useful in ecological and public health studies. Car cabin filters have previously been used to study particulates (Park et al. 2010; He et al. 2016; Wong et al. 2011), but this is the first report of their use for the study of bioaerosols that we are aware of. The results of the test system demonstrate that not all bioaerosols entering into the system are evenly distributed on the car filter or within the tube. This efficiency is most likely related to aerosol dynamics in the ventilation system and will be specific to each car model.

When a test system was used to assess DNA extraction methods, it was found that inclusion of a sonication step provided greater DNA yields than a standard DNA extraction kit based on bead beating. This supports the findings of Luhung et al. (2015) who found that high temperature sonication was essential for increasing the amount of DNA extracted from environmental samples collected on filters. The time and temperature used here were shorter and lower, respectively, than the optimum found by Luhung et al. (2015), but the mean amount of birch pollen quantified from filters by qPCR was slightly greater than the mean amount of birch impacting on the filter in the test system as determined by microscopy.

The test system itself could provide a valuable means of examining the collection of bioaerosols. More complex systems have been used in the examination of pollutants (Muala et al. 2014), and the test system described here could be further developed for studies of bioaerosols within controlled environments.

In translating the test system to samples collected from cars, it was expected that road samples would contain particles that could inhibit PCR. PCR inhibitors are prevalent in environmental samples and can take many forms, such as metal ions (Schrader et al. 2012; Combs et al. 2015). However, there was no inhibition observed in this study. The maximum DNA levels were extracted from the neat, unmodified samples. Using a commercially available clean-up kit resulted in DNA levels similar to that of a tenfold dilution of neat DNA. Diluting the DNA sample itself is sometimes sufficient to decrease the levels of inhibitors to allow PCR amplification when there is sufficient DNA present for detection; however, this was not observed here.

Although microscopic examination of samples remains the most widely used method of bioaerosol analysis, DNA-based methods are gaining ground and can provide a greater depth to the data obtained than basic quantification. For example, it has been possible to determine the structure of bioaerosol populations at different time points (Lee et al. 2010) and in different locations (Després et al. 2007) using real-time PCR and restriction fragment length polymorphism (RFLP) analysis. There are certain considerations to air sampling for molecular analysis, such as the substrate on which the sample is collected. This can vary depending on the type of air sampler used. Common samplers will impact particles onto coated glass slides for microscopic examination, while other methods impact into tubes or wells allowing subsequent analysis. It is also possible to collect samples directly onto agar plates, a method which has obvious advantages for the study of bacteria and fungi, while other samplers collect on to filters (Juozaitis et al. 1994; Radosevich et al. 2002).

It was possible to determine total protein concentration from the filters, suggesting that the quantity of airborne allergens could be determined if appropriate methods, such as enzyme linked immunosorbent assays (ELISA), were applied. Most common bioaerosol allergens are proteins and it has been possible to quantify these from air samples collected on filters using specialized sampling devices, such as the case of Bet v 1 (birch allergen) in a Europe-wide study (Buters et al. 2010). The variation observed in protein concentrations across the filters is likely to be due to the high variability that will exist between vehicles, the journeys travelled and the manner in which they

are driven. These factors will all affect the air flow and velocity on to the filters, and the localities covered by journeys where not known in this study. Further experimentation could examine the nature of this variation and the impact this has on human health, as it has been demonstrated that allergenicity does not form a linear relationship with pollen exposure (Buters et al. 2015).

An exploration of whether standard methods for extracting lipids by gas chromatography–flame ionization detection could be applied to the used car filters has been performed (unpublished data). The results suggest there may be potential to extract lipids from car air filters, as peaks were obtained which may, in theory, represent iso-fatty acids. Bashir et al. (2013) identified the main lipids in pollen (fatty acids, aliphatic hydrocarbons, fatty alcohols, sterols and terpenes), and bacteria and fungi will present different fatty acid profiles also. It has been demonstrated that it is possible to use lipid profiling to distinguish between gram positive and negative bacteria (Snyder et al. 1990) and to help identify bacteria to species level (Torkko et al. 2003), and the practice of using lipids to monitor environmental impact on micro-organisms is not new (Willers et al. 2015). To contribute to understanding of the role of the environment on allergenicity of airborne particles, future work in this area should focus on the lipids from pollen and the associated microbiomes, as well as fatty acids acting as PALMs. To achieve this, improvement of the methods used to study lipids from complex air samples will be needed.

The ability to identify species, allergens and lipids in bioaerosol samples will undoubtedly contribute to an improved understanding of airborne allergens as causative agents of respiratory conditions. In combination with improved sampling resolution, this will drive towards more personalized risk projections for predisposed individuals. Here the amount of birch pollen collected by CAF was greater than the amount measured by conventional method of using a Burkard 7-day volumetric spore sampler and microscopic counting. The air throughput of the Burkard sampler is 10 L min^{-1} . In cars many variables will affect air flow rate through the CAF, including the velocity of the vehicle, the time the air fan is turned on/off, whether air recirculation is activated, and the dimensions of the air inlet points. In a study of ultrafine particle concentrations in vehicle cabins, Xu and Zhu

(2009) estimated the mechanical airflow rate varied between 0 and $360 \text{ m}^3 \text{ h}^{-1}$ (6000 L min^{-1}), based on measurements of air velocity at different fan speeds. Park et al. (2010) used air flow rates between 57.6 and 172.8 L min^{-1} to represent air flow in their test system for studying PM_{2.5} trapped in CAF. Given this potential for a greater than tenfold increase in air flow between the Burkard volumetric sampler and the air flow in a vehicle, the expected amount of trapped pollen in a CAF would also be expected to increase by a similar proportion compared to the microscopic counts obtained from the Burkard sampler. A limitation of this study is that direct comparisons between the birch pollen counts and the CAF birch quantities cannot be drawn due to the number of variables unaccounted for during the 12 months over which the vehicles were driven, such as average speed, journey duration, driving style and fan use. A further expansion of this work should incorporate journey monitoring and driving data, to allow the necessary comparisons to be performed.

One of the greatest challenges in achieving an improvement in sampling resolution is having resources to monitor large areas at a local scale. Mobile air sampling offers this, but one of its limitations is sampling a large enough volume of air to obtain sufficient biological material to analyse. Traditional methods of air sampling, such as volumetric air samplers of the Hirst design (Hirst 1952), provide a good throughput of air and collect sufficient material, but are limited by being fixed in one location, while existing mobile sampling methods such as personal air samplers process a smaller volume but offer benefits of localized data. Success has been achieved in designing studies using personal air samplers (Agranovski et al. 2017; Tolchinsky et al. 2011) and, with the demand for advances in both meteorological forecasting and biological forecasting, recent technologies such as unmanned aerial vehicles (UAVs or 'drones') are emerging as potential sources of data through the collection of bioaerosols in the atmosphere (e.g. Savage et al. 2012) and collected geophysical data (Niedzielski et al. 2017). Collecting biological material with drones or other mobile units such as cars has the potential to provide data to support forecasting pollen distribution and the spread of plant disease, but the means with which the material is collected is not yet ergonomic. Although these technologies advance air sampling techniques, there are

limited studies which have utilized motor vehicles as potential sampling tools (West and Kimber 2015). The results presented in this study show that modern motor vehicles provide a resource that can be tapped into with respect to the knowledge of how bioaerosols are distributed in the environment.

In conclusion, there is the potential to utilize car cabin air filters as a means of studying the abundance and epidemiology of bioaerosols at a localized level. The benefits of this technique are abundance of samples, low cost, mobility, greater resolution providing constraints are met (routes recorded, etc.) and can act as an adjunct to existing methods of analysis. However, there are certain limitations, which would have to be considered in study design. These include variations in air flow between vehicles and different distances travelled, how recording routes driven is performed (spatial) and the infrequent assessment of data (lack of temporal effects). Providing these limitations are addressed in the design of future studies, there is scope for the incorporation of mobile air sampling into health and agricultural research.

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References

- Agranovski, I. E., Usachev, E. V., Agranovski, E., & Usacheva, O. V. (2017). Miniature PCR based portable bioaerosol monitor development. *Journal of Applied Microbiology*, 122(1), 129–138.
- Bashir, M. E. H., Lui, J. H., Palnivalu, R., Naclerio, R. M., & Preuss, D. (2013). Pollen lipidomics: Lipid profiling exposes a notable diversity in 22 allergenic pollen and potential biomarkers of the allergic immune response. *PLoS ONE*, 8(2), e57566. <https://doi.org/10.1371/journal.pone.0057566>.
- Brown, H. M. (1991). Mobile slit sampler for pollen and spore sampling on motorways. *Aerobiologia*, 7, 69–72.
- Brown, J. K. M., & Hovmöller, M. S. (2002). Aerial dispersal of pathogens on the global and continental scales and its impact on plant disease. *Science*, 297(5581), 537–541. <https://doi.org/10.1126/science.1072678>.
- Bublin, M., Eiwegger, T., & Breiteneder, H. (2014). Do lipids influence the allergic sensitization process? *Journal of Allergy and Clinical Immunology*, 134(3), 521–529. <https://doi.org/10.1016/j.jaci.2014.04.015>.
- Buters, J., Prank, M., Sofiev, M., Pusch, G., Albertini, R., Annesi-Maesano, I., et al. (2015). Variation of the group 5 grass pollen allergen content of airborne pollen in relation to geographic location and time in season. *Journal of*

- Allergy and Clinical Immunology*, 136(1), 87–95.e6. <https://doi.org/10.1016/j.jaci.2015.01.049>.
- Buters, J. T. M., Weichenmeier, I., Ochs, S., Kreyling, W., Pusch, G., Boere, A. J. F., et al. (2010). The allergen Bet v 1 in fractions of ambient air deviates from birch pollen counts. *Allergy*, 65, 850–858.
- Combs, L. G., Warren, J. E., Huynh, V., Castaneda, J., Golden, T. D., & Roby, R. K. (2015). The effects of metal ion PCR inhibitors on results obtained with the Quantifiler® human DNA quantification Kit. *Forensic Science International: Genetics*, 19, 180–189. <https://doi.org/10.1016/j.fsigen.2015.06.013>.
- D'amato, G., Cecchi, L., Bonini, S., Nunes, C., nnesi-Maesano, I., Behrendt, H., et al. (2007). Allergenic pollen and pollen allergy in Europe. *Allergy*, 62(9), 976–990.
- Despres, V. R., Nowoisky, J. F., Klose, M., Conrad, R., Andreae, M. O., & Pöschl, U. (2007). Characterization of primary biogenic aerosol particles in urban, rural, and high-alpine air by DNA sequence and restriction fragment analysis of ribosomal RNA genes. *Biogeosciences*, 4, 1127–1141.
- Douglas, P., Robertson, S., Gay, R., Hansell, A. L., & Gant, T. W. (2017). A systematic review of the public health risks of bioaerosols from intensive farming. *International Journal of Hygiene and Environmental Health*. <https://doi.org/10.1016/j.ijheh.2017.10.019>.
- Ghosh, D., Chakraborty, P., Gupta, J., Biswas, A., Roy, I., Das, S., et al. (2012). Associations between pollen counts, pollutants, and asthma-related hospital admissions in a high-density Indian metropolis. *Journal of Asthma*, 49(8), 792–799. <https://doi.org/10.3109/02770903.2012.716473>.
- Gilles, S., Mariani, V., Bryce, M., Mueller, M. J., Ring, J., Behrendt, H., et al. (2009). Pollen allergens do not come alone: pollen associated lipid mediators (PALMS) shift the human immune systems towards a TH2-dominated response. *Allergy, Asthma and Clinical Immunology*, 5, 3. <https://doi.org/10.1186/1710-1492-5-3>.
- Gleason, J. A., Bielory, L., & Fagliano, J. A. (2014). Associations between ozone, PM_{2.5}, and four pollen types on emergency department pediatric asthma events during the warm season in New Jersey: A case-crossover study. *Environmental Research*, 132, 421–429. <https://doi.org/10.1016/j.envres.2014.03.035>.
- Gonzalez-Barcala, F. J., Aboal-Viñas, J., Aira, M. J., Regueira-Méndez, C., Valdes-Cuadrado, L., Carreira, J., et al. (2013). Influence of pollen level on hospitalizations for asthma. *Archives Environmental Occupational Health*, 68(2), 66–71. <https://doi.org/10.1080/19338244.2011.638950>.
- Grinn-Gofron, A., Nowosad, J., Bosiacka, B., Camacho, I., Pashley, C., Belmonte, J., et al. (2019). Airborne Alternaria and Cladosporium fungal spores in Europe: Forecasting possibilities and relationships with meteorological parameters. *Science of the Total Environment*, 653, 938–946. <https://doi.org/10.1016/j.SCITOTENV.2018.10.419>.
- He, X., Brem, B. T., Bahk, Y. K., Kuo, Y., & Wang, J. (2016). Effects of relative humidity and particle type on the performance and service life of automobile cabin air filters. *Aerosol Science and Technology*, 50(6), 542–554.
- Heinzerling, L. M., Burbach, G. J., Edenharter, G., Bachert, C., Bindslev-Jensen, C., Bonini, S., et al. (2009). GA2LEN skin test study I: GA2LEN harmonization of skin prick testing: novel sensitization patterns for inhalant allergens in Europe. *Allergy*, 64(10), 1498–1506. <https://doi.org/10.1111/j.1398-9995.2009.02093.x>.
- Hirst, J. M. (1952). An automatic volumetric spore trap. *Annals Applied Biology*, 39, 257–265.
- Hwang, G. B., et al. (2015). Antimicrobial air filters using natural Euscaphis japonica nanoparticles. *PLoS ONE*, 10(5), 126481.
- Jones, A. M., & Harrison, R. M. (2004). The effects of meteorological factors on atmospheric bioaerosol concentrations: A review. *Science of the Total Environment*, 326(1–3), 151–180.
- Juozaitis, A., Willeke, K., Grinshpun, S. A., & Donnelly, J. (1994). Impaction onto a glass slide or agar versus impingement into a liquid for the collection and recovery of airborne microorganisms. *Applied and Environmental Microbiology*, 60(3), 861–870.
- Kim, K.-H., Kabir, E., & Jahan, S. A. (2017). Airborne bioaerosols and their impact on human health. *Journal of Environmental Sciences*. <https://doi.org/10.1016/j.jes.2017.08.027>.
- Lee, H., Lee, S., Lee, H. M., Kim, S., Kim, Y. P., & Kang, H. (2010). Identification of airborne bacterial and fungal community structures in an urban area by T-RFLP analysis and quantitative real-time PCR. *Science of the Total Environment*, 408(6), 1349–1357.
- Luhung, I., Wu, Y., Ng, C. K., Miller, D., Cao, B., & Chang, V. W. (2015). Protocol improvements for low concentration DNA-based bioaerosol sampling and analysis. *PLoS ONE*, 10(11), e0141158.
- Marchetti, P., Pesce, G., Villani, S., Antonicelli, L., Ariano, R., Attena, F., et al. (2017). Pollen concentrations and prevalence of asthma and allergic rhinitis in Italy: Evidence from the GEIRD study. *Science of the Total Environment*, 584–585, 1093–1099. <https://doi.org/10.1016/j.scitotenv.2017.01.168>.
- Muala, A., Sehlstedt, M., Bion, A., Osterlund, C., Bosson, J. A., Behndig, A. F., et al. (2014). Assessment of the capacity of vehicle cabin air inlet filters to reduce diesel exhaust-induced symptoms in human volunteers. *Environmental Health*, 13(1), 16.
- Müller-Germann, I., Vogel, B., Vogel, H., Pauling, A., Fröhlich-Nowoisky, J., Pöschl, U., et al. (2015). Quantitative DNA analyses for airborne birch pollen. *PLoS ONE*, 10(10), e0140949.
- Niedzielski, T., Skjøth, C., Werner, M., Spallek, W., Witek, M., Sawiński, T., et al. (2017). Are estimates of wind characteristics based on measurements with Pitot tubes and GNSS receivers mounted on consumer-grade unmanned aerial vehicles applicable in meteorological studies? *Environmental Monitoring and Assessment*, 189(9), 431. <https://doi.org/10.1007/s10661-017-6141-x>.
- Op De Beeck, M., Lievens, B., Busschaert, P., Declerck, S., Vangronsveld, J., & Colpaert, J. (2014). Comparison and validation of some ITS primer pairs useful for fungal metabarcoding studies. *PLoS ONE*, 9(6), 1–11.
- Park, J. H., Yoon, K. Y., Noh, K. C., Byeon, J. H., & Hwang, J. (2010). Removal of PM_{2.5} entering through the ventilation duct in an automobile using a carbon fiber ionizer-assisted cabin air filter. *Journal of Aerosol Science*, 41(10), 935–943.

- Pollock, J., Shi, L., & Gimbel, R. W. (2017). Outdoor environment and pediatric asthma: An update on the evidence from North America. *Canadian Respiratory Journal*, 2017, 1–16.
- Radosevich, J. L., Wilson, W. J., Shinn, J. H., DeSantis, T. Z., & Andersen, G. L. (2002). Development of a high-volume aerosol collection system for the identification of air-borne micro-organisms. *Letters in Applied Microbiology*, 34(3), 162–167.
- Saikin, A. M., et al. (2017). Air quality within vehicles. *Russian Engineering Research*, 37(5), 424–427.
- Savage, D., Barbetti, M. J., MacLeod, W. J., Salam, M. U., & Renton, M. (2012). Mobile traps are better than stationary traps for surveillance of airborne fungal spores. *Crop Protection*, 36, 23–30. <https://doi.org/10.1016/j.cropro.2012.01.015>.
- Schrader, C., Schielke, A., Ellerbroek, L., & John, R. (2012). PCR inhibitors – occurrence, properties and removal. *Journal of Applied Microbiology*, 113, 1014–1026. <https://doi.org/10.1111/j.1365-2672.2012.05384.x>.
- Sikoparija, B., Skjøth, C. A., Celenk, S., Testoni, C., Abramidze, T., Alm Kübler, K., et al. (2017). Spatial and temporal variations in airborne Ambrosia pollen in Europe. *Aerobiologia*, 33(2), 181–189. <https://doi.org/10.1007/s10453-016-9463-1>.
- Sim, K. M., et al. (2014). Development and evaluation of antimicrobial activated carbon fiber filters using *Sophora flavescens* nanoparticles. *Science of the Total Environment*, 493, 291–297.
- Skjøth, C. A., Baker, P., Sadyś, M., & Adams-Groom, B. (2015). Pollen from alder (*Alnus* sp.), birch (*Betula* sp.) and oak (*Quercus* sp.) in the UK originate from small woodlands. *Urban Climate*, 14(3), 414–428.
- Skjøth, C. A., Damialis, A., Belmonte, J., De Linares, C., Fernández-Rodríguez, S., Grinn-Gofroñ, A., et al. (2016). *Alternaria* spores in the air across Europe: Abundance, seasonality and relationships with climate, meteorology and local environment. *Aerobiologia*, 32(1), 3–22. <https://doi.org/10.1007/s10453-016-9426-6>.
- Skjøth, C. A., Ørby, P. V., Becker, T., Geels, C., Schlünssen, V., Sigsgaard, T., et al. (2013). Identifying urban sources as cause of elevated grass pollen concentrations using GIS and remote sensing. *Biogeosciences*, 10(1), 541–554.
- Smith, M., Jäger, S., Berger, U., Sikoparija, B., Hallsdóttir, M., Sauliene, I., et al. (2014). Geographic and temporal variations in pollen exposure across Europe. *Allergy*, 69(7), 913–923.
- Snyder, A. P., McClennen, W. H., Dworzanski, J. P., & Meuzelaar, H. L. (1990). Characterization of underivatized lipid biomarkers from microorganisms with pyrolysis short-column gas chromatography/ion trap mass spectrometry. *Analytical Chemistry*, 62(23), 2565–2573.
- Tolchinsky, A. D., Sigaev, V. I., Varfolomeev, A. N., Uspenskaya, S. N., Cheng, Y. S., & Su, W. (2011). Performance evaluation of two personal bioaerosol samplers. *Journal of Environmental Science and Health, Part A*, 46(14), 1690–1698.
- Torkko, P., Katila, M., & Kontro, (2003). Gas-chromatographic lipid profiles in identification of currently known slowly growing environmental mycobacteria. *Journal of Medical Microbiology*, 52(4), 315–323. <https://doi.org/10.1099/jmm.0.05113-0>.
- Werchan, B., Werchan, M., Mücke, H.-G., Gauger, U., Simoleit, A., Zuberbier, T., et al. (2017). Spatial distribution of allergenic pollen through a large metropolitan area. *Environmental Monitoring and Assessment*, 189(4), 169. <https://doi.org/10.1007/s10661-017-5876-8>.
- West, J. S., & Kimber, R. B. E. (2015). Innovations in air sampling to detect plant pathogens. *Annals of Applied Biology*, 166(1), 4–17.
- Willers, C., Jansen van Rensburg, P. J., & Claassens, S. (2015). Microbial signature lipid biomarker analysis: An approach that is still preferred, even amid various method modifications. *Journal of Applied Microbiology*, 118(6), 1251–1263.
- Wong, L. T., Mui, K. W., Cheung, C. T., Chan, W. Y., Lee, Y. H., & Cheung, C. L. (2011). In-cabin exposure levels of carbon monoxide, carbon dioxide and airborne particulate matter in air-conditioned buses of Hong Kong. *Indoor and Built Environment*, 20(4), 464–470.
- Xu, B., & Zhu, Y. (2009). Quantitative analysis of the parameters affecting In-cabin to On-roadway (I/O) ultrafine particle concentration ratios. *Aerosol Science and Technology*, 43(5), 400–410.