



Dissolved organic matter: Unraveling the role of organic carbon from the hypogeal cadaver decomposition island

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ABSTRACT

The biogeochemical processes occurring in soils beneath buried cadavers, here defined as hypogeal cadaver decomposition islands (HCDIs), remain poorly understood, particularly with respect to the role of organic carbon. This study investigated temporal variations in dissolved organic matter (DOM) within HCDI to evaluate its potential as a time-dependent decomposition marker. Twenty piglets were buried under controlled conditions at the non-human Forensic Taphonomy Facility Ticino-LEAF(s) (University of Milan, Italy), using four different concealment scenarios: unclothed, wounded, clothed in cotton, and covered with quicklime prior burial. Soil samples collected at successive post-burial intervals (PBIs) up to 467 days were analyzed for pH, DOM concentration, and molecular composition through Fourier Transform Infrared (FT-IR) spectroscopy. Results revealed consistent correlations between decomposition stage, soil pH, and DOM dynamics. In most groups, pH increased during early decay and declined in advanced stages, while DOM concentrations peaked at intermediate intervals before returning to baseline levels. FT-IR spectra highlighted molecular markers of lipids, fatty acids, and protein degradation, with adipocere-associated signals detectable at later stages. Quicklime-treated burials exhibited atypical chemical patterns, though DOM analyses remained informative. Other burial conditions, such as the presence of lesions or clothing, significantly influenced both decomposition processes and the expression of biochemical markers. This pilot study suggests that combined monitoring of DOM concentration and molecular characterization, supported by pH measurements, may contribute to a better understanding of decomposition processes. The approach shows potential for estimating the post-burial interval, suggesting a potentially rapid, cost-effective, and context-sensitive support tool for forensic investigations.

1. Introduction

The study of biogeochemistry related to the decomposition of a cadaver exposed to a natural environment and in contact with soil typically focuses on what is called cadaver decomposition island (CDI). It is defined as a dark-colored area immediately below the body characterized by a significant concentration of nutrients derived from putrefactive leachate and capable of affecting the normal physiology of the environmental matrix in a pointwise manner [1]. Despite the extensive chemical observation of these processes in both experimental [2,3] and uncontrolled studies [4,5] on surface depositions, numerous aspects

remain ambiguous, particularly concerning the dynamics of CDI in cadavers buried in clandestine graves (henceforth Hypogeal Cadaver Decomposition Island, or HCDI), which seem to be still poorly explored.

Among the various chemical markers considered diagnostic for understanding the dynamics affecting the matrix beneath a body are pH [6, 7], nitrogen [8,9] and two of its specific chemical species, nitrites and nitrates [8,10], phosphorus and phospholipids [9,11], and total organic carbon (TOC – [7,10]). However, the interaction between these compounds and both the mineral and organic components of the soil must also be considered, as it may lead to ambiguous results that are difficult to interpret, thereby limiting their utility for estimating the postmortem

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interval (PMI) or the post-burial interval (PBI). Among the parameters mentioned, variations in soil organic carbon content would logically be expected to provide a more accurate representation of the changes occurring within the context of a CDI. Nevertheless, most studies that have focused on monitoring organic carbon in association with a CDI have either failed to detect significant variations in the concentration of this element [7–10] or, when quantitative variations have been observed, have been unable to determine the origin of such changes [12].

For instance, Benninger et al. [9] investigated the impact of pig cadaver deposition on soil biogeochemistry over a 100-day period. Their results showed no significant differences in total carbon content between CDI and control soil samples. Similarly, Meyer et al. [10] examined the decomposition of swine carcasses during summer and winter, hypothesizing that more rapid and pronounced changes in grave-soil chemistry would occur during the summer. However, decomposition did not significantly affect total carbon concentrations, which were even higher during the winter period.

Another example is provided by Anderson et al. [7], who focused on chemical evidence of pig cadaver decomposition in CDI soils one and three years postmortem. Their study showed no significant differences in total carbon concentrations at either time point. Furthermore, Szelec et al. [8] sought to characterize the potential impact of pig cadavers (placed directly on the ground or suspended one meter above ground) on selected soil chemical markers over a one-year period. In this case as well, carbon content remained stable across all treatments, with no significant differences observed.

Finally, Martin et al. [12] analyzed chemical changes in loam and sandy loam soils surrounding buried animals after two months and found that organic carbon content was more strongly dependent on soil type than on decomposition products. Consequently, this marker was considered unreliable.

This issue may be attributed to the naturally high background levels of organic carbon in soils, largely resulting from inputs of plant-derived biomass, as well as to the limited ability of this parameter to reflect alterations associated with the presence of a decomposing body over the short to medium term. Furthermore, the low relevance of organic carbon as a marker in CDI studies may be hypothetically explained by the fact that carbon introduced into the soil undergoes multiple transformation processes, both biotic (e.g., reduction and oxidation) and abiotic (e.g., volatilization, leaching, and adsorption onto soil colloids).

Since the release of organic matter from a decomposing body is well documented by several chemical proxies [2,9], most notably through alterations in the natural soil pH [6,8], excluding the study of organic carbon may result in the omission of a key component in reconstructing decomposition dynamics. Consequently, it may be preferable to focus on a specific fraction of soil organic matter, namely dissolved organic matter (DOM), which has been proposed as an effective indicator of trends associated with the time-dependent input of decomposition products into the soil.

Soil DOM is operationally defined as the fraction of soil organic matter that can be extracted with water or other extracting solutions and passes through a 0.45 μm filter [13,14]. It consists of a complex mixture of organic compounds, including humic- and fulvic-like substances, phenolic compounds, carbohydrates, and nitrogen-containing molecules [13]. Despite its relatively small proportion in total soil organic matter, DOM is the most mobile and reactive fraction, playing a crucial role in nutrient cycling, microbial activity, and the transport of pollutants in soils [15]. From a dynamic perspective, putrefactive leachate originating from a buried decomposing body partially follows the same migration pathways as soil fluids. This observation supports the consideration of dissolved organic matter (DOM) as potential evidence of body concealment in clandestine graves.

Indeed, several previous studies have proposed focusing on dissolved organic matter (DOM) and dissolved organic carbon (DOC) as markers for the temporal evolution of a CDI beneath a decomposing cadaver. For

example, Fancher et al. [2] investigated the effects of surface human decomposition on soil characteristics over an approximately five-year period, suggesting that such changes may assist in estimating the post-mortem interval (PMI). In their study, DOC concentrations were higher in CDI soils than in control soils, although the observed abundance trends did not appear to be clearly time dependent.

Another application of this approach was reported by Aitkenhead-Peterson et al. [6], who evaluated the lateral spatial extent of decomposition products from the CDI of two human cadavers, depending on whether scavengers had access to the remains. Their results showed that mean DOC concentrations after approximately one year and eight months were significantly higher than those in control soils, differing by approximately one order of magnitude.

DOM was more recently investigated by Pecs et al. [16], who examined its relationship with soil microbial activity in CDI contexts. In their experiment, three adult domestic pigs were placed on the soil surface, and soil samples were collected at varying distances from the CDI up to 324 days post-deposition. Although the chemical analyses primarily focused on DOC concentrations, the results highlighted a localized increase in carbon associated with the decomposition of surface-deposited pig cadavers, extending to depths of up to 14 cm.

However, as also noted by the authors, the mere quantification of DOM within a soil sample is insufficient to distinguish cadaver-derived molecules from those naturally present in the soil system. For this reason, it may be more appropriate to perform a qualitative characterization of DOM, for example through Fourier transform infrared spectroscopy (FT-IR). This technique can facilitate a more detailed understanding of decomposition-related changes, as specific absorption bands can be associated with molecular subunits characteristic of individual biopolymers, such as lipids and proteins [17]. Moreover, variations in the intensity of these bands, driven by different stages of cadaver decomposition, may enable a semiquantitative assessment of the temporal enrichment or depletion of the corresponding biopolymers [16].

The treatment that the body may have undergone during concealment should also be taken into consideration: the presence or absence of clothing, the number and extent of injuries, or the use of caustic-acting substances may be able to alter normal decomposition processes [18–22] and, consequently, the influence that DOM may have on soil bacterial community [16].

Therefore, the present study aimed to detect the variation in DOM quantity and quality from the HCDI through time in four groups of simulated clandestine graves containing one piglet each at the non-human Forensic Taphonomy Facility (or FTF) Ticino-LEAF(s) (University of Milan, Italy). In particular, the goal of the experiment is to observe the relationship between the decomposition state of the cadavers and the corresponding pH anomalies, DOM concentrations, and organic compounds presence over time. The experiment underlined the effectiveness of the combined use of pH, DOM quantification, and spectroscopic characteristics (FT-IR spectroscopy) in monitoring decomposition processes, showing its potential forensic application in PMI estimation. A highly precise analytical approach capable of monitoring even minimal fluctuations in the concentration of dissolved organic matter (DOM) within the cadaveric decomposition island (CDI) over time shows promising potential in various forensic contexts.

In particular, in cases of clandestine burials, an increase in DOM concentration relative to the baseline biogeochemical conditions of the surrounding environment may support the estimation of the post-mortem interval (PMI). Broader application of this method alongside experimental research could also allow for the identification of consistent relationships between the physiological DOM levels of an environment and those observed within the CDI, serving as reliable indicators of time since death. Given its rapid execution and relatively low cost, this method could be effectively applied in real forensic investigations, providing timely information on the period a body has remained in a burial site or natural setting; an interval that can be cross-referenced with reports of missing persons or last known sightings.

2. Material and methods

2.1. Ticino-LEAF(s) and experimental setting

The experiment was conducted at the non-human Forensic Taphonomy Facility Ticino-LEAF(s) (Ticino Park-LABANOF facility for Environmental Analysis in Forensic Sciences) founded by LABANOF (Laboratory of Forensic Anthropology and Odontology) from the University of Milan, in collaboration with the Lombardy Park of the Ticino (PV – [23]).

The area is located in northern Italy (45°23' N 8°50' E) at 95 m a.s.l. The climate is sub-oceanic with the highest rainfall in spring and autumn and with an average rainfall of 1050 mm/year [11]. According to the Köppen & Geiger system [24], the experimental area could be defined as *Cfa*, which indicates humid temperate climates, characterized by long and hot summers, and cool to mild winters.

The vegetation within the study area is predominantly composed of lowland oak forests (*Quercus robur*), accompanied by a well-defined herbaceous layer including *Galeopsis pubescens* and *Aristolochia clematitis*. These woodlands are relatively young and situated in a landscape subject to significant anthropogenic use, which limits the structural development of the vegetation to specific zones. According to the "Land Use Map for Agricultural and Forest Soils," (DUSAF 7.0–2021) the area is classified as *broadleaved forests with medium to high density, managed as coppices*. Further floristic characterization was carried out based on previous ecological surveys related to the burial site, allowing for a more detailed identification of the local plant community [25]. Tree and shrub species identified include *Robinia pseudoacacia*, *Crataegus monogyna*, *Corylus avellana*, *Ailanthus altissima*, *Frangula alnus*. Additional plant species observed include *Rubus*, herbaceous taxa such as *Oxalis* spp. and *Brachypodium rupestre*, and climbing species like *Hedera helix*. The soils of the area are poorly developed on coarse fluvial deposits composed of crystalline rocks [11]. These soils are currently classified as Typic Udorthents sandy—skeletal, mixed, and mesic [26,27], characterized by highly permeable horizons with rapid drainage and sandy texture in the first meter.

Four sets of five simulated clandestine burials were established in a wooded area, each containing a piglet. Graves measuring 1 × 1.5 m were excavated to a depth of 50–60 cm and spaced 1.5 m apart. The entire experimental plot covered a compact area of approximately 950 m². All the specimens involved in this experiment, and in general all those used at Ticino-LEAF(s), had already died from natural causes or causes completely unrelated to the project [23]. This was made possible through partnerships established by the laboratory with a local breeder, who preserves the piglets in a freezer (−24 °C), rather than discarding them, in the unfortunate event that they die from natural causes (comparable to sudden infant death) or are accidentally crushed when the sow lies on them during nursing. The experiment was conducted in respect of the Animal Welfare Organization instituted by the University of Milan (Legislative Decree no. 26/2014), whose tasks include applying the "Principle of the 3Rs": Replacement (replace a model which uses an animal with an alternative model, when possible); Reduction (reduce the number of animals used in a given experiment); Refinement (take action to improve the techniques used to raise, house, care for, and utilize animals).

In the present case, current Italian legislation does not allow the establishment of human body donation programs for experimental research in the field of taphonomic studies; consequently, the animal model is the only one currently applicable. However, there is no scientific evidence supporting the necessity of causing the death of the animal in order for the simulation to be reliable; on the contrary, such a practice is widely considered unnecessarily violent and cruel [23,28,29]. Therefore, one approach that enables the use of animal models in forensic scientific research while upholding high ethical standards is the use of specimens that have already died from natural or otherwise unrelated causes. As stated by Mole and Heyns [30], the reuse of animals

can reduce costs (understood as the need for animal sacrifice) and help limit the overall number of animals used in research.

Prior to the burial, each of the 20 piglets was weighed (3–6 kg) and subjected to external and radiological examination to attest their integrity and suitability for the experiment. Each of the four groups of burials represented a method of body concealment: one group included five piglets buried naked (group GN); one group with five naked piglets wounded after death by gunshots, blunt-force wounds and stabbing; all three types of lesions were inflicted on the same subjects, in the ongoing effort to use the smallest possible number of animal specimens (group GW); one group including five piglets wrapped in 100 % cotton clothes (group GC); one group of five piglets completely covered with quicklime after deposition (CaO – group GQ). All piglets were inhumated on the same day in March 2023, while one piglet for group was exhumated at predetermined PBIs of 18, 49, 101, 206–220 and 397–467 days. Unfortunately, one of the piglets from the group GN was taken by the local wildlife, therefore data from the HCIDI for PBI 2 (49 days) are missing.

2.2. Weather conditions during the experiment

To assess the potential influence of weather conditions on DOM concentrations in the HCIDI, meteorological variables were monitored using data from the ARPA Piemonte weather station in Cerano (NO), located approximately 3 km north of the Ticino-LEAF(s) site at an elevation of 125 m a.s.l. Although temperature and humidity at a depth of 50–60 cm may differ from surface conditions, these parameters are generally less variable and buffered against short-term rainfall events and heat waves. Atmospheric parameters were therefore monitored using meteorological station data in order to identify any particularly intense events that may have altered the hypogeal microclimate during the experimental period.

During the experimental period (March 2023–June 2024), the mean daily air temperature (Fig. 1) was 14.8 °C, with a maximum of 35.8 °C recorded on August 28, 2023, and a minimum of −5.1 °C recorded on January 22, 2024. As shown in Fig. 1, a pronounced increase in air temperature was observed between PBI 3 and PBI 4, whereas the lowest temperatures occurred between PBI 4 and PBI 5.

Regarding precipitations (Fig. 2), the highest amount was recorded on May 15, 2024 (62.3 mm), while several dry periods (0 mm) occurred, notably in March, April and September 2023. As shown in Fig. 2, intense rainfall events were observed between PBI 3 and PBI 4 and again between PBI 4 and PBI 5.

2.3. Soil-HCIDI sampling and chemical analyses

During the fieldwork phase preceding burial, approximately 1000 g of soil was collected from the bottom of each grave, taking care not to exceed a sampling depth of 1–2 cm. Control soil samples were collected from the bottom of each individual grave rather than relying on a single generic control sample. This approach was adopted to account for the intrinsic spatial variability of soils, particularly in a woodland environment such as the experimental area considered here, which could otherwise reduce the precision of result interpretation. These samples were used as references for baseline pH values and DOM concentrations of the experimental area, unaffected by disturbances associated with grave excavation or cadaver decomposition.

Excavation for the exhumations was carried out following a stratigraphic method, with pairs of specialists assigned to each grave, each using their own trowels, which were employed exclusively for that single grave in order to avoid cross-contamination. After each recovery, soil samples were collected from the HCIDI, below the cadavers. Due to the small dimensions of the piglets, it was not possible to divide the HCIDI into sampling areas corresponding to the anatomical portions of the bodies, therefore only the center of it was considered, collecting circa 1000 g of soil. The samples collected at each phase were stored in clean plastic bags sealed hermetically.

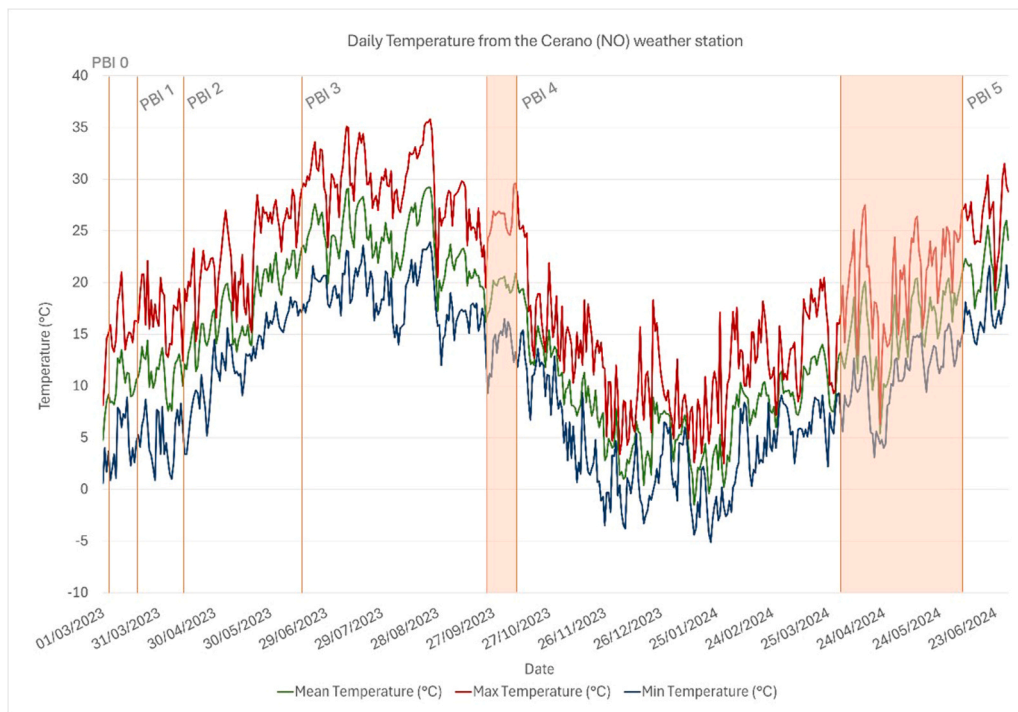


Fig. 1. Daily air temperature (°C) during the experimental period. The red, green, and blue lines indicate the trends of the mean maximum, mean, and mean minimum temperatures, respectively. Orange lines/rectangles mark the exhumation and sampling days for the corresponding PBIs (post-burial intervals). The final field operations required additional time due to accessibility issues at the FTF (Forensic Taphonomy Facility).

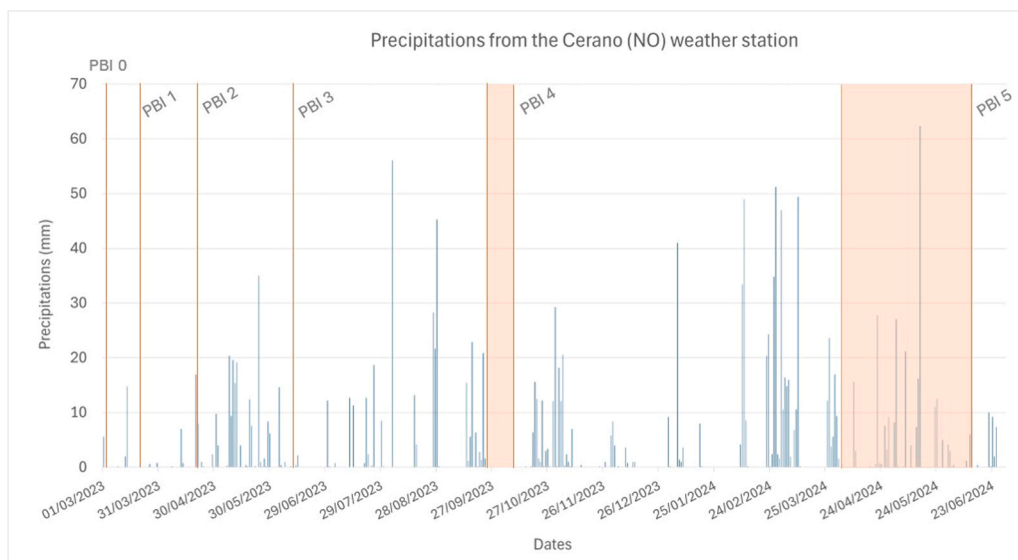


Fig. 2. Daily precipitation (mm) during the experimental period. Bars show cumulative rainfall for each day. Orange lines/rectangles mark the exhumation and sampling days for the corresponding PBIs (post-burial intervals). The final field operations required additional time due to accessibility issues at the FTF (Forensic Taphonomy Facility).

Once collected, the soil samples were air-dried in a protected environment in the laboratory within individual bags and kept separate from one another in order to avoid contamination. Once completely dried, the samples were prepared for chemical analyses. The samples' pH was determined using an aqueous solution prepared using a 1:2.5 soil-to-water ratio and measured with a benchtop multiparameter model PC8 +DHS Stirrer (Zetalab). Every measurement was repeated three times per sample.

DOM was extracted from all samples with distilled water using a solid-to-water ratio of 1:1.5 (w/v), on a fresh matter basis, shaking for

2 h at 25°C. The suspension was then centrifuged at 7000 rpm for 15 min, and the supernatant was filtered under vacuum through a 0.45 µm membrane. The organic carbon content in DOM extracts was determined using the dichromate method on an aliquot of the extracted volume and the final concentration was detected by a photometric detector, at around 620 nm, with PF-12Plus (Macherey-Nagel, Düren, DE; [31]). The remaining DOM extracts were frozen at −80 °C and subsequently lyophilized. Spectroscopic characterization was performed on the samples in powder form using Fourier Transform Infrared (FT-IR) spectroscopy. Spectra were acquired in Attenuated Total Reflectance

(ATR) mode with a Shimadzu IRAffinity-1S spectrometer equipped with a Miracle Pike ATR device (Shimadzu Italia srl, Milan, Italy). The wavenumber range investigated was 4000–400 cm^{-1} , with a resolution of 4 cm^{-1} and 32 scans per sample. The FTIR spectra were processed using LabSolutions IR Advanced Software (Shimadzu) for standard data treatment, including baseline correction, normalization, and smoothing, to improve spectral quality and facilitate interpretation without altering the original data.

3. Results

3.1. Decomposition state of the piglets

Following the various exhumation sessions of the piglet carcasses, an observation of their state of preservation was carried out. The results are summarized in Table 1.

For the GN group, after 18 days (PBI 1) the body appeared bloated, with slight green discoloration. By 101 days (PBI 3), active decay was evident, characterized by an abundance of putrefactive fluids beneath the carcass and the presence of adipocere. An advanced stage of decomposition was observed after 206 days (PBI 4), marked by the presence of skeletonized elements, such as the skull and limbs, and a substantial loss of mass in the remaining soft tissues. Adipocere was also present at this stage, and plant roots were more abundant and more extensively integrated with the body. Finally, after 399 days (PBI 5), the dry remains stage was observed, with only limited residues of adipocere still present, and the cadaver was completely enveloped by roots.

The decomposition pattern of the piglets in the GW group was similar to that observed in the previous group at PBI 1 (18 days) and PBI 5 (406 days). However, after 49 days (PBI 2), the carcasses exhibited clear signs of active decay, and by 101 days (PBI 3) the remains had already reached an advanced stage of decomposition. At this stage, anatomical structures were no longer recognizable during field recovery, and extensive liquefaction was observed, with some roots in direct contact with the torso. After 220 days (PBI 4), the soft tissues were extensively liquefied, with the presence of adipocere and roots enveloping the remains.

The GQ group, characterized by the presence of quicklime, showed a decomposition pattern that differed from the other groups. The formation of a lime cast was observed, which appeared to promote dehydration of the remains and may have contributed to partial physical protection. Progressive mass loss was recorded up to PBI 5 (467 days), at which point exposure of bone and cartilage was noted. At PBI 1 (18 days), bloating and epidermolysis were observed, with the skin presenting a desiccated appearance, increased rigidity, and detachment from the underlying epidermal layer. This layer exhibited a pronounced

reduction in thickness, becoming nearly transparent. From PBI 2 (49 days) onward, the external appearance of the skin remained broadly similar, although a gradual thickening was noted. Mass loss continued steadily over time, reaching its maximum at PBI 5, when the remains were dry, while cartilage and desiccated skin were still present.

Ultimately, the group GC presented a peculiar progression in the conservation state through time: at PBI 1 (18 days), the remains were in the full bloated stage, with slight discoloration in the abdominal area. At PBI 2 (49 days), on the other hand, the piglet's cadaver was in the active decay stage, with the skull and limbs already partially skeletonized and a large putrefactive stain in the abdominal region. In contrast, at PBI 3 (101 days), the cadaver was well preserved, with widespread discoloration but intact skin, although leathery. These characteristics are associated with humid mummification or corification (i.e., defined as a preservative decomposition process occurring in environments with high moisture and limited air exchange, resulting in arrested putrefaction and brownish, leathery skin with retained moisture; [32–34]).

At PBI 4 (206 days), the cadaver was already in an advanced stage of decay, with most body regions skeletonized and widespread presence of adipocere, while at PBI 5 (397 days), the remains were completely skeletonized. In both of these last two cases, the cotton t-shirts were mostly destroyed.

3.2. pH analysis

pH analysis of soil samples collected from the bottom of each grave prior to burial allowed the soils of the experimental area to be classified as acidic to subacidic, with values ranging from 4.2 to 6.1. Analysis of the HCDC soils (shown in Fig. 3 and summarized in Supplementary Material, Table S1) generally indicated an increase in pH starting at PBI 2, with a possible further rise at PBI 3, as observed for groups GN (Fig. 3a) and GW (Fig. 3b). Subsequently, pH values decreased, in some cases falling below those of undisturbed control soils. In contrast, group GQ (Fig. 3c) exhibited a marked increase in pH, exceeding twice the value of the control soil, although a decrease was observed at the final PBI. Finally, pH values in the HCDC soils of group GC (Fig. 3d) showed a peak at PBI 2, followed by a gradual decrease and stabilization over time at levels comparable to those of the control soils, until PBI 5, when pH declined to values lower than those measured in the control soils.

3.3. Dissolved organic matter (DOM) analysis

The mean DOM concentrations (shown in Fig. 4 and summarized in Supplementary Material, Table S2) generally increased relative to control soils starting at PBI 3, followed by a progressive decrease, eventually returning to values comparable to those considered physiological for the experimental area (37.39–74.85 mg/kg). This trend was observed in groups GN (Fig. 4a), GW (Fig. 4b), and GQ (Fig. 4c), in which DOM concentrations increased between PBI 3 and PBI 4, reaching values up to nearly eight times higher than those of the control soil (GW, PBI 4).

The only exception was group GC (Fig. 4d), for which DOM concentrations were generally comparable to those of the control soil, except at PBI 3. A pronounced peak was also observed at PBI 2, reaching a value nearly one hundred times higher than baseline levels (4647.95 vs 42.58 mg/kg).

3.4. Fourier transform infrared (FT-IR) spectroscopy

FT-IR analysis of the control soil DOM from the area revealed 20 superimposable spectra, represented by the example shown in Fig. 5, corresponding to the control sample from grave GQ1. These spectra are characterized by a broad absorption band in the single-bond region (4000–2500 cm^{-1}), with peaks around 3100 and 3030 cm^{-1} , attributable to C–H stretching vibrations in aromatic compounds. Additionally, two small sets of peaks were detected in the double-bond region (2000–1500 cm^{-1}), corresponding to aromatic ring vibrations, followed

Table 1

Decomposition state of the piglets as observed after the exhumations. GN=naked piglets; GW=wounded naked piglets; GC=clothed piglets; GQ=piglets covered with quicklime. PBI 1 = 18 days; PBI 2 = 49 days; PBI 3 = 101 days; PBI 4 = 206–220 days; PBI 5 = 397–467 days. “/” indicates that data of PBI 2 for the group GN is missing due to wildlife disturbance.

Group	Decomposition state				
	PBI 1	PBI 2	PBI 3	PBI 4	PBI 5
GN	Bloated	/	Active decay	Active decay Adipocere	Dry remains Adipocere
GW	Bloated	Active decay	Advanced decay	Advanced decay Adipocere	Dry remains Adipocere
GQ	Bloated	Desiccated	Desiccated Mass loss	Desiccated Mass loss	Dry remains Cartilage
GC	Advanced Bloating	Active decay	Humid mummification	Advanced decay Adipocere	Dry remains

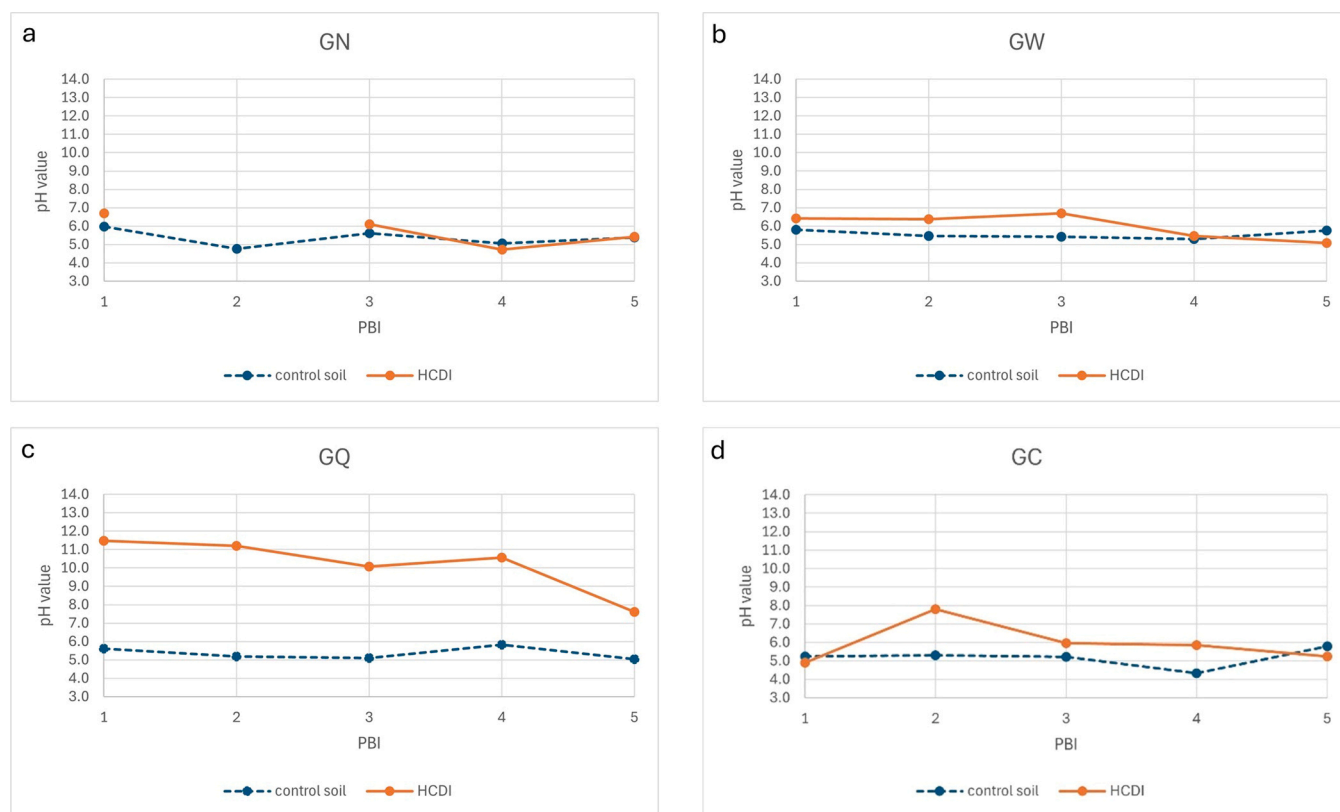


Fig. 3. Mean soil pH measured at the bottom of each grave for each group. The blue dotted line indicates the control soil pH, collected from each grave prior to inhumation, whereas the red line indicates the soil pH from each HCDI at different PBIs (post-burial intervals). GN = naked piglets; GW = wounded naked piglets; GC = clothed piglets; GQ = piglets covered with quicklime. PBI 1 = 18 days; PBI 2 = 49 days; PBI 3 = 101 days; PBI 4 = 206–220 days; PBI 5 = 397–467 days. Data for PBI 2 in group GN are missing due to wildlife disturbance.

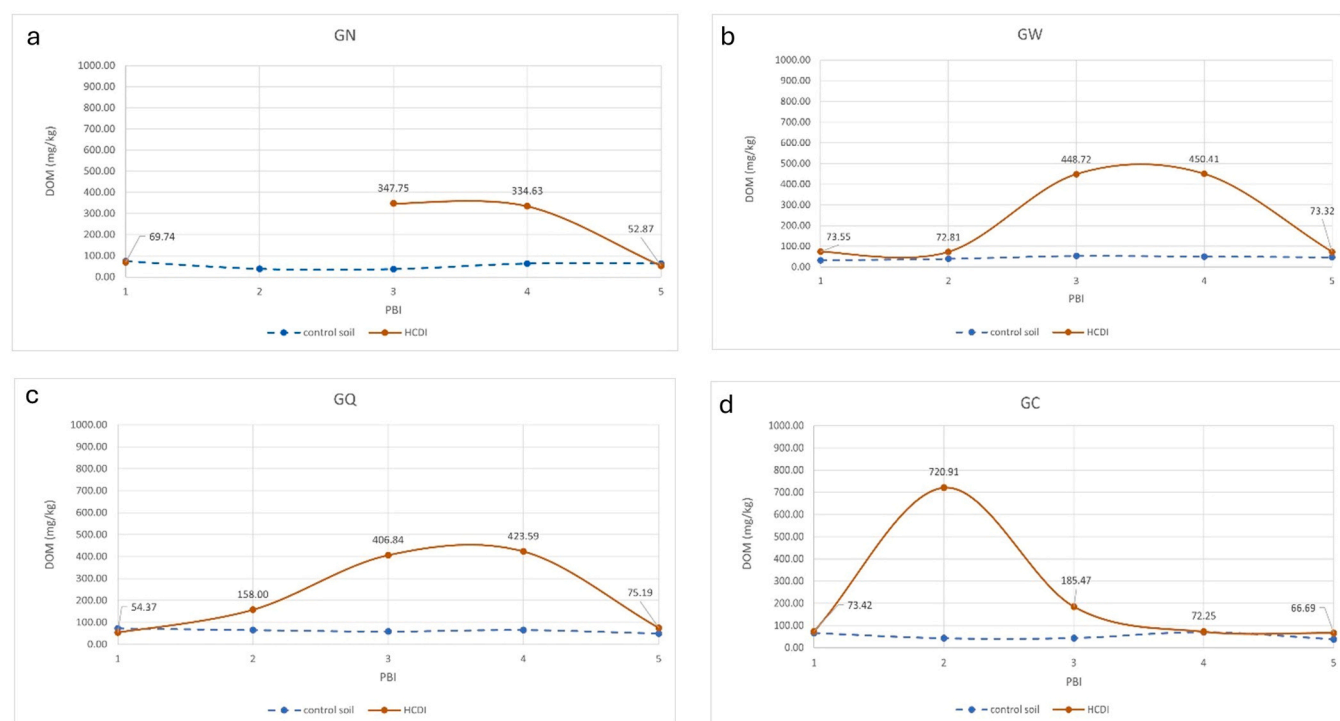


Fig. 4. Soil DOM concentrations measured at the bottom of each grave for each group. The blue dotted line indicates the DOM concentration in the control soil, collected from each grave prior to inhumation, whereas the red line indicates the DOM concentration in HCDI soil at different PBIs (post-burial intervals). GN = naked piglets; GW = wounded naked piglets; GC = clothed piglets; GQ = piglets covered with quicklime. PBI 1 = 18 days; PBI 2 = 49 days; PBI 3 = 101 days; PBI 4 = 206–220 days; PBI 5 = 397–467 days. Data for PBI 2 in group GN are missing due to wildlife disturbance.

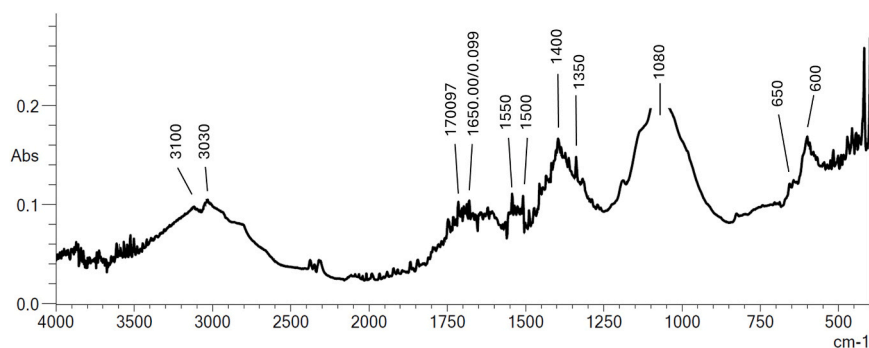


Fig. 5. Representative FT-IR spectrum of control soil DOM from the study area (GQ1), collected before inhumation.

by several very weak bands between 2000 and 1750 cm^{-1} . In the fingerprint region (1500–600 cm^{-1}), a prominent peak at approximately 1400 cm^{-1} , followed by another at around 1350 cm^{-1} , indicates the presence of three methyl groups ($-\text{CH}_3$), suggesting saturated aliphatic compounds such as trimethylamine ($\text{N}(\text{CH}_3)_3$). Finally, a strong peak near 1080 cm^{-1} , attributable to C–O stretching vibrations, suggests a substantial presence of polysaccharides or carbohydrates, while a

medium-intensity band between 650 and 600 cm^{-1} may be ascribed to C–H out-of-plane bending vibrations, further indicating the presence of organic material.

The FT-IR spectra of the group GN (Fig. 6a) showed no relevant alterations compared to the control soil, besides a progressive shifting of the absorption band in the single bond region towards higher wave-numbers, until reaching a splitting of the band in two separated peaks

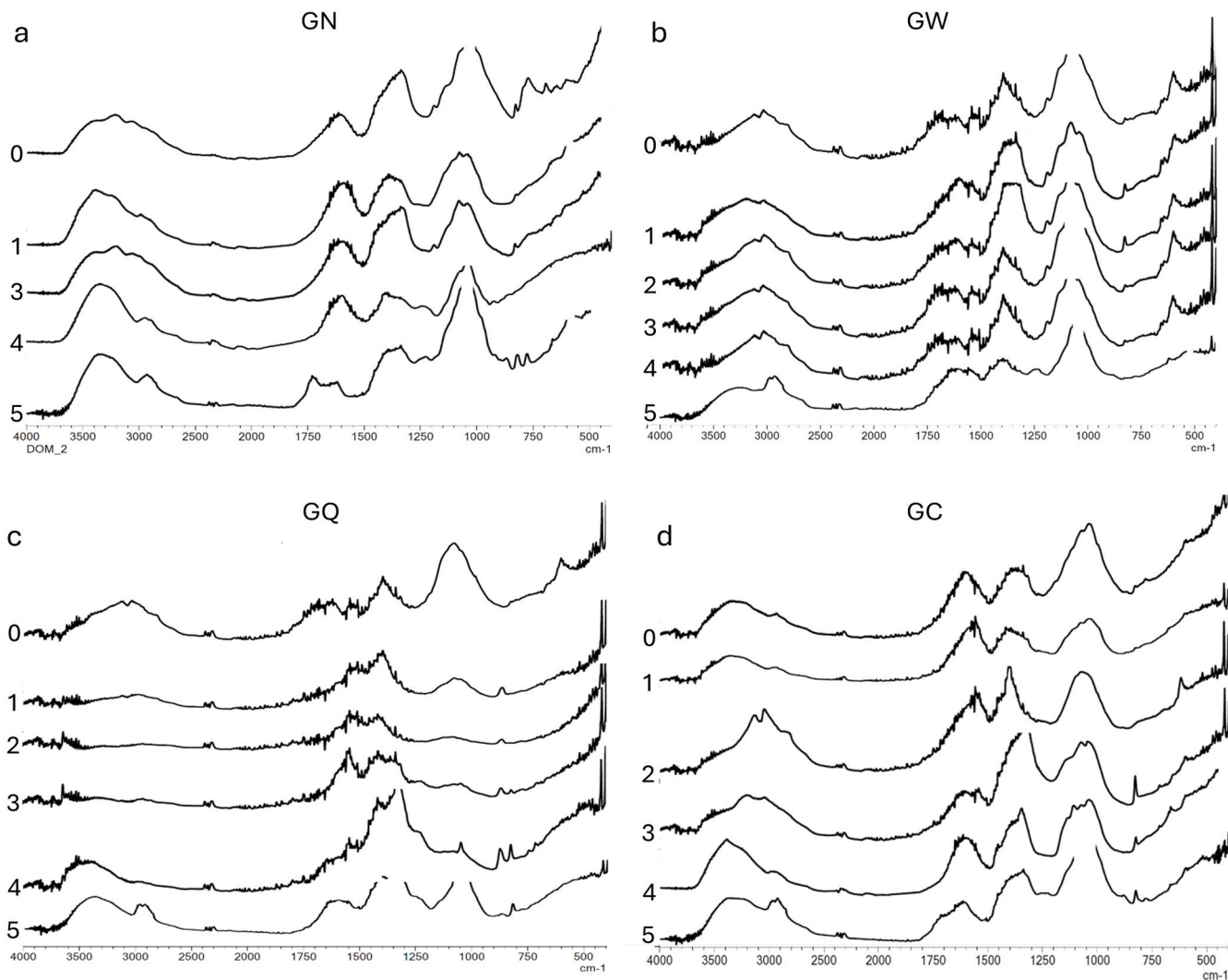


Fig. 6. FT-IR spectra of DOM samples collected from HCIDI soil for the four burial groups. Numbers to the left of each spectrum indicate the PBI (post-burial interval). PBI 0 = control soil spectrum from one grave prior to inhumation. PBI 1 = 18 days; PBI 2 = 49 days; PBI 3 = 101 days; PBI 4 = 206–220 days; PBI 5 = 397–467 days. GN = naked piglets; GW = wounded naked piglets; GC = clothed piglets; GQ = piglets covered with quicklime. Data for PBI 2 in group GN are missing due to wildlife disturbance.

(PBIs 4–5) attributable to unsaturated fatty acids ($3500\text{--}3000\text{ cm}^{-1}$) and hydroxy-fatty acids (2800 cm^{-1}). Moreover, from PBI 3 to PBI 5, two bands linked to amide I and II of proteins (around 1700 cm^{-1} and 1550 cm^{-1} respectively), and the emergence of a band near 1600 cm^{-1} , between the amide bands, and ascribable to free carboxylate groups are detectable. For group GW (Fig. 6b), a variation could be noticed in the intensity of the band around $1400\text{--}1250\text{ cm}^{-1}$ which increased from PBI 2 to later decrease and that could be linked to the C–N stretching of amide III. Moreover, from PBI 5 the spectra showed a different pattern of shifting to the right of the band in the single bond region, leading to the formation of a variation in the expression of lipids signals ($3500\text{--}2800\text{ cm}^{-1}$): O–H stretching bands (around $3400\text{--}3200\text{ cm}^{-1}$) attributed to fatty acids, and the well-defined absorbance bands around $3000\text{--}2800\text{ cm}^{-1}$ linkable to C–H stretching bands. On the other hand, group GQ (Fig. 6c) showed none to poor bands in the single bond region, with the only exception of PBIs 4 and 5, with a wide band ($3500\text{--}3100\text{ cm}^{-1}$) due to O–H stretching band of fatty acids and a well-defined absorbance band ($3000\text{--}2800\text{ cm}^{-1}$) attributed to C–H stretching bands of fats. Moreover, a small peak around 3630 cm^{-1} is observable from PBI 2 to PBI 4, detecting the O–H bond from water molecules. Conversely, triglycerides (1750 cm^{-1}) and unsaturated fatty acids (1670 cm^{-1}) absorbance bands were detected in samples from all the PBIs, until PBI 5 when they decrease. Furthermore, absorption bands around 1450 cm^{-1} , 1080 cm^{-1} and 870 cm^{-1} could be assigned to different vibration modes C–O between the oxygen atom of carbonate. However, the band in the area around 1450 cm^{-1} is characterized by a dense overlapping of absorbance bands, which could also be attributed to proteins or phospholipids. Finally, the FT-IR spectra of the group GC (Fig. 6d) presented a pattern similar to the ones of the groups GN and GW for what concerns the shifts of the band in the single bond region, attributed to =C–H stretching of lipids (3130 , 3050 , and 2800 cm^{-1}). Furthermore, bands related to the O–H stretching bands of fatty acids (around $3400\text{--}3200\text{ cm}^{-1}$) and the absorbance bands around $3000\text{--}2800\text{ cm}^{-1}$ linkable to C–H stretching bands, well detectable at PBI 5, are showing. In the double bonds region, two peaks around 1580 and 1550 cm^{-1} followed by modes between 1500 and 1400 cm^{-1} appeared at PBI 5, respectively ascribed to carboxylate bands of fatty acid calcium salts and lipid CH_2 scissoring. About the fingerprint region, the spectrum is characterized by the presence of a wide band between 1400 and 1250 cm^{-1} attributed to C–N stretching of amide III and, at PBI 2, another wide and strong absorbance band between 1080 and 1040 cm^{-1} , related to phospholipids. Ultimately, from PBI 3 it is possible to detect a fragmentation of the band between 1080 and 1020 cm^{-1} in three small peaks of C–O stretching of cotton cellulose, and a sharp peak at 850 cm^{-1} , attributable to the CH_2 rocking of lipids.

4. Discussion

This study aimed to investigate the concentration and temporal variation of DOM quantity and quality within the HCDI across four groups of simulated clandestine graves employing different concealment methods. The results indicate possible associations between the decomposition state of the remains, soil pH measured within the HCDI, and variations in both the concentration and molecular characteristics of DOM beneath the buried cadavers.

Considering the pH values observed in the GW group (Fig. 3b), a mean increase relative to control soils was recorded in samples collected from the HCDI of each grave during PBI 1, PBI 2, and PBI 3, followed by a decrease to average values comparable to, or even lower than, those of the control soils at PBI 4 and PBI 5. With the exception of PBI 2, for which data were unavailable, the GN group appeared to exhibit a comparable pH trend (Fig. 3a). The initial alkaline phase in both groups coincided with the exhumation periods corresponding to the bloating and active decay stages of decomposition. This pattern is consistent with findings from previous studies [8,9,35], which reported increases in soil pH during the early to intermediate stages of cadaver decomposition,

followed by a decline during the later stages of tissue breakdown. This trend was attributed to the release of ammonium ions (NH_4^+) resulting from protein ammonification processes occurring during autolysis and putrefaction phase, which includes bloating and active decay [1,9]. Similarly, in the more advanced decomposition stage (dry remains), a decrease in soil pH within the HCDI has been documented, as reported in other studies [8,35–37]. The secondary acidification may be attributed to the depletion of ammonium ions (NH_4^+) and other organic compounds [35], as a result of nitrification processes and the consequent production of H^+ [6,8,9], potentially indicating a shift in the microbial community inhabiting the CDI [38].

Accordingly, the DOM graphics of the samples from the two groups (Figs. 4a and 4b) showed a comparable trend with an increase of its concentration at PBIs 3 and 4, with a subsequent regression to values similar to the ones from the control soil (PBI 5). On the contrary, the FT-IR spectra revealed the presence of different compound signatures. In particular, the absorbance spectrum of group GW at PBI 2 (Fig. 6b) showed an increase in the intensity of the band in the $1400\text{--}1250\text{ cm}^{-1}$ region, which has been attributed to C–N stretching vibrations of the amide III band of blood in studies focusing on FT-IR characterization of human body fluids [39] and tissues [40]. Although these studies are not specifically related to forensic taphonomy or putrefactive leachates in soil, the presence of such molecular components in the present study cannot be excluded, particularly given that the GW group comprised individuals with multiple lesions that may have led to blood release into the surrounding environment. The absence of comparable spectral features at PBI 1 may be attributed to an insufficient accumulation of body fluids to produce signals distinguishable from background organic compounds, which may also exhibit band overlaps in the amide III region. It is therefore plausible that sufficient accumulation occurred only at PBI 2, after which these signals were progressively attenuated or lost as decomposition advanced or as compounds were leached by precipitation during subsequent PBIs.

In addition, it is possible to detect, at PBI 5, a variation in the expression of lipids signals ($3500\text{--}2800\text{ cm}^{-1}$): O–H stretching bands (around $3400\text{--}3200\text{ cm}^{-1}$) could be attributed to fatty acids [41,42] and the well-defined absorbance bands around $3000\text{--}2800\text{ cm}^{-1}$ could be linkable to C–H stretching bands, which were used as an indicator of the increase in the amount of lipid as the decomposition process proceeded, and of the presence of adipocere in other studies focused on the same topic [41,43,44].

In contrast, the spectra for group GN (Fig. 6a) showed no significant alterations until PBI 4, when a noticeable splitting of the absorption band between 3500 and 2800 cm^{-1} was observed. This could suggest the presence of hydroxy-fatty acids (2800 cm^{-1}) and the progressive formation of unsaturated fatty acids ($3500\text{--}3000\text{ cm}^{-1}$), better distinguishable at PBI 5, as evidence of the conversion to adipocere [41,44]. Furthermore, the evolution of the bands in the area $1700\text{--}1300\text{ cm}^{-1}$, attributable to proteins, was detectable from PBI 3 to PBI 5. According to Ueland et al. [22] two bands detected in the area could correspond to amide II (around 1550 cm^{-1}) and amide I (around 1700 cm^{-1}) and the respective evolution of the two through time or treatments could be useful for monitoring any change in protein of the decomposition fluid. Indeed, while the amide I peak is supposed to be sensitive to the protein conformation, which influences its location and intensity, amide II is normally more stable [22]. This relationship allows their ratio to be considered a marker of protein transformation [45]. The variation in the relative intensities and the broadening of the amide bands observed across the PBIs suggest that protein degradation and proteolysis occur throughout the decomposition process [43]. A reduction in the intensity of the amide I peak, accompanied by an increase in the intensity of the amide II, could serve as an indicator of protein breakdown, that could be employed to track the progress of proteolysis [46]. Additionally, as found in Collins et al. [43], the emergence of a band near 1600 cm^{-1} (situated between the amide bands) attributable to the formation of free carboxylate groups in the shorter peptide fragments

generated by proteolysis, could further contribute to the broadening of the amide bands as decomposition advances. In principle, the presence of analogous bands in the FT-IR spectra of the GW group was anticipated, given its comparable burial characteristics to those of the GN group. However, this was not observed. This discrepancy may be explained by the presence of a broad absorption band in the 1370–1200 cm^{-1} region in the GW spectra, attributed to C–N stretching vibrations of the amide III band of blood. Overlap of this band with the region of interest may have masked the corresponding signals, thereby hindering their detection over time.

About the group with quicklime (GQ), the pH graph (Fig. 3c) showed an atypical trend, reporting very high, despite decreasing values. This could be interpreted as the expression of the peculiar alkalinity of CaO, that being a basic oxide could have obliterated the pH signals attributable to NH_4^+ fluctuations due to putrefaction. The progressive acidification could suggest a corresponding hydration of the quicklime, obtaining lime (Ca(OH)_2), which is also typically alkaline. From this state on, once the hydration process is concluded, the carbonation due to the contact with CO_2 (being environmental or decomposition-related) leads to the formation of calcium carbonate (CaCO_3), whose pH is lower [47,48].

The cadavers of this group were recovered in a dehydrated state, potentially attributable to the hygroscopic capacity of quicklime, which may have created a microenvironment with reduced humidity, at least until complete hydration occurred. Nevertheless, the DOM analysis (Fig. 4c) appeared to be as effective as in the GN and GW groups, exhibiting a comparable temporal trend. These outcomes could be interpreted as suggestive of the analytical technique's potential to detect and monitor organic matter concentration, even in scenarios in which the amount of putrefactive leachate reaching the soil is minimal.

Accordingly, the FT-IR spectra (Fig. 6c) showed absorbance bands in the lipid-related region (3500–2800 cm^{-1}) at PBIs 4 and 5, similarly to those observed in groups GW and GN. In contrast, absorbance bands associated with triglycerides (1750 cm^{-1}) and unsaturated fatty acids (1670 cm^{-1}) were detected in GQ samples at all PBIs, with relatively constant intensity until PBI 5, when a decrease was observed. Forbes et al. [44] reported that the persistence of these compounds is indicative of minimal decomposition, which is consistent with the altered decay processes induced by lime. This interpretation is in agreement with the main findings of the present study.

Moreover, the same experiment reported by Forbes et al. [44] indicated that an absorbance band around 1450 cm^{-1} can be attributed to C–O stretching vibrations associated with lime. Although a signal in this region was observed in the FT-IR spectra of the GQ group, this spectral area was characterized by dense band overlaps, which may also reflect contributions from protein or phospholipid degradation products [39]. Nevertheless, it was possible to hypothesize trends related to the hydration and carbonation phases of quicklime. In particular, a small peak around 3630 cm^{-1} was observed from PBI 2 to PBI 4, corresponding to O–H stretching vibrations of water molecules [49], which may indicate progressive hydration of CaO, with a decrease in intensity over time. Furthermore, absorption bands around 1450, 1080, and 870 cm^{-1} can be assigned to different vibrational modes of carbonate (CO_3^{2-}) groups [50, 51]. The presence of these bands at later PBIs (4 and 5) may indicate a relative increase in CaCO_3 compared to Ca(OH)_2 .

Finally, pH analysis of soils from group GC (Fig. 3d) revealed a markedly different trend, characterized by a pronounced peak at PBI 2 followed by very low pH values. The latter were hypothetically attributed to the absorptive capacity of the cotton T-shirts in which the bodies were wrapped. The garments may have absorbed putrefactive leachate or acted as a physical barrier between the soil bacterial community and the soft tissues; however, the role of textiles as an obstacle to the diffusion of decomposition products remains unclear. According to Ueland et al. [22], whose study focused on the effects of decomposition fluids on textile degradation, cotton fabrics are capable of absorbing putrefactive leachate, which may also inhibit or delay textile

degradation. Similarly, Collins et al. [43] identified lipids, proteins, and other decomposition products within fluids retained in cotton clothes, demonstrating that textiles can absorb these substances throughout different stages of decomposition. In the present experiment, variations in decomposition rate were also observed for this group compared with unclothed individuals (Table 1), suggesting a potential influence of cotton clothing on decomposition dynamics. However, findings in the literature remain mixed. For example, Card et al. [52] reported that unclothed piglet cadavers decomposed significantly faster than those clothed in cotton, although clothing provided additional sites for Diptera colonization. Conversely, Matuszewski et al. [19] suggested that cadaver mass is a more influential factor in decomposition than clothing, with the latter exerting only a minor effect. Nevertheless, the authors emphasized that, even if textiles have a limited impact on decomposition rates, they should still be regarded as a key evidentiary element in forensic investigations, particularly due to their capacity to retain information on decomposition by-products that may be useful for PMI estimation.

The only exception to this assessment is the cadaver from the group GC exhumated at PBI 2: at that point, the decomposition state of the piglet was evaluated as active decay phase, being the cadaver worst preserved than the one analyzed at PBI 3, which underwent a conservative decomposition process, accentuating the steepness of the decline in pH values and DOM concentration between the two exhumations.

Accordingly, FT-IR analysis at PBI 2 (Fig. 6d) revealed strong C–H stretching bands, represented by a prominent double peak at 3130 and 3050 cm^{-1} , with a shoulder around 2800 cm^{-1} attributable to =C–H stretching vibrations. These features suggest the presence of unsaturated fatty acids [41,44]. Moreover, a wide and strong absorbance band between 1080 and 1040 cm^{-1} , utilized as a reference band for degradation products from phospholipid decomposition [53], was also present. Conversely, FT-IR spectrum of the sample from PBI 3 did not show an intensification of the signal in the single bond region, highlighting a weaker input of fatty acids in the grave soil, until PBI 5 when two sharp bands at 1580 and 1550 cm^{-1} appeared. They could be considered as characteristic of the carboxylate bands of fatty acid calcium salts [41, 43], while the modes expressing between 1500 and 1400 cm^{-1} could be attributed to lipid CH_2 scissoring [41,54].

The decomposition trends observed in the GC group suggest variable interactions between buried bodies wrapped in cotton clothing and the surrounding hypogeal environment. GC2 showed an advanced decomposition state, accompanied by corresponding chemical signals, which may reflect localized alterations in grave conditions, potentially driven by atmospheric events that increased air and water permeability. In contrast, GC3 was recovered in a preservative state consistent with wet mummification, indicative of sustained moisture and restricted air exchange, possibly facilitated by the close adherence of clothing and the maintenance of an anoxic microenvironment. GC4 exhibited a decomposition state comparable to GW4 but with substantially lower DOM concentrations, likely related to the absorbent properties of the cotton garment. Collectively, these observations emphasize the inherent variability of burial environments and the importance of a comprehensive, multidisciplinary assessment of factors influencing decomposition.

Moreover, from PBI 3 to PBI 5, a progressive fragmentation of the absorption band between 1080 and 1020 cm^{-1} was observed, resulting in three smaller peaks that shifted toward higher wavenumbers and became more clearly defined as the PBI increased. This trend may be tentatively attributed to the progressive degradation of the 100 % cotton T-shirts wrapping the piglets in this group, which may have resulted in the release of cellulose degradation products into the HCIDI soil, as suggested by Collins et al. [43]. As the spectral signal associated with fabric degradation became more pronounced, other bands related to decomposition products also became more detectable, including those associated with O–H stretching vibrations of fatty acids (approximately 3400–3200 cm^{-1} ; [41,42]) and C–H stretching vibrations of lipids (approximately 3000–2800 cm^{-1} ; [43,44]).

Considering the overall results of the experiment (summarized in Table 2), a correlation appeared to exist across all groups between trends in soil pH within the HCDI and changes in DOM concentration. However, deviations from this pattern were observed in groups GQ and GC, where the method of concealment appeared to significantly influence the chemical state of the soil matrix, particularly pH. Previous studies investigating biomarkers within CDIs as tools for PMI estimation [55] have proposed assessing soil alkalinity as an indicator of successful sampling, based on the alkaline effect exerted by ammonium (NH_4^+) ions on soil pH, as also discussed in the present study. Therefore, it is crucial to consider both cadaver treatment and concealment methods, as these factors may substantially alter soil chemical signals and potentially lead to erroneous interpretations.

Another example highlighting the extent to which body treatment and concealment methods can influence taphonomic analyses is provided by the FT-IR spectroscopy results presented here. Despite the

expectation of detecting comparable decomposition-derived organic compounds across the groups, with differences hypothesized primarily in terms of timing of appearance or absorbance band intensity, the findings instead revealed distinct molecular profiles. These differences appear to be driven more by the specific treatments applied than by time-dependent decomposition processes.

Nevertheless, it must be acknowledged that this is a pilot study conducted on a relatively small sample size, and further investigation is necessary. Conversely, what is consistent across all the experimental groups is the detection of spectral bands attributable to lipids and fatty acid salts, compounds typically found in adipocere [56], which was also macroscopically observed during examination of the remains in PBI 4 and 5. This finding could be significant, not only because it shows how these molecules are capable of passing through the DOM filter threshold ($0.45 \mu\text{m}$) and thus remain detectable even in analyses conducted for different purposes, but also because they may serve as useful indicators

Table 2

Resume of the highlights of the study. DOM and pH values are to be intended as compared to the control soil values. GN=naked piglets; GW=wounded naked piglets; GC=clothed piglets; GQ=piglets covered with quicklime. PBI 1 = 18 days; PBI 2 = 49 days; PBI 3 = 101 days; PBI 4 = 206–220 days; PBI 5 = 397–467 days. “/” indicates that data of PBI 2 for the group GN is missing due to wildlife disturbance.

		PBI 1	PBI 2	PBI 3	PBI 4	PBI 5
GN	Decomp. state	Bloated	/	Active decay	Active decay	Dry remains
	pH	+ 0.7	/	+ 0.5	-0.4 ± 0.0	Adipocere
	DOM (mg/kg)	-5.11	/	+ 310.36	+ 271.08	+ 0
	FT-IR		/	1700 cm^{-1} : amide I 1550 cm^{-1} : amide II (start of proteolysis)	Splitting of: 3500–3000 cm^{-1} : unsaturated fatty acids AND 2800 cm^{-1} : hydroxy-fatty acids.	3500–3000 cm^{-1} : unsaturated fatty acids (adipocere).
					Advanced decay	
GW	Decomp. state	Bloated	Active decay	Advanced decay	Adipocere	Dry remains
	pH	+ 0.6	+ 0.9	+ 1.3	+ 0.2	Adipocere
	DOM (mg/kg)	+ 40.9	+ 33.09	+ 395.16	+ 399.93	-0.8
	FT-IR		1400–1250 cm^{-1} : C–N stretching of amide III (blood)			+ 27.89
						3400–3200 cm^{-1} : O–H stretching bands of fatty acids. 3000–2800 cm^{-1} : C–H stretching bands of lipids (adipocere).
GQ	Decomp. state	Bloated	Desiccated	Desiccated	Desiccated	Dry remains
	pH	+ 5.9	+ 6.0	+ 5.0	+ 4.8	Cartilage
	DOM (mg/kg)	+ 18.96	+ 92.72	+ 348.08	+ 357.88	+ 2.6
	FT-IR	1750 cm^{-1} : triglycerides 1670 cm^{-1} : unsaturated fatty acids	3630 cm^{-1} : O–H bond of water molecules 1750 cm^{-1} : triglycerides 1670 cm^{-1} : unsaturated fatty acids	3630 cm^{-1} : O–H bond of water molecules 1750 cm^{-1} : triglycerides 1670 cm^{-1} : unsaturated fatty acids	3630 cm^{-1} : O–H bond of water molecules. 3500–3100 cm^{-1} : O–H stretching of fatty acids 1750 cm^{-1} : triglycerides. 1670 cm^{-1} : unsaturated fatty acids. 1450 cm^{-1} , 1080 cm^{-1} and 870 cm^{-1} : vibration modes C–O of carbonate groups CO_3^{2-} .	3000–2800 cm^{-1} : C–H stretching bands of lipids (adipocere). 1450 cm^{-1} , 1080 cm^{-1} and 870 cm^{-1} : vibration modes C–O of carbonate groups CO_3^{2-} .
					Advanced decay	
GC	Decomp. state	Advanced Bloating	Active decay	Humid mummification	Adipocere	Dry remains
	pH	-0.3	+ 2.5	+ 0.8	+ 1.6	-0.6
	DOM (mg/kg)	+ 7.21	+ 678.33	+ 141.93	+ 2.25	+ 28.41
	FT-IR		3130 and 3050 cm^{-1} : C–H stretching bands. Shoulder at 2800 cm^{-1} : =C–H stretching of unsaturated fatty acids. 1080 and 1040 cm^{-1} : Phospholipid degradation products.	1400–1250 cm^{-1} : C–N stretching of amide III (proteins/phospholipid degradation). 3 small peaks between 1080 and 1020 cm^{-1} : cotton cellulose. 850 cm^{-1} : CH_2 rocking of lipids.	3400–3200 cm^{-1} : O–H stretching bands of fatty acids. 3 small peaks between 1080 and 1020 cm^{-1} : cotton cellulose. 850 cm^{-1} : CH_2 rocking of lipids.	3400–3200 cm^{-1} : O–H stretching bands of fatty acids. 3000–2800 cm^{-1} : C–H stretching bands of lipids (adipocere). 1580 and 1550 cm^{-1} : carboxylate bands of fatty acid calcium salts. Modes 1500 and 1400 cm^{-1} : lipid CH_2 scissoring. 3 small peaks between 1080 and 1020 cm^{-1} : cotton cellulose. 850 cm^{-1} : CH_2 rocking of lipids.

for identifying a CDI that is no longer macroscopically visible. For example, in cases where a body is recovered in a skeletonized state, the chromatic alterations typically associated with the CDI may no longer be discernible. However, the application of chemical analyses may still yield signals attributable to cadaveric material, as observed in the case of FT-IR analyses. Furthermore, in scenarios involving the transfer of a body to a secondary deposition site, the original burial location may nonetheless remain informative. In such contexts, an interdisciplinary approach is essential, involving archaeologists capable of identifying the original grave bottom through stratigraphic interpretation and pedology experts able to recognize the natural soil profile of the area.

Finally, the potential influence of atmospheric conditions on the results must be considered. As shown in Figs. 1 and 2, marked temperature fluctuations and several episodes of heavy precipitation occurred during different sampling phases. These factors may have influenced the results, with precipitation in particular potentially affecting soil chemical dynamics, especially given the characteristics of the soils in the Ticino-LEAF(s) experimental area. These soils are shallow and derived from unconsolidated parent material and are therefore characterized by high porosity, which may predispose them to fluid leaching.

However, based on the observed trends in pH and DOM concentrations within the HCDCI, the overall pattern appears to reflect an initial peak in organic matter input followed by a gradual depletion, suggesting a limited influence of precipitation events on the measured parameters. A possible explanation may be related to burial depth (50–60 cm) and to soil disturbance associated with grave excavation and backfilling, which likely displaced the organic soil horizon, typically characterized by lower porosity, into deeper layers. Nevertheless, these interpretations remain speculative and warrant further investigation.

5. Conclusions

The study of biogeochemical processes associated with cadaver decomposition in natural environments and in contact with soil has traditionally focused on the cadaver decomposition island (CDI); however, several underlying mechanisms remain insufficiently explored. Among these, the behavior of organic carbon has yielded ambiguous and, at times, difficult-to-interpret results, leading previous studies to suggest limited utility of bulk organic carbon measurements for estimating the post-burial interval (PBI) of cadavers concealed in clandestine graves, largely due to the naturally high background levels of organic carbon in soils. In this context, the present study suggests that analyzing both quantitative and qualitative variations in dissolved organic matter (DOM), assessed through FT-IR spectroscopy within the HCDCI over time, may provide a potentially informative, time-dependent, and decomposition stage-dependent indicator for PBI estimation.

Across most experimental groups, an initial alkaline phase was observed, which appeared to correspond to active decay and the release of ammonium ions, followed by a gradual acidification during the later stages of decomposition. Trends in DOM concentration generally mirrored this progression, with increases during intermediate PBIs (3–4) followed by subsequent decreases, likely reflecting both the input of decomposition-derived organic matter and its progressive depletion or transformation. The group treated with quicklime exhibited atypical chemical behavior, likely attributable to the strong alkalinity of CaO and its reaction products, which may have masked typical pH signals and altered decomposition processes. Nevertheless, DOM remained detectable in this group, suggesting that the analytical approach may retain sensitivity even under chemically altered conditions. In contrast, the group of piglets wrapped in cotton T-shirts yielded results that diverged from those observed in the other groups, raising questions regarding the role of clothing as a potential barrier to the diffusion of putrefactive fluids into the surrounding environment.

FT-IR spectroscopy further revealed group-specific molecular features. Lipid- and fatty acid-related signals, often associated with adipocere formation, were consistently detected during later stages of

decomposition across all groups, alongside spectral indicators of protein degradation and blood-derived compounds. Variations in body treatment and concealment conditions appeared to influence decomposition dynamics and the expression of biochemical markers, underscoring the importance of considering burial context when interpreting forensic soil data.

Overall, the combined application of pH measurements, DOM quantification, and FT-IR spectroscopy appears to represent a promising approach for monitoring decomposition processes and may hold forensic relevance for PMI and PBI estimation. The findings highlight the potential value of high-resolution analytical techniques for tracking temporal changes in DOM within CDIs, thereby providing additional insight into decomposition-related soil alterations.

Further experimental investigation is needed to determine whether reproducible DOM concentration patterns can be reliably associated with specific postmortem intervals, thereby improving the robustness and forensic applicability of this approach. Given its relative speed, low cost, and operational simplicity, the proposed method may be suitable for real-case forensic contexts, where it could complement existing investigative tools by providing additional temporal information. Nevertheless, considering the pilot nature of this study and the limited sample size, the results should be interpreted with caution, and further research is required to validate and refine the proposed methodology.

CRedit authorship contribution statement

Fulvia Tambone: Writing – review & editing, Resources, Methodology, Investigation, Formal analysis. **Alex Golinelli:** Methodology. **Anna Masseroli:** Writing – review & editing, Investigation. **Giulia Tagliabue:** Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Cristina Cattaneo:** Writing – review & editing, Resources, Project administration, Funding acquisition. **Luca Trombino:** Writing – review & editing, Supervision, Resources, Funding acquisition.

Institutional review board statement

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Declaration of Competing Interest

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

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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.2026.112887](https://doi.org/10.1016/j.forsciint.2026.112887).

Data availability

The authors declare that all data supporting the findings of this study are available within the paper.

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