

## **Changes to soil bacterial profiles as a result of *Sus scrofa* domesticus decomposition**

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## Highlights

- Bacterial diversity and richness changed due to *Sus scrofa domesticus* decomposition
- Temporal and spatial community shifts evidenced relative to buried material
- Shifts linked also to temporal changes in taphonomic and environmental parameters
- DGGE can potentially become another forensic tool for clandestine grave location
- Applicability potential may be tested/advanced with *in situ* studies and sequencing

## Abstract

The importance of cadaver decomposition knowledge for clandestine grave location cannot be over emphasized. Notwithstanding this, only a limited understanding is available on the resulting soil microbial community dynamics. To address this paucity, a pig leg (*Sus scrofa domesticus*; 5 kg) was buried in freshly weighed (20 kg) sandy loamy soil in a sealed microcosm (40 cm height) in parallel with a soil only control. Both microcosms were perforated nine times at equal distances and maintained outside. Soil samples were collected through these perforations from the top (0–10 cm), middle (10–20 cm) and bottom (20–30 cm) segments every three days for the first two weeks, and then weekly up to 14 weeks. PCR-DGGE gels quantified by 1D Phoretix showed increases in the cumulative soil community richness values of 43, 66 and 106 for the top, middle and bottom segments, respectively, in the presence of *Sus scrofa domesticus*. Shannon-Wiener's ( $H'$ ) and Simpson's ( $D$ ) indices confirmed corresponding species diversity increases in the middle ( $H' = 1.58 - 2.33$ ;  $D = 0.79 - 0.91$ ) and bottom ( $H' = 2.48 - 3.16$ ;  $D = 0.85 - 0.95$ ) depths between days 10 and 71 compared with the control. In contrast, similar evenness was recorded for all segments in both the *Sus scrofa domesticus* and control soils.

*Keywords: Cadaver decomposition; Soil bacterial community; Sus scrofa domesticus; PCR-DGGE*

## 1. Introduction

Billions of microorganisms are found in terrestrial habitats with bacteria often the most prevalent [1]. Bacteria and fungi are responsible for almost 90% of organic matter breakdown and play significant roles in the carbon and nitrogen cycles [2]. Less than 1% of microorganisms have, however, been characterised by culture-based methods [1, 3]. Despite this, the use of molecular techniques to study soil microbial communities has proven to be very successful [4]. Extraction of nucleic acids from microbial cells in soil samples by molecular techniques and their various applications in polymerase chain reaction (PCR) amplification and hybridization experiments has allowed researchers to identify and characterise numerous microorganisms in different environments [5, 6]. The importance of cadaver decomposition knowledge cannot be over emphasized hence emergent studies have analysed changes in the

postmortem microbiome or necrobiome of the abdominal, interior anal and buccal cavities and skin of decomposing carcasses [7-10]. Nonetheless, the majority of research has focused on above ground decomposition [6, 11-13]. As a result, very little is known of soil microbial community changes following cadaver decomposition within grave sites [4, 14-16].

Cadaver decomposition is a complex process and is affected by enzymatic catabolism, microbial activity and changes in different environmental variables. It begins approximately four minutes after death and progresses through different stages namely: autolysis; putrefaction; and decay [14, 16-18]. Of fundamental importance to these is the unique roles played by microorganisms although, overall, decomposition is still largely a “Black Box” [12, 19-21]. Characterisation and identification of changes in microbial diversity can facilitate the location of clandestine or secondary graves and provide additional intelligence in cases of complete decomposition (as with juvenile remains). Hence, identification of specific catabolic biochemical and molecular markers in soil microbial communities represents an essential additional tool for forensic practitioners.

The hypothesis for this decomposition study was that soil microbial community changes would result with time and would be identified by targeting specific biochemical and molecular markers and, subsequently, quantified by different ecological measures. Also, soil microbial community changes were anticipated for the different microcosm depths relative to the buried material. This hypothesis was tested by identifying temporal and spatial soil community profile changes through DNA-based polymerase chain reaction (PCR) and denaturing gradient gel electrophoresis (DGGE) targeting the 16S rRNA gene.

## **2. Materials and Methods**

### *2.1 Sus scrofa domesticus burial and sampling*

Two sealed microcosms filled with sandy loamy garden soil (20 kg fresh weight) were maintained outside for 98 days with (experimental) and without (control) a 5 kg leg of domesticated pig (*Sus scrofa domesticus*). This species has been used for decomposition studies by various researchers because of its close similarities to the human body [14, 22]. The microcosms were 40 cm in height and were divided into

three depths (top, middle and bottom). Perforations at equal distances and heights facilitated aeration, moisture migration, sampling and hygiene maintenance. The mean temperature for the study duration was 14.9 °C and the average rainfall was 3.5 mm (Fig. 1). Composite soil samples for pH determination, DNA extraction and PCR-DGGE profiling were collected from the three segments on days 0, 3, 7, 10 and 14 of the first two weeks and then subsequently at weekly intervals.

## 2.2. Soil characterisation

Soil physicochemical characteristics (Derwentside Environmental Testing Services Ltd., County Durham, U.K.) were Al (28 g kg<sup>-1</sup>), Ca (18 g kg<sup>-1</sup>), Mg (9.2 g kg<sup>-1</sup>), K (5.3 g kg<sup>-1</sup>), Na (0.37 g kg<sup>-1</sup>), total organic C (2.8 %), total S (0.03 %), aqueous extractable NO<sub>3</sub><sup>-</sup> (1.5 mg L<sup>-1</sup>), ortho PO<sub>4</sub><sup>-</sup> as P (<0.10 mg kg<sup>-1</sup>), pH (7.6), calorific value (1.2 MJ kg<sup>-1</sup>) and electrical conductivity (250 uS cm<sup>-1</sup>).

## 2.3 pH measurement

The soil samples were mixed thoroughly with deionised water at a ratio of 1:5 (w/v) prior to pH determination with a pH 213 Microprocessor (Hanna Instruments, Bedfordshire, U.K.) fitted with a Fisher electrode according to the method described by Stokes et al (2009) [13].

## 2.4 DNA extraction

DNA extractions were made with FastDNA<sup>®</sup>Spin kits for Soil (MP Biomedicals, U.K.) according to the manufacturer's instructions and stored at -20 °C until needed. Mixtures of 5 µL DNA template and 1 µL 6X loading buffer were analysed on 1.5% (w/v) agarose gels which contained 6 µL SYBR Safe (Invitrogen, U.S.A.). The gels were electrophoresed in 1X TBE buffer for 90 min at 150 V and viewed (AlphaImager HP<sup>®</sup>, Alpha Innotech, Braintree, U.K.) under UV light.

## 2.5 Polymerase chain reaction (PCR) – denaturing gradient gel electrophoresis (DGGE)

The V3 region of the bacterial 16S rRNA gene from position 356 to 519 was targeted for amplification according to Manefield et al. (2002) [23], with the forward primer (5'

CGCCCGCCGCGCCCCGCCCCGGCCCGCCGCCCCGCCCCACTCCTACGGGAGGCAG

C 3') and the reverse primer (5' GTATTACCGCGGCTGCTG 3'). The 25  $\mu$ L PCR reaction mixture consisted of 12.5  $\mu$ L of 2X PCR master mix (Promega, Southampton, U.K.), 0.5  $\mu$ L of the forward and reverse primers (0.2  $\mu$ M), 1.25  $\mu$ L BSA (0.5 mg mL<sup>-1</sup>), 8.25  $\mu$ L molecular grade water (Promega, Southampton, U.K.) and 2  $\mu$ L of DNA templates. The thermo-cycling program (Primus 96 Plus, MWG-Biotech, Ebersberg, Germany) consisted of 1 cycle at 95 °C for 2 min, 35 cycles of: denaturation at 95 °C for 1 min; annealing at 60 °C for 1 min; and extension at 72 °C for 1.5 min, and final extension at 72 °C for 30 min. The amplicons were checked on 1.5% (w/v) agarose gels as above prior to separation (20  $\mu$ L) on 10% (w/v) polyacrylamide gel (acrylamide/bisacrylamide gel 37.5:1) with a 30% to 65% denaturing gradient (PHOR-U Ingeny System, Leiden, the Netherlands) at 60 °C and 110 V for 18 h. The gels were stained with SYBR Gold (Invitrogen, U.S.A.) and viewed (AlphaImager HP®, Alpha Innotech, Braintree, U.K.) under UV light.

## 2.6 Image detection and statistical data analysis

The DGGE images were analysed with Phoretix 1D software (TotalLab, Newcastle, U.K.) for community fingerprint quantification. Similarities between lanes were investigated by the un-weighted pair-group by the arithmetic average (UPGMA) method with a synthetic reference lane. The richness (S) and Shannon-Wiener (H') diversity index were determined based on the number of bands in a lane with H' then calculated (Equation 1). The Simpson (D) diversity index accounted for the intensity (relative abundance) of each band and was calculated (Equation 2). The evenness was determined by rank of the log<sub>10</sub> of abundance [24]. All data were analysed with single factor Anova ( $p < 0.05$ ).

$$\text{Shannon-Wiener index (H')} = - \sum P_i \ln P_i \quad \text{Equation 1}$$

$$\text{Simpson index (D)} = 1 - \frac{\sum n_i(n_i-1)}{N(N-1)} \quad \text{Equation 2}$$

## 3.0 Results

Both treatments recorded increased pH values in the top layer until day 42 when a decrease to near neutral (pH ~ 6.8) resulted in the experimental microcosm. Also, the bottom layer of this microcosm was more alkaline (pH~8.5) than the control (pH~7.6) (Fig. 2).

The PCR-DGGE fingerprints were analysed with 1D Phoretix software to determine the lanes, bands, band intensities and their positions and to create dendrogram representation of different operational taxonomic units (OTUs) present in the soils (Fig. 3). The un-weighted pair-group by arithmetic average (UPGMA) measured similarities between and within the control (C) and experimental (E) microcosms for the three segments: top (T); middle (M); and bottom (B). For the top segment the lowest similarity (22%) between the control and experimental microcosm was observed on day 71 while the highest (99%) was recorded on days 28 and 63 (Fig. 3a). The middle depths recorded the lowest similarity (19%) on day 49 and the highest (67%) on days 0 and 14 (67%) (Fig. 3b). Finally, the bottom depths recorded the lowest similarity (18%) on day 77 and the highest (87%) on days 10 and 14 (Fig. 3c).

Species richness (S) was determined by the number of OTUs observed in each lane while the relative abundance of individual species was assessed by individual band (band volume) intensity. These were then used to calculate the Shannon–Wiener (H') and Simpson's (D) diversity indices and evenness. No significant differences in richness between the top and middle segments of the control ( $p = 0.39$ ) and experimental ( $p = 0.44$ ) microcosms were apparent in contrast to the bottom depths ( $p = 0.0069$ ; Fig. 4).

As recorded for community richness, the Shannon-Wiener's diversity did not reveal significant differences between the control and experimental microcosms for the top ( $p = 0.54$ ) and middle ( $p = 0.14$ ) segments as was apparent ( $p = 0.0015$ ), particularly from day 28, for the bottom segments. In the presence of *Sus scrofa domesticus*, a significant depth difference ( $p = 0.032$ ) was observed but not for the control ( $p = 0.46$ ). In general, an average of the Shannon-Wiener's diversity determinations for the top, middle and bottom depths for the control and experimental soils showed a significant difference ( $p = 0.00004$ ) between the total communities due to the presence of the carcass (Fig. 5a).

Simpson's diversity revealed a significant difference ( $p = 0.0057$ ) between the two microcosms for the bottom depths (Fig. 5b) but not the top ( $p = 0.79$ ) and middle ( $p = 0.12$ ) depths. Overall, an average of the Simpson's diversity measurements for the

three layers exemplified a significant difference ( $p = 0.000029$ ) between the total communities due to carcass decomposition.

The evenness distribution was determined by taking the rank of the  $\log_{10}$  of abundance of the OTUs, with the inverse log of the slope then used to calculate the evenness value [24]. The values for the experimental top, middle and bottom segments were 0.788, 0.823 and 0.749, respectively (Fig. 6a) while the equivalents for the control were 0.873, 0.759, and 0.942 (Fig. 6b).

#### **4.0 Discussion**

Cadaver decomposition rate, as described by various scholars [16, 18, 19, 21], is influenced largely by the method of body disposal (dumped, buried, frozen, submerged), which subsequently affect its interactions with the fauna, flora and microbiota in its surroundings. As reported by Belle et al. (2009) [14], the active decay stage for a subterranean cadaver occurs within a period of three months. This conclusion was supported in the current research where the active decay period, as evidenced by extensive tissue decomposition, was recorded between days 10 and 42. On day 56 the soft tissue of the *Sus scrofa domesticus* leg had decomposed completely, thereby creating a void between the tibia, fibula and skin, with the skin forming a thick barrier against the sandy loamy soil. According to Gill-King (1997) [20], in the order of tissue decomposition, connective tissue and integuments are usually the last to decompose because of their high collagen content. Again, this was in agreement with observations in the experimental microcosm on day 56.

A further well reported parameter of cadaver decomposition is the formation of adipocere [19, 22, 25, 26], which is considered to result from bacterial activity in warm, moist and anoxic environments [19-21, 25]. According to Ubelaker and Zarenko (2011) [27], adipocere formation is an essential taphonomic phenomenon due to its soft tissue preservation and its power to reveal constitutional and environmental factors that may prove valuable in forensic investigation with its complicating role in the determination of postmortem interval. Also, Forbes (2008) [19] reported that soil pH can affect adipocere formation with a mildly alkaline environment resulting in an increased rate. A similar trend was recorded in the current study where adipocere formation on the *Sus scrofa domesticus* leg was recorded on day 45, with an alkaline pH value of 7.55.



The transition from aerobic to anaerobic bacteria (*Bacteroides*, Clostridia, Streptococci, Staphylococci and Enterobacteriaceae) during putrefaction has been reported widely [4, 19, 21, 28, 29]. This triggers the catabolism of lipids, carbohydrates and proteins into organic acids (*e.g.* butyric, lactic, propionic) and gases (*e.g.* ammonia, methane, sulphur dioxide, hydrogen sulphide), which results in a foul odour, colour change and cadaver swelling [11, 16, 18, 19]. Furthermore, the seepage of these by-products changes temporarily both the microbiota and soil chemistry [30]. As a result, soil microbial profiling at various grave depths is essential where understanding and estimating microbial community changes can be potential forensic tools. As observed in this study, different microcosm segments gave an insight of temporal microbial community changes with decomposition and relative to the position/depth of the buried material. These changes can be attributed to vertical nutrient leaching in the *Sus scrofa domesticus* treatment compared to the control. The PCR-DGGE analysis showed temporal community profile changes with decomposition from days 10 to 77 with some distinct numerically dominant OTUs recorded for the three depths compared to the control.

The diversity indices in the two microcosms showed that carcass decomposition resulted in significant differences ( $p < 0.05$ ) in the three segments with the top section recording the lowest total cumulative richness value of 43 in comparison with 65 and 106 for the middle and bottom segments, respectively. Also, as suggested by McGuire and Treseder (2010) [2], a rise in microbial species richness is linked to an increase in decomposition. This was observed for the three soil depths with the bottom layer in particular having the highest increase in richness from 11 (day 10) to 26 (day 70) in the experimental microcosm.

According to Bandeira et al. (2013) [31], the use of a single ecological index to define a community overgeneralises its actual biodiversity so the application of different indices is recommended. The Shannon-Wiener index, as described by various workers [31-33], is a function of evenness and species relative abundance, while the Simpson's index is a function of richness and relative abundance. Despite the use of different, but complementary, ecological measurements of the bacterial community dynamics,  $H'$  and  $D$  still showed similar trends of significant diversity differences for the three segments of the experimental microcosm compared to the control. This suggested a vital role of sample collection and subsequent community profiling of

clandestine grave soils relative to the position or depth of the buried material. Furthermore, both indices evidenced distinct differences in bacterial community diversity in the absence and presence of *Sus scrofa domesticus*. Therefore, DGGE profiling can be used potentially to differentiate between grave and non-grave soils.

Several authors [34-36] have reported that the closer the evenness number gets to 1, the more homogeneously are the species distributed in a community. As recorded for both microcosms, independent of soil depth, there was no significant difference ( $p > 0.05$ ) for the evenness values, which indicated that the bacterial communities were evenly distributed. Also, the relatively shallow burial (30 cm) and subsequent decomposition of the *Sus scrofa domesticus*, during the 98-day study, did not result in a significant shift in the bacterial community distribution.

## 5. Conclusion

Soil pH changes, coupled with adipocere formation, during *Sus scrofa domesticus* decomposition showed trends comparable to those recorded in other temporal taphonomic studies. In contrast to previous reports, this study then linked these taphonomic changes to pronounced shifts in soil bacterial community profiles as measured particularly by species richness and diversity. It is suggested, therefore, that despite their known limitations, the use of molecular techniques, such as PCR-DGGE, to identify clandestine graves will be of considerable importance and advantage as an additional tool for forensic practitioners. Thus, the findings of this research justify an extensive field study with subsequent PCR-DGGE profiling and sequencing to determine the response of different key microbial communities (bacterial, fungal and archaeal) to changes in environmental and taphonomic variables that occur *in situ*.

## References

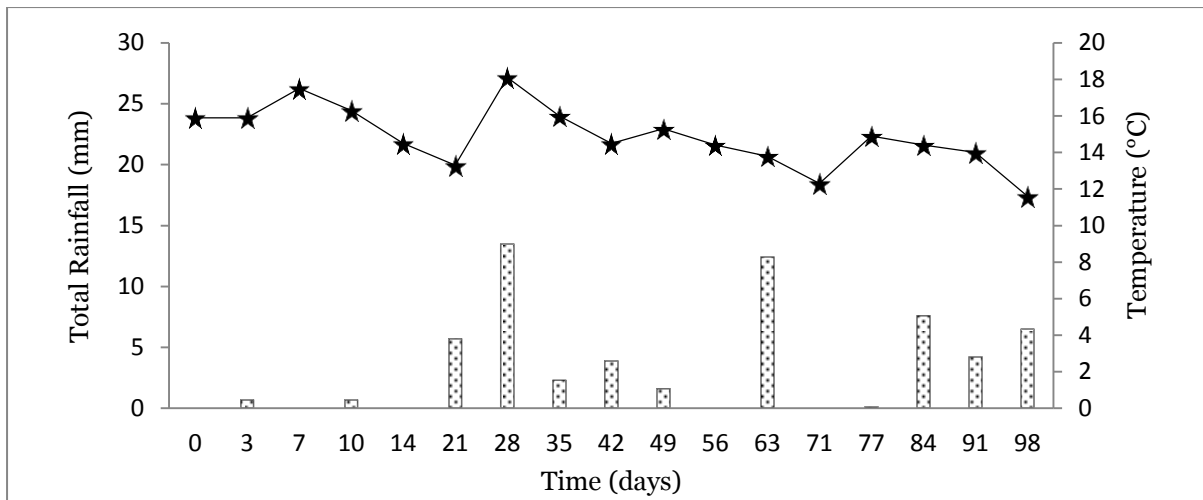
1. R.M. Maier, I.L. Pepper, Bacterial communities in natural ecosystems, in: R.M. Maier, I.L. Pepper, C.P. Gerba (Eds.), Environmental Microbiology (2nd ed.), Academic Press, Burlington, 2009a, pp. 347 - 356.

2. K.L. McGuire, K.K. Treseder, Microbial communities and their relevance for ecosystem models: Decomposition as a case study, *Soil Biol. Biochem.* 42 (2010) 529 - 535.
3. M.T. Madigan, J.M. Martinko, D.A. Stahl, D.P. Clark, Methods in microbial ecology, in: M.T. Madigan, J.M. Martinko, D.A. Stahl, D.P. Clark, *Brock Biology of Microorganisms* (13 ed.), Benjamin Cummings, Boston, 2012, pp. 641 -668.
4. D.O. Carter, D. Yellowlees, M. Tibbett, Moisture can be the dominant environmental parameter governing cadaver decomposition in soil, *Forensic Sci. Int.* 200 (2010) 60 - 66.
5. G. Muyzer, E.C. De Waal, A.G. Uitterlinden, Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA, *Appl. Environ. Microbiol.* 59 (3) (1993) 695 - 700.
6. J.E. Thies, Molecular methods for studying soil ecology, in: E.A. Paul (Ed.), *Soil Microbiology, Ecology, and Biochemistry* (3rd ed.), Academic Press, Oxford, 2007, pp. 86 - 118.
7. E.R. Hyde, D.P. Haarmann, A.M. Lynne, S.R. Buchell, J.F. Petrosino, The living dead: bacterial community structure of a cadaver at the onset and end of the bloat stage of decomposition, *PLoS One* 8 (2013), 1 - 10.
8. J.L. Metcalf, L.W. Parfrey, A. Gonzalez, C.L. Lauber, D. Knights, G. Ackermann, G.C. Humphrey, M.J. Gebert, W.V. Treuren, D. Berg-Lyons, K. Keepers, Y. Guo, J. Bullard, N. Fierer, D.O. Carter, R. Knight, A microbial clock provides an accurate estimate of the postmortem interval in a mouse model system, *eLIFE* 2 (2013) e01104.
9. J.L. Pechal, T.L. Crippen, A.M. Tarone, A.J. Lewis, J.K. Tomberlin, M.E. Benbow, Microbial community functional change during vertebrate carrion decomposition, *PLoS One* 8 (11) (2013) e79035.
10. J.L. Pechal, T.L. Crippen, M.E. Benbow, A.M. Tarone, S. Dowd, J.K. Tomberlin, The potential use of bacterial community succession in forensics as described by high throughput metagenomic sequencing, *Int. J. Legal Med.* 128 (1) (2014) 193 - 205.
11. C.P. Campobasso, G.D. Della, F. Introna, Factors affecting decomposition and Diptera colonization, *Forensic Sci. Int.* 120 (2001) 18 - 27.

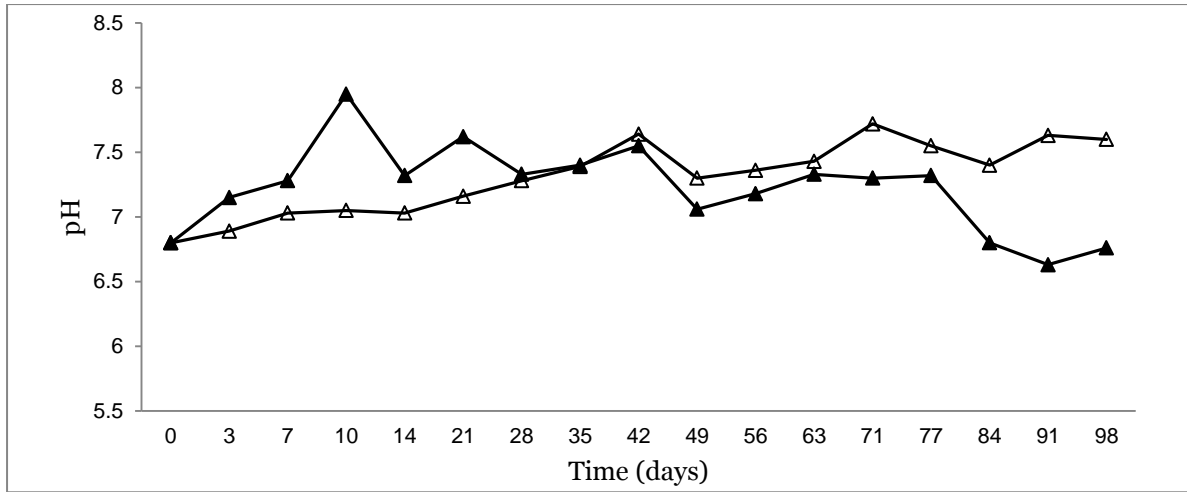
12. G.T. Howard, B. Duos, E.J. Watson-Horzelski, Characterization of the soil microbial community associated with decomposition of swine carcass, *Int. Biodeterior. Biodegradation* 64 (2010) 300 – 304.
13. K.L. Stokes, S.L. Forbes, M. Tibbett, Freezing skeletal muscle tissue does not affect its decomposition in soil: evidence from temporal changes in tissue mass, microbial activity and soil chemistry based on excised samples, *Forensic Sci. Int.* 183 (2009) 6–13.
14. L.E. Van Belle, D.O. Carter, S.L. Forbes, Measurement of ninhydrin reactive nitrogen influx into gravesoil during aboveground and belowground carcass (*Sus domesticus*) decomposition, *Forensic Sci. Int.* 193 (2009) 37 - 41.
15. D.O. Carter, M. Tibbett, Microbial decomposition of skeletal muscle tissue (*Ovis aries*) in a sandy loam soil at different temperatures, *Soil Bio. Biochem.* 38 (2006) 1139 - 1145.
16. D.O. Carter, M. Tibbett, Cadaver decomposition and soil processes, in: M. Tibbett, D.O. Carter (Eds.), *Soil Analysis in Forensic Taphonomy: Chemical and Biological Effects of Buried Human Remains*. CRC Press, Boca Raton, 2008, pp. 29-51.
17. I.D. Bull, R. Berstan, A. Vass, R.P. Evershed, Identification of a disinterred grave by molecular and stable isotope analysis, *Sci. Justice* 49 (2009) 142–149.
18. A.A. Vass, S.A. Barshick, G. Sega, J. Caton, J.T. Skeen, J.C. Love, J.A. Synsteliën, Decomposition chemistry of human remains: a new Methodology for Determining the Postmortem Interval, *J. Forensic Sci.* 47 (3) (2002) 542 - 553.
19. S.L. Forbes, Decomposition chemistry in a burial environment, in: M. Tibbett, D.O. Carter (Eds.), *Soil Analysis in Forensic Taphonomy: Chemical and Biological Effects of Buried Human Remains*. CRC Press, Boca Raton, 2008, pp. 203 - 223.
20. H. Gill-King, Chemical and ultrastructural aspects of decomposition, in: W. Haglund, M. Sorg (Eds.), *Forensic Taphonomy: The Post Mortem Fate of Human Remains*. CRC Press, Boca Raton, 1997, pp. 93 - 108.
21. R.C. Janaway, S.L. Percival, A.S. Wilson, Decomposition of human remains, in: S.L. Percival (Ed.), *Microbiology and Aging: Clinical Manifestations*. Springer, New York, 2009, pp. 313 - 334.
22. E.M. Schotsmans, W.V. Voorde, J.D. Winne, A.S. Wilson, The impact of shallow burial on differential decomposition to the body: a temperate case study, *Forensic Sci. Int.* 206 (1-3) (2011) e43 - e48.

23. M. Manefield, A.S. Whiteley, R.I. Griffiths, M.J. Bailey, RNA stable isotope probing, a novel means of linking microbial community function to phylogeny, *Appl. Environ. Microbiol.* 68 (2002) 5367 - 5373.
24. D. Ager, S. Evans, H. Li, A.K. Lilley, C.J. Van der Gast, Anthropogenic disturbance affects the structure of bacterial communities, *Environ Microbiol.* 12 (2010) 670 - 678.
25. A. Gunn, The decay, discovery and recovery of human bodies, in: *Essential Forensic Biology* (2nd ed.). John Wiley & Sons, West Sussex, 2009, pp. 11 - 35
26. D. Schoenen, H. Schoenen, Adipocere formation—The result of insufficient microbial degradation, *Forensic Sci. Int.* 226 (1-3) (2013) 301-306.
27. D.H. Ubelaker, K.M. Zarenko, Adipocere: What is known after two centuries of research, *Forensic Sci. Int.* 208 (2011) 167 - 1732.
28. F.E. Damann, D.O. Carter, Human decomposition ecology and postmortem microbiology, in: J. Pokines, S.A. Symes (Eds.), *Manual of Forensic Taphonomy*, CRC Press, Boca Raton, 2013, pp. 37 - 49.
29. M. Micozzi, Experimental study of postmortem change under field conditions: effects of freezing, thawing and mechanical injury, *J. Forensic Sci.* 31 (1986) 953 - 961.
30. L.I. Moreno, D. Mills, J. Fetscher, K. John-Williams, L. Meadows-Jantz, B. McCord, The application of amplicon length heterogeneity PCR (LH-PCR) for monitoring the dynamics of soil microbial communities associated with cadaver decomposition, *J. Microbiol. Methods* 84 (2011) 388 – 393.
31. B. Bandeira, J.-L. Jamet, D. Jamet, J.-M. Ginoux, Mathematical convergences of biodiversity indices, *Ecol. Indic.* 29 (2013) 522–528.
32. J. Barry, S. Birchenough, B. Norris, S. Ware, On the use of sample indices to reflect changes in benthic fauna biodiversity, *Ecol Indic.* 26 (2013) 154–162.
33. A.V. Strien, L. Soldaat, R. Gregory, Desirable mathematical properties of indicators for biodiversity change, *Ecol. Indic.* 14 (1) (2012) 202–208.
34. C. Heipp, A new index measuring evenness, *J. Mar. Biol. Ass. U. K.* 54 (1974) 555-557.
35. L. Jost, The relation between evenness and diversity. *Diversity*, 2 (2010) 207-232.
36. C. Ricotta, G. Avena, On the relationship between Pielou's evenness and landscape dominance within the context of Hill's diversity profiles, *Ecol. Indic.* 4 (2) (2003) 361–365.



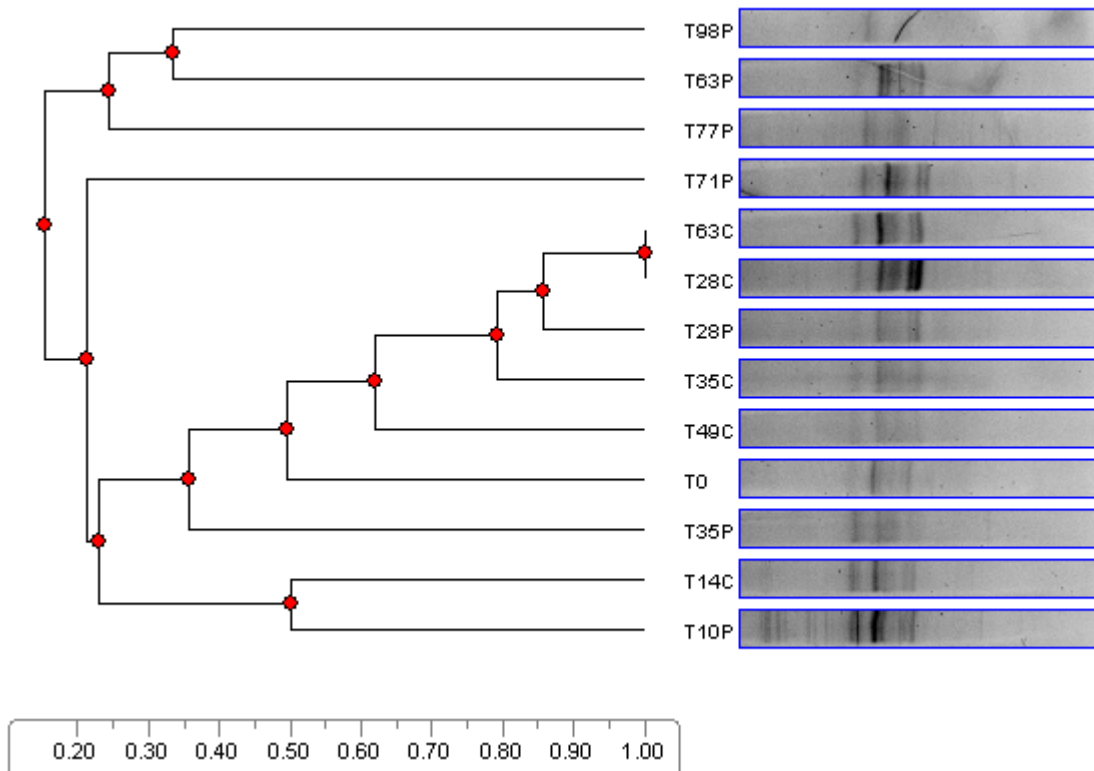


**Fig. 1.** Average temperature (\*) and rainfall (▨) during the 98-day study from June to September 2010 in Middlesbrough, United Kingdom (data from <http://www.worldweatheronline.com>).

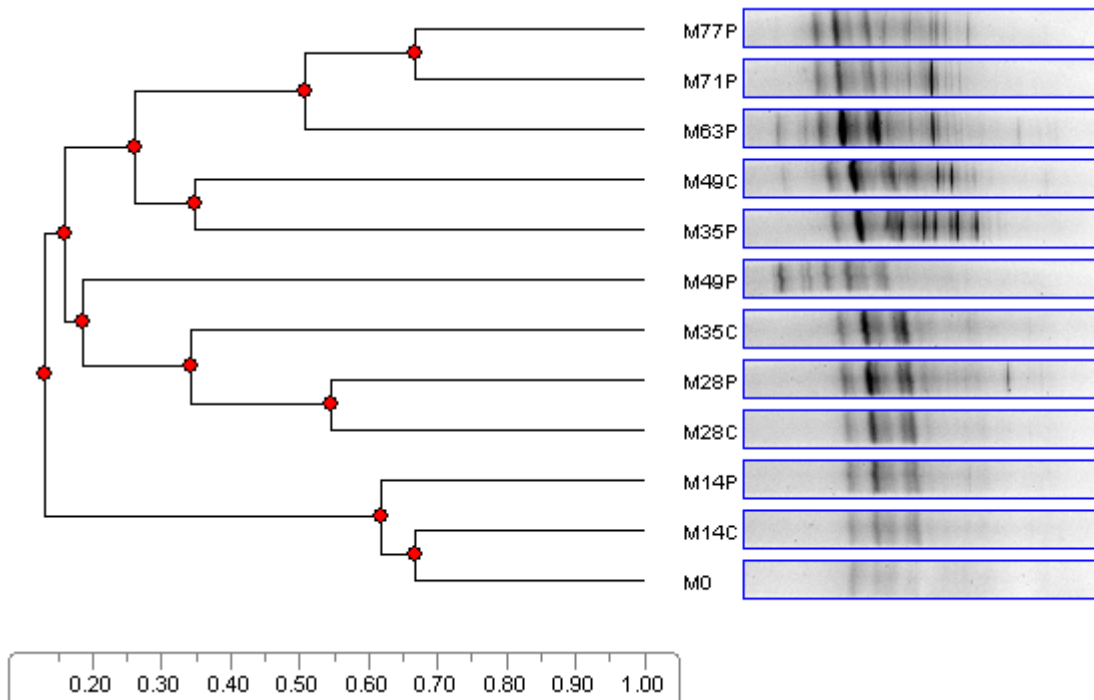


**Fig. 2.** pH readings for the top 10 cm soil segment in the *Sus scrofa domesticus* (▲) and control (Δ) microcosms during 98 days of study.

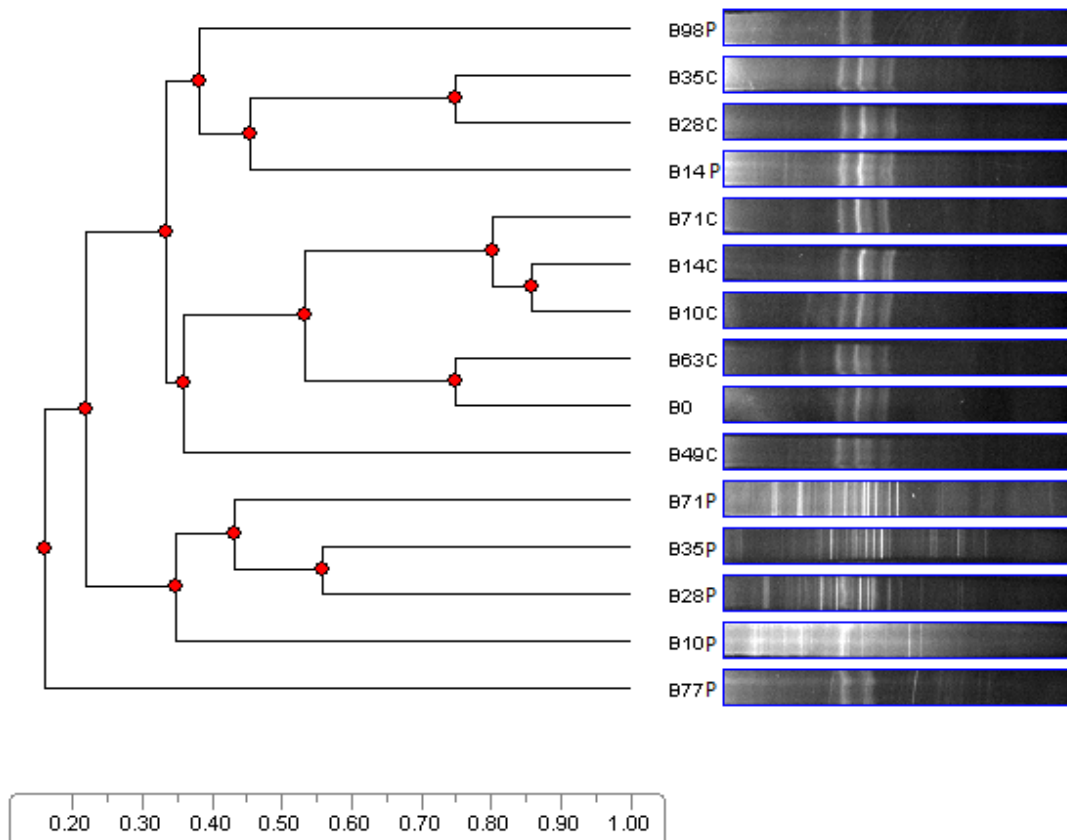




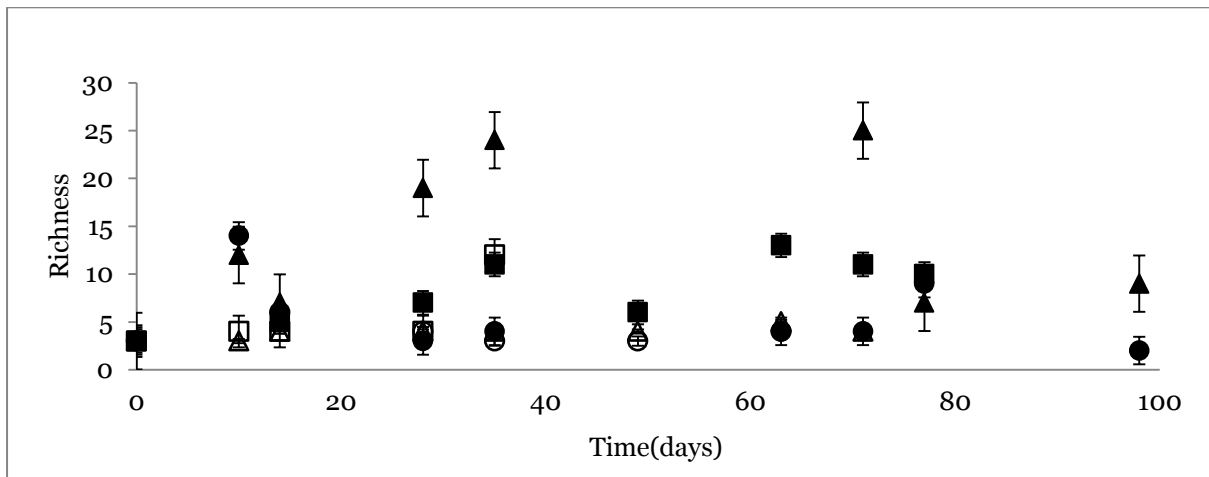
**Fig. 3a.** UPGMA cluster analysis for the top (T) soil depth in the absence (control; C) and presence (experimental; P) of *Sus scrofa domesticus*.



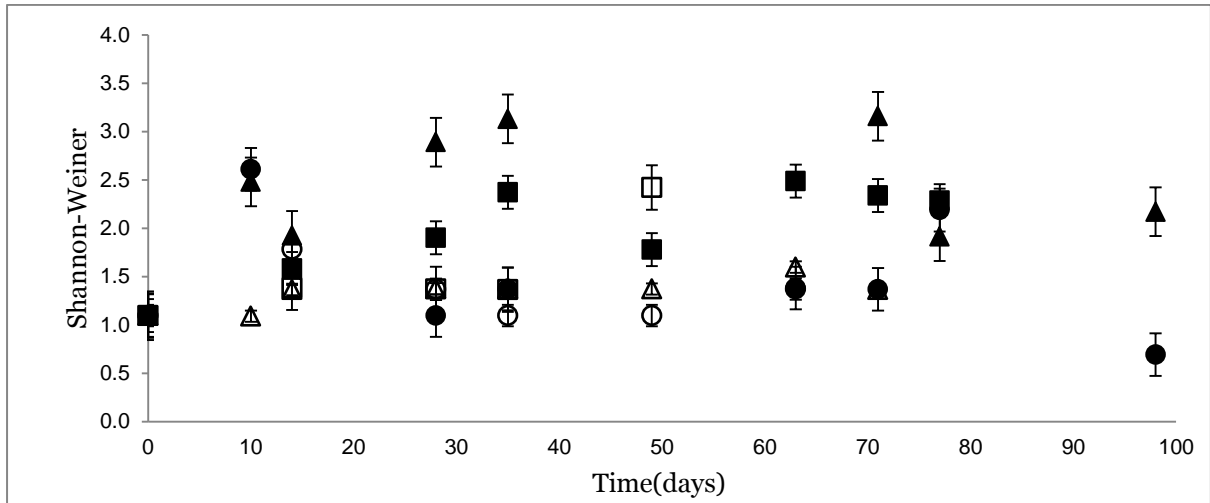
**Fig. 3b.** UPGMA cluster analysis for the middle (M) soil depth in the absence (control; C) and presence (experimental; P) of *Sus scrofa domesticus*.



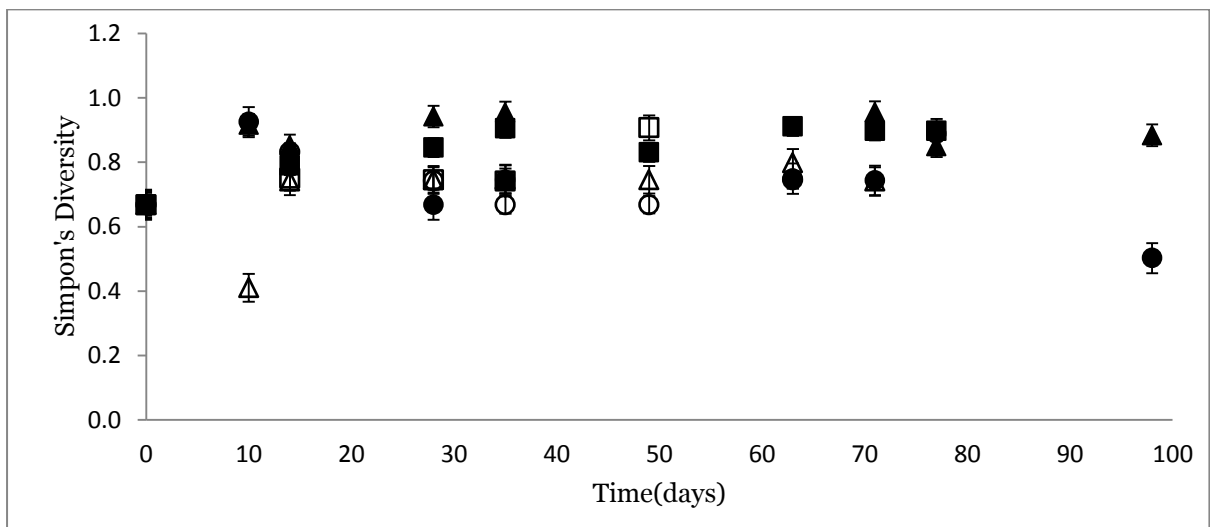
**Fig. 3c.** UPGMA cluster analysis for the bottom (B) soil depth in the absence (control; C) and presence (experimental; P) of *Sus scrofa domestica*.



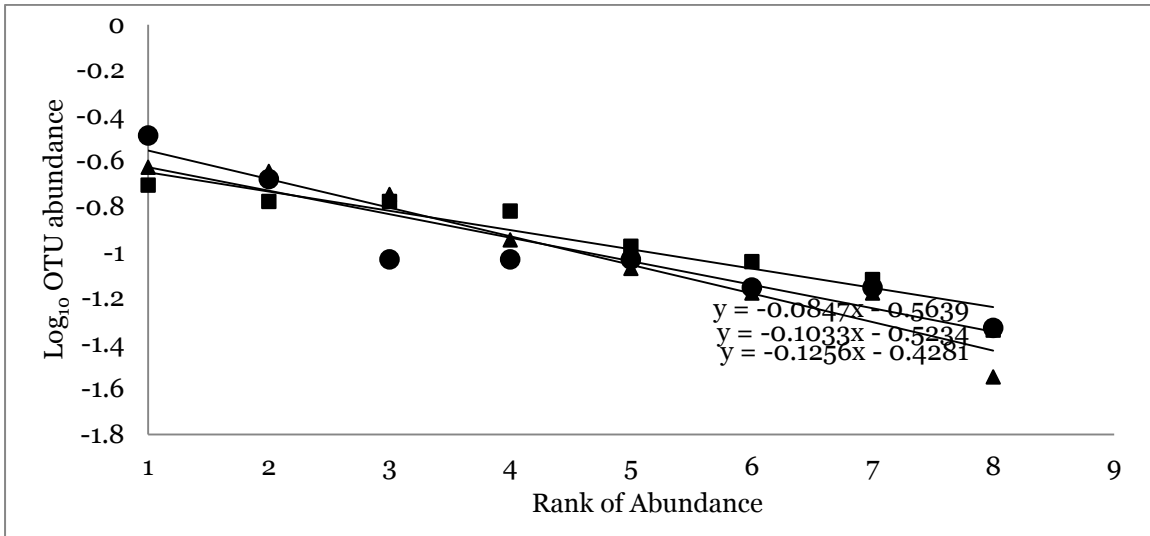
**Fig. 4.** Richness for the control (top ○, middle □, bottom Δ) and experimental (top ●, middle ■, bottom ▲) microcosms.



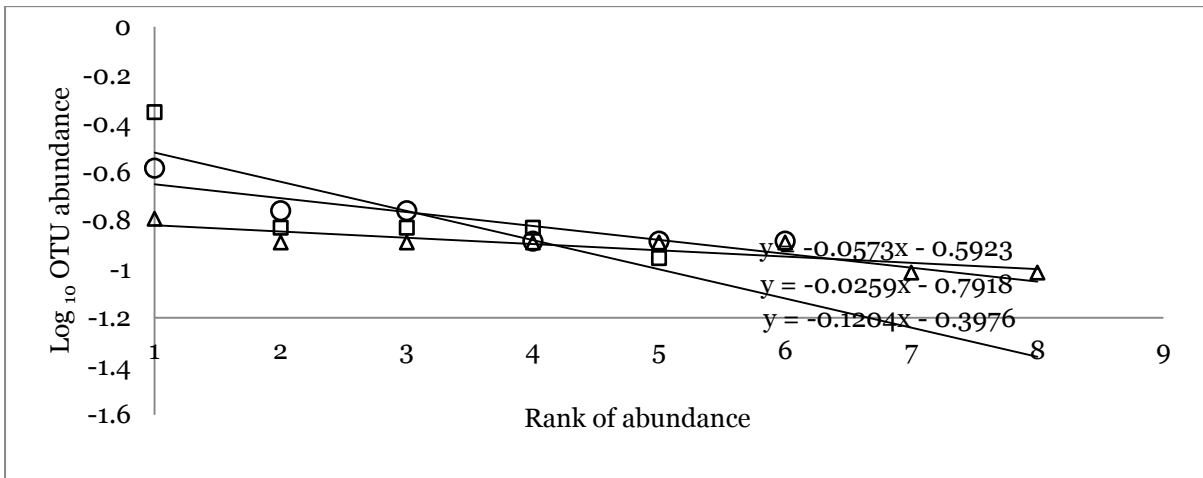
**Fig. 5a.** The Shannon-Weiner diversity index ( $H'$ ) for control (top ○, middle □, bottom △) and experimental (top ●, middle ■, bottom ▲) microcosms.



**Fig. 5b.** The Simpson's diversity index ( $D$ ) for the control (top ○, middle □, bottom △) and experimental (top ●, middle ■, bottom ▲) microcosms.



**Fig. 6a.** Rank of the log<sub>10</sub> OTU abundance for community evenness (E) for the top (●), middle (■) and bottom (▲) soil segments in the presence of *Sus scrofa domesticus*.



**Fig. 6b.** Rank of the log<sub>10</sub> OTU abundance for community evenness (E) for the top (○), middle (□) and bottom (△) soil segments in the absence of *Sus scrofa domesticus*.