Soil fungal community shift evaluation as a potential cadaver decomposition indicator

Monica Chimutsa, Ayodeji O. Olakanye, Tim J.U. Thompson, *T. Komang Ralebitso-Senior

School of Science and Engineering, Teesside University, Borough Road, Middlesbrough, Teesside, TS1 3BA, United Kingdom

M.Chimutsa@tees.ac.uk

A.Olakanye@tees.ac.uk

T.Thompson@tees.ac.uk

*Corresponding author. Tel.: +44 1642 342525; Fax: +44 1642 342401; E-mail address: K.Ralebitso-Senior@tees.ac.uk

Abstract

Fungi metabolise organic matter *in situ* and so alter both the bio-/physico-chemical properties and microbial community structure of the ecosystem. In particular, they are responsible reportedly for specific stages of decomposition. Therefore, this study aimed to extend previous bacteria-based forensic ecogenomics research by investigating soil fungal community and cadaver decomposition interactions in microcosms with garden soil (20 kg, fresh weight) and domestic pig (*Sus scrofa domesticus*) carcass (5 kg, leg). Soil samples were collected at depths of 0-10 cm, 10-20 cm and 20-30 cm on days 3, 28 and 77 in the absence (control -Pg) and presence (experimental +Pg) of *Sus scrofa domesticus* and used for total DNA extraction and nested PCR-DGGE profiling of the 18S rRNA gene. The Shannon-Wiener (H^{l}) community diversity indices were 1.25 ± 0.21 and 1.49 ± 0.30 for the control and experimental microcosms, respectively, while comparable Simpson species dominance (S) values were 0.65 ± 0.109 and 0.75 ± 0.015. Generally, and in contrast to parallel studies of the bacterial 16S rRNA and 16S rDNA profiles, statistical

analysis (t-test) of the 18S dynamics showed no mathematically significant shifts in fungal community diversity (H'; p = 0.142) and dominance (S; p = 0.392) during carcass decomposition, necessitating further investigations.

Key words: *Forensic ecogenomics, Decomposition, Fungal communities, Soil, PCR-DGGE*

1. Introduction

The term 'microbial forensics' was defined by Budowle [1] as "a scientific discipline dedicated to analysing evidence from a bioterrorism act, biocrime, or inadvertent microorganism/toxin release for attribution purposes". Petrisor et al. [2] subsequently described it as "the focusing of microbiology, virology, biochemistry and molecular biology for use in environmental forensic investigations". We propose the term 'forensic ecogenomics' where the application of molecular microbial ecology techniques encapsulates and extends the earlier definitions [3]. Independent of definition and context, this relatively novel approach focusses on microbial analysis to provide molecular fingerprints of different and phylogenetically complex ecosystems/sources such as soil, sediment, water and food to, potentially, aid criminal investigations [1, 2, 4, 5].

Although crime scene cadavers often contact soil, their interactions with indigenous microbial communities remain largely unexplored. For example, mass grave location is dependent predominantly on eye witness testimony, geophysical resistivity, magnetometry and ground penetrating radar [6]. Specifically, forensic investigation of soil has focused predominantly on particle comparison [7] by physical techniques to determine mineralogy and morphology [8, 9] while spectroscopic techniques provide further discrimination by chemical composition identification [6, 10]. In addition, physico-chemical and biological characteristics have been used to estimate postmortem interval (PMI) and determine clandestine grave location [11-13].

Elegant (micro) ecological and geological studies have started to establish a knowledge base of the vast numbers and types of microbial communities and the factors that influence and/or are affected by cadaver decomposition in soil [e.g. 5, 12-14]. They include: body size/mass; microbial activity; soil pH and resistivity/conductivity; temperature; redox potential; and humidity/moisture/water

activity. Soils are particularly complex and heterogeneous habitats that support a tremendous diversity of bacteria, fungi and archaea. These characteristics can be specific for a single location [15, 16] and so, potentially, may be used to differentiate between crime and non-crime sites [16]. Recently, soil evidence in criminal trials [17] has linked the victim and the crime scene [18] thus exemplifying the key role of soil origin in gathering forensic evidence.

Mycology, the study of fungi, including mushrooms, yeasts, human and plant pathogens and moulds [19], has established their ecological significance in plant growth promotion, nutrient cycling, soil aggregation, disease suppression and organic matter decomposition [20, 21]. Specifically, the use of mycology in criminal investigations has been demonstrated in cases of poisoning, through PMI estimation and linking a suspect to a crime [19]. Hawksworth and Wiltshire [19] observed microorganisms, including fungi, in the initial stages of cadaver decomposition when two groups of closely related ammonia and post-putrefaction fungi were recorded as visual markers. Nonetheless, forensic ecogenomic studies have, to date, focused on bacteria with fungi given little attention. To address this paucity, established and accessible ecogenomic tools, polymerase chain reaction and denaturing gradient gel electrophoresis (PCR-DGGE), were used in our study to characterise changes in soil microbial community genetic markers in response to cadaver burial.

In microbial ecology, culture dependent methods have characterised soil fungal communities but these detect only a fraction (*ca* 1%) of the total populations [22, 23]. Biochemical techniques are time consuming, labour intensive, prone to selective baiting [24], and depend heavily on cultivation, while morphology-based taxa identification is limited by biases. Consequently, as adopted by other researchers [24-26], PCR-DGGE was used in this study to gain a more descriptive and comparative analysis of soil fungal community richness, structure, composition and diversity in experimental and control soils. According to Gerber [27] species diversity is the number of species present and the evenness with which the individuals are distributed among the community.

The principal objective of the wider research programme was to elucidate the responses of indigenous soil microbial communities to cadaver presence with *Sus scrofa domesticus* used as a human analogue [28]. RNA-based DGGE analysis

identified statistically significant diversity and richness divergence in the metabolically active bacterial associations during decomposition [29]. Also, DNA-based probing recorded increased community richness and diversity in the presence of *Sus scrofa domesticus* particularly in relation to proximity to the decomposing material [28]. To complete the study, and address a key knowledge gap in forensic ecogenomics, this investigation was made to target the 18S rRNA gene and so determine whether changes in soil fungal communities could also be used as cadaver decomposition indicators.

2. Materials and Methods

2.1. Experimental design, sampling and soil characterisation

The experimental design, sample collection and soil analyses were as described by Olakanye et al. [28] and Bergmann et al. [29]. Briefly, a 5 kg leg of S*us scrofa domesticus* was buried in 20 kg of a sandy loam and maintained in a sealed microcosm parallel to a soil only control. Soil samples for analysis were collected from the top (0-10 cm), middle (10-20 cm) and bottom (20-30) layers of the microcosms on days 3, 28 and 77.

2.2. Total DNA extraction and PCR amplification

Total DNA was extracted from soil samples (1 g) with FastDNA® Spin Kit for Soil (MP Biomedicals, Santa Ana, U.S.A.). The extracts (5 µL) were then amplified by nested PCR with the forward primer NS1 (5'-CCAGTAGTCATATGCTTGTC-3') and the reverse primer NS8 (5'-TCCGCAGGTTCACCTACGGA-3') (18S rRNA gene of *Saccharomyces cerevisiae*) [30] (Stage 1). Stage 2 amplification used the forward primer NS1 (5'-CCAGTAGTCATATGCTTGTC-3') and reverse primer NS210-GC (5'-CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG G GAA TTA CCG CGG CTG CTG GC-3') [30, 31] with the first stage amplicons (1 µL) as templates. Both PCR stages (25 µL reaction volume) were made with a Primus 96 Plus thermal cycler (MWG Biotech, Ebersberg, Germany) at 94°C initial denaturation for 2 minutes followed by 30 cycles of: 94°C for 30 seconds; 50°C for 45 seconds; 72°C for 2 minutes; and final extension at 72°C for 5 minutes. Amplification products (5 µL) were then visualised by electrophoresis in 1.5 % (w/v) agarose gels stained with SYBR Safe DNA Gel Stain (Molecular Probes, Eugene, U.S.A.).

2.3. Denaturing gradient gel electrophoresis

The amplicons (20 µL) were separated in 0.5X TAE buffer (20 mmolL^{-1} Tris, 10 mM acetate, 0.5 mM EDTA, pH 7.4) on 6% (w/v) polyacrylamide gel (acrylamide/bisacrylamide, 37.5:1) with a 25% to 45% 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.

2.4. Detection and statistical analysis of DGGE profiles

Band quantification and cluster analysis, by the un-weighted pair group method with arithmetic averages (UPGMA) [30], were made with Phoretix 1D Pro gel analysis software (TotalLab, Newcastle, U.K.). Soil fungal community diversity was estimated by the Shannon-Wiener diversity index (H') while the index of dominance (S) was calculated by the Simpson formula [32, 33] with the probability of drawing two individuals from the same species either high (1) or low (0). Statistical analysis was made by a two tailed t-test (Microsoft Office Excel 2007; Microsoft, Redmond, U.S.A.), where $p < 0.05$ was considered to be statistically significant.

3. Results

The nested NS1/NS8 and NS1/NS201 primer sets generated reproducible amplicons of *ca* 1700 bp and 600 bp, respectively. 18S rRNA gene-based DGGE profiles showed averages of 10 ± 2 and 8 ± 2 operational taxonomic units (OTU) in the absence and presence, respectively of *Sus scrofa domesticus* carcass, together with temporal shifts in OTU presence/absence and relative abundance. The Shannon-Wiener species diversity (H') values for the control microcosm were 2.09 (day 3), 1.64 (day 27) and 1.68 (day 77) (p = 0.256) while the corresponding values for the experimental microcosm were 1.72, 1.47 and 1.68 (p = 0.135) (Fig. 1). Generally, comparable diversity decreases resulted with time and the two microcosms could not be separated statistically. The species dominance (S) values were 0.771 and 0.763 for the control and experimental soils, respectively (Fig. 2), so, again, no statistical difference (p = 0.392) was calculated.

DGGE band similarities were determined by the un-weighted-pair group method with the arithmetic average (UPGMA) clustering algorithm and these are shown as a

dendogram (Fig. 3). Although three main clusters with 54, 59 and 61% similarities were observed, the OTUs in each cluster were generally identical or closely related, which indicated low fungal diversity [34]. The top soil segment (0-10 cm) recorded the lowest similarity (59%; Arrow 1) between the control (Tp-Pg) and experimental (Tp+Pg) microcosms while the highest (67%; Arrow 2) resulted on days 3 and 77. Although a 53% similarity (Arrow 3) was recorded on days 3 and 28 in the presence of *Sus scrofa domesticus*, the middle (10-20 cm) layers revealed a maximum 47% similarity (Arrow 4) for all sampling times independent of the presence of decomposing material. In particular, a 60% similarity (Arrow 5) was recorded on day 28 for both the burial (Mid+Pg) and non-burial (Mid-Pg) soils. This contrasted parallel analyses [28, 29], which revealed distinct differences in the bacterial communities for this segment. Specifically, Bergmann et al. [29] recorded a 14% similarity (day 28) for the functional bacterial communities in the middle layers of the *Sus scrofa domesticus* treatment and control microcosm.

The bottom segments (20-30 cm) of the control soil (Bt-Pg) recorded the highest fungal community profile similarity (64%; Arrow 6) for days 28 and 77 and the lowest (43%; Arrow 7) for all three sampling times. For days 3 and 77, which represented the early and late decomposition phases of this study, the highest similarity (77%; Arrow 8) was recorded for the middle and bottom segments of the control microcosm while a lower similarity, as indicated above (67%; Arrow 2), resulted for the top layer of the experimental microcosm. Overall, a 43% divergence in the 18S rRNA gene profiles resulted from *Sus scrofa domesticus* decomposition compared to 1-12% and 18% for the 16S rRNA [29] and 16S rRNA gene [28] profiles, respectively. Thus, although providing sufficient differentiation, other fungal genes and/or ecogenomic techniques [e.g. 5] could enhance resolution between burial and non-burial soils.

4. Discussion

Current soil forensic investigations focus on linking, by physical and chemical techniques, a suspect to a crime scene. Despite the fact that bodies are often found in contact with soil, interactions between cadaver decomposition and soil microbial communities are only now being explored. Soil is phylogenetically and phenotypically diverse hence samples from different locations are characterised

typically by different species and community profiles [16, 20]. 'Forensic ecogenomics' combines the strengths of key pertinent disciplines including forensics, microbiology, molecular ecology and archaeology [3, 28, 29], and so should, potentially, provide forensic practitioners with another powerful investigative tool.

Decomposition is a complex temporal sequence of 'fresh', 'bloat', 'decay' and 'dry' stages that begins with autolysis shortly after death, concomitant with organism succession (bacteria, fungi and invertebrates) and changes in the environmental bio- and physico-chemical variables [5, 14, 35]. In particular, carcass inflation and bursting release decomposition compounds and associated microflora to form a cadaver decomposition island (CDI), which is dependent on the body size, soil type and maggot mass. Hawksworth and Wiltshire [19] identified fungi as major decomposers that can, therefore, be found in the CDI. Hence, soil samples were collected from three bands of the shallow (40 cm) carcass burial at three different times to estimate species diversity and dominance relative to the buried material. Statistically, no significant differences were recorded between the different bands: 0–10 cm, p = 0.524; 10-20 cm, p = 0.214; and 20-30 cm, p = 0.805, in both the presence and absence of *Sus scrofa domesticus*.

High diversity characterises the 'bloated' stage but decreases progressively towards the 'decay' phase, which is marked by increased microbial and insect competitive activity. The final 'dry' stage of decomposition then, typically, is nutrient limited [14, 36]. In our study, 18S rRNA gene DGGE analysis showed that cadaver decomposition impacted the soil ecosystem possibly due to localised nutrient concentration as also recorded by Macdonald et al. [14]. Thus, with *Sus scrofa domesticus* decomposition, the species diversity (H') averaged 1.61 ± 0.30 while 1.80 ± 0.21 was calculated for the control (Fig 1). In contrast to RNA-based analysis of bacterial communities [29], temporal decreases in fungal species diversity and numerical dominance were recorded in both the presence and absence of the carcass but the two could not be separated statistically (p = 0.142; H', p = 0.771; S).

Although using 18S next generation sequencing to determine decomposition-based seasonal (summer/winter) differences, Carter et al. [5] did not resolve statistically significant shifts (p = 0.364) in postrupture gravesoil eukaryotic community structure during 60-day *in situ* swine burials. Nonetheless, a cascading community

structure was suggested where bacterial community changes impacted eukaryotic (nematodes) clade composition. Temporal changes in gravesoil physico-chemical properties such as labile nutrients, moisture content, temperature and pH [14] can, however, result in successional microbial (bacterial) community dominances and so account for the differences in our 16S and 18S trends.

Community succession and changes in structure have been reported for bacteria following high throughput sequencing investigations [e.g. 37]. As a consequence, the need for comprehensive databases for bacterial populations characteristic of decomposing cadavers and gravesoils were highlighted [5, 37]. Our study illustrates the requirement for a similarly rigorous impetus to understand fungal community dynamics in gravesoils, including the use of accessible ecogenomics techniques in protracted further work.

5. Recommended Further Investigations

Nested PCR-DGGE profiling of the 18S RNA gene gave a preliminary descriptive and comparative analysis of the shifts in soil fungal communities in response to cadaver decomposition. Despite its known limitations [30, 38, 39], such profiling is both rapid and inexpensive and capable of visualising microbial community structure changes [25, 26, 39]. It can be repeated easily, even for small samples, but still give a range of statistically valid results as required by courts [13]. Its sensitivity and robustness for soil fungal community analysis does, however, require further validation before its adoption as a new technique that can be used confidently in criminal procedures.

Therefore, to obviate current limitations, comprehensive fungal profiling-based decomposition studies, underpinned by the strict forensic science code for evidence collection and preservation [2], and optimised recovery of high quality/quantity nucleic acids, could also include complementary tools such as: nucleic acid hybridisation and microscopy [19]; fluorescent *in situ* hybridization (FISH) for simultaneous identification and visualization in natural ecosystems [40]; terminal restriction fragment length polymorphism, which has been reported by Quaak and Kuiper [15] as applicable for small samples and easy to implement in forensic laboratories; and ecogenomics/next-generation sequencing metagenomics, whose potential application for the necrobiome, grave sites and criminal investigations has

been illustrated and highlighted by several researchers [3-5, 37, 41-44]. Also, such studies should investigate the interactions of carcass decomposition in different soil types and in response to pH, temperature, moisture content and burial depth with fungal communities. These data could then inform decisions on fungal profiling inclusion in the forensic toolkit for reliable application in crime scene investigations.

Acknowledgements

The authors would like to thank Madelynne Hopper and Karen Bradley for their technical assistance and the Teesside University Research Fund for supporting this work.

References

[1] B. Budowle, Genetics and attribution issues that confront the microbial forensics field, Forensic Sci. Int. 146 (2004) S185-S188.

[2] I.G. Petrisor, R.A. Parkinson, J. Horswell, J.M. Waters, L.A. Burgoyne, D.E.A. Catcheside, W. Dejonghe, N. Leys, K. Vanbroekhoven, P. Pattnaik, D. Graves, Microbial forensics, in: R.D. Morrison, B.L. Murphy (Eds.), Environmental Forensics: Contaminant Specific Guide, Academic Press, 2006, pp. 227-257.

[3] T.K. Ralebitso-Senior, T.J.U. Thompson, H.E. Carney, Microbial ecogenomics and forensic science: new methods for investigating clandestine gravesites, Human Remains Violence, In press.

[4] S.J. Finley, M.E. Benbow, G.T. Javan, Potential applications of soil microbial ecology and next-generation sequencing in criminal investigations, Appl. Soil Ecol. 88 (2015) 69 - 78.

[5] D.O. Carter, J.L. Metcalf, A. Bibat, R. Knight, Seasonal variation of postmortem microbial communities, Forensic Sci. Med. Pathol. 11 (2015) 202 – 207.

[6] C.S. Lee, T.M. Sung, H.S. Kim, C.H. Jeon, Classification of forensic soil evidences by application of THM-PYGC/MS and multivariate analysis, J. Anal. Appl. Pyrolysis 96 (2012) 33-42.

[7] R. Sugita, Y. Marumo, Validity of color examination for forensic soil identification, Forensic Sci. Int. 83 (3) (1996) 201-210.

[8] K. Pye, D. Croft, Forensic analysis of soil and sediment traces by scanning electron microscopy and energy-dispersive x-ray analysis: an experimental investigation, Forensic Sci. Int. 165 (1) (2007) 52-63.

[9] S.J. Mooney, A.R. Tams, P.M. Berry, A reliable method for preserving soil structure in the field for subsequent morphological examinations, Geoderma 133 (3-4) (2006) 338-344.

[10] L. Cécillon, N. Cassagne, S. Czarnes, R. Gros, J.-J. Brun, Variable selection in near-infrared spectra for the biological characterization of soil and earthworm casts, Soil Biol. Biochem. 40 (7) (2008) 1975-1979.

[11] D.O. Carter, D. Yellowlees, M. Tibbet, Moisture can be the dominant environmental parameter governing cadaver decomposition in soil, Forensic Sci. Int. 200 (1-3) (2010) 60-66.

[12] J.R. Jervis, J.K. Pringle, G.W. Tuckwell, Time-lapse resistivity surveys over simulated clandestine graves, Forensic Sci. Int. 192 (1-3) (2009) 7-13.

[13] J. Horswell, S.J. Cordiner, E.W. Maas, T.M. Martin, K.B.W. Sutherland, T.W. Speir, Forensic comparison of soils by microbial community DNA profiling, Sci. Justice 41 (3) (2001) 236.

[14] B.C.T. Macdonald, M. Farrell, S. Tuomi, P.S. Barton, S.A. Cunningham, A.D. Manning, Carrion decomposition causes large and lasting effects on soil amino acid and peptide flux, Soil Biol. Biochem. 69 (2014), 132 – 140.

[15] F.C.A. Quaak, I. Kuiper, Statistical data analysis of bacterial T-RFLP profiles in forensic soil comparisons, Forensic Sci. Int. 210 (1-3) (2011) 96-101.

[16] A. Lerner, Y. Shor, A. Vinokurov, Y. Okon, E. Jurkevitch, Can denaturing gradient gel electrophoresis (DGGE) analysis of amplified 16S rDNA of soil bacterial populations be used in forensic investigations? Soil Biol. Biochem. 38 (6) (2006) 1188-1192.

[17] N. Petraco, T.A. Kubic, N.D.K. Petraco, Case studies in forensic soil examinations, Forensic Sci. Int. 178 (2-3) (2008) e23-e27.

[18] News.bbc.co.uk (2011) BBC NEWS | England | Cambridgeshire | Asphyxia 'likely cause' of Soham deaths. [online] Available at: http://news.bbc.co.uk/1/hi/england/cambridgeshire/3249887.stm [Accessed: 20 October 2014].

[19] D.L. Hawksworth, P.E. Wiltshire, Forensic mycology: the use of fungi in criminal investigations, Forensic Sci. Int. 206 (1-3) (2011) 1-11.

[20] S. Schneider, M. Hartmann, J. Enkerli, F. Widmer, Fungal community structure in soils of conventional and organic farming systems, Fungal Ecol. 3 (3) (2010) 215-224.

[21] D.L. Hawksworth, The magnitude of fungal diversity: the 1.5 million species estimate revisited, Mycol. Res. 105 (12) (2001) 1422-1432.

[22] M. Oros-Sichler, N.C.M. Gomes, G. Neuber, K. Smalla, A new semi-nested PCR protocol to amplify large 18S rRNA gene fragments for PCR-DGGE analysis of soil fungal communities, J. Microbiol. Methods 65 (1) (2006) 63-75.

[23] M. Balázs, A. Rónavári, A. Németh, Z. Bihari, E. Rutkai, P. Bartos, I. Kiss, A. Szvetnik, Effect of DNA polymerases on PCR-DGGE patterns, Int. Biodeter. Biodegr. 84 (2013) 244-249.

[24] A.V. Marano, F.H. Gleason, F. Bärlocher, C.L. Pires-Zottarelli, O. Lilje, S.K. Schmidt, S. Rasconi, M. Kagami, M.D. Barrera, T. Sime-Ngando, S. Boussiba, J.I. de Souza, J.E. Edwards, Quantitative methods for the analysis of zoosporic fungi, J. Microbiol. Methods 89 (1) (2012) 22-32.

[25] P. Singh, C. Raghukumar, R.M. Meena, P. Verma, Y. Shouche, Fungal diversity in deep-sea sediments revealed by culture-dependent and culture-independent approaches, Fungal Ecol. 5 (5) (2012) 543-553.

[26] G. Muyzer, K. Smalla, Application of denaturing gradient gel electrophoresis (DGGE) and temperature gradient electrophoresis (TGGE) in microbial ecology, Antonie van Leeuwenhoek 73 (1998) 127–141.

[27] N. Gerber, Biodiversity measures based on species-level dissimilarities: a methodology for assessment, Ecol. Econ. 70 (2011) 2275-2281.

[28] A.O. Olakanye, T. Thompson, T.K. Ralebitso-Senior, Changes to soil bacterial profiles as a result of *Sus scrofa domesticus* decomposition, Forensic Sci. Int. 245 (2014) 101-106.

[29] R.C. Bergmann, T.K. Ralebitso-Senior, T.J.U. Thompson, An RNA-based analysis of changes in biodiversity indices in response to *Sus scrofa domesticus* decomposition, Forensic Sci. Int. 241 (2014) 190-194.

[30] T.J. White, T. Bruns, S. Lee, J. Taylor, Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, in: M.A. Innis, D.H. Gelfand, J.J. Sninsky, T.J. White (Eds.), PCR Protocols: A Guide to Methods and Applications, Academic Press, 1990, pp. 315-322.

[31] G.A. Kowalchuk, J.R. Stephen, W.E. De Beer, J.I. Prosser, T.M. Embley, J.W. Woldendorp, Analysis of ammonia-oxidizing bacteria of the beta subdivision of the class Proteobacteria in coastal sand dunes by denaturing gradient gel

electrophoresis and sequencing of PCR-amplified 16S ribosomal DNA fragments, Appl. Environ. Microbiol. 63 (1997) 1489-1497.

[32] I.F. Spellerberg, Shannon-Wiener index, in: S.E. Jorgensen, B. Fath (Eds.), Encyclopaedia of Ecology, Academic Press, 2008, pp. 3249-3252. http://dx.doi.org/10.1016/B978-008045405-4.00132-4.

[33] E.H. Simpson, Measurement of diversity. Nature 163 (1949) 688.

[34] E.F. Arcuri, A.F. El Sheikha, T. Rychlik, I. Piro-Métayer, D. Montet, Determination of cheese origin by using 16S rDNA fingerprinting of bacteria communities by PCR-DGGE: preliminary application to traditional Minas cheese, Food Control 30 (1) (2013) 1-6.

[35] R.A. Parkinson, K.-R. Dias, J. Horswell, P. Greenwood, N. Banning, M. Tibbett, A.A. Vass, Microbial community analysis of human decomposition in soil, in: K. Ritz, L. Dawson, D. Miller (Eds.), Criminal and Environmental Soil Forensics, Springer, Dordrecht, 2009, pp. 379-394.

[36] C. Zhou, R.W. Byard, Factors and processes causing accelerated decomposition in human cadavers - an overview, J. Forensic Leg. Med. 18 (2011) 6-9.

[37] E.R. Hyde, D.P. Haarmann, J.F. Petrosino, A.M. Lynne, S.R. Bucheli, Initial insights into bacterial succession during human decomposition, Int. J. Legal Med. 129 (2015) 661 – 671.

[38] G.P. Gafan, D.A. Spratt, Denaturing gradient gel electrophoresis gel expansion (DGGEGE) – an attempt to resolve the limitations of co-migration in the DGGE of complex polymicrobial communities, FEMS Microbiol. Lett. 253 (2) (2005) 303-307.

[39] W.K. Ma, S.D. Siciliano, J.J. Germida, A PCR-DGGE method for detecting arbuscular mycorrhizal fungi in cultivated soils, Soil Biol. Biochem. 37 (2005) 1589-1597.

[40] D. Mikkelson, U. Kappler, A.G McEwan, L.I. Sly, Probing the archaeal diversity of mixed thermophilic bioleaching culture by TGGE and FISH, Syst. Appl. Microbiol. 32 (2009) 501-513.

[41] 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 decompostion, PLos One 8 (2013), 1 - 10.

[42] 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.

[43] 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.

[44] 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.

HIGHLIGHTS:

- *Sus scrofa domesticus* decomposition effected changes in fungal diversity.
- Decreased Simpson dominance index was recorded for the experimental microcosm.
- Unlike bacterial profiles, no statistically significant diversity shifts resulted.
- Longer on-going studies are testing 18S-DGGE applicability in forensic ecogenomics.
- Applicability is also being advanced with sequencing and *in situ* studies.

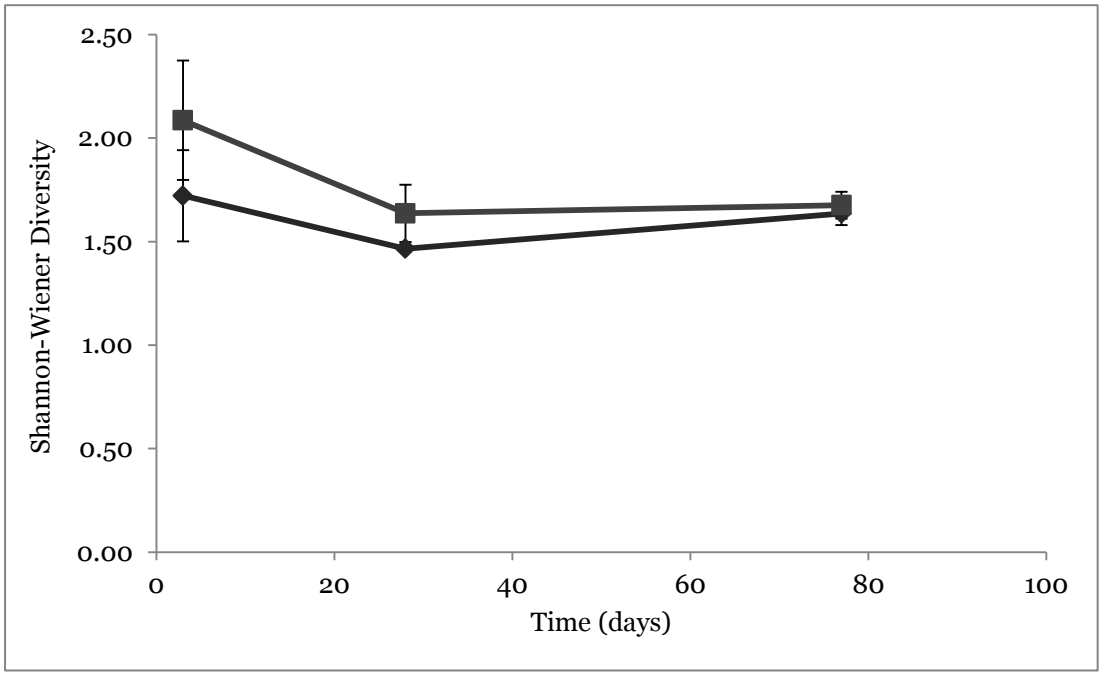

Fig. 1. Changes in Shannon-Wiener diversity (H') of control (■) and experimental (◆) microcosms during incubation at ambient temperature.

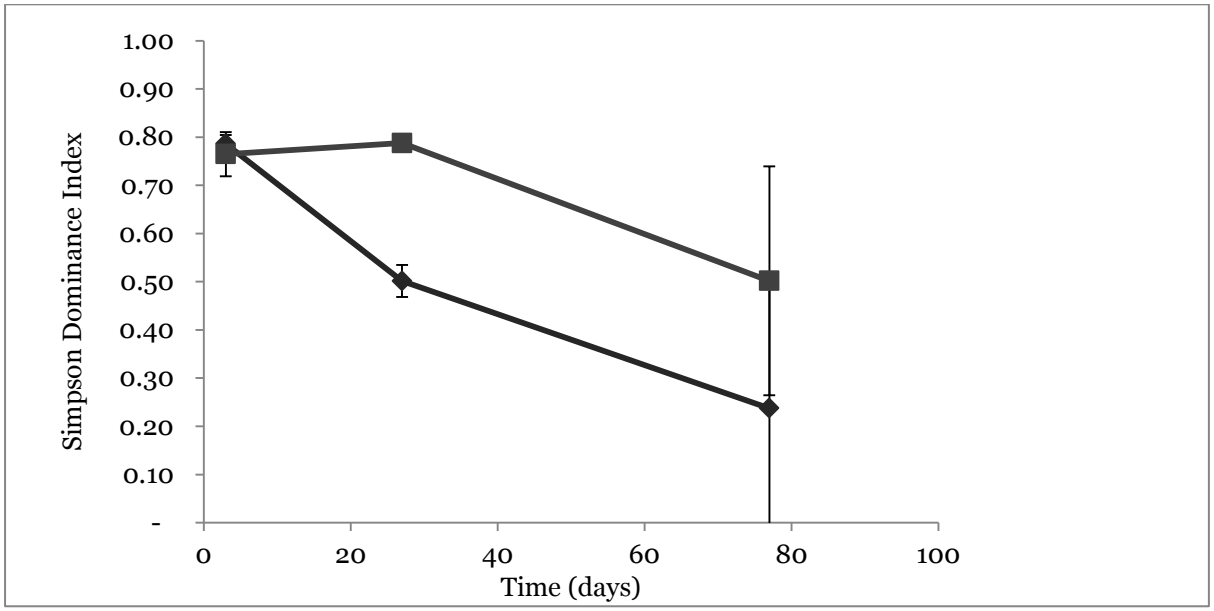

Fig. 2. Changes in Simpson dominance index (S) for control (■) and experimental (◆) microcosms during incubation at ambient temperature.

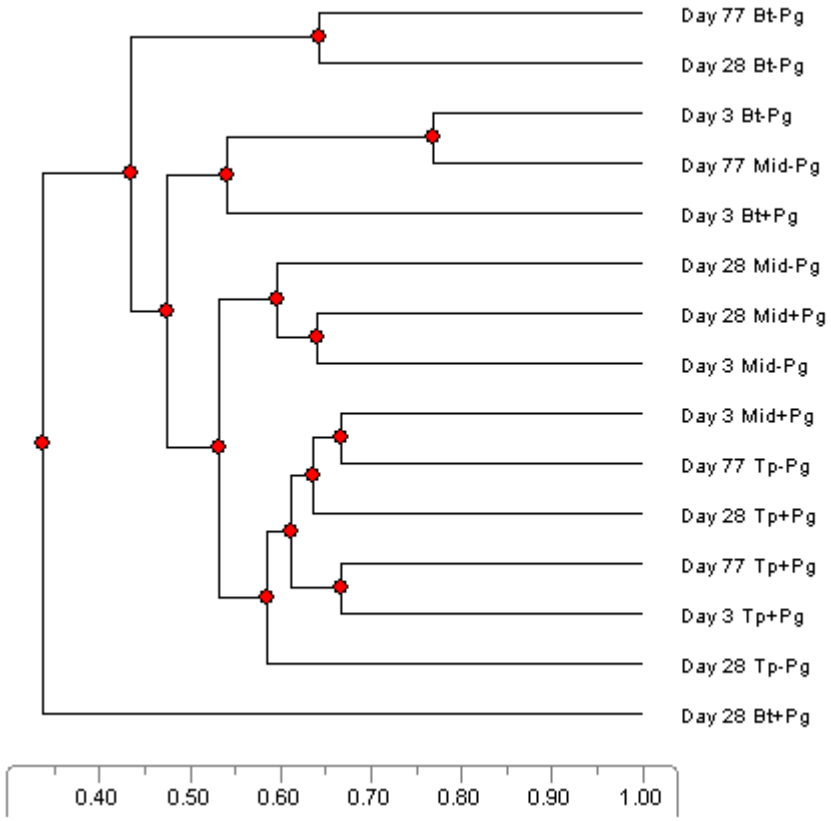

Fig. 3. UPGMA dendogram of DGGE profiles of the top (Tp), middle (Mid) and bottom (Bt) layers of control (-Pg) and experimental (+Pg) microcosms during incubation at ambient temperature. Arrows 1 - 8 identify specific % similarities for the soil layers of the control and *Sus scrofa domesticus* microcosms.