Science of the Total Environment 719 (2020) 137542

Contents lists available at ScienceDirect

Science of the Total Environment

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

Fingerprinting ambient air to understand bioaerosol profiles in three different environments in the south east of England

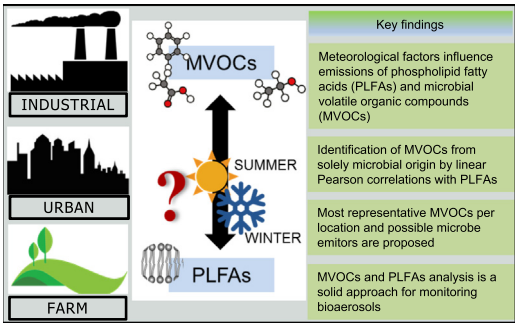
Sonia Garcia-Alcega [a], Zaheer Ahmad Nasir [a], Sabrina Cipullo [a], Robert Ferguson [b], Cheng Yan [a,c], Corinne Whitby [b], Alex J. Dumbrell [b], Gillian Drew [a], Ian Colbeck [b], Sean Tyrrel [a], Frederic Coulon [a,*]

[a] Cranfield University, School of Water, Energy and Environment, Cranfield MK43 0AL, UK
[b] University of Essex, School of Biological Sciences, Wivenhoe Park, Colchester CO4 3SQ, UK
[c] China University of Geosciences, School of Environmental Studies, Wuhan 430074, PR China

HIGHLIGHTS

- Meteorological factors influence both MVOCs and PLFAs emissions reflecting bacterial dynamics.
- Linear Pearson correlations between MVOCs and PLFAs suggest a set of MVOCs from solely microbial sources.
- Most representative MVOCs per location and possible microbe emissors are proposed.
- Combination of MVOCs and PLFAs analysis is a solid approach for bioaerosols monitoring.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 24 January 2020
Received in revised form 23 February 2020
Accepted 23 February 2020
Available online 24 February 2020

Editor: Damia Barcelo

Keywords:
Bioaerosols
Air quality
Outdoor environments
PLFAs
MVOCs
Metabolomics

ABSTRACT

Molecular and chemical fingerprints from 10 contrasting outdoor air environments, including three agricultural farms, three urban parks and four industrial sites were investigated to advance our understanding of bioaerosol distribution and emissions. Both phospholipid fatty acids (PLFA) and microbial volatile organic compounds (MVOC) profiles showed a different distribution in summer compared to winter. Further to this, a strong positive correlation was found between the total concentration of MVOCs and PLFAs ($r = 0.670$, $p = 0.004$ in winter and $r = 0.767$, $p = 0.001$ in summer) demonstrating that either chemical or molecular fingerprints of outdoor environments can provide good insights into the sources and distribution of bioaerosols. Environment specific variables and most representative MVOCs were identified and linked to microbial species emissions via a MVOC database and PLFAs taxonomical classification. While similar MVOCs and PLFAs were identified across all the environments suggesting common microbial communities, specific MVOCs were identified for each contrasting environment. Specifically, 3,4-dimethylpent-1-yn-3-ol, ethoxyethane and propanal were identified as key MVOCs for the industrial areas (and were correlated to fungi, *Staphylococcus aureus* (Gram positive bacteria) and Gram negative bacteria, $R = 0.863$, $R = 0.618$ and $R = 0.676$, respectively) while phthalic acid, propene and isobutane were key for urban environments (correlated to Gram negative bacteria, fungi and bacteria, $R = 0.874$, $R = 0.962$ and $R = 0.969$ respectively); and ethanol, 2-methyl-2-propanol, 2-methyl-1-pentene, butane, isoprene and methyl acetate were key for farms (correlated to fungi, Gram positive bacteria and bacteria, $R = 0.690$ and 0.783, $R = 0.706$ and $R = 0.790$, 0.761 and 0.768). The combination of MVOCs and PLFAs markers can assist in rapid microbial fingerprinting of distinct environmental influences on ambient air quality.

* Corresponding author.
E-mail address: f.coulon@cranfield.ac.uk (F. Coulon).

https://doi.org/10.1016/j.scitotenv.2020.137542

1. Introduction

Bioaerosols in ambient air have a significant societal impact ranging from climate change to public health (Mubareka et al., 2019; Polymenakou, 2012). The impact of bioaerosol emissions from urban, agricultural and industrial sites on ambient air quality is an ongoing public concern. In particular, emissions from industrial sources, such as intensive agriculture, biowaste facilities and wastewater treatment plants (WWTPs) have raised public and environmental health management challenges. However, the evidence base on nature, composition and spatio-temporal variation of bioaerosol emissions from such sources is limited (Bing-Yuan et al., 2018; Douglas et al., 2018; Pearson et al., 2015; Walser et al., 2015). While a range of collection and analytical methods have been used to characterise the emissions from diverse environments both indoors and outdoors, there are still uncertainties in the nature and composition of bioaerosols in ambient air (Garcia-Alcega et al., 2016). Determining the specific chemical fingerprints for bioaerosols in outdoor environments can, not only, advance the knowledge of their composition and dynamics, but also provide fundamental knowledge for rapid chemical characterisation from different environmental sources (Garcia-Alcega et al., 2018). This could result in the development of tools for the rapid monitoring of bioaerosols in urban, agricultural and industrial environments.

The characterisation and analysis of volatile secondary metabolites and phospholipid fatty acids (PLFAs) provides information on their occurrence, fate and behaviour, and also helps to identify and quantify shifts in the microbial community. This could lead to determination of specific chemical fingerprints for bacteria in outdoor environments, and potentially, a phenotypic 'airborne emissions fingerprint'. Microbial volatile organic compounds (MVOCs), which are the metabolic products of bacterial and fungal activities (Schenkel et al., 2015a) vary depending on multiple factors such as microbial species and growth phase (exponential, stationary or senescence), substrate and environmental conditions (e.g. temperature and water activity) (Konuma et al., 2015; Misztal et al., 2018). Volatilome studies are increasingly used to characterise and quantify volatiles in different fields including food industry (food aromas, cheese, beer, yoghurt and food control), medicine (as diagnostic tools for animal and human diseases), agriculture (sustainable and eco-friendly alternatives to pesticides and fertilizers), as well as to assess indoor contamination (Lemfack et al., 2018). However, their application in investigating microbial volatiles for ambient air, especially where the levels of biological material are higher, is limited. Similarly, PLFAs are critical components of the cell membranes of living organisms and are indicators of microbial biomass, composition of microbial communities and microbial stress (Wallander et al., 2013; Willers et al., 2015). Although most of the PLFAs are common to all the living organisms, there are some which are unique to fungi, bacteria, archaea and even specific genera (Cydzik-Kwiatkowska and Zielińska, 2016; Taha et al., 2007).

Our preliminary study focusing on the combined analysis of molecular and chemical biomarkers (PLFAs and MVOCs) from industrial, urban and farm sources (a WWTP a composting facility, and a park) suggested the presence of likely biochemical fingerprints in contrasting outdoor environments (Garcia-Alcega et al., 2018). However, MVOCs are not solely from microbial origin, and can also be produced by plants, animals and anthropogenic sources such as perfumes, chemicals, industrial processes, and so on (Garcia-Alcega et al., 2016), reducing the level of certainty to assign microbiological taxonomic information to MVOCs and PLFAs. In addition, MVOCs can be transformed to other volatile organic compounds (VOCs) by atmospheric reactions resulting from oxidation/reduction processes (Garcia-Alcega et al., 2018), as well as being taken up by other plants, microorganisms or animals and metabolised to other metabolites (Lemfack et al., 2018). Hence, the present study aims to advance our understanding on the inter- and intra- variability of biochemical markers from bioaerosols in different environments (a pool of industrial and urban environments and farms from different locations in the UK) and under different meteorological conditions (summer and winter) to inform source-specific airborne biochemical signatures and their occurrence.

2. Material and methods

2.1. Weather data and sampling locations

Ten different sites have been investigated comprising industrial environments (a composting facility in Colchester, a small WWTP in Cranfield, a large WWTP in Milton Keynes and a waste management facility in London), urban environments (parks in Colchester, Milton Keynes and London) and farms (in Colchester, Milton Keynes and London). The objective was to compare a pool of industrial and urban environments and farms from different locations in the UK (a dense urban area, i.e. London; a rural village, i.e. Cranfield; and two less dense urban areas, Colchester and Milton Keynes) (see Fig. 1). Each site was sampled twice, once in summer 2016 (June/July) and again in winter 2017 (February/March) over a 6-hour period. Meteorological data including temperature, relative humidity and wind speed were recorded during the whole sampling period with a Kestrel® weather station. The sampling was carried out downwind of the source. Weather data and details of each sampling location are described in Table S1.

2.2. Sample collection

2.2.1. MVOCs collection and analysis

Triplicate air samples were collected every 2 h over a 6-hour period using thermal desorption (TD) tubes packed with Tenax and carbotrap 50/50 *v*/v (Markes, Llantrisant, UK) attached to a GilAir® plus air sampling pump (Sensidyne, LP-Clear water, Florida, US). The sampling settings used were 100 mL min^{-1} for 10 min as previously described by Garcia et al. (Garcia-Alcega et al., 2017). The TD tubes were conditioned before sampling at 330 °C for 45 min at 20 psi. Prior to analysis, 0.5 ng of toluene-d_8 (internal standard) was loaded into the TD tubes using a helium flow rate of 400 mL min^{-1}. Air samples were analysed by TD gas chromatography coupled to mass spectrometry-Time of Flight (TD-GC/MS-TOF) using an Almsco TOF coupled to a TD autosampler (Markes International Limited, Llantrisant, UK) and a 6890 N Network GC System (Agilent Technologies, Palo Alto, USA). External multilevel calibrations ranging between 0.001 and 1 ng μL^{-1} were carried out using a mix of 8 individual MVOCs including dimethyl sulphide, 2-methyl-furan, 2-pentanol, 2-methyl-1-butanol, dimethyl disulphide, 2-heptanone, 2-pentyl furan and 2-methyl-1-propanol (Sigma-Aldrich, Dorset, UK). 1 μl of each standard concentration was loaded into conditioned TD tubes and additionally 50 ng of toluene-d_8 were added. The GC–MS was coupled with a 78 m × 250 μm × 0.5 μm Agilent column. The GC oven temperature was set as follows: initial temperature 35 °C, ramp 2 °C min^{-1} to 75 °C, ramp 2 °C min^{-1} to 140 °C and 10 °C min^{-1} to 10 °C min^{-1}. Ions were monitored in full scan mode. An empty TD tube and a blank containing 50 ng of toluene-d_8 as internal standard were run as QC every 15 samples. Chemometrics from the chromatograms obtained from GC–MS analysis were processed in batch using OpenChrom. Total VOCs were identified by NIST mass spectral library. Semi-quantitation of the compounds was carried out with the internal standard toluene-d_8 and when possible, a full quantitation with pure standards was undertaken.

2.2.2. PLFAs collection and analysis

Two-hour air samples were collected in triplicate over 6 h (9 samples in total) with 3 vacuum pumps attached to filter holders containing 47 mm polycarbonate filters with 0.8 μm pore size (225-1601, SKC Ltd., Dorset, UK) at 28 L min^{-1} flow rate. Polycarbonate filters were removed from the sampling heads after the sampling with sterile tweezers, deposited into sterile Eppendorf tubes (Fisher Scientific, Loughborough, UK) and stored at −20 °C (Macnaughton et al., 1999). PLFAs were

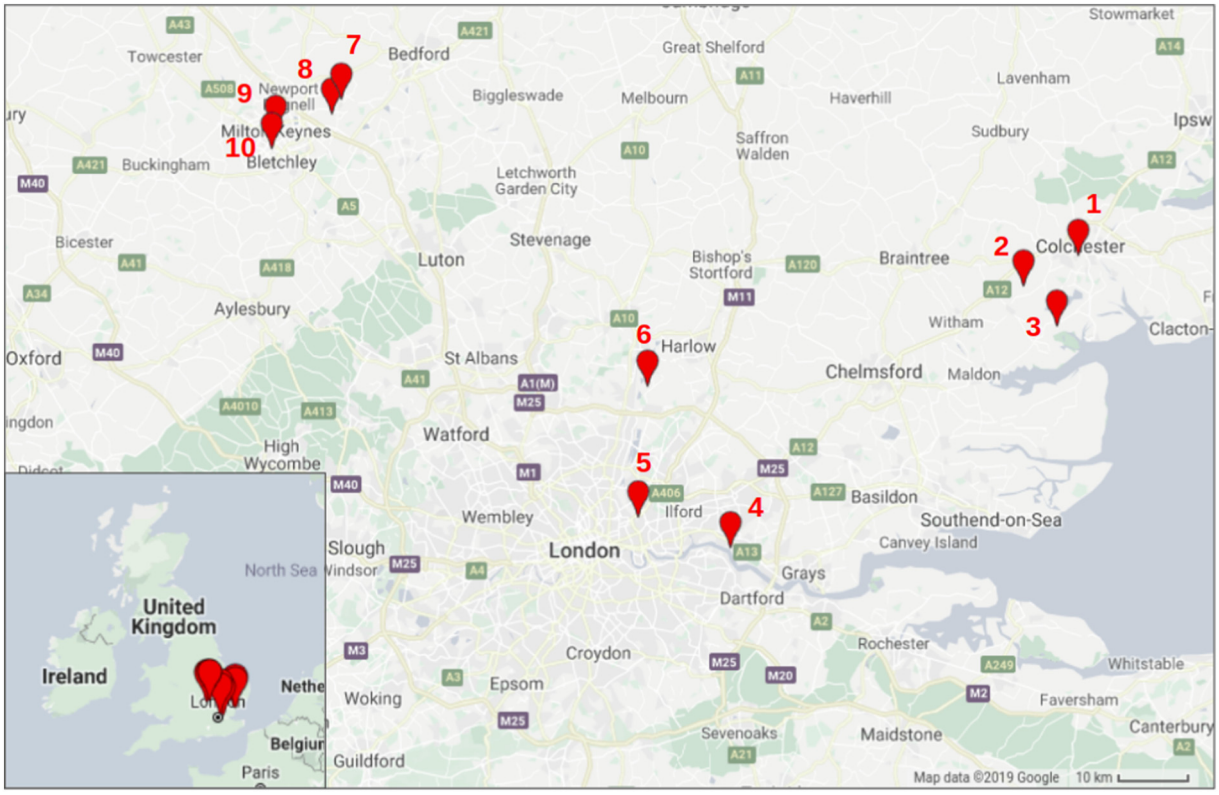


Fig. 1. Map of the UK showing the locations in the south-east part where the sampling was performed (1, 2 and 3 are a compost facility, urban park and a farm in Colchester; 4, 5 and 6 correspond to waste management facility, farm and a park in London; 7 and 8 are a farm and a wastewater treatment plant (WWTP) in Cranfield; 9 and 10 are a WWTP and a park in Milton Keynes).

extracted as described previously (Garcia-Alcega et al., 2018) following a modified version of the method developed by Frostegård et al. (Frostegard et al., 1991; Frostegård et al., 1993). Sterile polycarbonate filters were extracted for PLFAs in triplicate as a procedural blank. PLFAs were extracted from the filters by adding 15 mL Bligh Dyer solution which comprised of citrate buffer (31.5 g L^{-1} citric acid dehydrated with 44.1 g L^{-1} trisodium citrate), chloroform, and methanol at a ratio of 0.8:1:2 (*v*/v/v) and butylated hydroxyl toluene (50 mg L^{-1} Bligh Dyer solution). Filters were sonicated during 2 × 15 min and centrifuged at 2000 rpm for 10 min (Falcon 6/300; Sanyo, UK). The upper layer was placed into a clean glass vial and 4 mL chloroform and 4 mL 0.15 M citrate buffer were added followed by 10 min centrifugation at 2000 rpm. The upper phase was discarded, and the lower layer was dried under constant N_2 stream at <37 °C. The extracts were resuspended in 200 μL hexane and analysed by GC-FID (Agilent Technologies 6890 N) fitted with a HP-5 (Agilent Technologies) fused silica capillary column (30 m length, 0.32 mm ID, 0.25 μm film). Helium was used as a carrier gas at 1 mL min^{-1} flow rate. The oven program for separating fatty acid methyl esters (FAMEs) was as follows: 50 °C for 1 min; 160 °C at 25 °C min^{-1}; 240 °C at 2 °C min^{-1}; 310 °C at 25 °C min^{-1}; 1 μL sample was injected in splitless mode and the injector temperature was set at 310 °C. PLFAs data from the chromatograms obtained from the GC-FID were processed in ChemStation.

2.3. Data analysis

Individual PLFAs were assigned to microbial groups based on the studies of Pankhurst et al. and Willers et al. (Pankhurst et al., 2012; Willers et al., 2015) (see Table S2). MVOCs were differentiated from VOCs using the KEGG Pathway (Kanehisa Laboratories, 1995) and the MVOC (Lemfack et al., 2014) metabolic databases as well as recent literature (Hertel et al., 2016; Micheluz et al., 2016; Schenkel et al., 2015a). MVOCs were grouped according to Schenkel et al. (2015a) by alcohols, aldehydes, alkanes, esters, ethers, organic acids and ketones (MVOCs). Descriptive statistics, principal component analysis (PCA) and pie chart distributions based on the concentration of PLFAs and MVOCs were performed with Excel 2010. Permutational Analysis of Variance (PERMANOVA) was used to test the significance ($p \leq 0.05$) of meteorological conditions (categorical variable) and environment (categorical variable), on (1) microbial community structure using PLFA markers, and (2) MVOCs, on samples collected at industrial and urban sites and farms. PERMANOVA was performed using the "adonis" function of the vegan library in R Studio (Oksanen et al., 2011) with Euclidean distance. The similarities analyses (ANOSIM) were used to examine the PLFAs and MVOCs patterns between groups. For similarity analyses the abundance data were $\log_{10}$ transformed and Bray-Curtis coefficient used as the measure of similarity. The level of significance was set by 999 permutations between groups (999 permutations are setting a rejection zone of the null hypothesis at a significance level of at least 1 in 1000). Redundancy analysis (RDA) was applied to extract and summarise the variation of the PLFA and/or MVOC fingerprints due to environmental and meteorological conditions. Linear Pearson regression analysis was performed to determine correlations between PLFAs and MVOCs for both individual compounds, and compounds groups. All tests were computed with R Studio (Version R-3.5.1 for Windows 32/64 bit) (R Core Team *(2018) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna*, 2018).

3. Results and discussion

PCA analyses performed including the MVOCs and PLFAs found in the locations of the study indicated that meteorological factors influence on microbial abundance. PCA groups were similar in samples collected in summer when including (Fig. S1.a) or excluding (Fig. S1.b) weather conditions (temperature, relative humidity and wind speed). In winter, for instance, could be observed that the PCA groups were

different, probably due to the stronger winds (see Table S1) which were interfering in the sample collection. Therefore, weather conditions were included for the data analysis.

3.1. MVOCs profile and distribution across the 10 contrasting outdoor environments

Sites from the same environment (industrial, farms or urban) but from different locations within the UK (Cranfield, Milton Keynes, London or Colchester) differed in the presence/absence of several of PLFAs and MVOCs. These differences among samples from the same environment but different geographical locations are represented via Venn diagrams in Fig. 2. The four industrial locations (a composting facility, small WWTP, large WWTP and MBT facility) had 15 compounds in common in summer and 11 in winter. The three farms (in London, Colchester and Cranfield) had 17 and 15 common compounds in summer and winter respectively. The three urban locations (parks in London, Colchester and Milton Keynes) had 17 and 25 common compounds in summer and winter, respectively. To study the MVOCs and PLFAs emissions from the three different environments (industrial, farms and urban) the concentrations of the compounds from the sampling sites corresponding to the same environment were averaged.

MVOCs concentrations (ng m^{-3}), grouped by chemical groups, from the 10 sites are detailed in Fig. 3. Industrial sites had greater concentration of ketones (4900–15,100 ng m^{-3}), as reported by several authors as a consequence of incomplete aerobic degradation during the fermentation of organic matter (Chiriac et al., 2011; Gallego et al., 2012; Lasaridi et al., 2010). Farms samples were dominated by aldehydes and alkanes (up to 36,000 and 32,100 ng m^{-3}, respectively). These results are in good agreement with the findings of Zhao et al. (2016) who reported ethene, propene, ethanol, propanol, 2-butanol, acetaldehyde, acetone, 2-butanone, 2-pentanone and acetophenone as the 10 most abundant MVOCs in agricultural soils (Zhao et al., 2016). In urban sites, esters (up to 3350 ng m^{-3}), organic acids (38,600 ng m^{-3}) and alcohols (1780–7200 ng m^{-3}) were more abundant. Concentrations of grouped MVOCs at each of the individual locations separated into summer and winter are detailed in Table S3. The composition of MVOCs based on the chemical classification of the individual compounds was relatively similar across the different environments. The concentration of the compounds and/or the total concentration of some chemical groups, for instance was different. The MVOC % was higher in summer in all the locations compared to winter, although this difference was less obvious in the composting facility. As indicated in our previous study (Garcia-Alcega et al., 2018), the major contributors to the total VOCs in summer are therefore from microbial sources, whereas in winter these are associated with anthropogenic sources related to combustion processes.

PERMANOVA results indicated that the concentration of MVOCs was influenced by the nature of the environment (industrial, farms or urban), temperature and wind speed ($p = 0.028$, $p = 0.006$ and $p = 0.005$, respectively). The meteorological conditions when the sampling was undertaken was found to significantly affect the MVOCs in urban environments ($p = 0.042$) while no significant effect was observed for the farms. ANOSIM analysis further revealed significant differences between the concentration of MVOCs at each environment ($p = 0.001$) (Fig. S3).

Redundancy analysis of individual MVOCs showed two distinct clustering of compounds in summer and winter (Fig. 4a). Due to the large number of different MVOCs, these are often classified by their functional groups in order to study the volatile patterns (Schenkel et al., 2015b; Sun et al., 2014; Wilkins, 2002). Analysis of the MVOC classification by functional groups demonstrated MVOCs to be more diverse in summer (being comprised of alcohols, aldehydes, alkanes, alkenes, esters, ethers, ketones and organic acids) than in winter (aldehydes, alkanes, alkenes, esters, ketones and organic acids). However, RDA plots of the MVOCs classified by environments (Fig. 4b) were not as clear as when classified

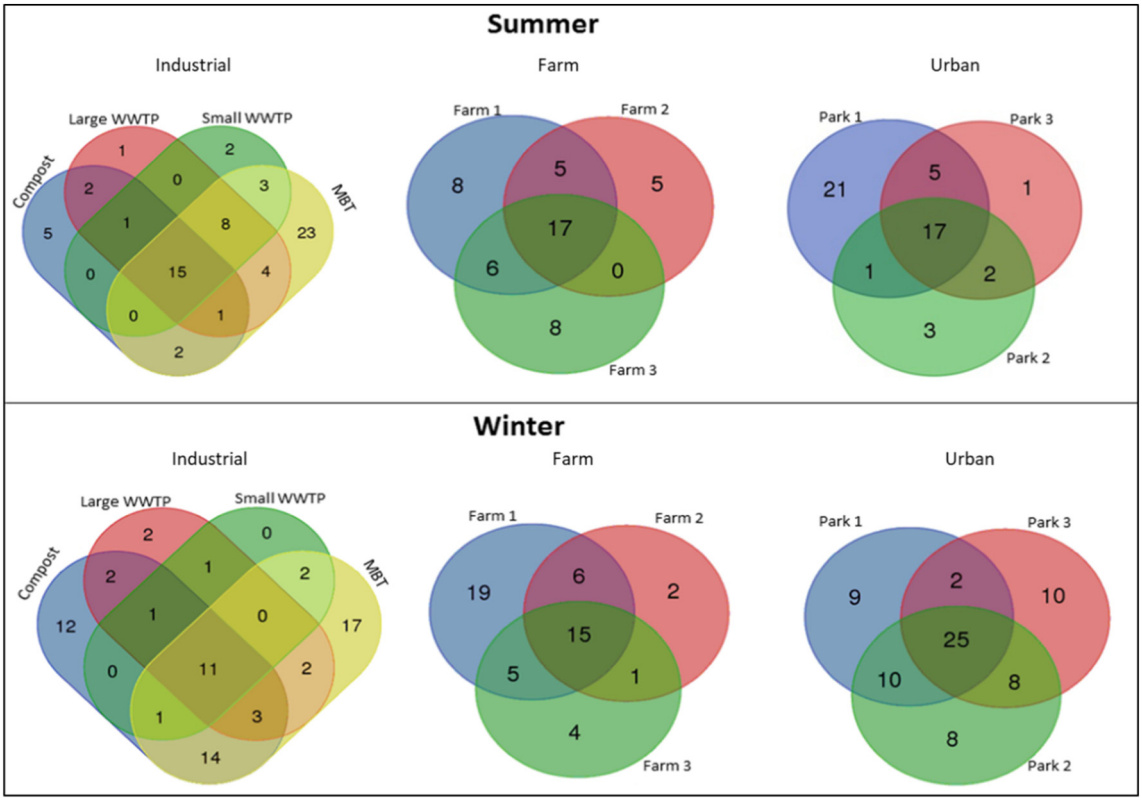


Fig. 2. Venn diagrams of overlapping MVOCs and PLFAs in common between locations within the same environment, industrial, farms or urban. Farms 1, 2 and 3 correspond to the farms in London, Colchester and Cranfield and Parks 1, 2 and 3 to the parks in London, Colchester and Milton Keynes, respectively.

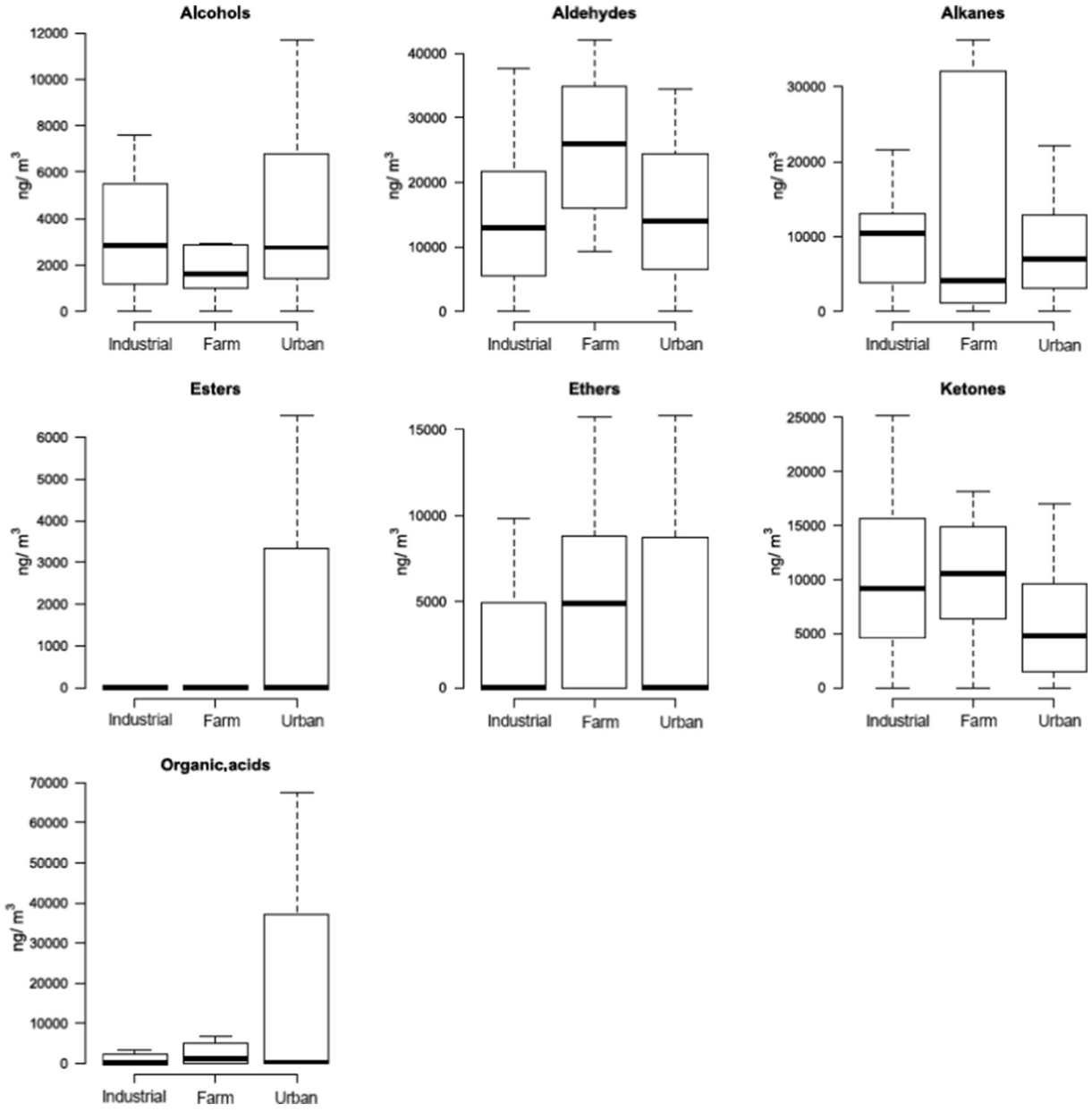


Fig. 3. Boxplot for grouped MVOCs concentrations expressed in ng m^{-3} in the three environments studied: industrial, farms and urban.

by meteorological conditions encountered during the sampling, as several MVOCs were present in all three environments, suggesting that the concentration of MVOCs emitted from different environments are very similar. Farms had a distinct and smaller MVOCs emission profile compared to urban and industrial environments. Industrial environments exhibited a more complex profile but there was an overlap with urban environments and farms. 1-butene, butane 2-methyl and acetone were the most representative MVOCs for industrial areas. The most representative MVOCs for urban sites were ethanol, 1,2 benzenedicarboxylic acid, propane 2-methoxy 2-methyl, formaldehyde, nonanal, 2-propane 1-hydroxy and 1-pentane.

3.2. PLFA profile and distribution across the 10 contrasting outdoor environments

The PLFAs concentrations (ng m^{-3}) grouped by taxonomic groups for all samples across the industrial, farms and urban sites are summarised in Fig. 5. At industrial sites, PLFAs associated with *Bacillus sp/Arthrobacter* and with Cyanobacteria/diatoms/green algae were the most predominant. Zhao et al. (2016) also identified *Bacillus* species in WWTPs (*B.niacini* and *B. persicus*). In contrast, the dominant PLFA groups were associated with general bacteria, followed by saprotrophic fungi and unknown microbes in the urban sites. Gram positive bacteria and fungi were the two most abundant. The distribution of the microbial communities characterised from this study were in agreement with our previous findings (Garcia-Alcega et al., 2018). Details on the individual PLFA concentrations at each site are provided in Table S4. During the summer, all locations, except the farm in Colchester, had higher amounts of bacteria compared to fungi, as previously reported by Garcia-Alcega et al. (2018). The higher fungi content in winter was predominantly Saprotrophic fungi, which increase their activity at low temperatures and with humidity (Vorıskova et al., 2014).

ANOSIM analysis revealed that there were significant differences ($p = 0.001$) between the concentration of PLFAs between different environments (industrial, farms and urban) and different meteorological conditions during summer and winter (Fig. S3).

RDAs analysis for PLFAs further showed two distinct differentiated groups for winter and summer (Fig. 6a). Similarly to the MVOCs,

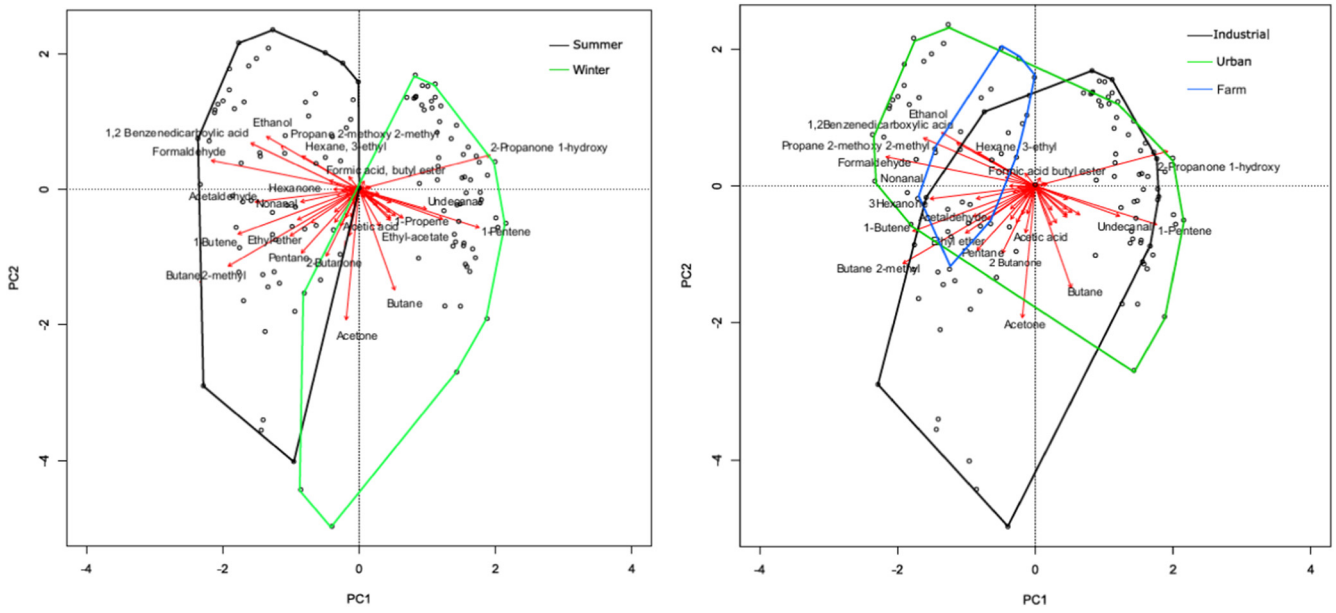


Fig. 4. Redundancy Analysis (RDA) plot explained by meteorological conditions encountered during the sampling in winter and summer (a) and environmental conditions (b) for individual MVOCs.

PLFAs did not show a clear differentiation between outdoor environments as most of the compounds were common in all three. Industrial sites and farms were again very similar, and farms had a narrower PLFA profile compared to the other two environments.

3.3. Correlation between MVOCs and PLFAs profiles

A good linear correlation was observed between $\log_{10}$ of the total concentration of MVOCs and total concentration of PLFAs both in summer ($r^2 = 0.767$, $p = 0.001$) and winter ($r^2 = 0.670$, $p = 0.004$) (Fig. S2.), suggesting a major but not solely common source between the chemical and molecular biomarkers. Some MVOCs have anthropogenic sources or are emitted by trees, plants and fruits (Garcia-Alcega et al., 2016). In order to specifically focus on VOCs from microbial origin and exclude the rest of the sources, only those strongly correlated with PLFAs were studied.

Linear Pearson correlation results of MVOCs and PLFAs grouped by chemical and taxonomic groups from all the air samples divided into industrial and urban environments and farms (Fig. S3) indicated that Gram negative bacteria were negatively correlated with aldehydes ($r = -0.630$) and ethers ($r = -0.650$) in industrial sites. This was probably because they take up these VOCs (Zhao et al., 2016). In urban sites, for instance, Gram negative bacteria were positively correlated with organic acids ($r = 0.861$), probably because of the acetic acid bacteria that are widely distributed in plants and air, and which produce acetic acid oxidizing sugars and ethanol (Yamada and Yukphan, 2008). *Bacillus*/*Arthrobacter* were positively correlated with alcohols in farms ($r = 0.790$), and probably, in this case, the bacteria present is *Bacillus* as Zhao et al. (2016) reported that alcohols were among the most common MVOCs emitted by *Bacillus* species. A positive correlation means that the MVOCs are produced by microbes or that those MVOCs enhance the microbial growth (Mayrhofer et al., 2006; Zhao et al., 2016). A negative correlation, for instance, means that the VOCs are taken up by that microorganism, or that another microorganism(s) is inhibiting the growth of the microbe that produces that VOC or group of VOCs (Zhao et al., 2016).

Linear Pearson correlations from individual MVOCs and PLFAs (Fig. 7) showed a positive correlation between the PLFA unknown 4 and the alcohol 1-pentyn-3-ol, 3,4-dimethyl ($r = 0.863$), 2OH-16:0, and negatively with ethoxyethane ($r = -0.676$) and formaldehyde ($r = -0.684$). The PLFA unknown 4 was positively correlated with propanal ($r = 0.618$) and i14:0 was correlated with pentanoic acid ($r = 0.578$) in the industrial samples. The high concentration of ketones represented in Fig. 1 and the lack of correlation with any microbial group suggest that they are a product of secondary alcohols by oxidation processes or by reduction of ethers (Garcia-Alcega et al., 2018), or anthropogenic sources (Han et al., 2019; Huang et al., 2019).

Urban samples showed a positive correlation between C18:3n3 and propene ($r = 0.962$), isobutane and C15:0 ($r = 0.969$), 16:0cis ($r = 0.704$), and C20:5n3 ($r = 0.940$). In addition, a negative correlation between 2OH-16:0 and formaldehyde ($r = 0.909$) and 1,2 benzene dicarboxylic acid ($r = -0.874$) was also observed. Urban sites were characterised by a high concentration of organic acids (up to 3000 ng m^{-3}) (Fig. 1), which had a strong correlation with Gram negative bacteria ($r = 0.861$) (Fig. S3). Ester compounds were not correlated with any microorganism, suggesting that they were produced by other sources or by oxidation processes of alkanes and alcohols (Garcia-Alcega et al., 2018). Alcohols were weakly correlated with an unknown PLFA ($r = 0.419$) suggesting that these were not only emitted by microbes.

In samples collected at the farms, C15:0 had a significant positive correlation with the alcohols, ethanol ($r = 0.790$) and 2-methyl-2-propanol ($r = 0.786$), 2-methyl-1-pentene ($r = 0.768$), and isoprene ($r = 0.794$). The PLFA 18:1w9 was correlated with the alcohols, ethanol ($r = 0.691$) and 2-methyl-2-propanol ($r = 0.706$); 2OH-16:0 and butane ($r = 0.787$) and C18:3n6 and butane ($r = 0.783$); C14:0 and methyl acetate ($r = 0.761$). A strong correlation between *Bacillus* or *Arthrobacter* and alcohols ($r = 0.790$) and alkanes ($r = 0.643$) was also observed. The production of alcohols could be associated with *Bacillus* species (Zhao et al., 2016) and the production of alkanes to some species of *Arthrobacter* (Frias et al., 2009). There was no correlation between aldehydes and any microorganism suggesting that they might come from another source. In a laboratory study Mancuso et al. indicated that acetaldehyde was emitted from plant roots (Mancuso et al., 2015).

3.4. Environment specific MVOCs

Based on the RDA and individual correlations between PLFAs, the most representative MVOCs in each of the environments have been

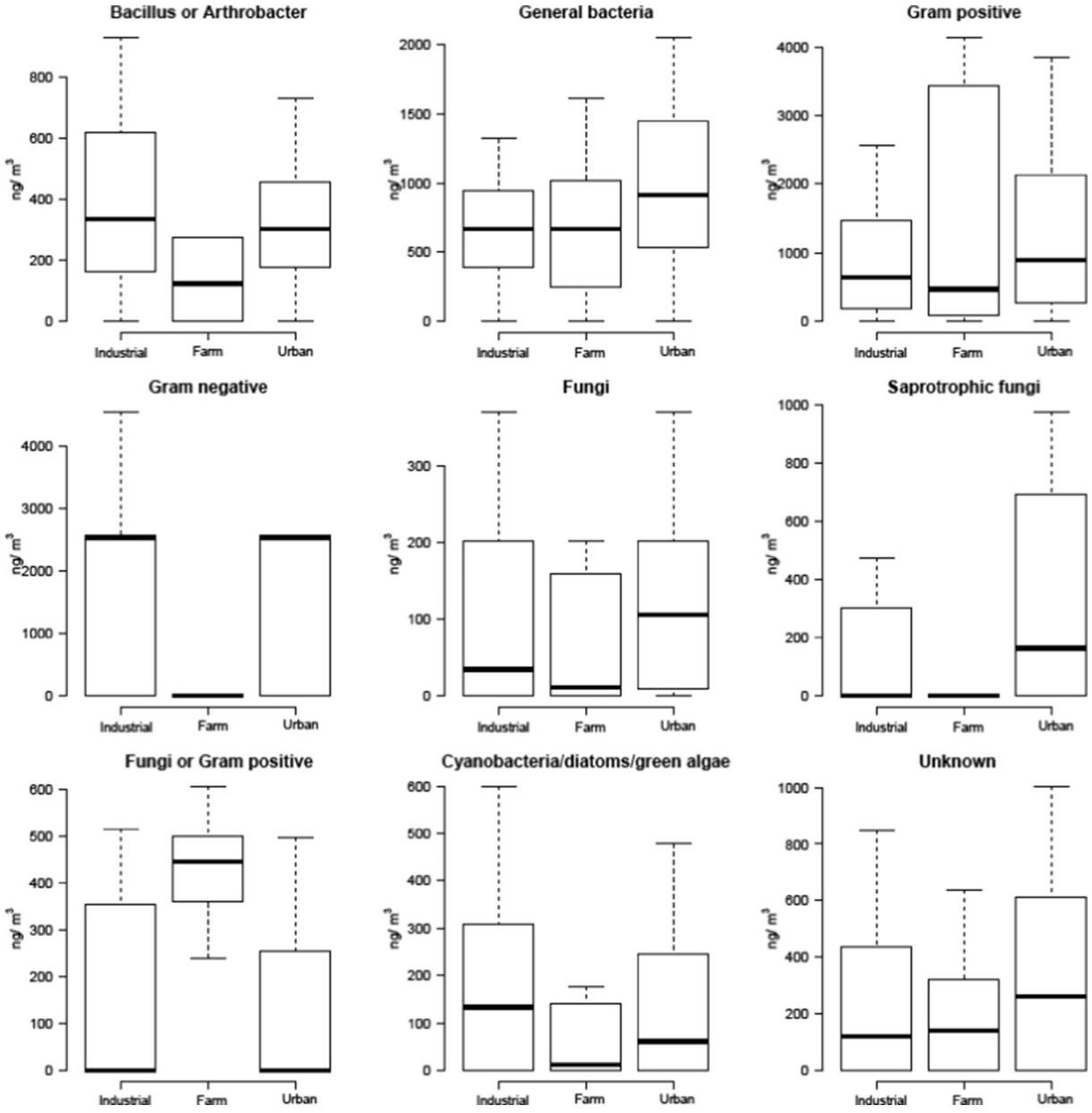


Fig. 5. Boxplot for grouped PLFAs concentrations expressed in ng m^{-3} in the three environments, namely industrial, farms and urban environments.

detailed in Table 1. All microbial species known to date that produce the MVOCs of interest have been listed by examining the MVOC database from Lemfack et al. (2018, 2014). The microbial origin (bacterial and/or fungal) from the PLFAs correlated with the specific MVOCs was identified according to the PLFA classification (Table S2) (Pankhurst et al., 2012; Willers et al., 2015).

Most of the MVOCs identified in this study from each outdoor environment matched with the ones reported in our previous research (Garcia-Alcega et al., 2018). For example, formaldehyde, propanal and pentanoic acid, were again identified as potential markers at industrial sites There was a PLFA not identified at this site (unknown 4), but which showed correlations with 3,4-dimethylpent-1-yn-3-ol and with propanal. In addition to our previous research, ethoxyethane was found which was correlated with 2OH-16:0 ($r = 0.676$) and is linked to *Klebsiella pneumoniae*, a Gram negative bacteria.

Phtalic acid, propene and isobutane were identified in urban parks. Phtalic acid was considered as an anthropogenic VOC in our previous research (Garcia-Alcega et al., 2018) but based on the recent MVOC database version 2.0 (Lemfack et al., 2018) it can also be a MVOC emitted by the Gram negative bacteria *Pseudomonas simiae* and *Pseudomonas putida* and *Serratia sp*.

This MVOC is negatively correlated with the PLFA 2-OH-16:0 ($r = -0.874$), which is present in Gram negative bacteria. Propene is emitted by the fungi *Penicillium digitatum* and was correlated with C18:3n3 ($r = 0.962$), which is associated with fungi. Isobutane is emitted by *Helicobacter pylori* and was strongly correlated to the PLFA C15:0 ($r = 0.969$), which is present in general bacteria. This MVOC was also correlated with other PLFAS, so there may be other microbes that produce this MVOC.

Thermoactinomycetes vulgaris, Streptomyces sp, Thermonospora sp, which are Gram positive bacteria and were correlated with the PLFAs 18:1w9 and C15:0, were only found in farms. These are associated with farmer's lung syndrome (Barrera et al., 2019; Brinkmann and Kurtböke, 2018). 2-methyl-1-pentene, which is produced by *Mycobacterium avium* and Helicobacter pylori, was detected in our two studies (Garcia-Alcega et al., 2017, 2018). This MVOC was correlated with the PLFAs C15:0 and 2OH-16:0, corresponding to general bacteria ($r = 0.768$) and Gram negative bacteria ($r = 0.768$).

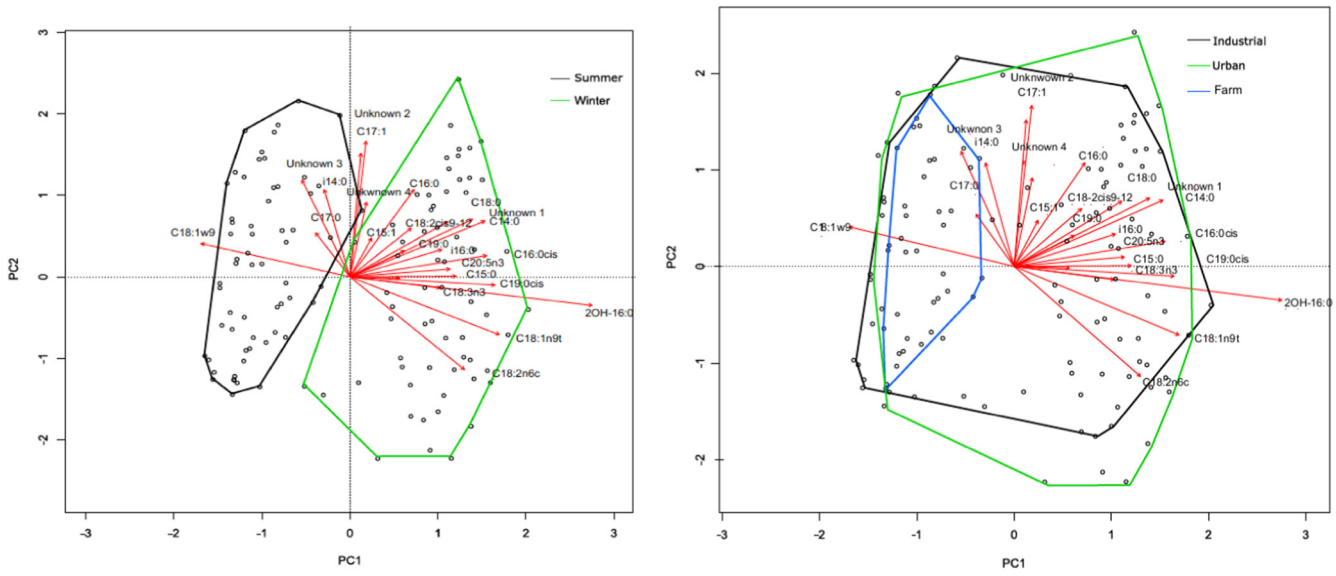


Fig. 6. Redundancy Analysis (RDA) plot explained by meteorological conditions encountered over summer and winter months (left) and environment (right) for individual PLFAs.

One limitation of this study is the occasional mismatch of the specific fungi or bacteria assigned by the MVOC database and the microbial PLFA classification. For example, methyl acetate, which according to the MVOC database is emitted by fungi, showed a strong correlation with C14:0 (which is associated with general bacteria). This could be explained by the fact that the volatilomes are constantly under research and the databases are continuously being updated. PLFAs analysis has been described as a rapid and sensitive method to study the changes in microbial communities (Frostegård et al., 2011), and it has also been used to assess the microbial community changes in bioaerosols from green waste composting (Pankhurst et al., 2012). However, as Frostegård et al. (2011) noted PLFA data should not be used as indicators of certain taxa on their own, as good PLFA markers are very limited. Thus, this technique is a good screening tool to understand the changes in a community, but for more detailed characterisation these data should be linked with additional molecular data.

Additionally, there were some MVOCs that differed between our two studies. This was expected as the air samples were taken at different times and air sample collection is affected by weather conditions. Moreover, MVOCs molecules can change by reaction/oxidation processes in the atmosphere in addition to up take and metabolisation by other organisms. Next generation sequencing from the air samples would help to identify species specific MVOCs and PLFAs more accurately. In parallel, the advances in volatilome studies will contribute to the development of the current volatilome databases.

4. Conclusion

Results from PLFAs and MVOCs markers show that meteorological factors influence microbial abundance. Despite many of the identified PLFAs and MVOCs being common across the 10 sites, some specific markers for farms, urban and industrial environments were identified. By linking the taxonomical information obtained from chemical and molecular markers, it is possible to have a more accurate identification of the microbes present in the air than solely analysing the PLFAs. In addition, the air fingerprint from combining MVOCs and PLFAs markers can help to differentiate microbial characterisation from urban and industrial environments, and farms. Even though environmental conditions may influence the rate of PLFA degradation and UV light may have an effect on MVOCs, a strong correlation was obtained between total concentration of MVOCs and PLFAs ($r = 0.670$, $p = 0.004$ in winter and $r = 0.767$, $p = 0.001$ in summer), suggesting that MVOCs analysis is a promising approach for bioaerosol monitoring. Some MVOCs had weak correlations or no correlations at all with any of the PLFAs, suggesting other sources such as anthropogenic, plants or being a product of oxidation/reduction processes. Apart from this, the chemical processes occurring in the air are complex and make the environmental characterisation of MVOCs challenging. MVOCs databases are constantly being updated and characterisation of the origin of the compounds is improving. Some of the most representative MVOCs per environment identified by RDAs and the microbial species producers assigned from MVOCs databases sometimes did not match with the microbial PLFA classification. Further volatilome studies in different ambient environments will advance the atmospherically relevant volatilome databases, and next generation sequencing can offer supplementary information to accurately identify the microbial species present in the different environments. In particular, the function of seasonality in microbial volatile organic compounds distribution in ambient air needs to be investigated in order to elucidate the underlying mechanisms affecting environmental volatilomics.

CRediT authorship contribution statement

Sonia Garcia-Alcega: Data curation, Investigation, Writing - original draft, Visualization, Formal analysis.**Zaheer Ahmad Nasir:**Data curation, Investigation.**Sabrina Cipullo:**Investigation, Methodology, Formal analysis.**Robert Ferguson:**Data curation, Investigation.**Cheng Yan:**Investigation, Methodology, Formal analysis.**Corinne Whitby:**Writing - review & editing.**Gillian Drew:**Writing - review & editing.**Ian Colbeck:** Writing - review & editing.**Sean Tyrrel:**Writing - review & editing. **Frederic Coulon:**Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by NERC Environmental Microbiology & Human Health Programme under the grant agreement NE/M010961/1 and NE/M010813/1, the National Natural Science Foundation of China (51608497), the Fundamental Research Funds for the Central Universities, China University of Geosciences (Wuhan) (CUGL170409), Hubei Chenguang Talented Youth Development Foundation (2018 B), and

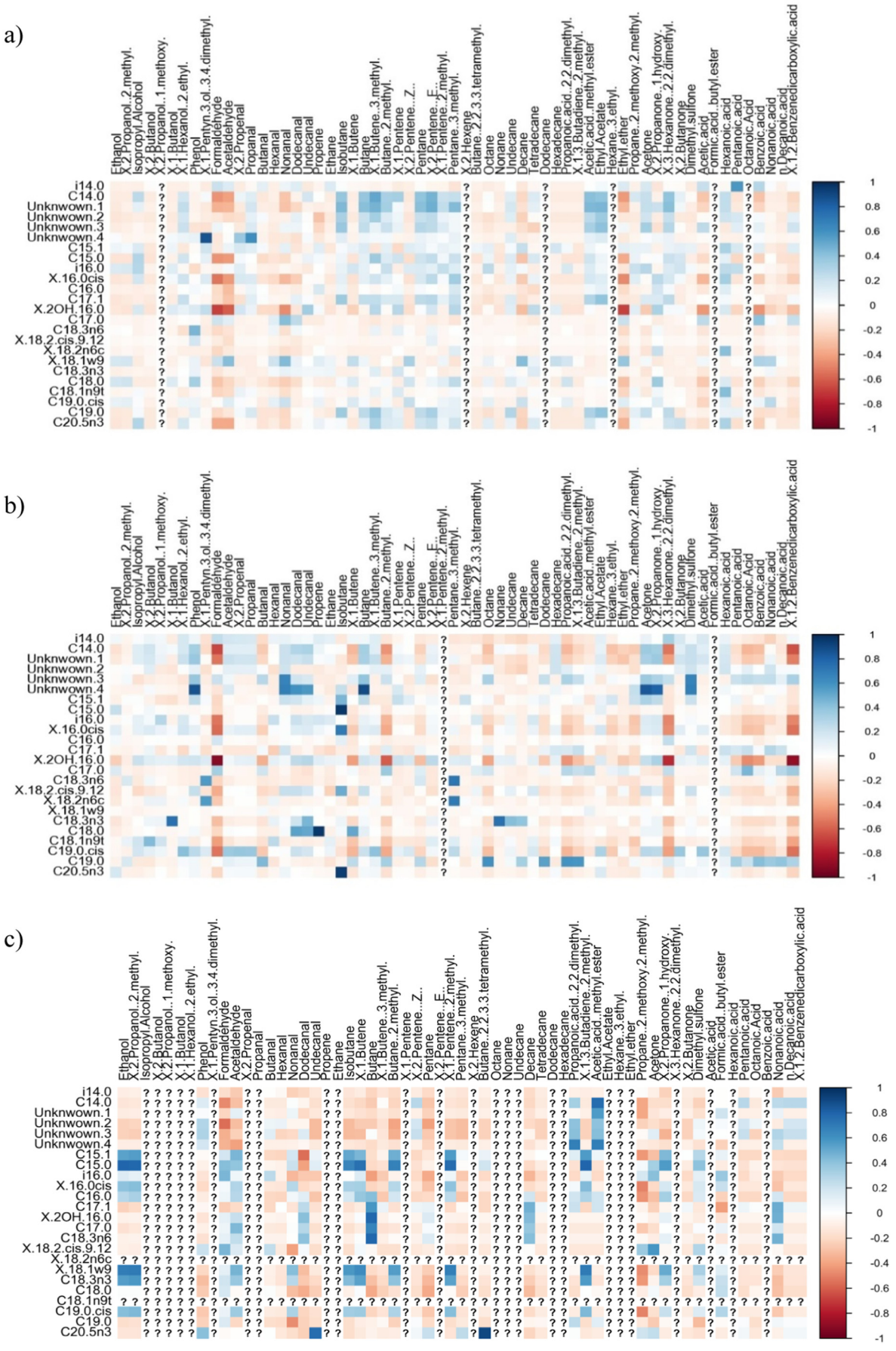


Fig. 7. Linear Pearson correlation between individual PLFAs and MVOCs at a) industrial, b) urban and c) farms. (? = all responses were equal, no correlation identified).

Table 1
Most representative MVOCs and PLFAs found for each outdoor environment, the microbes precursors and the correlation between each MVOC with a specific PLFA. G− and G+ are Gram negative and Gram positive bacteria.

Environment	MVOCs			PLFAs		MVOC-PLFA (r)
	Chemical group	Compound	Microbial origin (Lemfack et al., 2018; Sohrabi et al., 2014)	Compound	Microbial origin (Willers et al., 2015)	
Industrial environments	Alcohol	3,4-Dimethylpent-1-yn-3-ol	Fungi: *Aspergillus flavus, Aspergillus niger, Aspergillus parasiticus, Penicillium glabrum, Rhizopus stolonifer*	Unknown 4	Unknown	(+) 0.863
	Ether	Ethoxyethane	Bacteria: *Klebsiella pneumoniae (G−)*	2 OH-16:0	Gram negative bacteria	(−) 0.676
	Aldehyde	Formaldehyde	Fungi: *Ceratocystis Sp* and *Thielaviopsis basicola*	2 OH-16:0	Gram negative bacteria	(−) 0.684
		Propanal	Bacteria: *Staphylococcus aureus* (G+) Fungi: *Trichodema viride, Tuber aestivum, Tuber melanosporum*	Unknown 4	Unknown	(+) 0.618
	Organic acid	Pentanoic acid	Fungi: *Candida albicans* Bacteria: *Lactobacillus casei (G+), Lactobacillus plantarum (G+), Lactococcus lactis (G+), Leuconostoc cremoris (G+), Leuconostoc dextranicum (G+), Leuconostoc mesenteroides (G+), Leuconostoc oenos((G+)), Leuconostoc paramesenteroides (G+) Pediococcus damnosus (G+), Clostridium sp (G+), Fusobacterium necrophorum (G−), Fusobacterium nucleatum (G−), Fusobacterium simiae (G−), Peptococcus niger, Peptostreptococcus anaerobicus (G+), Peptostreptococcus asaccharolyticus (G+), Peptostreptococcus prevotii,(G+), Porphyromonas gingivalis (G−), Prevotella loescheii (G−)*	i14:0	Gram positive bacteria	(+) 0.578
Urban environments	Organic acid	Phtalic acid	Bacteria: *Pseudomonas putida (G−), Pseudomonas simiae (G−), Serratia spp (G−).* Fungi: *Lentinula edodes, Saccharomyces cerevisiae*	2 OH-16:0	Gram negative bacteria	(−) 0.874
	Alkane	Propene	Fungi: *Penicillium digitatum*	C18:3n3	fungi	(+) 0.962
		Isobutane	Bacteria: *Helicobacter pylori (G−)*	C15:0	general bacteria	(+) 0.969
				16:0 cis	Gram positive bacteria	(+) 0.704
				C20:5n3	cyanobacteria, diatoms, green algae	(+) 0.940
	Aldehyde	Formaldehyde	Fungi: *Ceratocystis sp* and *Thielaviopsis basicola*	2 OH-16:0	Gram negative bacteria	(−) 0.909
Farms	Alcohol	Ethanol	Fungi: *Tuber sp, Aspergillus fumigatus, Penicillium sp, Phoma sp, Boletus variegatus, Fomes sp, Fusarium sp, Aerobasidium pullulans, Ascocoryne sarcoides, Candida shehatae, Candida tropicallis, Ceratocystis sp, Muscodor albus, Muscodor crispans, Neurospora sp, Ogataea pini, Saccharomyces cerevisiae, Scolulariopsis basicola, Trichoderma sp* Bacteria: *Arthrobacter agilis (G+), Bacillus amyloliquefaciens (G+), Bacillus subtilis (G+), Clostridium sp (G+), Enterobacter cloacae (G−), Escherichia coli (G−), Klebsiella pneumoniae (G−), Listeria monocytogenes (G+), Lactobacillus fermentum (G+), Paenibacillus polymyxa (G+), Pseudomonas simiae (G−), Salmonella enterica (G−), Salmonella enteritidis (G−), Serratia sp (G−), Shigella flexneri (G−), Staphylococcus aureus (G+), Thermoactinomycetes vulgaris (G−)*	18:1w9	fungi/Gram positive bacteria	(+) 0.691
				C15:0	general bacteria	(+) 0.790
		2-Methyl-2-propanol	Fungi: *Tuber sp, Ascocoryne sarcoides, Muscodor albus, Muscodor crispans, Phoma sp, Phomosis sp, Penicillium sp,Saccharomyces cerevisiae, Xylaria sp,Pleurotus eryngii, Rhizoctonia solani, Trichodema pseudokoningii, Trichodema viride, Aspergillus candidus, Aspergillus versicolor, Ampelomyces sp, Geotrichum candidum, Candida shehatae,Mortierella isabellina, Paecilomyces variotii* Bacteria: *Streptomyces sp (G+), Enterobacter cloacae (G−), Bacillus amyloliquefaciens (G+), Bacillus subtilis (G+), Paenibacillus polymyxa (G+), Mycobacterium bovis (G+), Serratia sp (G−), Thermoactinomyces vulgaris (G+), Thermomonospora fusca (G+)*	C15:0	general bacteria	(+) 0.786
				18:1w9	fungi/Gram positive bacteria	(+) 0.706
	Alkane	2-Methyl-1-pentene	Bacteria: *Mycobacterium avium (G+), Helicobacter pylori (G−)*	C15:0	general bacteria	(+) 0.768
		Butane	Bacteria: *Ralstonia solanacearum (G−)*	2 OH-16:0	Gram negative bacteria	(+) 0.787
				C18:3n3	fungi	(+) 0.783
	Ester	Isoprene	Fungi: *Tuber borchii, Tuber mesentericum, Aspergillus fumigatus* Bacteria:*Burkholderia sp (G−), Paenibacillus sp (G+), Bacillus sp (G+), Enterobacter cloacae (G−), Pseudomonas aeruginosa (G−), Pseudomonas fluorescens (G−), Pseudomonas putida (G−), Pseudonocardia sp (G+), Saccharomonospora sp (G−), Serratia liquefaciens (G−), Streptomyces sp (G+), Thermoactinomyces sp (G+), Thermomonospora sp (G+)*	C15:0	general bacteria	(+) 0.794
		Methyl acetate	Fungi: *Tuber sp, Paecilomyces variotii bain, Penicillium commune pitt*	C14:0	general bacteria	(+) 0.761

the the UKRI Natural Environment Research Council (NERC) Environmental Microbiology and Human Health programme. The underlying data can be accessed at doi: 10.17862/cranfield.rd.11888880.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.137542.

References

Barrera, C., Rocchi, S., Degano, B., Soumagne, T., Laurent, L., Bellanger, A.-P., Laplante, J.-J., Million, L., Dalphin, J.-C., Reboux, G., 2019. Microbial exposure to dairy farmers' dwellings and COPD occurrence. Int. J. Environ. Health Res. 29, 387–399.

Bing-Yuan, Zhang, Y.H., Leung, N.H.L., Cowling, B.J., Yang, Z.F., 2018. Role of viral bioaerosols in nosocomial infections and measures for prevention and control. J. Aerosol Sci. 117, 200–211.

Brinkmann, C., Kurtböke, I., 2018. Extrinsic allergic alveolitis-causing actinomycetes in indoor and farm environments. Microbiology Australia 39, 149–152.

Chiriac, R., Morais, J., Carré, J., Bayard, R., Chovelon, J.-M., Gourdon, R., 2011. Study of the VOC emissions from a municipal solid waste storage pilot-scale cell: comparison with biogases from municipal waste landfill site. Waste Manag. 31, 2294–2301.

Cydzik-Kwiatkowska, A., Zielińska, M., 2016. Bacterial communities in full-scale wastewater treatment systems. World J. Microbiol. Biotechnol. 32, 66.

Douglas, P., Robertson, S., Gay, R., Hansell, A.L., Gant, T.W., 2018. A systematic review of the public health risks of bioaerosols from intensive farming. Int. J. Hyg. Environ. Health 221, 134–173 (h).

Frias, J.A., Richman, J.E., Wackett, L.P., 2009. C 29 Olefinic Hydrocarbons Biosynthesized by Arthrobacter Species. 75(6) pp. 1774–1777.

Frostegard, A., Tunlid, A., Baath, E., 1991. Microbial biomass measured as total lipid phosphate in soils of different organic content. J. Microbiol. Methods 14, 151–163.

Frostegård, Å., Tunlid, A., Bååth, E., 1993. Phospholipid fatty acid composition, biomass, and activity of microbial communities from two soil types experimentally exposed to different heavy metals. Appl. Environ. Microbiol. 59, 3605–3617.

Frostegård, Å., Tunlid, A., Bååth, E., 2011. Use and misuse of PLFA measurements in soils. Soil Biol. Biochem. 43, 1621–1625.

Gallego, E., Roca, F.J.J., Perales, J.F.F., Sánchez, G., Esplugas, P., 2012. Characterization and determination of the odorous charge in the indoor air of a waste treatment facility through the evaluation of volatile organic compounds (VOCs) using TD–GC/MS. Waste Manag. 32, 2469–2481.

Garcia-Alcega, S., Nasir, Z.A., Ferguson, R., Whitby, C., Dumbrell, A.J., Colbeck, I., Gomes, D., Tyrrel, S., Coulon, F., 2016. Fingerprinting outdoor air environment using microbial volatile organic compounds (MVOCs) - a review. Trends Anal. Chem. 86, 75–83.

Garcia-Alcega, S., Coulon, F., Tyrrel, S., 2017. Sampling microbial volatile organic compounds: optimisation of flow rate and sampling time. WIT Trans. Ecol. Environ. 211, 69–76.

Garcia-Alcega, S., Nasir, Z.A., Ferguson, R., Noël, C., Cravo-Laureau, C., Whitby, C., Dumbrell, A.J., Colbeck, I., Tyrrel, S., Coulon, F., 2018. Can chemical and molecular biomarkers help discriminate between industrial, rural and urban environments? Sci. Total Environ. 631–632, 1059–1069.

Han, Y., Huang, X., Wang, C., Zhu, B., He, L., 2019. Characterizing oxygenated volatile organic compounds and their sources in rural atmospheres in China. J. Environ. Sci. 81, 148–155.

Hertel, M., Preissner, R., Gillissen, B., Paris, S., 2016. Detection of signature volatiles for cariogenic microorganisms. Eur. J. Clin. Microbiol. Infect. Dis. 35, 235–244.

Huang, X., Wang, C., Zhu, B., Lin, L., He, L., 2019. Exploration of sources of OVOCs in various atmospheres in southern China. Environ. Pollut. 249, 831–842.

Kanehisa Laboratories, 1995. KEGG PATHWAY Database. Retrieved from. http://www.kegg.jp/kegg/pathway.html.

Konuma, R., Umezawa, K., Mizukoshi, A., Kawarada, K., Yoshida, M., 2015. Analysis of microbial volatile organic compounds produced by wood-decay fungi. Biotechnol. Lett. 37, 1845–1852.

Lasaridi, K., Katsabanis, G., Kyriacou, A., Maggos, T., Manios, T., Fountoulakis, M., Stentiford, E.I., 2010. Assessing odour nuisance from wastewater treatment and composting facilities in Greece. Waste Manag. Res. 28, 977–984.

Lemfack, M.C., Nickel, J., Dunkel, M., Preissner, R., Piechulla, B., 2014. mVOC: a database of microbial volatiles. Nucleic Acids Res. 42, D744–D748.

Lemfack, M.C., Gohlke, B.O., Toguem, S.M.T., Preissner, S., Piechulla, B., Preissner, R., 2018. MVOC 2.0: a database of microbial volatiles. Nucleic Acids Res. 46 (D1), D1261–D1265.

Macnaughton, S.J., Jenkins, T.L., Davis, G.A., White, D.C., 1999. Quantitative sampling of indoor air biomass by signature lipid biomarker analysis. J. Ind. Microbiol. Biotechnol. 22, 80–87.

Mancuso, S., Taiti, C., Bazihizina, N., Costa, C., Menesatti, P., Giagnoni, L., Arenella, M., Nannipieri, P., Renella, G., 2015. Soil volatile analysis by proton transfer reaction-time of flight mass spectrometry (PTR-TOF-MS). Appl. Soil Ecol. 86, 182–191.

Mayrhofer, S., Mikoviny, T., Waldhuber, S., Wagner, A.O., Innerebner, G., Franke-Whittle, I.H., Märk, T.D., Insam, H., 2006. Microbial community related to volatile organic compound (VOC) emission in household biowaste. Environ. Microbiol. 8, 1960–1974.

Micheluz, A., Manente, S., Rovea, M., Slanzi, D., Varese, G.C., Ravagnan, G., Formenton, G., 2016. Detection of volatile metabolites of moulds isolated from a contaminated library. J. Microbiol. Methods 128, 34–41.

Misztal, P.K., Lymperopoulou, D.S., Adams, R.I., Scott, R.A., Lindow, S.E., Bruns, T., Goldstein, A.H., 2018. Emission factors of microbial volatile organic compounds from environmental bacteria and fungi. Environ. Sci. Technol. 52, 8272–8282.

Mubareka, S., Groulx, N., Savory, E., Cutts, T., Theriault, S., Scott, J.A., Roy, C.J., Turgeon, N., Bryce, E., Astrakianakis, G., Kirychuk, S., Girard, M., Kobinger, G., Zhang, C., Duchaine, C., 2019. Bioaerosols and transmission, a diverse and growing community of practice. Front. Public Health 7, 23.

Oksanen, A.J., Blanchet, F.G., Kindt, R., Legen-, P., Minchin, P.R., Hara, R.B.O., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., 2011. Package 'vegan' September 1, 2019. https://cran.r-project.org/web/packages/vegan/vegan.pdf.

Pankhurst, L.J., Whitby, C., Pawlett, M., Larcombe, L.D., Mckew, B., Deacon, L.J., Morgan, S., Villa, R., Drew, G.H., Tyrrel, S., Pollard, S.J.T., Coulon, F., 2012. Temporal and spatial changes in the microbial bioaerosol communities in green-waste composting. FEMS Microbiol. Ecol. 79, 229–239.

Pearson, C., Littlewood, E., Douglas, P., Robertson, S., Gant, T.W., Hansell, A.L., 2015. Exposures and health outcomes in relation to bioaerosol emissions from composting facilities: a systematic review of occupational and community studies. Journal of Toxicology and Environmental Health. Part B, Critical Reviews 18, 43–69.

Polymenakou, P.N., 2012. Atmosphere: a source of pathogenic or beneficial microbes? Atmosphere 3, 84–102.

R Core Team, 2018. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna (2018). Retrieved from. https://www.r-project.org.

Schenkel, D., Lemfack, M.C., Piechulla, B., Splivallo, R., 2015a. A meta-analysis approach for assessing the diversity and specificity of belowground root and microbial volatiles. Front. Plant Sci. 6, 707.

Schenkel, D., Lemfack, M.C., Piechulla, B., Splivallo, R., 2015b. A meta-analysis approach for assessing the diversity and specificity of belowground root and microbial volatiles. Front. Plant Sci. 6, 1–11.

Sohrabi, M., Zhang, L., Zhang, K., Ahmetagic, A., Wei, M., 2014. Volatile organic compounds as novel markers for the detection of bacterial infections. Clinical Microbiology 3, 1–6.

Sun, D., Wood-jones, A., Wang, W., Vanlangenberg, C., Jones, D., Gower, J., Simmons, P., Baird, R.E., Mlsna, T.E., 2014. Monitoring MVOC profiles over time from isolates of Aspergillus flavus using SPME GC-MS. Journal of Agricultural Chemistry and Environment 3, 48–63.

Taha, M.P.M., Drew, G.H., Tamer Vestlund, A., Aldred, D., Longhurst, P.J., Pollard, S.J., 2007. Enumerating actinomycetes in compost bioaerosols at source—use of soil compost agar to address plate 'masking. Atmos. Environ. 41, 4759–4765.

Vorıskova, J., Brabcova, V., Cajthaml, T., Baldrian, P., 2014. Seasonal dynamics of fungal communities in a temperate oak forest soil. New Phytol. 201, 269–278.

Wallander, H., Ekblad, A., Godbold, D.L., Johnson, D., Bahr, A., Baldrian, P., Björk, R.G., Kieliszewska-Rokicka, B., Kjøller, R., Kraiger, H., Plassard, C., Rudawska, M., 2013. Evaluation of methods to estimate production, biomass and turnover of ectomycorrhizal mycelium in forests soils - a review. Soil Biol. Biochem. 57, 1034–1047.

Walser, S.M., Gerstner, D.G., Brenner, B., Bünger, J., Eikmann, T., Janssen, B., Kolb, S., Kolk, A., Nowak, D., Raulf, M., Sagunski, H., Sedlmaier, N., Suchenwirth, R., Wiesmüller, G., Wollin, K.-M., Tesseraux, I., Herr, C.E.W., 2015. Evaluation of exposure-response relationships for health effects of microbial bioaerosols - a systematic review. Int. J. Hyg. Environ. Health 218, 577–589.

Wilkins, K., 2002. Microbial VOC (MVOC) in buildings, their properties and potential use. Proceedings Indoor Air 2002, 431–436. https://www.irbnet.de/daten/iconda/CIB6164.pdf.

Willers, C., Jansen van Rensburg, P.J., Claassens, S., 2015. Phospholipid fatty acid profiling of microbial communities–a review of interpretations and recent applications. J. Appl. Microbiol. 119, 1207–1218.

Yamada, Y., Yukphan, P., 2008. Genera and species in acetic acid bacteria. Int. J. Food Microbiol. 125, 15–24.

Zhao, J., Wang, Z., Wu, T., Wang, X., Dai, W., Zhang, Y., Wang, R., Zhang, Y., Shi, C., 2016. Volatile organic compound emissions from straw-amended agricultural soils and their relations to bacterial communities: a laboratory study. J. Environ. Sci. 45, 257–269.