

CRANFIELD UNIVERSITY

Shuaib Kamza Mahmud

Rapid Characterization Of Bioaerosols From Rural, Industrial and
Agricultural Environments

School of Energy Environment and Agrifood
Environmental Engineering

MSc

Academic Year: 2015 - 2016

Supervisor: Prof Frédéric Coulon
September 2016

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the degree of MSc

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ABSTRACT

There are significantly growing health concerns associated with exposure to bioaerosols. As culture methods for detecting bioaerosols can be time-consuming and results can be highly variable between samplings, it is important to have a rapid, low-level detection and reproducible method to quantify bioaerosols in the environment. Also, as the risk of exposure to bio aerosols is directly linked to the concentration and type of microorganisms, a much clearer quantification and characterization mechanism are needed if the temporal and spatial trends of infectious species are to be evaluated in different environments.

The aim of this study was twofold: 1) to compare two different bio aerosol sampling techniques, polycarbonate filters, and Coriolis®µ and 2) characterise bioaerosols by the analysis of PLFA biomarkers in order to identify the microbial communities present in contrasting outdoor environments (a WWTP, compost facility, three different farms, 2 parks and an urban area). Sample collection efficiency was 34% greater when collecting air samples with polycarbonate filters than with Coriolis in recreational parks where there is a low microbial activity. Areas with higher diversity index in the microbial communities corresponded to the ones with a higher amount of PLFAs such as the WWTP and the composting facility (1.986 and 1.624 respectively). Actinomycetes were only found in industrial and urban areas. No site specific PLFA was found and only the PLFA profile from the composting facility was different from the rest of the sites. This chemical approach therefore might not be enough to differentiate different bioaerosol profiles from outdoor environments.

Keywords: Chemical biomarkers; bioaerosols; PLFA;

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LIST OF ABBREVIATIONS

PLFA	Phospholipid fatty acid
MVOC	Microbial Volatile organic compounds
IS	Internal standards
CE	Collection Efficiency
RH	Relative Humidity
GC	Gas Chromatography
FID	Flame Ionization Detector
MDS	Multidimensional Scaling
G ⁺	Gram positive
G ⁻	Gram negative
CFU	Colony Forming Units
HEPA	High-efficiency particulate air

1 Introduction

Bioaerosols are aerosolized materials of particulate matter which are composed of fungal spores, wood dust, plant and animals debris, bacteria, viruses, and pollen (Douwes et al., 2003; Kummer and Thiel, 2008; Xu et al., 2011). The impact of bioaerosol emissions from urban, agricultural and industrial scenarios such as bio waste processing facilities on local air quality is of growing policy concern (Pearson *et al.* 2015). Deleterious health effects can arise following exposure to infective bacteria and viruses (NIOSH, 1997). Examples of known occupational health hazards related to bioaerosol exposure include asthma and chronic respiratory symptoms in the cotton and waste industry, acute and chronic decline in lung function in livestock farming (pigs and poultry grain), airway inflammation in the fiberglass industry, bronchial hyper responsiveness in slaughter houses, flu-like symptoms, fever and airway symptoms in waste and compost industry (Douwes *et al.* 2003). Further to this, there has been a lot of interests in bioaerosol science research over the past decade due to the threat of biological warfare, where pathogens are used intentionally with malicious intent to cause human fatalities as well as the destruction of plants and animals (Soomro et al. 2015). These biological pathogens such as anthrax (*Bacillus anthracis*) and small pox can be devastating if directly exposed to concentrations greater than 10^5 CFU m^{-3} (Soomoro et al 2015).

As the risk of exposure is directly linked to the concentration and type of microorganisms, much clearer quantification and characterization mechanisms are needed if the temporal and spatial trends of infectious species are to be evaluated in different environments. Most of the uncertainties surrounding personal exposure to microorganisms are also magnified by the ease with which bacteria and viruses can permeate through the air due to their fluidity and movements. Microorganisms are subject to transport and to turbulence in the same way as non-biological particles of similar physical characteristics (Baron, 2001). The measurement of the mass, or particle number concentration, of total inhalable particulate aerosol (particulate matter with diameter <10 μm , or PM_{10}) does not correlate with microbial concentration, and thus setting legislative limits on PM_{10} levels will not necessarily result in a reduction of bioaerosol loadings (Coker 2006).

The first hurdle in bioaerosol monitoring is to capture sufficient amounts of material to statistically generate distinguishable and reproducible patterns for bioaerosol identification

and classification. Studying such patterns requires the collection of data and environmental field sampling. During a sampling campaign, the three most common methods for collecting bioaerosols are impaction, impingement, and filtration (Macher 1999, Ginder 1990). Impaction uses centrifugal forces to transfer air into the sampler. Due to this velocity, the particles stick to the collection surface. A notable issue with direct impaction when using the Anderson sampler occurs when there are high concentrations of microorganisms transferred to the agar plate. This concentration makes bioaerosol enumeration difficult and might yield unreliable results (Henningson 1994, Macher 1999). Impingement uses a similar method as impaction but the key difference is that the samples are collected over a liquid suspension at a predetermined flow rate (Pillai 2002). Another common method is using a filter media. In this method, air is drawn during filtration by passing it through a porous media (polycarbonate filters) and the particles are bound to the filter media (Pillai 2002).

Microbial fingerprinting methods are used to provide a general overview of the microbial community studies in an environmental sample (Willers *et al.* 2015). Of particular relevance to this study, phospholipid fatty acids (PLFA) analysis is often employed as a molecular fingerprinting technique of microbial communities from the environment (Willers *et al.* 2015). PLFA are major cell components of Bacteria and Eukaryotes and can be found in most living organisms (Brock 2006). The fatty acid structure contains a lipid bilayer consisting of hydrophobic tails and a hydrophilic phosphate head joined by a glycerol molecule (Brock 2006, McClements & Gumus 2016). As bacteria and fungi die, the phospholipids degrade quickly and as such, the remaining contents of the sample left are assumed to be from living cells. The phospholipids of different groups of bacteria and fungi contain a variety of fatty acids that can serve as useful biomarkers in studying microbial communities from contrasting environments (Willers *et al.* 2015). Table 2 summarises the most common PLFAs found in bio aerosol samples and their correspondent microbial group. Extracting and analyzing the PLFA distribution allow a phenotypic 'airborne emissions fingerprint', and contributes to identify and quantify shifts in community giving us a snapshot of the entire airborne microbial community. This method has already been tested by Pankhurst *et al.* (2012) where the authors demonstrated that it is possible to distinguish microbial community composition of upwind and downwind air samples of composting facilities. This finding opens up the possibility that the bio aerosols generated from different sources (e.g. agriculture, woodland,

industrial or indoor environments) and operations such as composting, harvesting, and grass-cutting may be characterized in the form of a molecular profile or “fingerprint”. Ultimately, such techniques could be used to quantify the relative bio aerosol exposures residents experience from composting facilities and another emission from other sources.

In this study, PLFA analysis was used to characterize airborne microbial community fingerprint from 5 contrasting outdoor environments including urban (a park), two rural (farms) and two industrial (a compost facility and a WWTP) settings. Sampling was carried out using a combination of polycarbonate filters and Coriolis impinger to compare the sampling efficiency for bioaerosol biomass and community fingerprinting of ambient air samples.

2 Materials and Methods

2.1 Sampling locations

Air samples were collected from 5 contrasting environments including a waste water treatment plant (WWTP), a composting facility, a recreational park and two farms (see details in Table 1).

2.2 Air sample collection

Outdoor air samples were collected using two different sampling techniques including polycarbonate filters and Coriolis®µ impinger. Since polycarbonate filters consist of a dry collection method and the Coriolis®µ impinger is a wet collection method, it is important to compare the collection efficiency of both air sampling techniques.

2.2.1 Air sample collection with polycarbonate filters

Air samples were collected using polycarbonate filters (47 mm Whatman™ Cyclopore™ Polycarbonate Membranes; Fischer Scientific, Loughborough, UK) mounted on filter holders and SKC pumps (SKC Universal XR Pump Model PCXR8, SKC Ltd, Dorset, UK). Air samples were collected in triplicate using a flow rate of 40 L/min for 1 hour. After sampling, the filters were removed from the sampling heads with sterile tweezers and deposited into a sterile Eppendorf tubes (Fisher Scientific, Loughborough, UK). Samples were stored under dry ice during the sampling and at -20°C in the lab prior PLFAs analysis.

2.2.2 Air sample collection with Coriolis®µ impinger

The cyclone sampler Coriolis®µ (Bertin; Montigny le Bretonneux, France) was used to collect air particles onto 10 mL of deionized water. The duration of sampling was 20 min and a flow rate of 300 L/min was applied. After sampling, water samples were transferred into sterile plastic tubes and stored under dry ice during the sampling and at -20°C in the laboratory.

Table 1: Overview of the outdoor sampling sites and weather conditions.

Site		T(° C)	Wind Speed (m/s)	RH (%)	Dates	Description
Industrial	WWTP	5.22	4.7	80	25/02/16	Cranfield University waste water treatment plant. (Pilot hall). Sampling carried out at 50 m, 100 m and 200 m from the digester.
	Composting facility	6.30	5.2	82	9/02/16	The compost facility was medium size and it was located in Colchester. To access the facility there is a motorway. Closest receptors (houses) were at 200 m distance.
Recreational park	Colchester	9.16	1.8	71.4	8/02/16	The park was outside the city center of Colchester and was surrounded by a road and houses.
Agricultural	Colchester	6.7	2.3	76.3	10/02/16	The farm is located within a natural park. There is a small road with access to cars and a pathway transited by people and dogs. There are buildings of architectural or historic interest and conservation areas as well as small office buildings for people who work in the park. There were growing wheat and rapeseed.
	Silsoe	12.63	3.1	66.4	3/02/16	The land was next to a rural pathway where dogs and people were walking around. The land is usually cultivated for wheat. However, at the time of the sampling, there was no cultivation.

2.3 PLFAs analysis

Prior to extraction, water suspensions obtained from the Coriolis were filtered on sterile 47 mm diameter glass microfiber filters (Sigma-Aldrich, Dorset, UK). Then all filters were spiked with 50 μl of d_6 -benzene (1000 $\text{ng } \mu\text{l}^{-1}$) (Sigma-Aldrich, Dorset, UK) as internal standard (IS). PLFAs were then extracted using a modified version of the method described by Frostegård *et al.* (1991; 1993a). Briefly 15 mL Bligh Dyer solution (200 mL citrate buffer (0.15M citric acid dehydrate with 0.15 M trisodium citrate), 250 mL chloroform, and 500 mL methanol) and 1 mg of butylated hydroxyl toluene were added to each filter. After 30 minutes sonication, the samples were centrifuged at 2000 rpm for 10 minutes (Falcon 6/300; Sanyo, UK). After separating into two immiscible phases, the upper layer was removed and placed into a clean glass vial with PTE caps. This extract was separated into two phases through the addition of 4 mL chloroform and 4 mL citrate buffer 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 $< 37^\circ\text{C}$. The extracts were re-suspended in 200 μL hexane and analysed by GC-FID (Agilent Technologies 6890N) fitted with an HP-5 (Agilent Technologies, Cheadle, UK) 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 started at 50°C for 1 minute (split less mode); increased at 25°C per minute to 160°C ; increased at 2°C per minute to 240°C ; increased at 25°C per minute to 310°C ; 1 μL sample was injected and the injector temperature was set at 310°C . Quantification was done with a set of BAME and FAME standards (Sigma-Aldrich, Dorset, UK) (0.25-50 $\text{ng } \mu\text{l}^{-1}$). PLFA nomenclature are designated as X:Y ω Z, X represents the carbon chain length, Y represents the corresponding number of double bonds and Z indicates the specific location of the double bond from the aliphatic methyl " ω " end of the molecule. The *i* (iso) and *ai* (anteiso) prefixes designate the methyl branching on the acyl chain at the ω -1 or the ω -2 carboxylic group. "*cyc*" refers to cyclopropanol functional groups and also stand for the cis and trans configurations are represented by *c* and *t* respectively (Pankhurst *et al.* 2012). The 10M represents the methyl group on the tenth carbon atom of the carboxylic molecule. OH-hydroxyl fatty acids contain an additional alcohol group on the acyl chain, these are designated as α - or $-\beta$ when bonded to the second or third carbon from the carboxylic end of the chain (Sebastian *et al.* 2005). As an example 10-OH-C15:0, but if elsewhere on the chain it becomes α -hydroxy-C15:0 or 2-OH-C15:0. The most common PLFAs used as biomarkers are shown in Table 2.

Table 2: Phospholipid fatty acids used as a biomarker (adapted from Willers et al. 2015).

PLFA Biomarkers	Designation	References
Straight-chain saturated fatty acids 14:0; 15:0; 16:0; 17:0; 18:0	General bacterial marker	Balasooriya et.al. (2014); Lange et.al. (2014)
Methyl- branched fatty acids 10Me 16:0; 10Me 17:0; 10Me 18:0	Actinomycetes (Actinobacteria)	Hogberg et.al. (2013); Berulmann et.al. (2014); Dong et.al. (2014); Merchri et.al. (2014); Reinsch et.al (2014)
Monosaturated fatty acids 14:1 ω 5c; 15:1; 15:1 ω 6c; 16:1 ω 7t; 16:1 ω 9c; 16:1 ω 11c; 17:1; 18:1 ω 5c; 19:1 ω 9c; 19:1 ω 12c; 20:1 ω 9c; 20:1 ω 9t; 22:1 ω 9c; 22:1 ω 9t; 16:1 ω 5c;	Gram-negative bacteria Arbuscular mycorrhizal fungi	Buckeridge et.al. (2013); Djukic et. Al. (2013); Zheng et.al. (2013); Banks et.al. Banks et.al. (2014); Lange et.al. (2014); Zhang et.al. (2014)
16:1 ω 7c	Gram-negative bacteria Cyanobacteria; diatoms	Tavi et.al. (2013); Reinsch <i>et.al</i> (2014) Dijkman <i>et al.</i> (2010); De Carvalho and Caramujo (2014)
16:1 ω 5t; 16:1 ω 8c;	Type 1 methanotrophs (Gammaproteobacteria)	Knoblauch et.al (2008); Bodelier et.al. (2009);
17:1 ω 8; 17:1 ω 5;	Sulphate reducing bacteria	Buhring et.al. (2014)
18:1 ω 7c;	Cyanobacteria; diatoms Gram-negative bacteria	Dijkman et al. (2010) Tavi et al. (2013);
18:1 ω 7t	Gram- negative bacteria	Mechri et.al (2014);
18:1 ω 8c	Type II methanotrophs (alphaproteobacteria) Methylocystaceae & Beijerinckiaceae	Knoblauch et al. (2008); Bodelier et al.(2009)
18:1 ω 9c	Cyanobacteria; green algae Fungi	Buhring et al. (2014); De carvalho and Caramujo (2014) Dong et al. (2014); Fichtner et al. (2014); Reinsch et al.(2014)
Hydroxy-substituted fatty acids 2OH 12:0; 3OH 12:0; 2OH 14:0; 3OH 14:0; 2OH 16:0; 2OH 18:0	Gram-negative bacteria	Buckeridge et al. (2013); Fichtner et al. (2014); Wei et al. (2014)

2.4 Data analysis

PLFA chromatograms were integrated using Chemstation software. Linear regression of each PLFA compound was plotted in Excel 2010 for quantitation. Furthermore, the PLFA concentrations were corrected with the extraction efficiency of the internal standard. The similarity between PLFA profiles was calculated using binary data for the presence of particular peaks or bands respectively (Jaccard coefficient). A 2-dimensional non-metric

multidimensional scaling ordination (2D-nMDS) was produced from the resulting proximity matrix using Primer 6 Software (Primer-E Ltd., UK; Clarke & Warwick, 2001). The diversity of microbial species was determined using the Shannon index (Equation 1) and PLFAs evenness (Equation 2), respectively:

$$H' = - \sum_{i=1}^S P_i \ln P_i \dots \dots \dots \text{Eq. 1}$$

where H' = shannon index; S = the number of PLFA's Encountered, P_i = Individual vs total PLFA's ratio, \sum = Total Sum of PLFA's encountered, \ln = natural log

$$E = \frac{H'}{(\ln H)} \dots \dots \dots \text{Eq. 2}$$

where E = PLFA evenness, H' = Shannon Index, \ln = natural Log

3 Results

3.1 Sample collection method efficiency

Two sites, a WWTP and a recreational park in Colchester were sampled simultaneously with both sampling techniques, polycarbonate filters, and Coriolis to test the sampling efficiency.

From the WWTP samples collected with the filters, 12 PLFAs were identified with a total average concentration of 62,286 ng m⁻³. whereas with the Coriolis 11 PLFAs with a total biomass of 49,959 ng m⁻³, meaning that sampling in the WWTP with polycarbonate filters was 20% more efficient than with Coriolis impinger (Table 3). The 5 additional PLFAs identified with the SKC polycarbonate filter device included 11:0, i13:0c, i15:0, Me17:0 and 17:0c.

At the recreational park in Colchester, 16 PLFAs were identified when sampling with polycarbonate filters (59,986 ng m⁻³) and 10 PLFAs with the Coriolis (39,605 ng m⁻³), thus, filters were 34% more efficient than the Coriolis in the recreational park (Table 3). Excluding the unknown PLFAs, the PLFA's recovered in the filters revealed an extra 8 PLFA's including i15:0, 16:1w5, 17:0br, 17:1, i17:0, a17:0, 18:2w6, 18:0c.

Due to the loss of PLFAs when sampling with the Coriolis, this technique underestimated the total biomass present in the air, this is most noticeable in the park, where there is a

lower microbial activity (background levels). The microbial community structure was underestimated when sampling with the Coriolis in the park as some microbial species were missing (G+, G- bacteria and saprotrophic fungi respectively). In the WWTP for instance, similar microbial communities were identified when sampling with both, Coriolis and filters.

Since sampling with filters was considered to be a better approach for outdoor environments, the next studies were carried out using this technique.

3.2 Comparison of the Bioaerosol biomass and community fingerprints of the outdoor environments

Among the 5 sites sampled with polycarbonate filters, the compost facility had the highest number of PLFA's (40) and higher average biomass $178,719 \text{ ng m}^{-3}$ (Table 4). Colchester farm was the second location with higher biomass ($70,147 \text{ ng m}^{-3}$) and here 16 PLFA peaks were identified. The next location with higher biomass was the WWTP ($62,286 \text{ ng m}^{-3}$), with 11 PLFA peaks. In Colchester park, 16 PLFA peaks were identified with an average biomass of $59,986 \text{ ng m}^{-3}$.

Silsoe Farm was the location with lower biomass $21,397 \text{ ng m}^{-3}$ and only 5 PLFAs were identified, probably because this farm was not in use as there was no activity such as plowing or mowing of the fields to agitate movement of debris and particles. The farm also had a narrow footpath with minimal pedestrian use.

The PLFAs i15:0, 16:1w5 and 18:2w6c were common in all the sites. There were not found any specific PLFA for a single location but in the composting site, there were 10 unknown PLFAs. All the sites had G⁺ bacteria, saprotrophic bacteria, and methanotrophs bacteria. The WWTP, compost facility and the recreational park were the only ones where actinomycetes were found. These bacteria are associated with contamination and these sites were the ones with a higher concentration of PLFAs. Actinomycetes are frequently found in compost facilities and WWTP (Taha et al. 2007; Cydzik-Kwiatkowska & Zielińska 2016). In cleaner sites such as Silsoe farm and Colchester farm, this bacteria was not identified.

Table 3: Sample collection efficiency comparison

Nb	PLFA	Taxonomy	WWTP		Recreational park Colchester	
			filter	impinger	filter	impinger
1	11:0		5876			4098
2	12:0				3151	
3	unknown		5845	4709	3657	2925
4	13:0				5844	2944
5	i13:0c		5849			
6	a13:0				3665	
7	15:0	bacteria				4677
8	i15:0	Gram positive bacteria	5847		3440	
9	a15:0	Gram positive bacteria	5459		3670	
10	16:1w5	Type 1 methanotrophs Methylococcaceae		4687	3649	
11	16:1c		5858	4681		
12	16:0	bacteria			3026	4366
13	16:0c	bacteria		4685		
14	Unknown					4681
15	Me17:0 i	Actinomycetes		4671	3649	2921
16	Me17:0 br	Actinomycetes	5849		3652	
17	unknown				3225	
18	unknown					
19	unknown					
20	17:0c	bacteria				
21	17:1w8t	Sulphate reducing bacteria (stressed)	5421			
22	17:1w8c	Sulphate reducing bacteria		4682		
23	17:1	Gram negative bacteria			3968	
24	17:1w5	Sulphate reducing bacteria				
25	i17:0	Gram positive bacteria			3163	
26	a17:0	Gram positive bacteria			3163	
27	18:0					
28	18:2w6c	Saprotrophic fungi			3648	
29	18:0c	bacteria	5415		5416	
30	19:10c			4653		
31	unknown		5411	4515		
32	unknown			4681		
33	unknown			4697		4341
34	unknown		5456	3277		4327
35	unknown					4325
36	unknown					
37	unknown					
38	unknown					
39	19:0					
40	20:00					
Amount of PLFA Collected (ng m⁻³)			62286	49938	59986	39605
Difference			12327		20381	
			20%		34%	

Table 4: PLFAs (ng m⁻³) identified across 5 different sites using polycarbonate filters

Nb	PLFA	WWTP	Composting Facility	Recreational park Colchester	Colchester Farm	Silsoe Farm	Taxonomy
1	11:0	5876	4747		3152		
2	12:0		3651	3151			
3	unknown	5845	3676	3657	3650	5843	
4	13:0		5121	5844	3649		
5	i13:0c	5849	3661		5859		
6	a13:0		2920	3665	5413		
7	15:0	5847	3714		4752		bacteria
8	i15:0	5459	2951	3440	2908	3225	Gram positive bacteria
9	a15:0		4700	3670	5372		Gram positive bacteria
10	16:1w5	5858	5901	3649	3652	5846	Type 1 methanotrophs Methylococcaceae
11	16:1c		3650				
12	16:0		5420	3026		3241	bacteria
13	16:0c		5428				bacteria
14	Unknown		5926				
15	Me17:0 i	5849	4372	3649			Actinomycetes
16	Me17:0 br		5859	3652			Actinomycetes
17	unknown		5851	3225			
18	unknown		5918				
19	unknown		5861				
20	17:0c	5421	3650		3275		bacteria
21	17:1w8t		3017		3652		Sulphate reducing bacteria (stressed)
22	17:1w8c		2986		5414		Sulphate reducing bacteria
23	17:1		2952	3968			Gram negative bacteria
24	17:1w5		3675		5406		Sulphate reducing bacteria
25	i17:0		3653	3163			Gram positive bacteria
26	a17:0		3653	3163			Gram positive bacteria
27	18:0		5455				
28	18:2w6c	5415	3761	3648	5420	3241	Saprotrophic fungi
29	18:0c		5416	5416			bacteria
30	19:10c	5411	5417				
31	unknown		2962		5421		
32	unknown		5458				
33	unknown	5456	5406				
34	unknown		5415				
35	unknown		3656				
36	unknown		3665				
37	unknown		5410				
38	unknown		5411				
39	19:0		2902		3152		
40	20:00		5472				
Amount of PLFA (ng m⁻³)		62286	178719	59986	70147	21396	

3.3 Bioaerosol community structure

A cluster diagram was elaborated to compare the similarities in PLFAs identified in the environmental samples (Figure 1). WWTP and Colchester farm were very close to each other sharing 60% of similarity on the PLFA profile. WWTP, the 2 farms and Colchester park had 40% of similarity on the PLFAs. All the locations together only shared 20% similarity. According to the MDS plot, only the compost facility was the site which was distinguishable from the other sites. The WWTP, farms and the recreational park could not be differentiated. Molecular fingerprint obtained with the PLFA analysis on its own therefore did not provide a good resolution to distinguish rural, urban and industrial sites as there were no specific PLFA profiles according to environment type apart from the compost facility.

The two farms were big open fields but only in Colchester farm there were growing wheat and rapeseed. At Silsoe farm, there were farming activities at that time, so it was being used as a park as it had footpaths with accessibility to people. The park in Colchester contained only had trees and there were no site activities such as concerts or grass maintenance that could generate bioaerosols in high concentrations, which is possibly the reason why this location shared more similarity with Silsoe farm. Surprisingly, the WWTP and Colchester farm shared high similarity (60%), which could be attributed to the low activity at the WWTP at that moment and because the WWTP was surrounded by fields. The composting facility was the site that differed most from all the rest as it was a location that contained high levels of organic waste.

Since the collected data are affected by weather conditions, and each site was only sampled once on a different day, probably different profiles would have been obtained in another period of time. During this sampling campaign, temperature varied from 5.22-12.63°C, relative humidity of 71.4-82% and wind speed 1.8-5.2 m/s between the sampling dates (see Table 1).

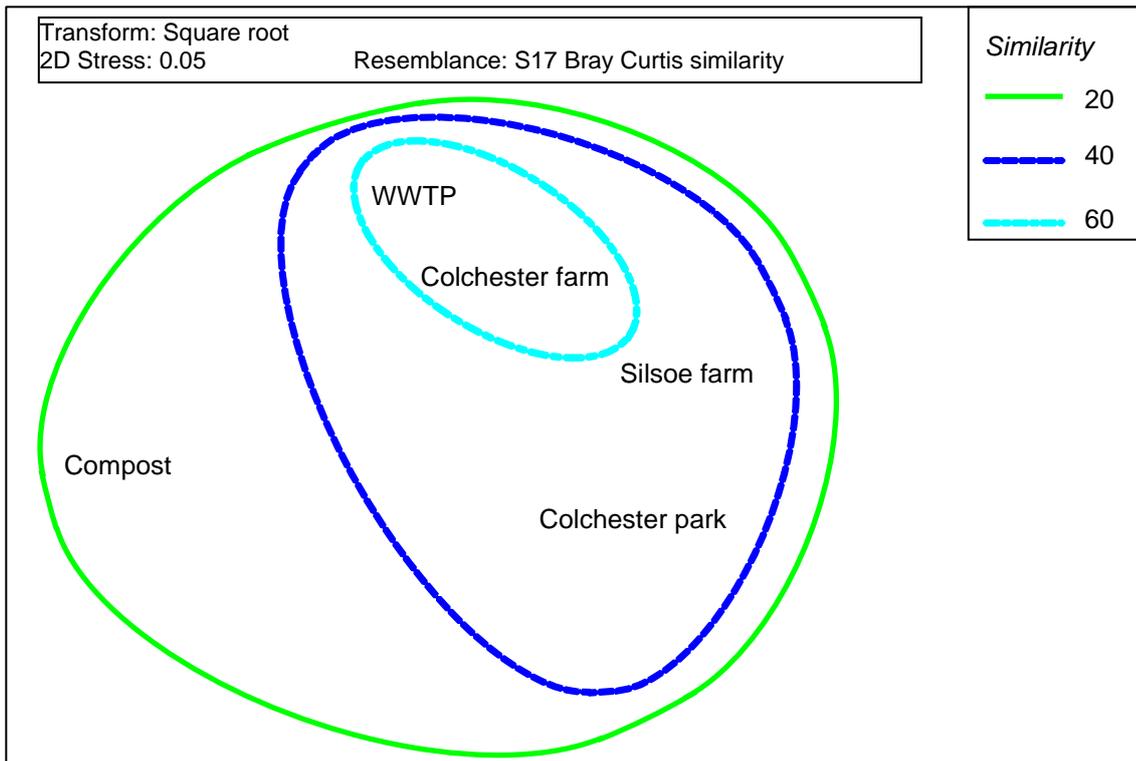


Figure 1. Microbial community structure assemblage of the different sites based on PLFA profiles analysis obtained using polycarbonate filters.

Shannon's index was used to quantitatively evaluate the diversity of the microbial communities per sampling location (Table 5). The evenness quantifies how equal the community is numerical. The less evenness in communities between the species (and the presence of a dominant species), the lower diversity in the community is and *vice versa*. All the locations shown have similar evenness index (0.999-1) indicating that the microbial communities were distributed equally. The diversity was higher in the sites with more concentration of PLFAs such as the composting facility and the WWTP (1.956 and 1.624 respectively). The locations with lower levels of PLFAs, i.e. the farms and the recreational park had less diversity (Colchester farm = 1.374, recreational park = 1.184, Silsoe farm = 1.047).

Table 5: Shannon index and evenness across all environmental PLFA profiles.

Sites	Shannon Index	Evenness	Total no of PLFAs	ΣPLFAs (ng m ⁻³)
Composting facility	1.986	0.9999	21	8275
WWTP	1.624	0.9999	8	3116
Colchester Farm	1.374	1.0000	5	1933
Recreational park in Colchester	1.184	1.0000	3	1161
Silsoe Farm	1.047	1.0000	2	775

4 Discussion

The sampling technique that captured higher concentration of PLFAs was the polycarbonate filters, which were connected to the pumps operating at a flow rate of 40 L/min for 1h. The settings for the Coriolis were 300 L/min flow rate for 20 min. This flow rate is very high and previously has been reported that there is a sample loss when using this technique (Taha et al. 2007; Cydzik-Kwiatkowska & Zielińska 2016). These preliminary results suggested that when sampling at sites with high microbial activity such as a WWTP, there was only a 20% of the difference in sample collection efficiency between polycarbonate filters and Coriolis, being the filters capturing a higher amount of microbes. At these locations, the use of the Coriolis impinger could be suitable as samples can be obtained in a short period of time compared with the filters (20 min vs. 1h). In sites with background levels of bioaerosols such as recreational parks, for instance, the sampling efficiency of the Coriolis resulted in 34% less compared to the filters. Therefore, the background levels can lead to the underestimation of microbial biomass present in the air when sampling with Coriolis and for this type of locations sampling with polycarbonate filters would be more appropriate.

The results of PLFAs analysed across all the environmental samples using polycarbonate filters were plotted in cluster diagrams and MDS plots in order to identify and compare differences between different rural, urban and industrial environments. The use of fatty acids as molecular fingerprint allowed the identification of bioaerosol biomass and microbial characterization but only the PLFA profile from the compost facility was distinct from the rest of the environments. The location with the highest concentration of PLFAs was the composting site (178,719 ng m⁻³). PLFA concentrations in the farm in Colchester, the WWTP and Silsoe farm were 70,147 ng m⁻³, 62,286 ng m⁻³ and 21,397 ng m⁻³ respectively. The microbial species diversity calculated from the Shannon index showed

that the sites with the highest diversity were the ones with a higher amount of PLFAs. The diversity index for the composting site was 1.986, followed by the WWTP, 1.624, the farm in Colchester, 1.374, the recreational park, 1.184 and Silsoe farm, 1.047.

The common PLFAs in all the sites consisted of 15:0i, 16:1w5 and 18:2w6. These PLFAs mainly corresponded to G⁺ bacteria, methanotroph bacteria, and saprotrophic fungi respectively. Microbial species common to all the sites were saprotrophic bacteria, G⁺, G⁻ and methanotroph bacteria. Actinomycetes were only present in industrial and urban sites, this implies that they are indicators of contaminated areas. There were also not found any site specific PLFA for a particular location. The compost facility had additionally 10 unknown PLFAs.

As a difference with indoor environments, outdoors factors such as stage of compost, type of crop, farming activity on the piece of agricultural land and changes in meteorological patterns would affect the microbial community as well as the sample collection. These results were obtained from sampling in winter and probably a different PLFA profile can be obtained in summer.

5 Conclusion

The project focused on determining a method to compare the microbial footprint in order to obtain a community snap shot and characterise bioaerosols from contrasting environments. To achieve this, two experimental setups using the Coriolis and Filters were used in the project in order to determine their collection efficiencies whilst sampling bioaerosols in five contrasting environments. The filters had a higher collection efficiency than the Coriolis and hence is better suited for the sampling campaign.

PLFA sampling on 5 different sites (a WWTP, a Composting facility, a recreational park and 2 farms) using the Coriolis and filters to capture PLFA's prior to laboratory analysis. The sampling technique that is best suited for sampling in the environment is the Filters as it had a 34% higher efficiency than the Coriolis. This is because the filters were able to capture a greater number of PLFAs than the Coriolis. There were no site specific PLFAs. Actinomycetes were found in the Composting site and WWTP. These industrial sites are highly contaminated sites compared to the rural farms in Colchester and Silsoe.

Distinguishing PLFA profiles from contrasting environments was possible on the composting site as it had the highest concentration of microbes and PLFA diversity. The sites have to have therefore, a considerable amount of PLFAs to differentiate outdoor bioaerosols. Although it is possible to differentiate bioaerosols from contrasting sites, sampling at different seasons (winter vs. summer) might give a better description of the microbial community as the microbial species can vary.

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