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Abstract: This study represents a preliminary, quantitative approach to the examination of differential decomposition patterns in mass graves. Five pairs of mass graves, each containing the carcasses of 21 rabbits, were used to examine decomposition extent at four fixed positions within the burial. A pair of graves was exhumed at approximately 100 accumulated degree day (ADD) intervals. At exhumation the total body score (TBS) and internal carcass temperature of each rabbit were recorded. Although there was no significant difference between decomposition extent for core and deep-positioned carcasses (p = 0.13), all other position differences were significant (p < 0.001). Decomposition occurred fastest in shallow carcasses, followed by mid-outer carcasses; both deep and core carcasses exhibited a lesser extent. Internal carcass temperature was significantly influenced by carcass location within the mass grave (p<0.001); there was a mean internal temperature difference of ca. 1 oC between deep and shallow carcasses (30 cm apart). Adipocere formation was minimal and confined, with the exception of a single individual in the mid- periphery, to the deepest level. Decomposition extent may be as affected by the compactness of a mass as by interment depth and/or peripheral substrate contact, and further investigation into the role of oxygenation is required.

Suggested Reviewers:

A preliminary examination of differential decomposition patterns in mass graves

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Running Head: Differential decomposition patterns in mass graves

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2 This study represents a preliminary, quantitative approach to the examination of differential 3 decomposition patterns in mass graves. Five pairs of mass graves, each containing the carcasses 4 of 21 rabbits, were used to examine decomposition extent at four fixed positions within the 5 burial. A pair of graves was exhumed at approximately 100 accumulated degree day (ADD) 6 intervals. At exhumation the total body score (TBS) and internal carcass temperature of each 7 rabbit were recorded. Although there was no significant difference between decomposition 8 extent for core and deep-positioned carcasses (p = 0.13), all other position differences were 9 significant (p < 0.001). Decomposition occurred fastest in shallow carcasses, followed by mid-10 outer carcasses; both deep and core carcasses exhibited a lesser extent. Internal carcass 11 temperature was significantly influenced by carcass location within the mass grave (p<0.001); 12 there was a mean internal temperature difference of ca. 1 °C between deep and shallow 13 carcasses (30 cm apart). Adipocere formation was minimal and confined, with the exception of 14 a single individual in the mid-periphery, to the deepest level. Decomposition extent may be as 15 affected by the compactness of a mass as by interment depth and/or peripheral substrate 16 contact, and further investigation into the role of oxygenation is required. 17 18 19

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22 depth

23 Introduction

24 The application of taphonomy to the investigation of mass graves provides assistance in 25 establishing context and reconstructing a broad sequence of events to achieve the ultimate goal 26 of victim identification [1, 2]. One fundamental aspect of such investigations is to establish the 27 post mortem interval (PMI) for remains within the grave and document associated taphonomic 28 evidence [3, 4] in order to corroborate witness statements, limit the number suspects involved, 29 link the grave to a particular event or perpetrator and increase chances of positive identification 30 [4]. Although several researchers (e.g. [5], [6], [7], [8]) have published accounts pertaining to 31 mass grave exhumations, classification systems [9], and guidelines/strategies for excavation and 32 maximisation of data retrieval [10, 11, 12, 13, 14], data concerning the decomposition rate, 33 extent and pattern of remains within mass graves are scant, and experimental approaches to 34 the issue have not been undertaken. 35 Accurate PMI estimations are generally based on degree of soft tissue decomposition, 36 identifiable stages of tissue alteration and loss that occur in a predictable, sequential and semi-37 continuous pattern at a rate that is dependent on both accumulated temperature over time 38 (measured in Accumulated Degree Days (ADD)) [15, 16] and insect access [17, 18, 19]. ADD 39 constitutes the accumulation of thermal energy (degrees Celsius) over time (days) and is related 40 to the rate of the chemical and biological processes of decomposition [19, 20, 21]. 41 Characteristic features of decomposition have been categorized into phases for the purpose of 42 soft tissue taphonomy [22], most recently by Megyesi et al. [15] and previously by Reed [23], 43 Payne [24], Johnston [25] and Galloway et al. [26]. Megyesi et al. [15] assigned numerical values 44 to three anatomical regions (head and neck, abdomen and limbs) by visually evaluating the

45 state of decomposition according to macroscopic criteria. These values are summed to 46 generate a Total Body Score (TBS) that is used to predict Accumulated Degree Days (ADD), which, in turn, provides an accurate and reliable method of estimating the PMI [15]. 47 48 Buried remains however, generate unique microenvironments quite different to those of surface 49 remains with consequences for the rate and pattern of decomposition; the interactions of 50 various biological, geological and environmental variables (i.e. temperature, insect access, 51 surrounding substrate, etc.) results in a slower rate of decomposition. When such biological, 52 geological and environmental factors are eliminated and temperature remains relatively 53 constant, decomposition of buried remains is frequently stated to take approximately eight 54 times as long [15, 27, 28, 29, 30]. This delay is primarily attributed to the limitation of insect 55 access (which eliminates insect-mediated degradation of soft tissue) and ambient 56 temperature. Simmons et al. [18, 19] demonstrated that where insects can freely access a 57 carcass, decomposition progresses faster than where they are excluded by any mechanism, 58 whether indoors, in water, or buried. Likewise, the speed at which decomposition progresses 59 increases as temperature rises. The mechanism by which this occurs is through the metabolic 60 activity of micro-organisms in soil; as temperature of the soil decreases, so does the rate that 61 cellular processes occur within the microbial cells, retarding microbial activity and ultimately, 62 decomposition [30, 31]. Fiedler and Graw [27] note greater interment depths can produce a 63 cooling effect resulting in lower internal burial temperatures contributing to a reduced rate of 64 decomposition in deeply buried remains. Simmons et al. [17, 18, 19] demonstrated that 65 decomposition (as measured by TBS) is strongly correlated with the accumulation of soil

temperature at burial depth, and ADD predictive equations can be produced for burials (with
and without insect access) in addition to surface remains.

68 The nature of the surrounding also soil bears influence on the rate and pattern of 69 decomposition. Moisture rich or wetter environments are conducive to adjocere formation, 70 well drained or dry soils can promote mummification, and extreme soil acidity or alkalinity has 71 been reported to decrease microbiological activity and, subsequently, decomposition [31, 32, 72 33]. Soil moisture, modified by the soil texture and structure, is understood to control microbial 73 motility, the diffusion of nutrients and waste, and the activity of extracellular enzymes [28]. 74 Where the host soil environment exhibits moisture content which exceeds optimal matric 75 potential (suction with which water is held between soil particles) decomposition processes can 76 be retarded [28]. Moreover, in areas where soil is poorly drained or seasonally waterlogged 77 (wetter soils), levels of free oxygen are low and gas diffusion is slower, limiting microbial activity 78 resulting in low bioactivity [16, 28, 32, 34].

79 Though there is published literature pertaining to decomposition rate of buried remains [17,

80 18, 30, 35] with and without insect access [17, 36], the majority of the literature concerning

81 mass graves is instead primarily focused upon excavation technique [10, 11, 12, 13, 14].

82 Although Haglund [5] considers the taphonomic properties of mass burials, a comprehensive,

83 quantitative examination of how decomposition processes differ within mass graves has not

84 been satisfactorily undertaken since Mant's [37] initial work.

Mass graves have been most recently defined as a single burial unit containing two or more victims who have died as a result of extra-judicial, summary or arbitrary executions [9] and are characterized by two main components: a body mass and a periphery. The body mass consists

of bodies which are only in contact with one another and often forms a dense contiguous
aggregate, whereas the periphery is a zone comprised of bodies in contact with both the mass
and the surrounding substrate [5, 33, 37].

91 Mass graves present a unique micro-environment where decomposition differs from single 92 burials, as a number of bodies within a single grave unit can result in complex interactions 93 among a wide range of variables (i.e. climate, depth, oxidisation, soil environment, hydrology, 94 clothing, size/weight individuals) [38]. Mant [37], who exhumed 150 World War II graves in 95 North West Europe, reported that bodies decomposed at various rates within a mass grave 96 based on their relative position to the body mass; bodies positioned towards the centre mass 97 decomposed at a slower rate than those towards the periphery [33, 37]. This differential 98 decomposition/preservation, a phenomenon termed the "feathered edge effect" [37], has been 99 attributed to the unique taphonomic microenvironments which are created in such 100 circumstances. According to Haglund [5], the peripheral bodies bridge two taphonomic 101 interfaces (i.e. the bodies of the mass and surrounding substrate) and are affected by the 102 porosity and percolation of the soil. Conversely, bodies of the mass generate their own 103 synergistic environment, separate from that of the soil [5]. This phenomenon however, remains 104 largely anecdotal and there is a little in the published literature to wholly confirm its presence, 105 the degree of differential appearance and the frequency of occurrence. 106 The aim of this research was to conduct a preliminary experiment, to determine whether the 107 position of a carcass within a mass grave affects its extent of decomposition over time. 108 Consistent with what Mant [33, 37] proposed, it was hypothesized that there would be a 109 significant difference in the extent of decomposition over time between carcasses situated in

110	various positions within the grave. In particular, it was predicted that carcasses positioned more
111	superficially and along the periphery would decompose to a greater extent than those
112	positioned in the centre of the mass (surrounded only by other carcasses) and those situated
113	deeper along the periphery.
114	
115	Materials and Methods
116	This research was conducted at the University of Central Lancashire's TRACES ('Taphonomic
117	Research in Anthropology: Centre for Experimental Studies') facility, located in Northwest
118	England. TRACES consists of 13 acres of semi-improved grade 3 rough pastureland surrounded
119	by a thin mixed native tree line and is situated approximately 270 m above average mean sea
120	level [39]. The soil is approximately 50 cm of slow-permeable, wet upland soil over clay and
121	milnow sandstone that supports vegetation of wet unimproved pasture [40].
122	A total of 210 wild rabbits (Oryctolagus cuniculus), with an average weight of 1.59 kg and an
123	accrued ADD of 8 since death, were used in this study. The rabbits exhibited projectile trauma
124	resulting from a 12 gauge shotgun with which they were culled, the most common type of
125	weapon used to hunt rabbits. Detailed information concerning the location the gunshot
126	wounds could not be determined as it would have require removal of the fur and skin.
127	Penetrating trauma and location has been previously shown to have no influence on the extent
128	of decomposition in either surface or buried remains [33, 37, 41] and gunshot trauma is
129	common within mass graves [8]. Twenty-one rabbits were interred within ten graves
130	(dimensions of each: 60 cm X 60 cm X 60 cm). The rabbits were arranged in a circular fashion so
131	as to establish concrete positions, i.e. the location, or position, of a carcass within a mass grave.

132	The four zones include: Shallow (Periphery - 5 rabbits), Mid-Outer (Periphery - 5 rabbits), Core	
133	(Center Mass - 5 rabbits) and Deep (Periphery - 6 rabbits) (Figure 1).	
134	The graves were dug manually 48 hours prior to burial in order to facilitate rapid inhumation	
135	and avoid pre-burial insect access [17]. All graves had flat bases (to prevent the localized	
136	collection of water from rainfall and/or of bodily fluids which may encourage the formation of	
137	adipocere in specific locations) and a surface depth of 30 cm (to inhibit insect access and	
138	scavenging for the duration of the experiment). The graves were positioned in a grid formation	
139	of five columns and two rows with graves situated two meters apart, generating a total surface	
140	area of approximately 10 meters X 3 meters. A pair of graves was exhumed at every data	
141	collection interval.	
142		
143	Internal Carcass Temperature	
144	The internal carcass temperature of each rabbit was measured in °C upon exhumation. These	
145	were manually recorded using a probe thermometer placed immediately into the rabbit's	
146	abdomen when it was extracted from the ground.	
147		
148	Total Body Score	
149	Decomposition was measured by recording Total Body Scores of each rabbit upon exhumation	
150	using a refined scale from Bachmann and Simmons [17] for rabbit carcasses. This scale is a	
151	modification of White's [42] scale for scoring buried rabbit remains, a revision created from	
152	Megyesi et al. [15] and Adlam and Simmons [43]. Numerical values were attributed to the head	
153	and neck, abdomen and limbs of each rabbit by visually evaluating the state of decomposition	
154	according to macroscopic criteria described by Megyesi et al. [15]. These values were summed	

to generate the TBS. Additionally, visual characteristics of decomposition were recorded for
each carcass and photographs of every rabbit were taken using a Nikon D80 digital camera.

158

159 Data Collection

160 The duration of the experiment was determined based on an experiment conducted at a 161 different site with different soil conditions by Bachmann and Simmons [17], where the 162 maximum slope of single rabbit burials' decomposition curve was used to predict 163 skeletonisation at 500 ADD. The average ambient soil temperature in North West England is 164 10°C [17, 42] resulting in an expected experimental duration of 50 days. Data were collected 165 from five paired grave exhumations; one pair of graves (i.e. Grave 1A/1B) was exhumed and the 166 carcasses evaluated at each of five data collection points approximately every ten days (100 167 ADD). Upon exhumation of the third set of graves (Grave 3A/3B) it was determined that this 168 study would benefit extending the duration of interment for the remaining two sets of graves. 169 Therefore, for the purpose of collecting additional data and allowing for a greater level of 170 decomposition, the timing of the remaining exhumations was extended. Hence the final two 171 pairs of graves (4A and 4B; 5A and 5B) were exhumed 120 ADD and 240 ADD after the 172 exhumation of 3A and 3B, respectively.

173

174 Statistical Analysis

All statistical analyses were performed using the open access software R [44]. A mixed-effects
model was used to compare Total Body Scores (TBS) of different positions over time using the
nlme package [45], and treating TBS as a continuous variable. Internal exhumation temperature

data were found to be non-normally distributed, restricting the possible analyses available, so
data were converted to differences from the mean temperature for each exhumation period
and non-parametric statistics were generated across all exhumations using the Kruskal-Wallis
test. Follow-up pairwise comparisons were performed using Steele's test [46] using the npmc
package [47].

183

184 **Results**

185 Unfortunately, ADD for the four positions could not be calculated as settling of the carcasses 186 within the mass had caused movement of the dataloggers; instead of the dataloggers recording 187 temperatures within each zone of rabbits, the final positions recorded the temperature between 188 each layer. These data could not be corrected for use in the analysis as it was not possible to 189 ascertain at which point during the experiment the loggers ceased to record correctly with 190 regard to their initial position. As a result, the following analyses are based solely on the 191 relationship of TBS to day since burial. 192 There was a marked levelling off of Total Body Scores after the penultimate sampling period, so 193 the final period (Day 64) was not included in this analysis. This produced a model which fit the 194 data sufficiently well, as indicated by diagnostic plots, despite the suggestion of a sigmoid 195 pattern (Figure 3). The mixed-effects model showed a clear relationship between TBS and day 196 across all grave positions (t = 17.4, df = 6, p < 0.001) with a unit increase in TBS taking 3.45 days. 197 There was no significant difference between the decomposition extent in the core- and the 198 deep positioned carcasses (t = 1.53, df = 154, p = 0.13), but all other differences were highly

significant (p < 0.001 in each case). As Figure 2 reflects, decomposition was fastest in shallow

200 carcasses, followed by mid-outer carcasses with the deep and core carcasses showing the lesser201 extent.

202

202	There were significant differences amongst the internal temperatures of rabbits at different
204	positions (Kruskal-Wallis: χ^2 = 121, df = 4, p < 0.001). While there was no difference between the
205	core and outer middle position temperatures (Steel's Test: p = 0.999), there were significant
206	differences between all other pairs of positions (p < 0.001 in every case). Figure 3 (lower axis)
207	illustrates that the shallower carcasses were at a higher temperature (mean difference = 0.58
208	$^{\circ}$ C) and the deeper carcasses at a lower temperature (mean difference = 0.56 $^{\circ}$ C) than those of
209	the middle layer (whose values are combined for parsimony). There was a mean temperature
210	difference of a little over 1 $^{\circ}$ C between the deep and shallow carcasses, a distance of
211	approximately 30 cm. Thus, the location of a carcass within a mass grave made a significant
212	difference to internal carcass temperature (p<0.001).
213	
214	Discussion

While the results suggest that position of a carcass within a mass grave can influence its extent 215 216 of decomposition, there appears to be more than one environmental factor influencing this. The 217 more rapid decomposition at the top of the grave was expected, since temperature is a main 218 driver of decomposition [27, 34, 48, 49, 50, 51] and carcasses nearer the surface experienced 219 higher temperatures than those beneath. However, differences were found in decomposition 220 extent of deeper carcasses buried at the same depth and at similar temperatures between 221 those at the periphery and those in the centre. This phenomenon has been noted before by 222 others, e.g. Haglund [5]. Furthermore, carcasses that experienced different temperatures (e.g.

those deepest or in the mass centre) actually showed similar extents of decomposition. Clearly,
factors other than temperature must be responsible for these discrepancies.

225 We speculate that the amount of oxygen available to the carcasses may be important to the 226 extent of decomposition (and we intend to follow this up with experimental investigation in the 227 near future). For other types of biological matter, it has long been held that decomposition 228 occurs faster in an aerobic environment [52, 53]. It seems reasonable to suggest that oxygen 229 availability would have been greater with proximity to the soil surface and without other 230 carcasses lying intermediate. At the shallowest layer, carcasses are subject to the percolation of 231 air and water through the superficial layer and/or surrounding substrate [5, 54]. Moreover, the 232 periphery of the middle layer tends to be less densely compacted than those of the deep layer. 233 Compactness of both soil and carcasses coupled with the temperature difference would likely 234 inhibit gaseous diffusion and moisture availability which would limit microbial activity and 235 prolong the course of decomposition therefore, better preserving the remains [16, 28, 31, 34, 236 50, 51]. This is consistent with the results presented here, where more decomposition was seen 237 in the periphery of the middle layer than in either the centre of the middle layer or the entirety 238 of the layer beneath. Carcasses at the bottom of a mass may become increasingly more 239 compressed and compact, with reduced oxygen and moisture availability resulting in a higher 240 proportion of anaerobic decomposition. While anaerobic conditions have been shown to slow 241 decomposition rates in a number of systems, the interaction between decomposition, body position and oxygen availability in mass graves is unclear and requires further investigation. 242 243 Moreover, the surrounding substrate may inhibit various organisms' microbiological activities; 244 as the natural limits of bacteria to function in hyper-acidic/alkali environments are met,

245 microbiological function can be inhibited, resulting in a reduced extent of decomposition and in 246 some cases the formation of adipocere [55]. Small guantities of adipocere formation were 247 observed throughout the duration of this study, most commonly observed at the deepest layer 248 of the mass and on one carcass of the mid-outer periphery. The translocation of the liquefied 249 fat content via gravity and its influence on decomposition [27, 56] may result in higher levels of 250 adipocere located deeper within a mass contributing to the lesser extent of decomposition of 251 carcasses situated deeper within a grave. Due to the compact nature of the body mass in 252 comparison to the periphery, which potentially inhibits water entry percolating from above, 253 adipocere formation deeper in the mass is more likely. Nevertheless, given that there were few 254 carcasses (mostly of the deeper layer, one of the mid-outer periphery) exhibiting adipocere 255 formation, this speculation requires further experimental observation.

256

257 Conclusions

258

259 This study took a preliminary, quantitative approach to the examination of differential 260 decomposition patterns in mass graves, a grey area in the realm of forensic anthropology with 261 few experimentally supported findings and some largely anecdotal reports. Results revealed 262 that decomposition proceeds differentially over time depending on the position of a carcass 263 within a mass; carcasses which were situated in the centre mass and/or deeper within the grave 264 were revealed to decompose to a lesser extent compared to those situated along the periphery 265 and/or more shallow within the grave. These findings confirm current assumptions reported in 266 academic literature. The core mass did decompose to a lesser extent than the peripheral 267 carcass excluding those of the deepest layer (feathered edge effect), suggesting that

- 268 decomposition extent may be as affected by compactness of a mass as interment depth and/or
- 269 peripheral substrate contact. These observations hold implications of a preliminary nature for
- 270 investigations involving the exhumation of mass graves. Further research that considers the
- 271 effect of oxygen access and compactness of a mass will be beneficial in advancing an
- 272 experimentally supported understanding of decomposition processes within mass graves.

References Cited

- M. Doretti, C. Snow, Forensic anthropology and human rights: the Argentine experience, in: D.W. Steadman (Ed.), Hard Evidence: Case Studies in Forensic Anthropology, Prentice Hall, New Jersey, 2003.
- [2] K. Burns, Forensic anthropology and human rights issue, in: K.J. Reichs (Ed.), Forensic Osteology: Advances in the Identification of Human Remains, second edition, Charles C. Thomas, Illinois, 1998.
- [3] M. Kaliszan, R. Hauser, G. Kernbach-Wighton, Estimation of the time of death based on the assessment of post mortem processes with emphasis on body cooling, Leg. Med. 11 (2009) 111-117.
- [4] P. Sledzik, Forensic taphonomy: postmortem decomposition and decay, in: K.J. Reichs (Ed.), Forensic Osteology: Advances in the Identification of Human Remains, second edition, Charles C. Thomas, Illinois, 1998.
- [5] W. Haglund, Recent Mass Graves, An Introduction, in: W. Haglund, M. Sorg (Eds.), Advances in Forensic Taphonomy: Method, Theory and Archaeological Perspectives, CRC Press LLC, London, 2002.
- [6] W.D. Haglund, M. Connor, D.D. Scott, The archaeology of contemporary mass graves, Hist. Arch., 35 (2001) 57-69.
- [7] S. Cordner, R. Coupland, Missing people and mass graves in Iraq, Lancet, 362 (2003) 1325-1326.

- [8] Baraybar, J, Pablo, M. Gasior, Forensic anthropology and the most probable cause of death in cases of violations against international humanitarian law: an example from Bosnia and Herzegovina, J. For. Sci., 51(2006) 103-108.
- [9] E. Jessee, M. Skinner, A typology of mass grave-related sites, For. Sci. Int. 152 (2005) 55-59.
- [10] C. Steele, Archaeology and the forensic investigation of recent mass graves: ethical issues for a new practice of archaeology. *Archaeologies: J. Wor. Arch. Cong.* 4 (2008) 414-428.
- [11] M. Cox, A. Flavel, I. Hanson, J. Laver, R. Wessling (Eds.) The Scientific Investigation of Mass Graves: Towards Protocols and Standard Operating Procedures, Cambridge University Press, Cambridge, 2007.
- [12] H. Tuller, M. Duric, Keeping the pieces together: comparison of mass grave excavation methodology, J. For. Sci. 156 (2006) 192-200.
- [13] M. Skinner, J. Sterenberg, Turf wars: authority and responsibility for the investigation of mass graves, For. Sci. Int. 151 (2005) 221-232.
- [14] M. Skinner, D. Alemppijevic, M. Djuric-Srejic, Guidelines for the international forensic bioarchaeology monitors of mass grave exhumations, For. Sci. Int.134 (2003) 81-92.
- [15] M. Megyesi, S. Nawrocki, N. Haskell, Using accumulated degree days to estimate the postmortem interval from decomposed human remains, J. Forensic Sci. 50 (2005) pp. 618-626.
- [16] A. Vass, W. Bass, J. Wolt, J. Foss, J. Ammons, Time since death determinations of human cadavers using soil solution, J. For. Sci. 37 (1992) 1236-1253.

- [17] J. Bachmann, T. Simmons, The influence of pre-burial insect access on the decomposition rate, J. For. Sci. 55 (2010) 893-900.
- [18] T. Simmons, P. Cross, R. Adlam, C. Moffatt, The influence of insects on decomposition rate in buried and surface remains, J. For. Sci. 55 (2010) 889-892.
- [19] T. Simmons, R. Adlam, C. Moffat, Debugging decomposition data: comparative taphonomic studies and the influence of insects and carcass size on decomposition rate, J. For. Sci. 55 (2010) 8-13.
- [20] C.Y Arnold, The determination and significance of the base temperature in a linear heat unit system, P. Am. Soc. Hortic. Sci. 74 (1959) 430-445.
- [21] C.Y Arnold, Maximum-minimum temperatures as a basis for computing heat units, P. Am. Soc. Hortic. Sci. 76 (1960) 682-692.
- [22] M. Micozzi, Postmortem Changes in Human and Animal Remains, Charles C. Thomas, Illinois, 1991.
- [23] H. Reed, A study of dog carcass communities in Tennessee, with special reference to the insects, American Midland Naturalist, 59 (1958) 213- 245.
- [24] J. Payne, A summer carrion study of the baby pig *Sus scrofa* Linnaeus, Ecology, 46 (1965) 592-602.
- [25] D. Johnson, Seasonal and microseral variation in the insect population on carrion, American Midland Naturalist, 93 (1975) 79-90.

- [26] A. Galloway, W.H. Birkby, A.M. Jones, T.E. Henry, B.O. Parks, Decay rates of human remains in an arid environment, J. For. Sci., 34 (1989) 607-616.
- [27] S. Fielder, M. Graw, Decomposition of buried corpses, with special reference to the formation of adipocere, Naturwissenschaften, 90 (2003) 291-300.
- [28] D.O Carter, D.Yellowlees, M. Tibbett, Moisture can be the dominant environmental parameter governing cadaver decomposition in soil, For. Sci. Int. 200 (2010) 60-66.
- [29] J. Dix, M. Graham, Time of Death, Decomposition and Identification: An Atlas, CRC Press, London, 2000.
- [30] W. Rodriguez, Decomposition of buried and submerged bodies, in: W. Haglund, M. Sorg (Eds.), Forensic Taphonomy: The Postmortem Fate of Human Remains, CRC Press LLC, London, 1997.
- [31] H. Gill-King, Chemical and ultrastructural aspects of decomposition, in: W. Haglund, M. Sorg (Eds.), Forensic Taphonomy: The Postmortem Fate of Human Remains, CRC Press LLC, London, 1997.
- [32] B. Turner, P. Wiltshire, Experimental validation of forensic evidence: a study of the decomposition of buried pigs in a heavy clay soil, For. Sci. Int. 101 (1999) 113-122.
- [33] A.K. Mant, Knowledge acquired from post-War exhumations, in: A. Boddington, A.N. Garland, R.C. Janaway (Eds.), Death, Decay and Reconstruction: Approaches to Archaeology and Forensic Science, Manchester University Press, Manchester, 1987.

- [34] A.N. Garland, R.C. Janaway, The taphonomy of inhumation burials, in: C.A. Roberts, F. Lee, J.L. Bintliff (Eds.), Burial Archaeology: Current Research, Methods and Developments, British Archaeological Reports, Oxford, 1989.
- [35] A.S. Wilson, R.C. Janaway, A.D. Holland, H.I. Dodson, E. Baran, A.M. Pollard, D.J. Tobin, Modelling the buried human body environment in upland climes using three contrasting field sites, For. Sci. Int. 169 (2007) 6-18.
- [36] H. Schroeder, H. Klotzbach, K. Püschel, Insects' colonization of human corpses in warm and cold seasons. Leg. Med. 5 (2003) S372-S374.
- [37] A.K. Mant, A Study of Exhumation Data, Ph.D. Thesis, London University, 1950.
- [38] J. Hunter, M. Cox, Forensic Archaeology: Advances in Theory and Practice, Routledge, New York, 2005.
- [39] Earth Tools (Data recorded Feb. 2000) : HYPERLINK "<u>http://www.earthtools.org</u>"<u>http://www.earthtools.org</u>" NASA.
- [40] National Soil Resources Institute, Cranfield University: HYPERLINK "<u>http://www.landis.org.uk/soilscapes/</u>"<u>http://www.landis.org.uk/soilscapes/</u>".
- [41] P. Cross, T. Simmons, The influence of penetrative trauma on the rate of decomposition, J.For. Sci. 55 (2010) 295-301.
- [42] R. White, Decomposition in a mass grave and the Implications for Post Mortem Interval Estimate, Proceedings of the American Academy of Forensic Sciences, Annual Meeting, San Antonio, TX, February 19-24 (2007) 367.

- [43] R. Adlam, T. Simmons, The effect of repeat physical disturbance on soft tissue decomposition – are taphonomic studies an accurate reflection of decomposition, J. For. Sci., 52 (2007) 1007-1014.
- [44] R Development Core Team (2010). R: A language and environment for statistical computing, R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/.
- [45] J. Pinheiro, D. Bates, S. DebRoy, D. Sarkar and the R Development Core Team, nlme: Linear and Nonlinear Mixed Effects Models. R package version 3. 1-97, 2010.
- [46] R. D. G. Steel, A rank sum test for comparing all pairs of treatments, Technometrics 2 (1960) 197–207.
- [47] J. Helms, U. Munzel, npmc, Nonparametric Multiple Comparisons, R package version 1. 0-7, 2008.
- [48] A. Vass, S.A. Barshick, G. Sega, J. Caton, J.T. Skeen, J.C. Love, J.A. Synstelien, Decomposition chemistry of human remains: a new methodology for determining the postmortem interval.
 J. For. Sci. 47 (2002) 542-553.
- [49] R. Mann, W. Bass, L. Meadows, Time since death and decomposition of the human body: variables and observations in case and experimental field studies, J. For. Sci. 35 (1990) 103-111.
- [50] D.O Carter, M. Tibbett, Microbial decomposition of skeletal muscle tissue (Ovis aries) in a sandy loam soil at different temperatures, Soil Biol. Biochem. 38 (2006) 1139-1145.
- [51] D.O Carter, D. Yellowlees, M. Tibbett, Temperature affects microbial decomposition of cadavers (Rattus rattus) in contrasting soils, Appl. Soil Ecol. 40 (2008) 129-137.

- [52] D.J. Greenwood, The effect of oxygen concentration on the decomposition of organic material in soil, Plant and Soil, 4 (1961) 360-376.
- [53] E. Kristensen, S.I. Ahmed, A.H. Devol, Aerobic and anaerobic decomposition of matter in marine sediment: which is fastest?, Limnology and Oceanography, 40 (1995) 1430-1437.
- [54] A. Vass, Beyond the grave understanding human decomposition, Microbiology Today, 28 (2001) 190-192.
- [55] S.L. Forbes, B.H. Stuart, B.B. Dent, The effect of the burial environment on adipocere formation, For. Sci. Int. 154 (2005) 24-34.
- [56] S.L. Forbes, B.H. Stuart, B.B. Dent, The effect of soil type on adipocere formation, For. Sci. Int. 154 (2005) 35-43.

Additional information and reprint requests:

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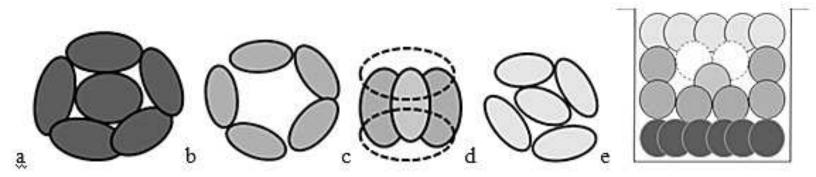
Figure Captions

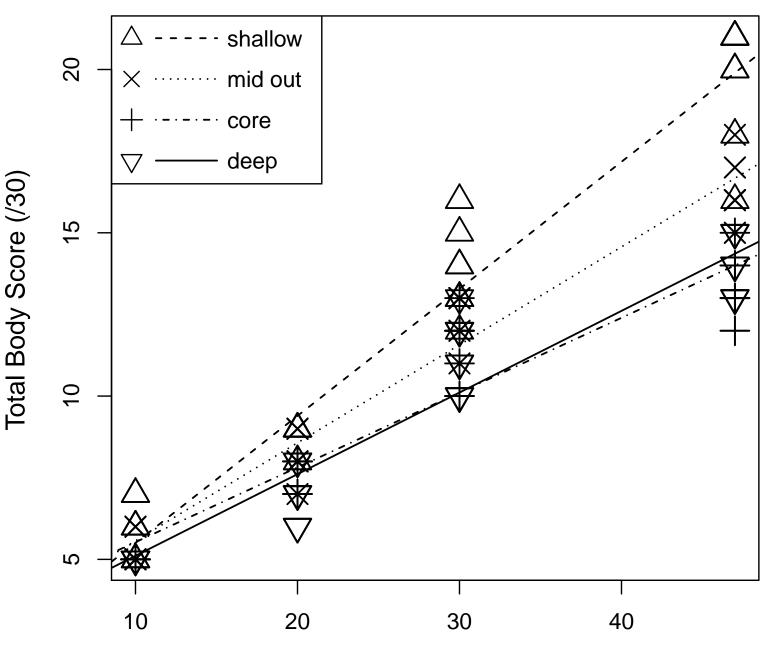
Figure 1. The layers comprising the circular arrangement of carcasses determining the internal grave positions designated in this study: a) Deep b) Mid-Outer c) Core d) Shallow e) Two-Dimensional, vertical cross-section representation of grave.

Figure 2. Total body scores for all carcasses against exhumation day (excluding final day – see text) with regression lines.

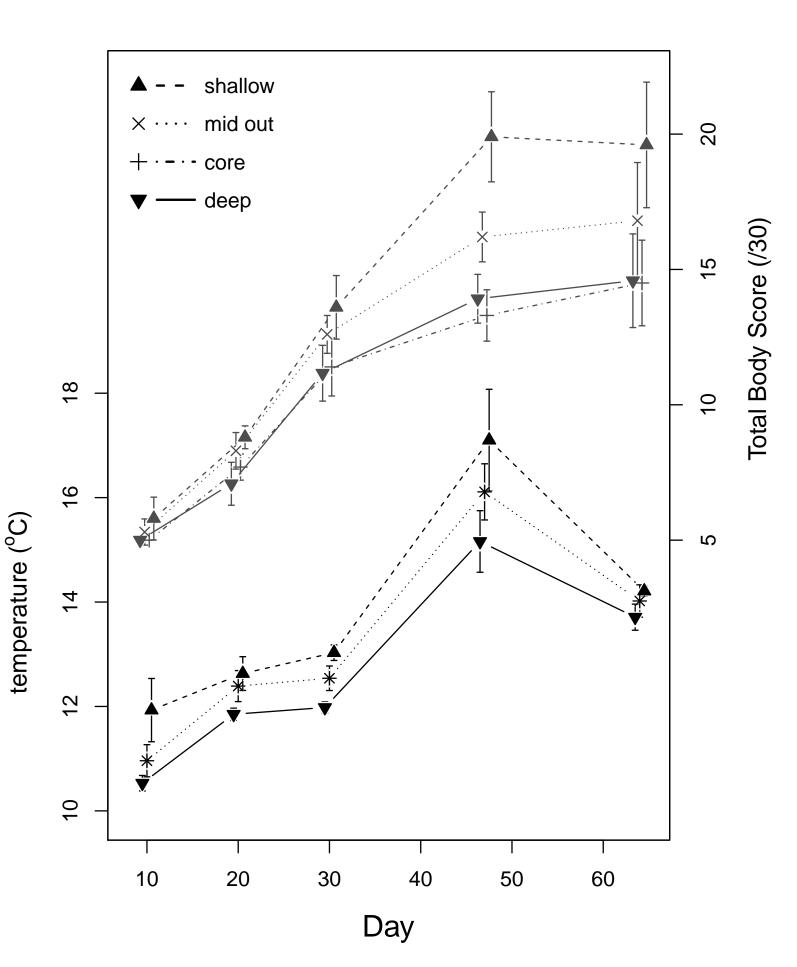
Figure 3. Mean total body scores (grey) and mean temperatures (black) for grave positions against day of exhumation. In the temperature plot, mid outer and mid inner temperatures have been combined as have their symbols. Points have been offset slightly on Day axis for the sake of ease of interpretation; there were five exhumation days.

Figure 1





Day



9.5.2012

Dear Editor,

We hereby submit the revisions to Manuscript **FSI-D-12-00060R1** in hopes that it now meets the required standard.

We sincerely apologise for the delay in re-submission. Ms Troutman, the senior author, was travelling in South America for much of the intervening time and subsequently suffered a death in the family, so her required input was, of necessity, deferred.

We urge you to review the table below that details how we have met each of the requirements specified in the reviewer's comments. We have done a very thorough job and responded to each one of the specifications made by the reviewer – and I have furthermore noted each line and page number on which the response/correction/explanation can be found within the revised manuscript. It is therefore hoped that you, in your editorial capacity, will find this acceptable without requiring further review and it can be published without further postponement.

With our thanks,

Tal Simmons, Colin Moffatt and Lauren Troutman

* * * * * * * * * *

The points raised by the reviewer have been addressed as follows:

How addressed
This has been done
This has been added
This has been done
The citations have been added and the phrase
"amongst others" has been removed
These have been identified in the text

synergistic biological and geological variables." that you refer to.	
Although the reference to Casper's Law has been acknowledged, as you state, recent work shows that it is not accurate. A corpse in soil does not necessarily decompose eight times more slowly than one in open air. Please modify this sentence.	This has been addressed by rewording lines 48- 51, page 2
The sentence that begins "This delay, with ADD constant," is redundant and poorly worded. Please modify.	This has been re-worded in lines 51-53, page 2
There exist no datasets to show that autolysis is the primary driver of buried corpse breakdown. There are certainly no datasets presented in the book chapter that you cite for this statement. I concede that it might be possible for autolysis to outstrip putrefaction in the early postmortem period when the internal microbiota are in a lag phase. But there is no evidence to support the claim that autolysis results in more decomposition than internal bacteria during exponential growth or the stationary phase. In fact, suggesting such a phenomenon goes against fundamental principles of the ecology of decomposition in terrestrial ecosystems: the vast majority of decomposition is mediated by organisms, not abiotic processes.	This is clearly a difference of opinion and remains as such
3rd paragraph - Anaerobic conditions are not always the result of high levels of carbon dioxide. Anaerobic simply means a lack of oxygen to be used as the terminal electron acceptor in the electron transport chain. Also, you should not discount an environment of low oxygen availability; the microaerophilic bacteria can thrive in these habitats. Again, you cite work that does not support your statements. Some work you should be familiar with, that would represent better citations are:	The suggested citations have been duly added
4th paragraph - The fourth paragraph restates many of the same points introduced in the third paragraph. Please merge these two paragraphs	This has been done

into one.	
Inhibition of postmortem change is not brought about by adipocere. Adipocere slows decomposition and acts as a resource for a number of bacteria. Also delete the term "spontaneous".	This has been done
Please modify "soils cater to." Perhaps, "soils promote" instead?	This has been done - Line 70, page 3
Be careful with the term "reduce". I suggest that you change it to "decrease" to avoid confusion for readers that will be prompted to think of oxidation-reduction reactions.	This has been done, Line 71, page 3
7th Paragraph - Please describe the "wide range of variables" among which a mass grave can function.	This has been done – lines 93-4, page 4
8th Paragraph - It is not necessary to tell the reader that your experiment is "scientifically robust". Please delete that clause.	The phrase "scientifically robust" has been removed
Materials & Methods (1st paragraph) - Please include the soil type. The World Reference Base classification is probably the most appropriate.	The soil has been described and duly referenced (lines 120-1, page 5)
2nd Paragraph - You imply that the rabbits were killed with a gunshot. Please make this explicit and describe the type of firearm used to kill the rabbits. Also describe the location of the gunshot wound.	The weapon has been described in line 124 and the reason that wound locations could not be described has been explained in line 125-6, page 5
Please change "manner" to "cause" in the 2nd sentence. Manner of death has a very strict definition; gunshot wound is not a manner of death.	This sentence non longer exists given the re- wording required above
Statistical Analysis - Please change text to state, "non-parametric statistics were generated."	This has been done in line 179, page 8
Results (2nd Paragraph) - You refer to Figure 2 in the 2nd sentence. Do you not mean Figure 3?	This has been corrected to read "Figure 3"
Please change all references to the calculation of rate of decomposition. You did not measure a rate, which is a function of time. You measured	All references to this experiment studying decomposition "rate" removed, replaced with reference to "extent of decomposition"

the output of decomposition at a series of the series	
the extent of decomposition at a series of times. Rather than say carcasses showed the "slowest rate" you must say that carcasses "decomposed less".	
In the last sentence of the Results you state "The location of a carcass within a mass grave made a significant difference." Please include a p value or modify this sentence.	The p-value has been included in line 211, page 9
Discussion – The Discussion presents several problems. Chief of which is that it provides more evidence to show that you are not familiar with the literature relevant to your study. You speculate that the presence of oxygen may be important to the rate of decomposition. This is fair, but you should be familiar with this work because it addresses many of your points. You also speculate about the role of pH in decomposition but fail to cite the following work.	Some of the requested references have been duly cited
You state, "In aerobic conditions respiration, synthesis of microbial material and rapid disappearance of simple organic compounds represents decomposition." This is true but these processes also occur during anaerobic respiration. It is not until fermentation is reached that the components of this sentence will change. Again, you refer to anoxic conditions without considering low-oxygen conditions. You must do so.	This paper is a preliminary investigation in to the differential decomposition within mass graves, it was never intended, nor presented, to be a conclusive discussion of this phenomenon. We indicate on page 10, lines 225-6, that this will be investigated in the future. As O2 levels were not measured in our experiment, we really cannot comment further on this at the present time.
One area of the Discussion that is clearly missing is the modification of the duration of your experiment. You wisely based the duration of your experiment on previous datasets generated at TRACES. Well done. However, those data did not seem to give you an accurate timeframe and your experiment had to be extended. Why was this? Why were the previous datasets not accurate? What implications do this have on PMI estimates using data from the TRACES facility? Without addressing these points it become easy for a reader to argue that the data are not reliable. I strongly suggest that you address	This has been discussed earlier in the paper now on page 7, lines 161-2 re. this being an experiment at a different location (i.e. not TRACES) with single burials in different soil conditions

these points and offer logical explanations.	
Conclusions - You again refer to the rate of decomposition without actually measuring a rate. Please modify this sentence. Overall, the latter half of your Conclusion section is not comprehensible. What does the "entirety" of the periphery represent? I am not sure how your results "both challenge and confirm" current assumptions. To me it appears as though your findings confirm previous work.	The phrase "entirety of the periphery" has been removed and re-phrased (see line 263, page 11) and the phrase "both challenge and confirm" has been removed to indicate "confirm" (line 264, page 11)
References - Citation 16 has an incorrect title. Please provide the correct title.	This has been corrected
Figure Legends - It is not appropriate to include "see text" in a figure legend. A figure legend must be able to stand alone without the manuscript text. A reader must be able to understand the nature of the experiment, its protocol, and the figure by reading a figure legend. As such, figure legends typically do not have a word limit. Please make your figure legends robust and include the details of your experiment in each one.	The phrase "see text" has been removed – the figure legends can stand alone s they were without this phrase.