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Can chemical and molecular biomarkers help discriminate between industrial, rural and urban environments?

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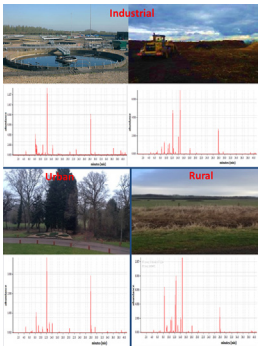
HIGHLIGHTS

- Higher concentration of VOCs and PLFAs were identified in winter compared to summer.
- Seasonal variations in the chemical distribution of MVOCs and in microbial communities.
- Temporal variations during 6 h sampling in the concentrations of VOCs and MVOCs.
- Combination of MVOCs and PLFAs allow the differentiation of outdoor air from a park, a farm, a compost facility and a WWTP.

GRAPHICAL ABSTRACT



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ABSTRACT

Air samples from four contrasting outdoor environments including a park, an arable farm, a waste water treatment plant and a composting facility were analysed during the summer and winter months. The aim of the research was to study the feasibility of differentiating microbial communities from urban, rural and industrial areas between seasons with chemical and molecular markers such as microbial volatile organic compounds (MVOCs) and phospholipid fatty acids (PLFAs). Air samples (3 l) were collected every 2 h for a total of 6 h in order to assess the temporal variations of MVOCs and PLFAs along the day. MVOCs and VOCs concentrations varied over the day, especially in the composting facility which was the site where more human activities were carried out. At this site, total VOC concentration varied between 80 and 170 μg m^{-3} in summer and 20–250 μg m^{-3} in winter. The composition of MVOCs varied between sites due to the different biological substrates including crops, waste water, green waste or grass. MVOCs composition also differed between seasons as in summer they are more likely to get modified by oxidation processes in the atmosphere and in winter by reduction processes. The composition of microbial communities identified by the analysis of PLFAs also varied among the different locations and between seasons. The location with higher concentrations of PLFAs in summer was the farm (7297 ng m^{-3}) and in winter the park (11,724 ng m^{-3}). A specific set of MVOCs and PLFAs that most represent each one of the locations was identified by principal component analyses (PCA) and canonical analyses. Further

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to this, concentrations of both total VOCs and PLFAs were at least three times higher in winter than in summer. The difference in concentrations between summer and winter suggest that seasonal variations should be considered when assessing the risk of exposure to these compounds.

1. Introduction

Outdoor environments containing high levels of biological material such as wastewater treatment plants, biowaste facilities or animal and agricultural farms, are of a great concern for the occupational workers carrying out continuous exposure to bioaerosols. Occupational workers are likely to develop respiratory diseases such as allergies, asthma or chronic obstructive pulmonary disease (Albrecht et al., 2008; Fischer et al., 2008; O'Connor et al., 2015). Currently there is a paucity of information on the microbial composition of bioaerosols in outdoor environments Elucidation of specific chemical and biomolecular markers that relate to different outdoor bioaerosol profiles may provide an alternative rapid, cost-effective approach to characterise outdoor bioaerosols that circumvents the limitations of cell culturing techniques (Oliver, 2005; Garcia-alcega et al., 2017a).

Phospholipid fatty acids (PLFAs) are present in the cell membranes of living organisms and are indicators of microbial biomass, composition of microbial communities and microbial stress (Willers et al., 2015). There are PLFAs common to all the living organisms but there are also specific ones which are unique for bacteria, archaea, fungi, or specific genera (Gram positive or Gram negative bacteria, saprotrophic fungi, etc.) (Taha et al., 2007; Cydzik-Kwiatkowska and Zielińska, 2016). Microbial volatile organic compounds (MVOCs) are side-products produced during the secondary metabolism of moulds, fungi and bacteria (Schenkel et al., 2015). The metabolic products produced by the microbes are different depending on the substrate they are utilising (Konuma et al., 2015). The combination of PLFAs and MVOCs biomarkers could potentially allow us to differentiate ambient outdoor air by chemical analysis and chemometrics (Mason et al., 2010; Lemfack et al., 2014).

In general, bioaerosol studies which include the analysis of MVOCs have been carried out in indoor buildings and focused on the detection of volatile metabolites produced by moulds (Ghosh et al., 2015). To date few studies of bioaerosols from outdoor air have investigated the use of MVOCs and PLFAs. Specifically, MVOCs concentrations have been monitored in composting sites (Fischer et al., 2008; Müller et al., 2004; Muller et al., 2004a) and in WWTPs (Lehtinen, 2012; Gallego et al., 2012) and PLFAs only in composting sites (Pankhurst et al., 2012). Outdoor sites including agricultural farms, anaerobic digestion plants or urban and recreational areas, therefore, are still lacking. Moreover, there are no studies that combine the chemical and molecular characterisation of outdoor air from urban, rural and industrial environments.

There are several challenges for conducting accurate measurements of bioaerosol emissions from outdoor environments. Sampling conditions such as the distance from the source, meteorological conditions (wind direction, wind speed, relative humidity and temperature) and the topography of the landscape all effect the collection of bioaerosol material (Muller et al., 2004b) and therefore, sample replicates from the same location can differ considerably. For this reason the reproducibility of the data is low and the inter-variability between replicates (samples collected at the same time) is often high.

In the present study, an outdoor sampling campaign was undertaken from 3 different contrasting environments, urban, rural and industrial sites, with the objective of investigating the possibility of differentiating outdoor air using chemical and molecular markers. The chosen sampling locations were a park and a farm in London, a composting facility in Colchester and a WWTP in Milton Keynes.

2. Material and methods

2.1. Outdoor sampling sites and weather data

The four locations were sampled twice, in summer 2016 and in winter 2017 in order to study the effects of seasonal variations. Air sampling took place at each site during a total of 6 h. For PLFAs analysis, air samples were collected in triplicate every 2 h each of 2 h duration (i.e. every 2 h 3 samples were collected so in total 9 samples). MVOCs were also sampled at the beginning of each 2 h period in triplicate so again 9 samples were collected. Weather data (wind speed, temperature and relative humidity) was monitored on site, throughout the 6 h period with a Kestrel® weather station. The description of each sampling site, dates, time and weather conditions are detailed in Table 1.

2.2. MVOCs collection and analysis

Air samples were collected in triplicate every 2 h over 6 h sampling using TD tubes packed with tenax and carbotrap 50/50 v/v (Markes, Llantrisant, UK) attached to a GilAir® plus air sampling pump (Sensydine, LP-Clear water, Florida, US). In total, 9 TD tubes were used at each location. Before proceeding with the sampling campaign, in situ preliminary tests at Cranfield waste water treatment plant (WWTP) were performed for the optimisation of sampling conditions (sampling time and sampling flow rate) for the analysis of MVOCs (Garcia-Alcega et al., 2017b). Different sampling settings were tested: fixed sampling time (10 min) and variable flow rates (100, 500 and 1000 ml min^{-1}) and fixed flow rate (100 ml min^{-1}) and variable sampling time (10, 20 and 30 min). Higher collection efficiency was obtained when a flow rate of 100 ml min^{-1} was applied during 10 min. The capacity of the TD tubes is relatively small and since the extraction efficiency of the technique is high, little volume of air sample is required for the analysis of MVOCs before the compounds start desorbing from the tubes (breakthrough) (Batterman et al., 2002). For this study, therefore, the sampling settings of 100 ml min^{-1} during 10 min were used.

TD tubes were conditioned before sampling at 330 °C for 45 min at 20 psi. After the sampling, 0.5 ng of toluene d_8 as internal standard were loaded into the TD tubes containing the air samples with helium at 400 ml min^{-1} flow rate. Air samples were analysed by 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} was carried out using a mix of 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 concentration was loaded in new conditioned TD tubes followed by 50 ng of toluene d_8 as an internal standard. The GC–MS was coupled with a 78 m × 250 μm × 0.5 μm Agilent column. The initial oven temperature was set at 35 °C and increased to 75 °C at 2 °C min^{-1}. Then the temperature was increased to 140 °C at 2 °C min^{-1}. The final ramp was set at 300 °C at 10 °C min^{-1}. Ions were monitored in full scan mode. MVOCs 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

Table 1
Description of sampling sites, dates and weather conditions (wind speed, WS; temperature, Ta and relative humidity, RH) in a park, farm, composting facility and WWTP.

Environment		Urban		Rural		Industrial			
Location		Park (London)		Farm (London)		Composting facility (Colchester)		WWTP (Milton Keynes)	
Description		Consists of 227 ha and 635 km of waterways. There is a busy highway behind the park. There is a high diversity of trees: London plane tree, oak, tulip trees, small leaved lime, wild cherry, white willow, common alder, common ash, aspen, mountain ash, elm, multi-stemmed birch, crab apple, hawthorn, acacia, golden rain tree, native black polar.		89 ha of organic and biodynamic farm with indoor and outdoor adventure play zones for kids. 20 ha consist of woodlands and gardens, 16 are arable fields, 53 permanent pasture and grass leys. There is a herd of native jersey cows and a native Angus-cross jersey cows, a flock of Castle milk Moorit sheep, Tamworth pigs and a small flock of free range chickens.		6.8 ha composting site located close to a motorway. The closest houses are at 200 m distance. The composting facility contains 8–10 open windrows.		A conventional 35 ha. WWTP which provides a treatment capacity for a population of 6 million, including industrial, domestic and commercial customers. It consists of 4 rectangular primary treatment tanks, 4 aeration lanes and 8 final settlement tanks.	
Date		August 2016	February 2017	August 2016	February 2017	July 2016	February 2017	August 2016	February 2017
Weather	WS (m/s)	2.2	3.1	6.4	4.5	1.8	3.4	2.5	6.1
	Ta (°C)	19.0	83.0	19.0	8.3	18.4	8.7	17.8	7.0
	RH (%)	63.0	85.0	66.0	85.7	65.1	83.3	54.3	81.0
Time		10:00–16:00	9:00–15:00	10:00–16:00	9:00–15:00	8:00–14:00	8:00–14:00	10:00–16:00	8:00–14:00
Human activity during sampling period		Normal activities	Normal activities	Potatoes, cabbages, carrots, onions	Cereal crops and stubble turnips	Turning & addition of green waste	Lorry deliveries (low activity)	Normal activities	Normal activities

undertaken. An empty TD tube and a blank containing 50 ng of toluene d_8 as internal standard were run as QC every 15 samples.

2.3. PLFA collection and analysis

Air samples were collected in triplicate 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) during 2 h at 28 l min^{-1} flow rate. In total, 9 polycarbonate filters were used at each location. After sampling, polycarbonate filters were removed from the sampling heads with sterile tweezers and deposited into a sterile Eppendorf tubes (Fisher Scientific, Loughborough, UK). Filters were stored at −20 °C prior to PLFA analysis (Macnaughton et al., 1999). PLFAs analysis was carried out following a modified version of the method described by Frostegård et al. (Frostegard et al., 1991; Frostegård et al., 1993). 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 dehydrate 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). After 2 × 15 min of sonication, samples were centrifuged at 2000 rpm for 10 min (Falcon 6/300; Sanyo, UK) and the upper layer was placed into a clean glass vial. Then, 4 ml chloroform and 4 ml 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 FAMEs was as follows: 50 °C for 1 min (splitless mode); 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 and the injector temperature was set at 310 °C. Sterile polycarbonate filters were extracted for PLFAs in triplicate as a procedural blank.

Cell biomass was determined using the following conversion factor: 2.0×10^4 cells = 1 picomole PLFA (Balkwill et al., 1988).

2.4. Data analysis

PLFAs data from the chromatograms obtained from the GC-FID were processed in ChemStation. Individual PLFAs were designated to microbial groups as reported by Willen et al. (Willers et al., 2015). Chemometrics from the chromatograms obtained from GC–MS analysis were processed in batch using OpenChrom. Prior to the identification and quantitation of the VOCs, first the background of the chromatograms was subtracted. MVOCs were differentiated from VOCs using the Kegg Pathway (Kanehisa Laboratories, 1995) and mVOC (Lemfack et al., 2014) metabolic databases as well as in the recent literature (Schenkel et al., 2015; Hertel et al., 2016; Micheluz et al., 2016). Descriptive statistics and pie charts were performed with Excel 2010. Correlations between MVOCs chemical groups were performed by Draftman's plots in Primer 6 Betta 11 and ANOVA analyses were performed with Statistica 12.5. Heat Maps were produced using ClustVis (Metsalu et al., 2015) to investigate the possibility of differentiating the 4 locations with MVOCs and PLFAs biomarkers. Relationships between PLFA and MVOC biomarkers were tested using correlation analysis carried out with the 'corrplot' R package (Wei and Simko, 2017). Spearman coefficient was computed to estimate a rank-based measure of the association. The significance of each correlation was evaluated using 'cor.mtest' function and the non-significant correlations were removed from the analyses. Correlations were represented in correlograms. Canonical correspondence analyses (CCA) were performed in order to identify multivariate relationship between weather data (wind speed, temperature and relative humidity) and biomarker concentrations (MVOCs and PLFAs). Only biomarkers measured in both summer and winter sampling times were used with the weather data of the different locations during these periods. The analyses were performed using the CCA function available in the 'vegan' R package (Oksanen et al., 2017). CCA biplots were made using symmetric scaling, both biomarker and

Table 2
Total VOCs and PLFAs (μg m^{-3}), %MVOC, % non-microbial VOCs and cell biomass (μmol m^{-3}) in 4 different outdoor environments (a park, composting facility, WWTP and a farm). Average of 3 replicates collected every 2 h over 6 h sampling.

Site		Total VOCs (μg m^{-3})	MVOCs (%)	VOCs (%)	PLFAs (ng m^{-3})	Cell biomass (μmol m^{-3})
Composting facility	S	172 ± 5	73	27	2702	963
	W	1235 ± 32	72	28	6951	4017
WWTP	S	90 ± 2	79	21	3267	1272
	W	176 ± 5	43	57	4250	2849
Farm	S	235 ± 5	73	27	7297	2466
	W	366 ± 63	56	44	8150	1468
Park	S	122 ± 2	82	18	3123	1584
	W	835 ± 13	76	24	11,724	4017

*S: summer; W: winter.

weather scores were scaled symmetrically by square root of eigenvalues to preserve the features of biplots without focusing on one set of scores.

3. Results and discussion

3.1. Sites overview

An overview distribution of the total concentration of VOCs, % MVOCs, %VOCs and concentration of PLFAs in summer and in winter for each location is shown in Table 2. All the locations had higher concentrations of total VOCs and PLFAs in winter compared to summer, which is in agreement with previous indoors and outdoors studies (Rehwagen et al., 2003; Matysik et al., 2010). The percentage MVOCs across sites were higher in the summer (WWTP 79%; farm 73%; park 83%) compared to the winter (WWTP 43%; farm 56%; park 76%). The composting facility was the site with similar %MVOCs between seasons (73% in summer and 72% in winter). The lower concentrations in summer can be explained by a faster degradation process of VOCs in the lower troposphere during the warm periods as a result of higher oxidation processes produced by an elevated UV light intensity (photochemistry) (Matysik et al., 2010) and by the higher levels of Reactive Oxygen species (ROx) (Xue et al., 2013). Moreover, high temperatures lead to the gas-particle shift equilibrium and VOCs become less available (Duan et al., 2006). In a laboratory based experiment, Schlink et al. (Schlink et al., 2004) studied the dispersion of VOCs depending on temperature variations and they concluded that VOCs adhere to the surfaces at high temperatures. Further to this, it has been shown that volatile compounds adhere to air particles (PM 2.5 is higher in winter) at low temperature so becoming easier for collection (Cheng et al., 2016). Higher seasonal variations were observed in the farm and the park compared to the WWTP and the composting facility (despite the working activities that were being carried out in the composting site). It was previously addressed that industrial areas have less seasonal variations (Matysik et al., 2010). The higher variations in rural and urban areas respect to the industrial ones, suggest that it is easier to detect and identify chemical and molecular profiles from sites influenced by high anthropogenic activities.

The composting facility and the park did not differ in the concentration of MVOCs between summer and winter ($p > 0.05$) (Table S1). The concentration of MVOCs present in the WWTP and farm for instance, were double in summer respect to winter ($p < 0.05$), probably due to higher microbial activity. The concentration of PLFAs was not significantly different between seasons indicating that there was similar microbial biomass in winter and in summer (Table S2). Given the differences in VOCs concentrations between summer and winter, seasonal variation factors should be applied to adjust the risk exposure estimates of these compounds to human health (Schlink et al., 2004).

MVOCs/VOCs ratio (Fig. 1) in the summer was >1 for the composting facility, the WWTP and the farm and park which indicated that the major contributors to the total VOCs were from microbial sources. In contrast, the MVOCs/VOCs ratio was <1 in the winter demonstrating that VOCs were more predominant. The higher amount of anthropogenic VOCs in winter compared to summer was probably associated to the major use of heating and combustion processes in the park which was surrounded by houses and by the smoke of the vehicles (Yli-Tuomi et al., 2015; Bari and Kindzierski, 2017; Duan et al., 2016). The cold temperatures in winter make the dispersion of VOCs less favourable (they are easily dispersed in the vapour phase at high temperatures) (Gupta et al., 2017). The WWTP and the composting facility were affected by the smoke of the heavy good vehicles (HGV) whereas the farm was only surrounded by fields without neither traffic nor buildings around. This explains why the farm had MVOCs/VOCs ratios >1in both seasons, although this ratio was higher in summer (2.1 vs 1.3).

3.2. Temporal variation

The total concentration of VOCs (the sum of VOCs and MVOCs), VOCs and MVOCs were plotted at 3 different sampling times (2 h of difference each one) and within the same day in order to study the temporal variation (Fig. 2). Less temporal variations were observed in summer compared to winter and also between replicates. The total concentrations of

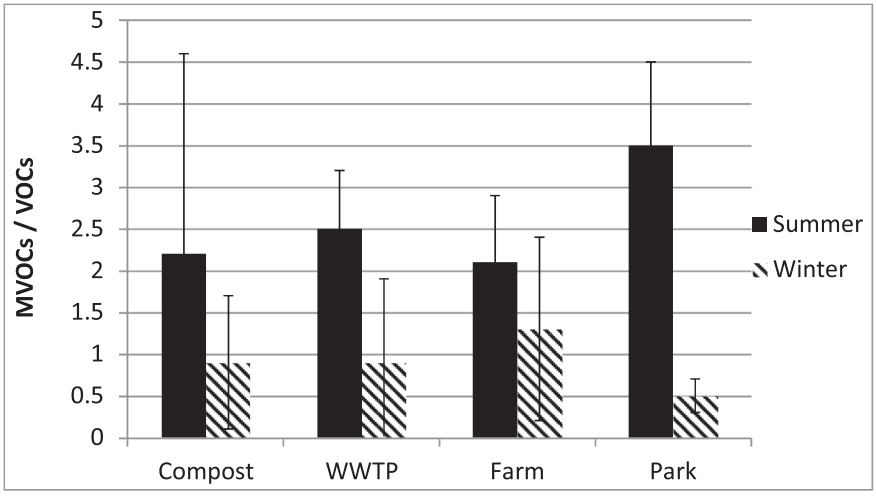

Fig. 1. MVOCs/VOCs average ratio of 9 individual samples during 6 h sampling in the composting facility, the WTP, and the farm and park in winter and summer.

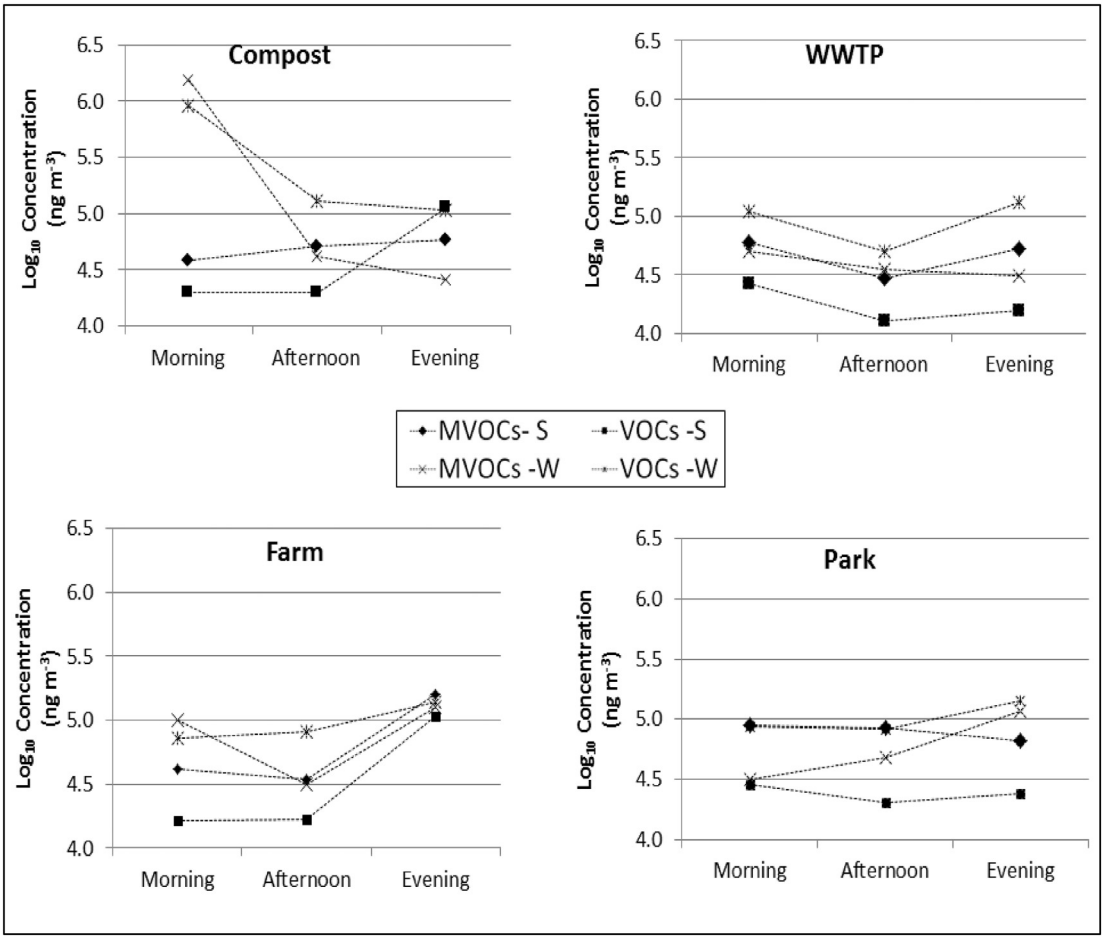

Fig. 2. Temporal variation of concentration (ng m^{-3}) of MVOCs, non-microbial VOCs and total VOCs identified in the composting facility, the WWTP and the park and farm in summer and winter.

VOCs in the composting facility in summer varied between 70 and 170 μg m^{-3} as a consequence of the works that were being carried out on site (i.e. turning and addition of green waste). In the farm, as it was in an open area and the wind speed was very high (average 6.39 m/s), the total concentration of VOCs varied between 75 and 400 μg m^{-3}. VOCs concentrations remained more constant at the WWTP (45–88 μg m^{-3}) and the park (100–120 μg m^{-3}).

In winter the temporal variations and the variations between replicates were higher as the wind speed in most of the locations was also higher compared to summer (the park, compost and WWTP) (see Table 1). The site with greatest variations in the total concentration of VOCs was the composting facility (30–2.500 μg m^{-3}) as there was a lorry in movement on site. Variations in the total concentration of VOCs in the WWTP, park and farm were 100–175 μg m^{-3}, 100–900 μg m^{-3} and 100–330 μg m^{-3} respectively. Given the reasonable differences in concentrations of VOCs at different times, sampling should be done at least at 3 different times in order to have representative measurements of each location along the day.

3.3. Classification of MVOCs and PLFAs

MVOCs identified in each location were classified according to their respective chemical groups (alcohols, alkanes, aldehydes, esters, ethers, organic acids and ketones) and their contribution (Fig. 3A). The % of the chemical groups was different in summer respect to winter at every location. In summer, the composting site and the WWTP shared similar % MVOCs groups (35–38% aldehydes, 25–26% ethers and 15–11% ketones, respectively). In the farm the predominant group were alcohols (50%) followed by aldehydes (14%), whereas in the park it was aldehydes (32%) and then ethers (24%). In winter, all the sites had different % MVOCs. The compost was dominated by alkanes (68%) and organic acids (12%), the WWTP by alcohols (29%) and aldehydes (17%), the farm by alcohols (55%) and alkanes (17%) and the park by alkanes (62%) and ketones (28%). The MVOCs are produced by fermentation processes which are substrate dependent. The high content of alcohols monitored in the farm in winter and summer were associated with the fermentation processes produced by the vegetation (Mellouki et al., 2015) or by the microbes (Alam and Saeed, 2013). The fermentation of organic waste, for instance, produces a high content of aldehydes, ketones and ethers, as it was observed in the WWTP in summer and winter and in the compost in summer. The high alkane emissions at the composting facility and the park in winter could be attributed to anthropogenic sources too as alkanes are released from residential heating and combustion activities (Gupta et al., 2017).

The same classification was done with the different microbial species based on the PLFA analysis (Fig. 3B). Seasonal variations in the microbial composition were observed in all the sites. In summer time, all the locations had a higher amount of bacteria compared to fungi (compost 44% G+ and 22% *Bacillus/Arthrobacter*, WWTP 36% *Bacillus/Arthrobacter*, park 51% bacteria and 9% G+ bacteria) whereas in the farm apart from G+ (44%) fungi also were at high levels compared to the rest of the locations (28%). In winter, for instance, the park contained a large amount of fungi (49%

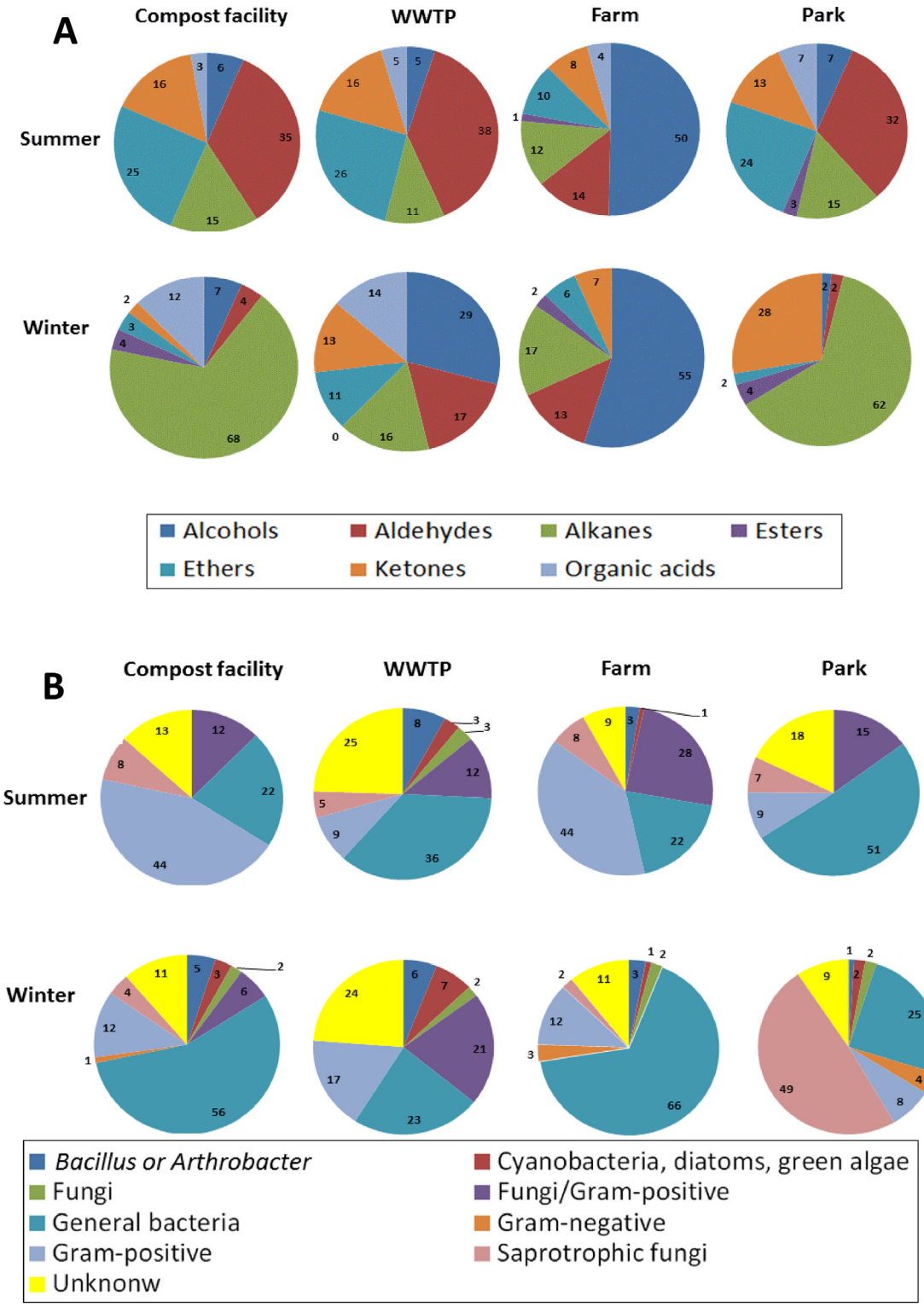

Fig. 3. a) Classification of MVOCs and b) classification of PLFAs identified in 4 outdoor air environments in summer and winter (composting site, WWTP, farm and park).

saprotrophic fungi) whereas in the composting site and the farm mainly were *Bacillus/Arthrobacter* bacteria (56% in the compost and 66% in the farm, respectively). The WWTP contained 24% of unknown PLFAs and 23% of PLFAs common to all bacterial taxon. The minimal changes observed in the PLFA composition respect to the MVOCs composition is because many of the PLFAs are present in most of the organisms (Pankhurst et al., 2012; Frostegård et al., 2011). The most characteristic difference is the high percentage of saprotrophic fungi in the park in winter, due to the higher activity of the extracellular enzymes (Voriskova et al., 2014).

3.4. Atmospheric chemistry of MVOCs

VOCs experience oxidation and reduction processes in the atmosphere (Koppmann, 2010; Guenther, 2015). When the compounds are oxidised they get an additional molecule of oxygen. In the opposite

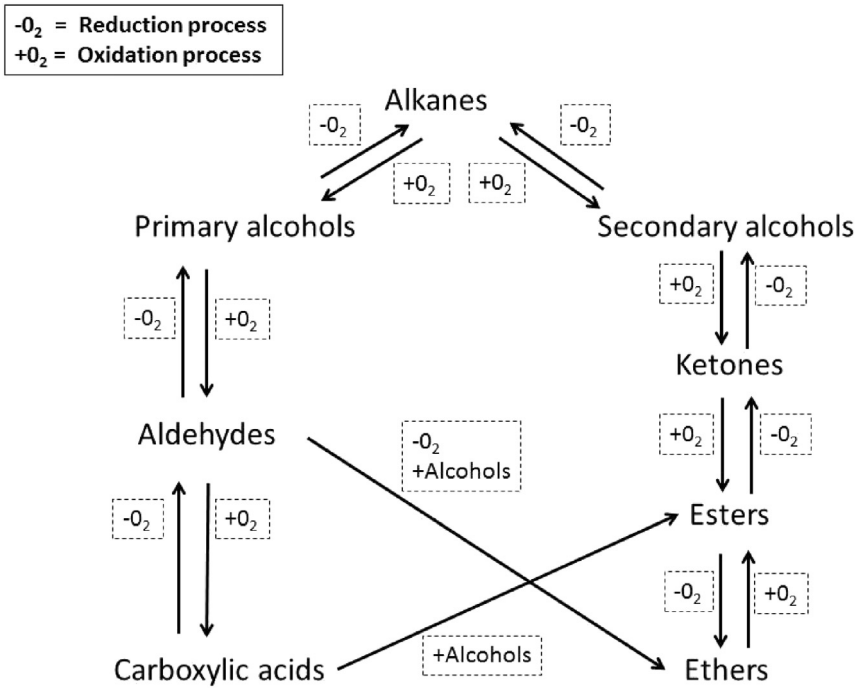

Fig. 4. Oxidation and reduction processes of the MVOC groups in the atmosphere.

way, when they are reduced they lose a molecule of oxygen. Additionally, some compounds can also react with other molecules and be converted to other chemical groups such as aldehydes are converted to ethers by an addition of an alcohol and a loss of a molecule of O_2 or carboxylic acids to ethers with an addition of an alcohol (Fig. 4). This process is reversible meaning that if by oxidation alkanes are converted to alcohols, the oxidation of alcohols lead to the production of aldehydes and the oxidation of these produce carboxylic acids. In the opposite way, the reduction of carboxylic acids produce aldehydes, these reduced form alcohols and the reduction of alcohols lead to alkanes. The biochemistry of MVOCs from the 4 locations of this work was studied taking into consideration the correlation of all the chemical MVOCs groups per location (compost, farm, WWTP and park) and the seasonal variation (summer/winter). In summer time MVOCs transformations were dominated by oxidation processes, i.e. the compounds were transformed by addition of a molecule of O_2.

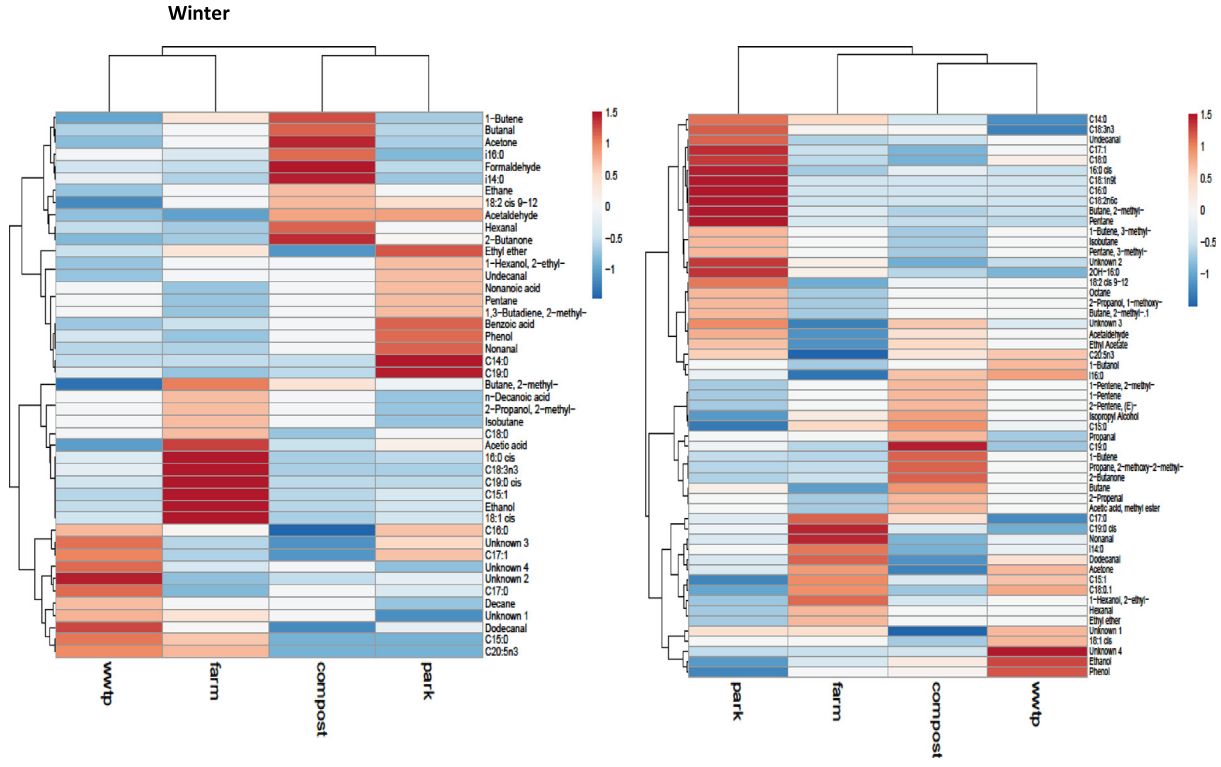

Fig. 5. Heatmap of MVOCs and PLFAs identified in a WWTP, farm, composting facility and park in summer and winter.

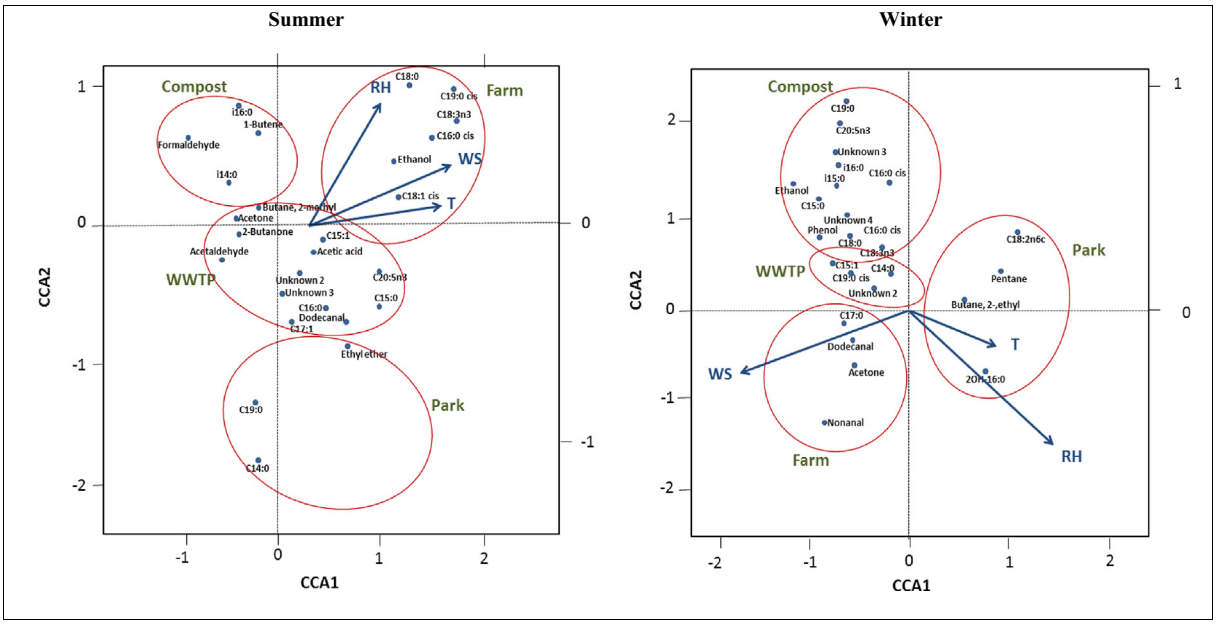

Fig. 6. Canonical correspondence analysis of MVOCs and PLFAs present in the WWTP, farm, composting facility and park linked to weather conditions (RH%, WS (m/s) and T^a (°C)) in summer and winter.

The predominant groups of MVOCs in the composting facility were aldehydes (35%) and ethers (26%), where alcohols were converted to ethers ($R^2 = 0.826$) and ethers to aldehydes ($R^2 = -0.600$) (Fig. S1). MVOCs in the WWTP predominantly consisted of aldehydes (38%), ethers (26%) and ketones (16%) and the most significant reactions were the conversion from alkanes to aldehydes ($R^2 = 0.839$) and from ketones to ethers ($R^2 = 0.732$). In the park mostly aldehydes (32%) and ethers (24%) were identified and the transformation from alcohols to ethers was the most significant one ($R^2 = 0.740$). The farm was the site which showed a reduction pattern converting ketones to alkanes ($R^2 = 0.864$). MVOCs in winter were for instance transformed by reduction processes, i.e., losing a molecule of O_2. The predominant group of MVOCs in the compost were alkanes (68%) and these were produced by the reduction of ketones ($R^2 = 0.995$) (Fig. S2). In the WWTP alcohols were present at high levels (29%) and they were formed by the reduction of organic acids which also were at high levels (14%) ($R^2 = -0.683$). The park mainly had alkanes (62%) and ketones (28%). Alkanes probably were formed by reduction of ketones ($R^2 = 0.985$) and the reduction of aldehydes ($R^2 = 0.942$). In the same case as in summer, ketones in the farm were reduced to alkanes ($R^2 = 0.715$). Reduction processes in winter were more usual than in summer because the O_3 is highly formed at low temperatures by the reduction of VOCs and NO_X (Carter and Seinfeld, 2012).

3.5. Sites characterisation

All the MVOCs and PLFAs present in the 4 environments were plotted in a heat map (Fig. 5). Compounds that are coloured in dark red mean that are strongly correlated with the specific location. At each location there was a different cluster comprised of a different set of PLFAs and MVOCs which also were different in summer and in winter. The cluster in the WWTP in summer was formed by the PLFAs C16:0, C17:1, C17:0, C15:0,

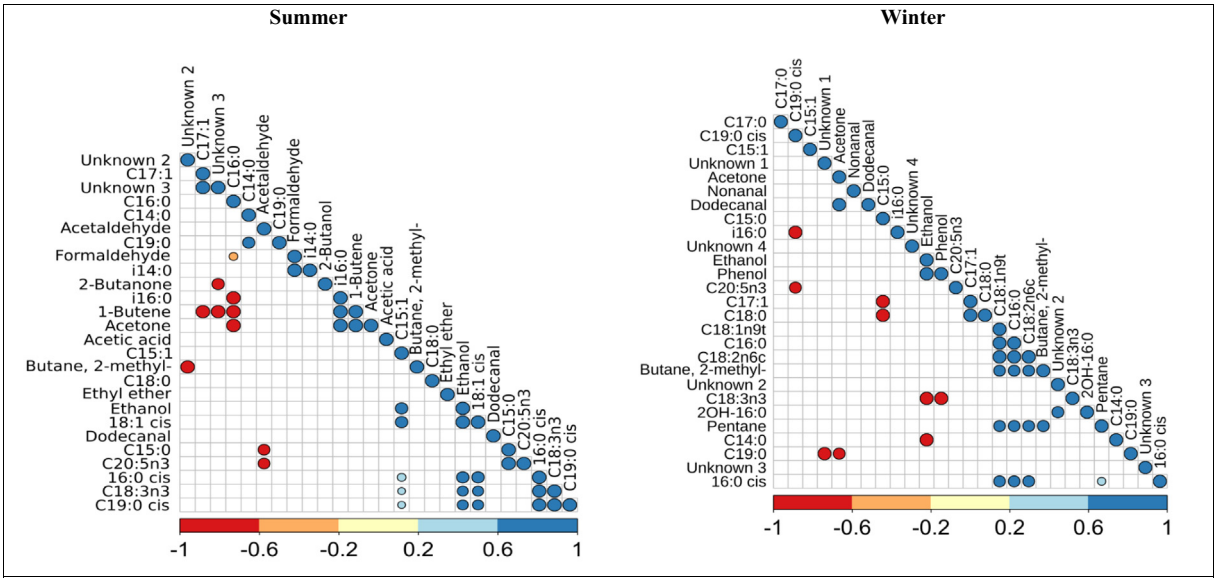

Fig. 7. Correlation (based on the Spearman coefficient) between MVOCs and PLFAs present in the WWTP, farm, composting facility and park in summer and winter.

C20:5n3 and 4 unknown and the MVOCs decane and dodecanal. In winter, for instance, the cluster was formed by C15:1, C18:0, an unknown PLFA and the MVOCs dodecanal, acetone, ethanol and phenol. The cluster from the farm in summer was comprised of C18:0, C16:0cis, C15:1, C18:1 cis, and C16:0 and the MVOCs 2-methyl butane, n-decanoic acid, 2-methyl-2-propanol, isobutene and acetic acid. In the farm during winter, for instance, were the PLFAs C17:0, C19:0 cis, i14:0, C15:1, C18:0 and the MVOCs nonanal, dodecanal, acetone, 2-ethyl-1-hexanol, hexanal and ethyl ether. The cluster in the composting facility in summer was formed by the PLFAs i16:0, i14:0, 18:2 cis 9–12 and the MVOCs 1-butene, butanal, acetone, formaldehyde, ethane, acetaldehyde, hexanal and 2-butanone. In winter, the cluster in the composting site was formed by lees compounds, the PLFA C19:0 and the MVOCs 1-butene, 2-methoxy,-2-methyl-propane and 2-butanone. The cluster in the park in summer was formed by the PLFAs C14:0 and C19:0 and the MVOCs ethyl ether, 2-ethyl, 1-hexanol, undecanal, nonanoic acid, pentane, 2-methyl-1,3-butadiene, benzoic acid, phenol, phenol and nonanal. In winter, the cluster in the park contained several compounds, the PLFAs C14:0, C18:3n3, C17:1, C18:0, C16:0cis and C16:0, C18:1n9t, C18:2n5c and the MVOCs undecanal, 2-methyl butane, pentane, 3-methyl, 1-butene, isobutene, 3-methyl pentane-2OH-16:0, 18:2cis 9–12 and octane. The different clusters per site suggest the possibility of differentiating distinct outdoor environments looking at chemical and molecular biomarkers. A more specific analysis of MVOCs and PLFAs present in the WWTP, farm, composting facility and park was performed by canonical correspondence analysis where the weather conditions (RH%, WS and T[a]) were also included (Fig. 6). The canonical analysis indicated that the farm was the only location affected by the weather conditions in both summer and winter, probably because it was the most exposed location as it was not protected by any building nearby. The test confirmed the clustering results obtained from the heat map. The most representative compounds for the farm in summer were 2-methyl butane, C18:0, C19:0cis, C18:3n3, C16:0 cis, ethanol and C18:1 cis, whereas in winter were the PLFA C17:0 and the MVOCs dodecanal, acetone and nonanal. The composting facility in summer was mostly represented by the PLFAs i16:0 and i14:0, and the MVOCs 1-butene, and formaldehyde. In winter, for instance, this location was mainly represented by PLFAs (C19:0, C20:5n3, i16:0, C15:0, 16:0cis, C18:0, C18:3n3, C15:1, C19:0cis, C14:0, 4 unknown PLFAs) and ethanol. Most representative compounds in the park in summer time were ethyl ether, C19:0 and C14:0 and in winter in contrast 2-methyl butane, pentane and C18:2n6c. The compounds that best described the WWTP in summer were the MVOCs 2-butanone, acetic acid, acetaldehyde and dodecanal and the PLFAs C20:5n3, C15:0, C17:1 and 2 unknown ones. In winter time the most characteristic compounds for this WWTP were the PLFAs C15:1, C19:0 cis, C14:0, C18:3n3, 2 unknown and the MVOCs ethanol and phenol. As anticipated, MVOCs productions were different in summer respect to winter. In summer the production of acetone, 1-butene and ethanol were positively correlated with PLFAs (i16:0 and C15:1 respectively) and moreover, acetone and 1-butene were strongly correlated, suggesting that they had been produced by the same microorganisms (Fig. 7). The MVOCs 2-butanone, 1-butane, acetone, and 2-methyl butane were negatively correlated with some PLFAs (2 unknown, C17:1, C16:0). These negative correlations could be related to the oxidation process of the MVOCs being alcohols converted to ketones and aldehydes to alkanes. In winter, for instance, 2-methyl butane and pentane were correlated with the PLFAs C18:1n9t, C16:0 and C18:2n6c. The pair of MVOCs 2-methyl butane and pentane, dodecanal and acetone as well as phenol and ethanol were strongly correlated between each other also indicating that are simultaneously produced by the same microbial species. Ethanol, phenol and acetone were negatively correlated to the PLFAs C18:3n3, C14:0 and C19:0, possibly because ketones and alcohols were reduced in winter to alkanes.

The results show therefore, that outdoor air from industrial, urban and rural origins have different chemical and molecular profiles and this air can be differentiated by MVOCs and PLFAs analysis. Chemical and molecular profiles are season dependent as microbial

Table 3
Specific VOCs, MVOCs and PLFAs found in the composting facility, WWTP, farm and park.

Compost		WWTP		Farm		Park	
Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter
1,4-Cyclohexadiene	Butane,	1,2-Benzenedicarboxylic	**Formic acid Butyl**	2,4-Hexadiyne	**Nonane**	Propane, 1-nitro	C18:2n6c
1-methyl-4-(1-methylethyl)	2,2,3,3,-tetramethyl	acid	**ester**				
Pentane, 3-methyl-	1,3,5-Cycloheptatriene	**Dodecane**	**Decane**	**Hexanoic acid**	Acetamide	**Nonane**	C18:1n9t
Eucalyptol	**Dimethyl sulfone**	Cyclopentane	**Dodecane**	**1-Butene, 3-methyl-**	**1-Octane**	1,3,5-Cycloheptatriene	**2-Hexene**
2-Butanol	**Acetic acid**		Methylamine	**2-Pentene, (Z)**	Carbon tetrachloride	**Ethyl Acetate**	**3-Hexanone 2,2-dimethyl**
Pentanoic acid	Hydrazine, methyl			**1-Pentene, 2-methyl-**	**2-Butanol**	Methyl vinyl ketone	Ethane 1,2-diethoxy
2-Propanone, 1-hydroxy				Methoxyacetyl chloride	**Undecane**	**Butane**	(*R*)-(+)-3-Methylcyclopentanone
				Propanoic acid, 2,2-dimethyl-	**3-Ethyl Hexane**	**Acetic acid, methyl ester**	Ethylbenzene
					Propene	**Octanoic Acid**	Cyclohexane
						Acetamide, 2-fluoro	Cycloheptane
						C14:0	Methoxyacetyl chloride
						Tetradecane	1-methyl-4-(1-methylethylidene)
						2,4-dimethyl, Hexane	C16:0
						Propene	Tetrachloroethylene

*Compounds in bold are **MVOCs** and underlined compounds are PLFAs. The rest of the compounds are VOCs.

communities' shift and also change their metabolic processes depending on the substrate.

3.6. Site specific markers

Site specific markers have been identified at each site (Table 3). The compounds in bold correspond to MVOCs, the compounds that are underlined are PLFAs and the rest are anthropogenic VOCs. None of the MVOCs can be attributed exclusively to microbial origin as the same compounds can also be emitted from anthropogenic sources, substrates or plants (Kuske et al., 2005; Schleibinger et al., 2008; Dunkel et al., 2009). In addition, the majority of MVOCs are common for most of the microorganisms and are not specific at species level because they share similar growing conditions and metabolism (Korpi et al., 2009). The limitation of this study is that it has only been done in 4 different locations, 2 industrial sites (a WWTP and a composting facility), a farm as a rural site and a park as an urban site. It would be recommended to evaluate whether some of these compounds are also specific in other set of farms, parks or industrial areas.

4. Conclusions

Higher concentrations of MVOCs and PLFAs were found in winter in comparison to summer. The composition of MVOCs based on their chemical classification was also different at each location. The farm predominantly produced a major concentration of alcohols respect to the rest of the locations in summer and winter (50–55%). The WWTP and composting facility contained higher concentrations of ketones, ethers and aldehydes. In winter, alkanes were predominant in the composting facility, farm and park. Seasonal variations on the classification of PLFAs were observed, being in summer time the proportion of bacteria respect to fungi higher. The differences on PLFAs between sites were minimal except in the park in winter were saprotrophic fungi were predominant. Temporal variations on the total concentration of MVOCs and VOCs were also observed along the same sampling day. These findings showed that chemical and molecular biomarkers can be used to distinguish bioaerosol fingerprints from contrasting outdoor environments (a rural, urban and 2 industrial) focusing on the concentration and presence/absence of chemical and molecular biomarkers. However, atmospheric emissions are very complex and the fingerprint of the source emissions is highly influenced by metabolic processes which are substrate dependent. Further to this, most of the MVOCs are not solely from microbial origin and can also be from plants and/or anthropogenic emissions. In addition, the contribution from background emissions interferes with the characterised and speciated MVOCs. The UV light plays an important seasonal role as its high intensity in summer accelerates the degradation of the MVOCs and increments their atmospheric oxidative capacity. To enhance the identification and assignation of specific MVOCs and PLFAs to bacterial or fungal species, a larger number of samples will be required and other techniques focusing on different constituents such as endotoxins, DNA or real time fluorescence based detection methods would give more insights in the characterisation of outdoor environments. In addition, the contribution of the UV light to the redox reactions of MVOCs is very complex and still needs to be explored. Lab based and more real world measurements are needed to generate data-sets on atmospherically relevant chemical and molecular profiles.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.03.062.

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