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Lime and odour: A preliminary investigation into the effect of hydrated lime on the volatiles emitted from human remains☆

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ABSTRACT

The location of human remains is performed primarily through the aid of cadaver detection dogs, which rely on the malodour produced through decomposition of decaying bodies. Malefactors will attempt to conceal these putrefactive odours through chemical additions such as lime, which is also wrongly believed to accelerate decomposition and prevent the identification of the victim. Despite the frequency of lime in forensic applications, to date no research has been performed to determine its effect on the volatile organic compounds (VOCs) released during human decomposition. This research was therefore conducted to ascertain the effects of hydrated lime on the VOC profile of human remains. Two human donors were used in a field trial at the Australian Facility for Taphonomic Experimental Research (AFTER): one donor was covered with hydrated lime, and the other had no chemical additions acting as a control. VOC samples were collected over a period of 100 days and analysed using comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GC×GC-TOFMS). The volatile samples were accompanied by visual observations of how decomposition progressed. The results showed that lime application decreased the rate of decomposition and decreased total carrion insect activity. Lime increased the abundance of VOCs during the fresh and bloat stages of decay, however the abundance of compounds plateaued during active and advanced decomposition and were much lower than those detected from the control donor. Despite this suppression of VOCs, the study found that dimethyl disulfide and dimethyl trisulfide, key sulfur-containing compounds, were still produced in high quantities, and can thus still be used to locate chemically altered human remains. Knowledge of the effects of lime on human decomposition can inform the training of cadaver detection dogs, and ensure a greater chance at locating victims of crimes or mass disasters.

1. Introduction

Canines (*Canis familiaris*) are used by law enforcement agencies and search-and-recovery teams to locate missing persons, who can be the victims of events such as crimes or mass disasters [1]. Cadaver-detection dogs (CDDs), also referred to as human remains detection dogs (HRDDs), are the most commonly employed method of locating decedents, as their highly developed olfactory system allows them to detect small amounts of human tissue and to find remains that may be hidden to human search teams [2]. CDDs are trained on a variety of decomposition odour sources, including human remains, blood, contaminated clothing, soil, decomposition fluids, and synthetic compositions made to imitate the scent of human decay [1,3,4]. The aim of the dogs' training is to familiarise them with the human-specific odour of decay to enable them to locate remains in the field [1]. To locate remains CDDs rely on the odour plume released by decomposition, which is a complex amalgam of volatile organic compounds (VOCs) that changes as the body degrades [1,5]. In brief, decomposing remains will progress through several distinct stages: fresh, bloat, active decomposition, advanced decomposition, and dry remains /skeletonisation [6], with the peak of VOC production normally occurring during active and advanced decomposition [7,8]. There are a number of compound groups and individual compounds (i.e. biomarkers) that have been known to be present during decomposition. In particular, dimethyl disulfide (DMDS), dimethyl trisulfide (DMTS), and other polysulfuric compounds are highly reported within the post-mortem human volatile profile [9,10].

Perpetrators often try to conceal a crime by destroying evidence, hiding a corpse, hampering identification, and suppressing decomposition

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odours. Often, chemicals such as alkalines or acids are used to avoid the detection of remains by police and CDDs [11]. One of the chemicals that is often used to mask malodours and hinder recovery is lime. Lime is a generic term that encompasses several derivatives of calcium carbonate ($CaCO_3$), including calcium oxide (CaO, or quicklime, unslaked lime, or burnt lime), calcium hydroxide ($Ca(OH)_2$, variously known as hydrated lime or slaked lime), and other non-pure derivatives such as silica-containing hydraulic lime [11–13]. Hydrated lime and quicklime are often employed by criminal organisations [14,15] and individual perpetrators [16–19] with the intention to increase the rate of decomposition and suppress smell [13,20]. Lime has also been found on human remains in single graves and mass burials associated with conflict [21–24]. Its use is evident in World War I [21], World War II [22], the Spanish Civil War [23], and during the Portuguese Colonial War [24]. In the past, lime has also been used in graves resulting from disease [25–27]. When associated with disease, it is thought to discourage bacterial growth associated with infection and to stop the spread of pathogens [13,23,26], which is assumed to go hand-in-hand with odour. Very recent examples are the COVID-19 outbreaks in Iran [28] and Denmark [29], where lime was added to mass graves to “manage decay and odour” [28] and “prevent virus transmission” [29].

Despite lime’s frequent use in both clandestine and mass burials, there is little research surrounding the effects of lime on human decomposition. The belief that lime accelerates decomposition is currently unsupported. In 1932 Laudermilk [30] was the first to disprove this theory by using pieces of beef coated in quicklime and showed the preservatory effects of the chemical. The effects of quicklime and hydrated lime on entire pig carcasses were studied by Thew [31] and Schotsmans et al. [12,13,20] over a period between 6 months and 3.5 years. The results showed that both hydrated lime and quicklime retard the rate of decomposition if present in a burial environment, but do not stop it. Quicklime instigated an initial acceleration in the rate of decay that was not observed for hydrated lime. This was due to the exothermic hydration reaction of quicklime with moisture and decomposition fluids, causing internal temperatures to increase, encouraging bacterial digestion and thus accelerating putrefactive change. Despite its initial acceleration, quicklime was observed to slow decay on the longer term, which is consistent with the effect of hydrated lime [13]. Since lime is only applied to the outside of a body, putrefactive decay continues from the inside by micro-organisms derived from the body itself. Bacterial population densities on the exterior of the carcass appeared to be initially reduced by lime, with the surfaces of the pigs exposed to the chemicals carrying fewer colonies than at the start of the experiment [13]. However, on the longer term bacteria appeared to flourish again due to restoring pH conditions [12]. The end result is skeletonisation [12,13,20,31].

In addition to the myth that lime accelerates decomposition, it is also believed to reduce odour production; hence its use to conceal putrid remains from dogs and search teams [13,23]. Only one study, published in 1986, explores the effect of lime on odour production, specifically on the odour produced by sludges [32]. Toogood and Diaper [32] examined the odour from sludge samples, and found that lime initially decreased the smell released, however after two weeks it increased beyond that of their control sludge. This suggests that lime’s effect on decomposition odour would cause a similar preliminary reduction, however as the study was not conducted on decomposing remains, an absolute statement on lime’s effect on the VOCs released from decay cannot be made.

The following study assessed the effects of hydrated lime on human decomposition rates and the production of volatile compounds. Two donated human cadavers were studied for 100 days post-placement, with one donor covered with hydrated lime and one donor without lime to act as a control. The human remains and lime were visually assessed, and VOC samples were actively collected and analysed using two-dimensional gas chromatography-time-of-flight mass spectrometry (GC×GC-TOFMS). The findings of this study can be used to aid in the location of missing persons and CDD training when concealment/chemical alterations are attempted.

2. Materials and methods

2.1. Field site

The experiment was performed at the Australian Facility for Taphonomic Experimental Research (AFTER), privately owned by the University of Technology, Sydney (UTS). The site is located in the Hawkesbury region of western Sydney and consists of 4.86 ha of Cumberland Dry Sclerophyll Forest [33].

Weather was monitored by a HOBO® U30 Weather Station erected near the entrance to the facility, and readings of temperature (°C) and rainfall (mm) were taken on an hourly basis. Additional hourly temperature (°C) readings were taken from local data loggers using HOBO MX2302 (Onset Computer Corporation) placed on and near the donors. These readings were used to capture the temperature change of the remains as decomposition progressed. On Donor 1 (Control), one sensor was placed on top of the torso and one underneath the torso, with both between the donor and the T-shirt. On Donor 2 (Lime), one sensor was placed on top of the torso and one underneath the donor, with lime added after the sensor placements.

2.2. Experimental design

Two donors were used for the purpose of this study (Table 1). Both donors were received through the UTS Body Donation Program overseen by the Surgical and Anatomical Science Facility (SASF), and consent was provided by each donor in accordance with the NSW Anatomy Act of 1977. The use of these donors was approved by the UTS Human Research Ethics Committee (UTS HREC REF NO. ETH18–2999).

To account for the seasonal disparity between the donor placements, accumulated degree-days (ADD) were used as a measure of time passing in addition to days post-placement. ADD are the cumulative sum of the average temperature per day starting from the date of placement, and were first proposed used by Vass et al. [34] to aid in post-mortem interval (PMI) determinations.

Both donors were stored at 4 ºC for four days prior to placement at AFTER. The donors were placed on the surface in a supine position in separate plots measuring 5 × 5 m^2 to avoid cross contamination from other donors [35]. Metal anti-scavenging cages measuring 1.2 m (W) × 2 m (L) × 0.9 m (H) were placed over each cadaver to prevent vertebrate scavenging activity whilst allowing insect activity.

Donor 1 was fully clothed due to their use in a concurrent textile study. This follows the AFTER policies on donor collaborations, where donors are employed across multiple studies in order to utilise the remains most effectively. The clothing consisted of a cotton t-shirt (Kmart, Wesfarmers Ltd., Sydney), cotton boxer briefs (Kmart, Wesfarmers Ltd., Sydney), denim jeans (Kmart, Wesfarmers

Table 1
Donor information (BMI: Body Mass Index; BSI: Body Surface Area).

Information	Donor 1 - Control	Donor 2 - Covered with lime
Date received	12 August 2020	2 October 2020
Age	96	83
Sex	Female	Male
Height	154 cm	175 cm
Weight	53 kg	68 kg
BMI and BSA	22.3 kg/m^2 and 1.50 m^2	22.2 kg/m^2 and 1.83 m^2
Cause of death	Pneumonia	Congestive cardiac failure, chronic kidney disease

Table 2
Post-placement sampling days of Donor 1 (Control) and Donor 2 (Lime).

Donor	Sampling days (post-placement)														
Donor 1 (Control)	0	1	3	5	7	9	11	14	17	21	27	41	62	82	102
Donor 2 (Lime)	0	1	3	7	11	14	17	21	25	27	40		61	82	102

Ltd., Sydney), one thick woollen sock (Kmart, Wesfarmers Ltd., Sydney), and one nylon sock (Kmart, Wesfarmers Ltd., Sydney). Donor 2 was placed without clothing in a separate plot and was completely covered with two 20 kg bags of hydrated lime (Independent Cement and Lime Pty Ltd., Bunnings Warehouse, Sydney) after an initial volatile sampling. The feet of the deceased were only partially covered with lime as they were unable to be covered completely due to the nature of the powder (Fig. S1). There has been no correlation found between clothing and a change in the abundance of VOCs detected from human donors [36], and therefore Donor 2 was placed unclothed. Clothing acts as a physical barrier that can have a minor effect on insect succession and consequentially the rate of decomposition [37], however as hydrated lime is toxic to insects that colonise human remains, the presence of the lime negates any effect clothing would have on possible insect activity.

VOCs samples (n = 3) were collected on each sampling day (Table 2), where the progression of decomposition for the cadaver being sampled was recorded through visual observations and photographs, then categorised according to the stage of decomposition. The state of the lime on Donor 2 was also recorded.

To remove any VOCs produced by the environment from the statistical analysis, environmental plots were established for both donors. These environmental plots were sampled simultaneously with their respective donor. The environmental plot for Donor 1 contained the same clothes the donor was dressed in (Environmental 1). Two more environmental plots were established for Donor 2: one without lime (Environmental 2) measuring the outdoor environmental VOCs in the trial area, and one containing 40 kg of hydrated lime from the same brand as used for Donor 2 (Environmental 3) to determine the lime's production of volatiles. Volatile samples of the lime (Environmental 3) were collected on Day 0, Day 22, and Day 41. When performing statistical analysis, the volatiles produced by Environmental 2 were removed from Donor 2, and the remaining compounds were compared to the VOCs released by Environmental 3. pH readings of the lime were also collected to find the initial average (n = 8) pH and to determine whether the pH changed over time. Readings from eight different locations in the lime were taken with a pH probe (Mettler Toledo Seven2Go S2) on Day 0, Day 22 and Day 41, and on Day 62, Day 84 and Day 102 for limed donor 2.

2.3. Volatile sample collection

An aluminium hood measuring 1.20 m (W) × 1.90 m (L) × 0.90 m (H) was used to sample the headspace above the decomposing remains. Prior to sampling, the hood was cleaned with acetone (POCD Scientific, Sydney, Australia) and vented for 10 min to ensure the acetone's complete evaporation. The hood was then placed over the remains and left for 15 min for headspace to accumulate. Once the headspace had accumulated, a dual sorbent tube packed with Carbograph 5TD/Tenax® TA (Markes International Ltd.) was connected to the hood's sampling port, and headspace samples were actively collected using an ACTI-VOC™ low-flow sampling pump (Markes International Ltd, Llantrisant UK) for 10 min at a flow rate of 100 mL/min. After collection, the sorbent tubes were sealed with brass Swagelok® style caps fitted with polytetrafluoroethylene (PTFE) ferrules (Swagelok Company, Caringbah, Australia), wrapped in aluminium foil, and transported to the laboratory in an air-tight container. They were then stored in the laboratory at 4 ºC until analysis.

2.4. Volatile sample analysis

Prior to analysis, an internal standard of 0.2 µL of 10 ppm chlorobenzene-d_5 (analytical standard, Sigma-Aldrich, Macquarie Park, NSW, Australia) was injected into each sorbent tube. After this, the samples underwent thermal desorption (TD) using a Markes Unity 2 Thermal Desorber (Markes International Ltd., Gold River, California, USA) and a Series 2 ULTRA multi-tube autosampler (Markes International Ltd.). Sorbent tubes were desorbed at 300 ºC for 4 min, upon which the analytes were carried at 25 mL/min to a general-purpose cold trap (Tenax® TA/Carbograph 1TD) using helium (high purity grade 5.0, BOC, Sydney, NSW, Australia) as the carrier gas. The cold trap was held at − 15 °C for 4 min then desorbed at 300 °C for 4 min with a flow of 25 mL/min. This connected to the Pegasus 4D GC×GC-TOFMS (LECO, Castle Hill, NSW, Australia) using a 1 m uncoated silica transfer line (Markes International Ltd.).

The GC×GC was equipped with a 30 m × 0.25 mm × 1.40 µm midpolarity Crossbond phase Rxi®− 624Sil Ms primary column (Restek Corporation, Bellefonte, PA, USA), and a 2 m × 0.250 mm × 0.5 µm film thickness polar phase Stabilwax® second column (Restek Corporation). The columns were connected using a SilTrite™ µ-Union (SGE Analytical Science, Ringwood, VIC, Australia), and helium was used as the carrier gas at a flow rate of 1.0 mL/min. The primary oven was held at 35 ºC for 5 min, then increased to 240 ºC at a rate of 5 ºC/min. The oven was held at 240 ºC for 5 min. The modulator offset was set to + 5 ºC, and the secondary oven offset was + 15 ºC, with a modulation period of 5 s. The transfer line was kept at 250 ºC, and mass acquisition was performed between 29 and 450 amu at 100 spectra/s. The ion source for the mass spectrometer was kept at 200 ºC and the electron ionisation energy was kept at − 70 eV with a 200 V offset detector voltage.

2.5. Data processing

Data processing was performed using ChromaTOF® (version 4.72.0.0, LECO) and the software's statistical compare function. A signal-to-noise ratio of 150 was used with a baseline offset of 0.8. Peak widths of 30 s and 15 s were used for the first and second dimensions respectively, and compounds were tentatively identified using the National Institute of Standards Technology (NIST) spectral library with a minimum similarity match of 80%. Peak alignment was performed between the samples with a spectral match of 75%. Samples were then classed according to their donor or environmental plot (Donor 1: donor n = 32, environmental n = 31; Donor 2: donor n = 32, environmental n = 32; Lime plot: n = 8). An analyte was retained if it was present in at least two samples or in 30% of samples within a class. After alignment, compound peak areas were normalised against the internal standard. Once the compound list was established, all analytes were exported into Microsoft Excel, where any column contamination, CO_2, oxygen, methanol, the internal standard, and field contamination were removed using the following method: compounds were retained if they were exclusive to the donor samples, or if the donor response was ≥ 50% when divided by the sum of the donor and environmental responses and multiplied by 100. This threshold accounts for extracting useful information from the donor headspace, whilst excluding any

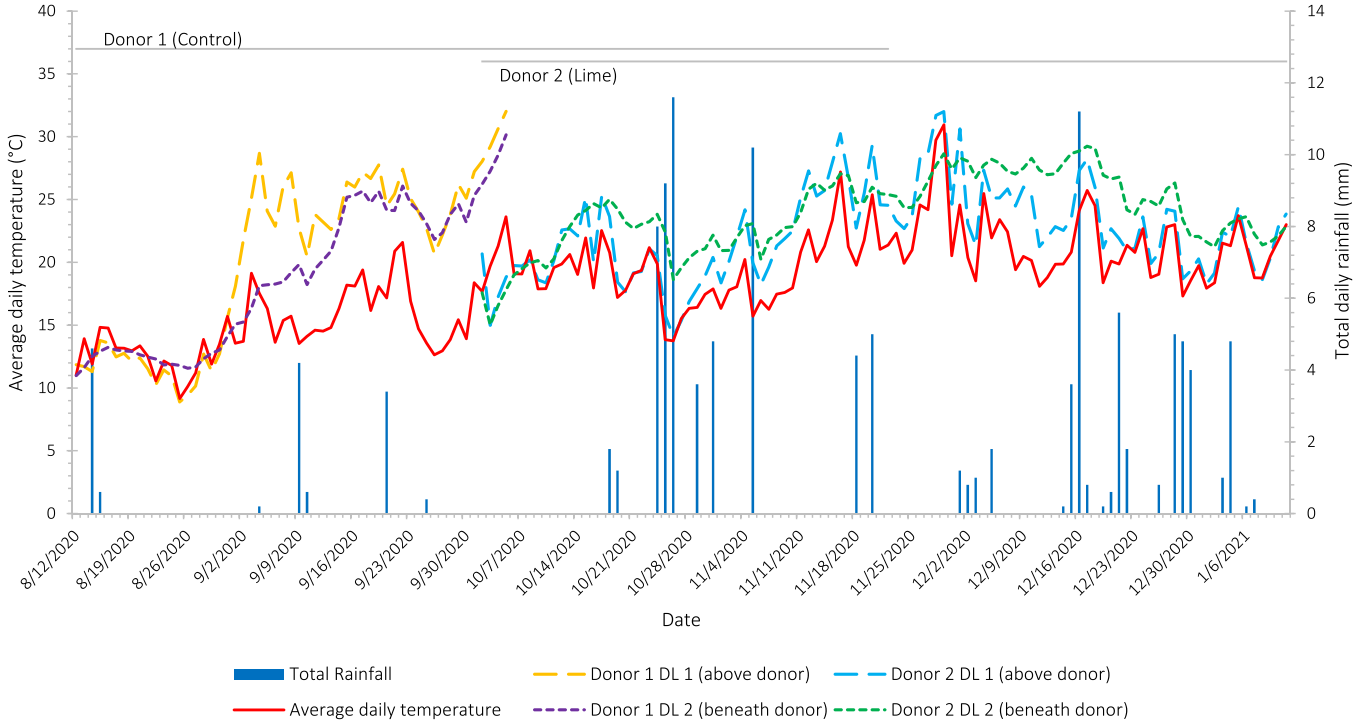

Fig. 1. Average temperature and total rainfall readings per sampling day (Day 0 – 100), and daily average temperature data collected from each data logger (DL) on both donors. *Gap in Donor 1 DL 1 and Donor 1 DL 2 is due to the HOBO MX2302 data logger running out of battery power on 6/10/2020.

environmental background contamination. Compounds were then sorted into the following classes: alcohols, aldehydes, anhydrides, aromatics, carboxylic acids, esters, ethers, halogenated compounds, hydrocarbons, ketones, nitrogen-containing (N-containing) compounds, sulfur-containing (S-containing) compounds, terpenes, and other, and then graphed.

3. Results and discussion

3.1. Environmental data

The ambient average daily temperate and total daily rainfall readings collected over the sampling schedules of Donor 1 and Donor 2 are presented in Fig. 1. In addition to the weather data, the average daily temperature reading for each data logger probe is also shown in Fig. 1 (i.e. two probes per donor - one above and one below the individual).

The trial using Donor 1 (Control) lasted from August 12th, 2020 (Day 0) until November 22th, 2020 (Day 102). The temperature recorded on the surface of the donor (data logger 1) began to increase after Day 21 and spiked at 28 °C on September 4th, 2020 (Day 23). This peak was due to the heat produced from increased larval aggregations on the remains [38] in addition to the rising temperature of the environment. The temperature recorded under the remains (data logger 2) experienced a delayed increase with the environmental temperature. The highest temperature recorded by both data loggers was on October 5th, 2020 (Day 54) due to an increase in environmental temperature, with a reading of 32 °C on the surface of the remains and 30 °C underneath. The average daily temperature was 24 °C. The lasting disparity between the environmental temperature and the temperatures recorded by the data loggers is surprising, as maggot activity causing an increase in the temperature of the remains was recorded as ceased on Day 40. This indicates that the body was retaining the heat produced by the larval masses long after the activity had stopped. The readings for Donor 1 on both data loggers stop on Day 54 due battery failure of the HOBO MX2302.

The trial using Donor 2 (Lime) lasted from October 2nd, 2020 (Day 0) until January 11th, 2021 (Day 101). After the addition of hydrated lime the temperature recorded above (data logger 1) and below the remains (data logger 2) decreased to 15 °C, which was much lower than the average daily temperature of 20 °C. This was due to the lime prohibiting sun exposure and insulating the remains. Over the course of the trial, the surface of the remains experienced the same daily fluctuations as the environment, which was observed to a lesser extent underneath the remains.

The temperature fluctuations recorded above and beneath Donor 1 and Donor 2 varied significantly. Donor 2 (Lime) did not experience a large increase in temperature during active decomposition, as there were not larval masses present to create excessive heat. Thus, the temperatures recorded both above and below the donor remained similar to the environmental temperature throughout the trial.

3.2. Observations of decomposition and lime

3.2.1. Donor 1 (Control)

Donor 1 was in the fresh stage of decomposition upon arrival to the field site. The donor remained in the fresh stage of decomposition until Day 7 (ADD 106) (Fig. 2), upon which the upper limbs and face entered the bloat stage. The mouth and forearms appeared green, and egg masses were present around the groin, lateral and medial sides of the lower limbs, mouth, nose, and eyes. Maggots were present under the epidermis on the forearms. The lower limbs entered the bloat stage on Day 9 (ADD 132).

The head of Donor 1 entered active decomposition on Day 14 (ADD 185) (Fig. 2), which was characterised by the swelling of the face due to maggot activity within the mouth and nasal cavity. Skin slippage occurred on the fingers of the right hand, feet, and stomach, and maggots were visible under the epidermis on the torso, upper limbs, left hand, and feet. The most visible change in appearance occurred on Day 27 (ADD 377) where donor's clothes were soaked with dark brown decomposition fluid and both upper limbs had

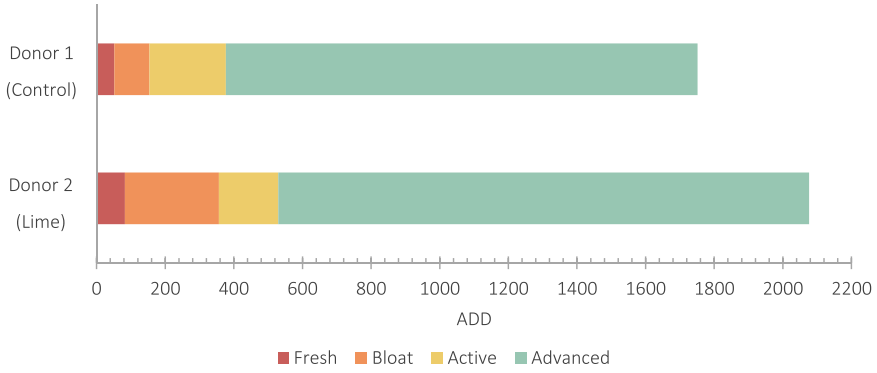

Fig. 2. The stages of decomposition vs ADD for Donor 1 and Donor 2.

orange discolouration. The soft tissue on the face had loosened and pulled away from the skull due to maggot activity. Skeletal elements could be seen in the head region and an abundance of maggots was present. A large cadaver decomposition island (CDI) surrounded the donor.

The deceased had entered advanced decomposition by Day 41 (ADD 614) (Fig. 2). The face, torso, and lower limbs had desiccated, the upper limbs were dark brown and had less soft tissue present. Some areas of the orbits and mandible was visible. On Day 82 (ADD 1346) the ilium and pubis became visible, and on Day 102 (ADD 1752) no skin remained on the nose or mandible. Blackened soft tissue connected the right side of the cranium to the right clavicle. The skin on both clavicles and the ribs was thin and fragile. The blackened soft tissue on the donor's upper limbs was fibrous showing soft tissue loss, and the lower limbs were skeletal. This was the end of the trial.

3.2.2. Donor 2 (covered with hydrated lime)

Donor 2 (covered with hydrated lime) was in the fresh stage of decomposition upon arrival and had swollen lower limbs due to oedema. Lime was manually added on top to cover the deceased on Day 0 (ADD 18). Small cracks appeared in the lime powder on Day 1 (ADD 37) due to the lime settling. These cracks grew on Day 3 (ADD 82) and horizontal cracks appeared across the face.

The first significant change in the donor's appearance occurred on Day 7 (ADD 159) when the bloat stage commenced (Fig. 2). The abdomen was distended and the thighs bloated, which caused large cracks to form in the lime. The largest opening appeared next to the donor's right thigh and measured 22 cm × 6 cm, followed by a crack at the right lower limb which measured 24 cm × 4 cm. The lower limbs and hips were discoloured a light yellow/green and the feet were blue. Some fly activity began near the cracks in the lime, and the purging of decomposition fluid was seen in the lime on the lateral side of the left ankle. On Day 11 (AD 237) an opening was observed in the lime over the mouth which was stained with brown decomposition fluid. This caused the number of flies around the donor to increase and focus around the head. Due to the distended abdomen the torso and lower limbs became more visible, and all exposed skin was discoloured a dark blue/black (Fig. S2). On Day 17 (ADD 357) the head became completely visible due to the effects of gravity, rain, and wind.

On Day 21 (ADD 434) Donor 2 was in active decomposition (Fig. 2). The lime had formed a solid cast during bloat, so when the body deflated a gap between the lime cast and the donor became visible (Fig. S3). The donor's slipped skin remained attached to the lime cast around the shoulders. Skin slippage occurred on the torso, lower limbs, and feet. The eyelids were desiccated and did not retract. The abdomen was beginning to lighten from a dark blue to brown. Fly activity increased on Day 27 (ADD 530) with a small number of maggots present in the mouth. The feet began to blacken. No CDI could be observed.

Active decomposition lasted from Day 21 (ADD 434) until Day 40 (ADD 760), when beetles began to appear and fly activity had ceased. The donor was in advanced decomposition (Fig. 2) as visible skin on the donor was mummified, and the inside of the mouth had hardened. The posterior of parietal had begun to skeletonise, and the maxilla and nasal cartilage were visible. The average pH of the lime cast was 11.91 and the cast was hard to the touch. The donor's feet began to blacken on Day 61 (ADD 1243). Bone became visible on the knees, shoulders, and feet on Day 101 (ADD 2076). The gap between the lime cast and the deceased measured 7 cm on the right side of the torso and 6 cm on the left side. 2–3 cm gaps were recorded at the head and lateral sides of the lower limbs. This was the end of the trial.

3.2.3. Comparison of donor decomposition

A notable difference in the decomposition of Donor 1 and Donor 2 was the length of each decomposition stage (Fig. 2). Donor 2 was still in bloat on ADD 357, when Donor 1 was reaching the end of active decomposition on ADD 376. The visual cues of each stage also varied, as the only indicator of Donor 2 entering active decomposition was the retraction from the lime cast. No purging fluid was visible, save in small patches on Day 7 and Day 11, and the donor appeared always dry to the touch, directly opposing Donor 2's wet decomposition. Donor 2 lost less soft tissue during active decomposition, and Donor 2 remained desiccated for longer than Donor 1. This effect of lime on Donor 2 is supported by previous studies by Schotsmans et al. [12,13,20], which found hydrated lime to slow the decomposition of pigs within the first six months.

The difference in the rate of decomposition between the donors can be partially attributed to the lack of insect activity, as maggots produce proteolytic enzymes and feed on the disintegrated tissue [39]. The lack of insect activity therefore greatly influenced decomposition. Insect activity surrounding Donor 2 was limited to the face, and no maggots were observed on other body parts throughout the course of decomposition. The decreased amount of insect activity was also notable due to the seasonal disparities between the deceased. Warm weather is known to increase the activity of maggots, and a wider variety of insect species are observed during spring [40,41], which was when Donor 2 was received. Despite the warmer weather, no flies were recorded on the body, and the flies only to laid eggs within the mouth and nasal cavities, which were more easily accessible through the natural holes in the lime. Detailed entomological analyses are currently being carried out.

3.3. VOC production and analysis

VOC samples were collected on each sampling day (Table 1), with a total of 786 compounds being detected from Donor 1, and 726

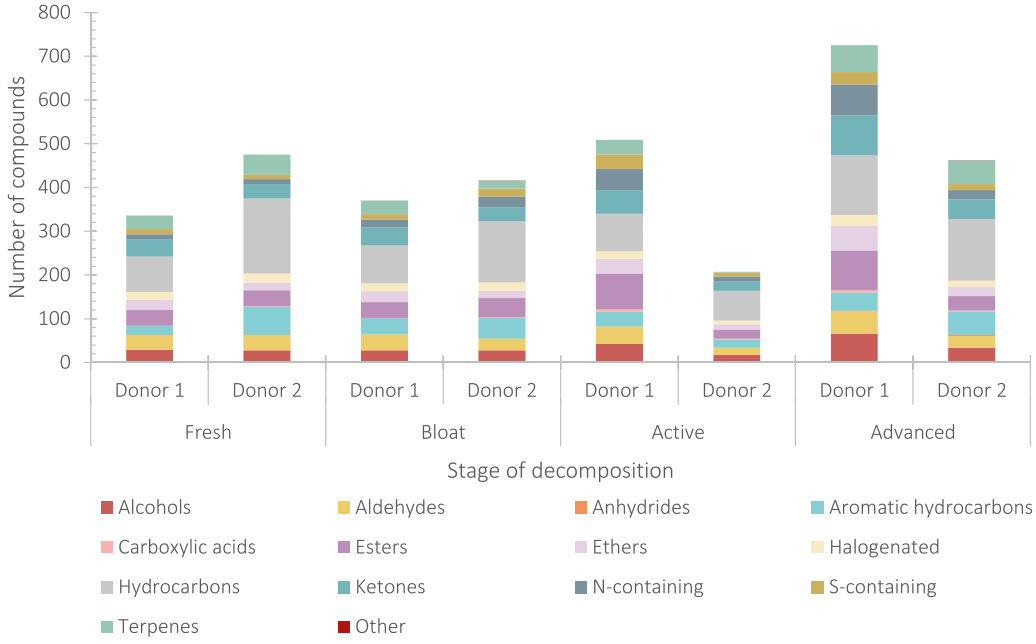

Fig. 3. Number of compounds for Donor 1 and Donor 2 per stage of decomposition.

compounds from Donor 2 (Fig. 3). The highest number of compounds produced by Donor 1 occurred during advanced decomposition with 725, followed by active with 509. Comparatively, 475 compounds was the highest number detected from Donor 2 during the fresh stage, followed by 462 during advanced decomposition.

3.3.1. Donor 1 (control)

The fresh stage of decomposition was characterised by a low abundance of VOCs (Fig. 4), with ketones, terpenes, and alcohols being classes with the highest intensity. S-containing compounds including dimethyl sulphide (DMS), DMDS, and DMTS were detected in low abundances, and a total of 336 different compounds were produced during fresh decomposition (Fig. 3). The compound with the highest intensity was methyl vinyl ketone (Table S1), which is an intermediate product produced by the photochemical oxidation of the plant metabolite isoprene [42]. The most predominant terpenes were limonene and α-pinene, which are aroma compounds produced by plants [43]. The decomposition-related compounds detected in the early decomposition sampling days included ethanol, which is derived from the anaerobic decay of carbohydrates [5], and hexanal, 2-pentanone, and 2,3-butanedione, which are all human metabolites found within saliva [44]. Previous studies have also found ethanol and 2-pentanone during the fresh stage of decomposition [7,45].

The bloat stage lasted from Day 7 until Day 11, and compound classes of highest abundance were hydrocarbons, alcohols and esters. Alcohols are frequently detected during the bloat of both human and animal remains [7,8], and previous research has found alcohols and esters to be produced through the degradation of carbohydrates, and hydrocarbons to be formed via lipid decay [46]. Polyunsaturated fatty acids are susceptible to oxidation and can form hydroperoxides upon contact with the air [47]. As

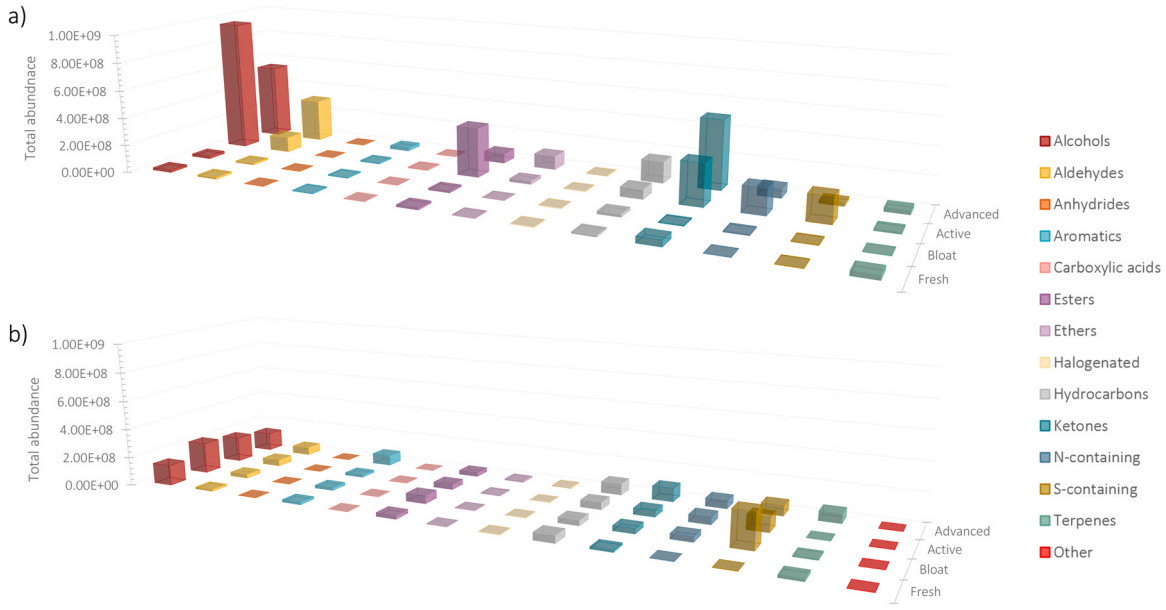

Fig. 4. Total abundance of compounds detected from a) Donor 1 (Control) and b) Donor 2 (Covered with lime).

decomposition progresses, liquified tissue is released from the orifices, which exposes lipids such as linoleic acid to oxygen and causes hydroperoxides to form. These hydroperoxides can further breakdown to produce VOCs including hydrocarbons, aldehydes, acids, and epoxides [47,48]. The intensity of S-containing compounds increased from the fresh stage, with carbon disulfide and DMDS being among the most abundant compounds produced during bloat (Table S1). Carbon disulfide is widely reported in decomposition studies using both humans and pigs, and it has been detected across all stages of decomposition [45,49–52]. The prevalence of S-containing compounds within the VOC profile is due to the degradation of S-containing amino acids, including cysteine, cystine, and methionine [48,53,54]. Benzaldehyde is also a product of amino acid decomposition, formed through bacterial degradation of phenylalanine [48]. Benzaldehyde was of high abundance during bloat but was not found in another other stages (Table 3). 370 compounds were produced during bloat with n-hexane having the highest abundance, followed by ethanol and ethyl acetate (Table S1). Rosier et al. [55] determined n-hexane to be a biomarker of human decomposition, which was concluded after comparing VOC profiles of various tissue types from human cadavers, pigs, roe deer, and lambs. The compound has also been found in studies on both human cadavers and pig remains during the fresh, bloat, and active stages of decomposition [46,50,56]. Ethanol was the second most abundant compound, and in addition to being produced by carbohydrates [5], it is also found in adipose and muscle tissue [57], and in skin, saliva, and most organs [44].

During active decomposition (Day 14–27) the most abundant compounds were alcohols, esters, and ketones, with Day 27 being the peak of odour production from Donor 1 (Fig. S4). The high abundance of alcohols within the active profile is supported by the studies of Hoffman et al. [58] and Deo et al. [36]. Research using pig cadavers by Forbes et al. [45] found high concentrations of alcohols in both summer and winter trials, which were discriminatory for active and advanced decomposition in both seasons. Stadler et al. [8], also using pigs, found alcohols to peak during early putrefaction in bloat and not in active decomposition. Hoffman et al. [58] used human remains, and found early stages of active decomposition to have higher levels of 1-propanol and 1-butanol, whereas the later stages had longer chain alcohols such as 1-octen-3-ol. They determined this shift to be the effect of an environmental change from an anaerobic to an aerobic decomposition environment. In the current study, the aromatic alcohol phenol was the second most abundant compound produced during active decomposition (Table S1). Phenol is a human metabolite found within the epidermis, adipose, muscle tissue, and several organs including the intestine, spleen, liver, and brain [44]. It is also found within the ante-mortem matrices of saliva, sweat, and urine, which means its presence is not indicative of human decomposition and can also be resultant of living persons. Phenol was also found during bloat and advanced decomposition (Table 2) Esters were the second most abundant class during active decomposition (Fig. 4) and were released from the decomposition of both carbohydrates and lipids [53]. The esters with the highest concentrations were butyl acetate, butyl butyrate, butyl propionate, and ethyl acetate (Table S1), which have previously been documented during bloat, active, and advanced decomposition[7,46] and are deemed the result of saponification [46]. Ethyl acetate was prevalent within the profile and was found from fresh to active decomposition (Table 3). Esters also prevalent in the headspace of muscle and fat [58], and were found primarily during active and advanced decomposition by Dekeirsschieter et al.[7]. Ketones were the third most abundant class, and have been reported in high concentrations in two studies by Statheropoulos et al. [46,59]. A study by Forbes et al. [45] also found ketones and alcohols to be discriminating classes for both active and advanced stages of decay. In the current trial, S-containing compounds increased in abundance during active decomposition, which can be attributed to an increased rate of soft tissue decomposition and insect activity [45]. DMDS and DMTS were among the most abundant compounds detected during active decomposition (Table S1). The temperature recorded above the donor increased 10 °C from Day 2 to Day 23, rising from 18 °C to 28 °C (Fig. 2). Over this period the average daily temperature of the environment rose 4 °C, therefore the large increase in the donor's temperature was not only due to the warming environment, but also due to the heat produced from the increased maggot activity associated with active decomposition [38] and the increased production of sulfides (Table 3).

Advanced decomposition was the peak of VOC production in terms of abundance and the number of compounds (Fig. 3 and Fig. 4). The most abundant class produced during advanced decomposition were alcohols followed by ketones and aldehydes. Ketones and aldehydes within the profile are caused by fat degradation, after which aldehydes will further decompose to form alcohols [60]. Aldehydes are also produced due to the breakdown of muscle tissue and the organic phase of bone, which is responsible for the increase in their concentration during the later stages of decomposition [57]. The presence of these compounds is supported by Forbes et al. [45], who found alcohols and ketones to be discriminating classes for the active and advanced stages of decay. The alcohols of the highest abundance were the short-chain 1-propanol, 2-butanol, and 1-butanol. These compounds were also present within active decomposition and 1-butanol has been heavily reported in previous studies [7,46,61–63]. 2-pentanone and 2-butanone were the ketones with the highest concentration during advanced decomposition, and were found in all stages of decomposition by Armstrong et al. [50], and in all stages except dry remains by Forbes et al. [45]. In the headspace of Donor 1, they were both found during active and advanced decomposition, and 2-pentanone was also found during the fresh stage. In addition to being the product of fat degradation, 2-pentanone and 2-butanone can be produced through the oxidation of 2-pentanol and 2-butanol, respectively [60,64]. The aldehydes with the highest concentration were hexanal, octanal, and nonanal, which have been found in blood, muscle, fat, adipocere, and bone [58,65]. Nonanal and hexanal are formed through the aerobic oxidation of linoleic acid [57], and all three compounds have been documented in previous studies profiling decomposition volatiles [7,46,58,62,66].

The abundance and number of compounds produced during decomposition peaked in the advanced stage. This finding is contradicted by a study by Dekeirsschieter et al. [7], which noted the decrease in the number of VOCs produced as decomposition progressed from the active to advanced. The study found the concentration of carboxylic acids to decrease and the predominant classes to be ketones and hydrocarbons, with hydrocarbon production increasing as decomposition progressed. High rainfall was recorded between Day 61 and Day 82 (Fig. 1), which could have increased the production of VOCs by rehydrating any dried tissue and creating a more suitable environment for insect populations [67].

3.3.2. Donor 2 (covered with lime)

During the fresh stage of decomposition (Day 0 – Day 3) the classes of the highest abundance were alcohols, hydrocarbons, and esters (Fig. 4). Although alcohols were the most abundant class, only 28 different compounds were produced, which is comparatively low to the 172 hydrocarbons detected. This illustrates the high concentration of alcohols within the volatile profile, as ethanol was the compound of the highest intensity during fresh stage of decomposition. The hydrocarbons with the highest abundance were primarily long-chain straight and branched alkanes, including undecane, decane, dodecane, 5-dodecane, 2,6-dimethyl undecane, and nonane. Previous studies have found long-chain hydrocarbons in the headspace of fresh animal tissue [65], and within stages of

Table 3
The top 20 most abundant compounds per stage of decomposition occurring in more than one stage of decomposition.

	Donor 1 (Control)				Donor 2 (Covered with lime)			
Classes and compounds	Fresh	Bloat	Active	Advanced	Fresh	Bloat	Active	Advanced
Alcohols								
1-Butanol			✓	✓		✓	✓	✓
3-Methyl-1-butanol			✓	✓				
2-Butanol			✓	✓				
1-Propanol			✓	✓		✓	✓	
2-Methyl-1-propanol			✓			✓		
Ethanol	✓	✓	✓	✓	✓	✓	✓	✓
Phenol	✓	✓	✓	✓		✓		
Aldehydes								
2-Methyl-propanal	✓	✓						
Acetaldehyde	✓				✓	✓	✓	
Benzaldehyde		✓			✓	✓		✓
Butanal			✓			✓		
Hexanal	✓	✓		✓				
Methacrolein					✓		✓	✓
Aromatics								
Benzene	✓				✓			
Toluene		✓			✓	✓	✓	✓
Esters								
Butyl acetate			✓			✓		
Ethyl acetate	✓	✓	✓		✓	✓		
3-Hexen-1-ol acetate	✓				✓			
Isopropyl palmitate	✓	✓						
Methyl acetate						✓	✓	✓
Ethers								
1-Methoxy-2-propanol	✓	✓			✓			
Hydrocarbons								
Ethylidene-cyclopropane					✓	✓	✓	✓
n-Hexane		✓				✓	✓	✓
Pentane		✓		✓			✓	
Ketones								
2,3-Butanedione	✓		✓					
2-Butanone			✓	✓	✓	✓	✓	✓
2-Pentanone	✓		✓	✓		✓	✓	✓
3-Methyl-2-butanone			✓	✓			✓	✓
Methyl vinyl ketone	✓		✓		✓	✓		✓
N-containing								
Acetonitrile							✓	✓
Methenamine		✓	✓			✓	✓	
Methyl isocyanide		✓						✓
N,N-Dimethyl-methylamine		✓	✓					
S-containing								
Dimethyl disulphide		✓	✓			✓	✓	✓
Dimethyl trisulphide			✓			✓		
Terpenes								
Limonene	✓				✓			✓
α-Pinene	✓				✓	✓		✓

decomposition ranging from fresh to advanced decomposition [7,61]. There is limited information regarding the production of hydrocarbons from decomposing remains, with one study stating they are produced from the decay of lipids [46]. As the donor was still fresh, lipid decay is an unlikely source of the high number of hydrocarbons present within the headspace, and instead they are more likely to have been produced the hydrated lime (Supplementary Information). The only hydrocarbons found in more than one stage of decomposition were ethylidene-cyclopropane and n-hexane, which do not include any long-chained species. Given the production of VOCs from lime decreases significantly after initial placement in the field (Fig. S5), the presence of long-chained hydrocarbons only in the early stages of decomposition supports their production by the hydrated lime.

Esters were also abundant within the volatile profile of Donor 2, including ethyl acetate and 3-hexen-1-ol acetate. Ethyl acetate is found within the cytoplasm and within biological matrices including saliva, faeces, and urine [44], and was also detected during bloat. Dekeirsschieter et al. [7] found ethyl acetate during bloat, active, and advanced decomposition, however not within the fresh stage. Other studies have found acetic acid, butanoic acid, hexanoic acid, and octanoic acid esters [46,58,63,65], however no studies have reported 3-hexen-1-ol acetate. This stage of decomposition also reported a high number of aromatics (Fig. 3), despite their low concentration within the headspace. Aromatics are typically found in later stages of decomposition [7,8], and have been shown to be produced by a wide range of tissue types including muscle, skin, fat, bone, blood, and adipocere [58,65].

During the bloat stage (Day 7–17) the most abundant classes produced were S-containing compounds, alcohols, and esters (Fig. 4). The compound with the highest concentration within the volatilome was DMDS, followed by 1-propanol and 1-butanol. Propanol and butanol are produced in early stages of decomposition through the degradation of glycerol and the anaerobic digestion of carbohydrates and were found from bloat until advanced decomposition. S-containing containing compounds including DMDS and DMTS have been heavily observed from bloat until advanced decomposition [8,45,68], and are associated with an increase in insect activity surrounding a cadaver [10,54]. Although insect activity did increase during this stage, all flies were concentrated around the opening in the lime cast. This indicates that the volatiles were being released from the gap in the lime and not from the areas of the

donor that remained covered, suggesting that the lime was hindering the dispersion of compounds.

Similar to the bloat stage of decomposition, active decomposition produced primarily alcohols, S-containing compounds, and hydrocarbons. The most abundant compounds were ethanol, DMDS, and 1-butanol. This stage of decomposition produced 209 less compounds than bloat and saw a drop in the abundance of compounds produced. These are notable decreases, as the peak of volatile production is typically seen between the end of bloat and the middle of advanced decomposition [7,8,45,68,69]. Active decomposition also has been found to produce an increase in nitrogen- and S-containing compounds [7]. Although the abundance of N-containing compounds increased by roughly 40%, the number of compounds decreased from 24 to 12. The number of S-containing compounds also decreased from 18 to 11, and the abundance decreased by roughly 50%. As Donor 2 appeared not to purge any fluids during active decomposition, this could have affected the volatiles produced in addition to the lime masking the odour. The most abundant hydrocarbons produced include cyclic compounds such as ethylidenecyclopropane, 3,3,5-trimethylcyclohexene, and 1-ethyl-2-methyl-cyclopropane, and acyclic compounds including n-hexane and tetradecane (Table S2). Ethylidenecyclopropane is a compound found in alveolar breath [70], and tetradecane has been found in the decomposition volatilome from bloat until advanced decomposition [7,61].

Advanced decomposition saw an increase in the abundance of compounds produced from active decomposition, however it was still lower than the abundance of bloat (Fig. 4). The total number of compounds produced increased significantly with 462 compounds, which is 255 more compounds than during active decomposition (Fig. 3). The most abundant compounds produced during this stage were alcohols, ketones and sulphur-containing compounds, of which there were 34, 45, and 14 respectively. The most abundant alcohols were ethanol and 1-butanol, which are both produced during the anaerobic decay of carbohydrates [5]. Ethanol was found in all decomposition stages for Donor 2, and 1-butanol was found in all stages excluding fresh (Table 3). The most abundant ketones produced were 2-butanone, 2-pentanone, 3-methyl-2-butanone, and methyl vinyl ketone. 2-butanone and 2-pentanone were found in bloat and active decomposition, and the 2-butanone was found in all stages excluding fresh in a study by Armstrong et al. [71]. Forbes et al. [45] found 2-butanone and 2-pentanone in all stages of decomposition except skeletonisation during the winter trial, and 2-butanone in active decomposition, advanced decomposition, and skeletonisation during the summer trial.

Donor 2 produced 3-methyl-2-butanone during active decomposition as well as during advanced, and the abundance increased as decomposition progressed. Methyl vinyl ketone was also found during the fresh stage, and the abundance doubled from fresh to advanced decomposition. Neither 3-methyl-2-butanone have been reported previously in studies examining human or human analogue decomposition volatiles. More terpenes were produced during advanced decomposition than any other stage, which can be attributed to the increase in plant life surrounding the lime plot. The most abundant terpenes are limonene, pinene, and o-cymene, which are all volatiles produced from plants [72].

3.3.3. Comparison of volatile production by Donor 1 and Donor 2

The VOC profile of the two donors varied significantly in the abundance of compounds produced, the number of compounds produced, and during which stage of decomposition the peak of volatile production occurred. For Donor 1 (Control), the highest abundance of VOCs occurred at the end of active decomposition on Day 27 (Fig. S4), however the cumulative sum of VOCs produced during advanced decomposition exceeded that of the active decomposition stage (Fig. S6). Both stages produced high concentrations of alcohols, and active decomposition also produced a high abundance of S-containing compounds.

The highest abundance of VOCs for lime Donor 2 also occurred on Day 27 (Fig. S5), however the highest cumulative sum of VOCs occurred during the bloat stage (Fig. S7). During the bloat stage, the high abundance of S-containing compounds is notable, as their concentration exceeded those released from the non-lime donor even during the peak of VOC production. As maggots release proteolytic enzymes [39] and decomposition of proteins releases S-containing compounds [48], it can be inferred that a high rate of maggot occupancy on a set of remains will increase the abundance of DMDS, DMTS, and other S-containing compounds. The low insect activity on Donor 2 was thus expected to lessen the prevalence of sulfur within the volatile profile, which was not observed. The seasonal variation could account for the production of sulfides and high abundance during initial decomposition, as Donor 2 was received in the middle of spring, and Donor 1 was received during winter (Table 1). The concentration of sulfides has been found to be much higher during periods of heat, and suppressed during winter, which is also observed for other compound classes including ketones and alcohols [45]. Despite Donor 2 being received in spring, the temperature both above and below the deceased only increased slightly during bloat and decreased dramatically during active decomposition (Fig. 1). The decrease in donor temperature during active decomposition corresponds to a decrease in environmental temperature, however the temperature differences between Donor 2 and Donor 1 is likely due to the decrease in maggot activity. Maggot activity on remains results in heat production [38], so the lack of oviposits and subsequent maggot populations on Donor 2 is likely the cause of the temperature change disparities.

Although the abundance of sulfides appeared largely unaffected, and even increased, by the addition of lime, other compound classes were suppressed by the chemical addition. The concentration of esters during active decomposition, aldehydes during advanced decomposition, and ketones during both stages were diminished by the lime. Thus, the lime appears to prohibit the release, or formation of, some compound classes, but not sulfides. This finding is significant, as S-containing compounds are biomarkers for human decomposition and are known to attract insect activity to carrion. Alcohol abundance was also very high during the bloat stage of decomposition, which was not observed for Donor 1. This suggests that the decomposition of carbohydrates was unaffected by the lime, which also results in gas production and subsequent abdomen distention (bloat). As the abdomen and face of Donor 2 (lime) both swelled during bloat, the internal fermentation appeared unaffected.

In contrast, esters, ketones, and aldehydes decreased in the number produced and their abundance, which are all produced through the decay of adipose tissue. Lipids decompose through the process of lipolysis, which involves the hydrolysis of triglycerides catalysed by lipolytic enzymes [5,57,62]. Intracellular lipases are pH dependent, performing in either neutral (pH 7) or acidic (pH 4–5) environments [73,74]. pH readings from the lime plot and lime on Donor 2 showed a strong alkaline pH of 13.19 at the time of placement, which decreased to 11.91 after 100 days (Fig. S8). A strongly alkaline environment was hence maintained throughout the experiment, and was hypothesised to denature the lipases, thus prohibiting them from catalysing the decomposition of the triglycerides and fatty acids within the donor's subcutaneous fat. Hydrated lime is also able to absorb water through a capillary effect [75], which further prevents the decomposition of lipids by removing a key component in the reaction mechanism. Thus, a combination of the pH and capillary effect is believed to have decreased the decomposition of lipids, explaining the lower number and concentration of esters, ketones, and aldehydes.

The effect of lime on the volatile profile of Donor 2 is dissimilar to the study of Toogood and Diaper [32]. Their research found that although there was an initial decrease in the odour released from sludges, after two weeks it increased beyond that of their control. This previous work indicates that the lime was effective at prohibiting the production and/or release of volatiles during initial exposure, however the chemical's efficacy diminished after two weeks. The findings of Toogood and Diaper contradict the findings of this study, in which the effects of lime became more pronounced as decomposition progressed, and a large decrease of VOC production was seen during active as well as advanced decomposition in comparison to Donor 1. This is a substantial finding as it shows CDDs may struggle when attempting to locate victims covered with chemical such as lime. It is currently unknown whether CDDs rely on the full profile of VOCs, individual biomarkers, or an amalgam of compounds to locate human remains [1], and they will only indicate to stimuli on which they are trained. The presence of S-containing compounds despite chemical additions thus demonstrates the need to emphasise DMDS, DMTS, and other sulfides when training the canines in order to maximise the dogs' capacity for human remains location.

4. Conclusions

Lime has been applied to human remains by malefactors and in times of war or disease, often in an attempt to reduce the malodourous production of volatile compounds. This research is the first study to offer insights into the effect of hydrated lime on odour using human remains. The application of hydrated lime appeared to slow the rate of decomposition and preventing/absorbing the release of fluids. The volatile profile was eminently affected, and the production and/or release of compound classes including alcohols, aldehydes, esters, and ketones was greatly inhibited by the hydrated lime. However, the abundance of S-containing compounds such DMDS and DMTS were not affected by the hydrated lime, which indicates that they can still be used to locate human remains that may be covered with this chemical and should be focused on when training CDDs. As decomposition progressed into the late stage of advanced decomposition, the remains covered with lime released a very low abundance of compounds, whereas the control donor was still producing a large number of VOCs. This shows that CDDs may struggle to detect human remains covered with hydrated lime after a larger post-mortem interval. It would be interesting to confirm these findings with police dog units.

While this study focussed on hydrated lime, it is hypothesed that quicklime might have similar reactions on odour than hydrated lime. All quicklimes go through the process of hydration to turn into hydrated lime. The initial exothermic reaction caused by quicklime will increase bacterial activity, thus might lead to a faster transition from fresh to bloat stage. During the active and advance decomposition stages quicklime would have turned into hydrated lime leading to similar VOC emissions as hydrated lime. This hypothesis, however, should be tested in future studies as it might affect post-mortem calculations.

Other further research should be conducted using donors placed at the same time in order to avoid seasonal disparities that significantly affect decomposition. A large limitation of studying human remains is a low number of replicates, and as such the study should be replicated to create a larger data set. Despite the limitations of the study, valuable insight was gained into the effect of chemicals on human decomposition, both in the rate of decay and in the volatiles produced. The findings of this study can potentially influence future training of CDDs and enhance their ability to locate victims of violent crimes.

Credit authorship contribution statement

Bridget Thurn: Conceptulisation; Methodology; Formal analysis; Investigation; Writing – original draft; Writing – review and editing; **Eline M.J. Schotsmans:** Conceptulisation; Methodology; Writing – review and editing; Supervision; **Maiken Ueland:** Conceptulisation; Methodology; Writing – review and editing; Supervision.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.forsciint.2023.111745.

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