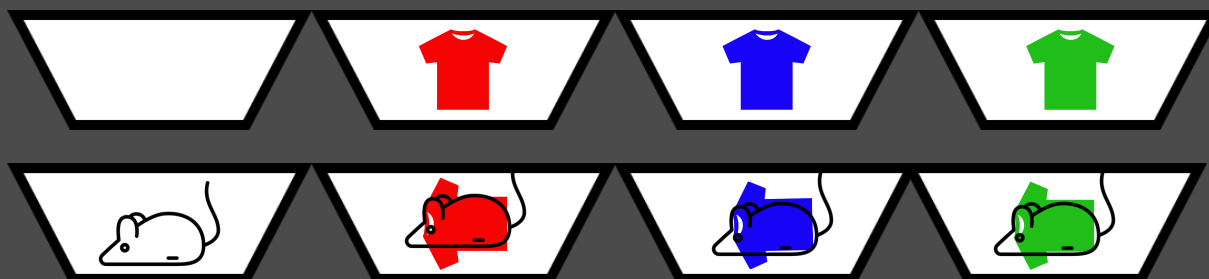


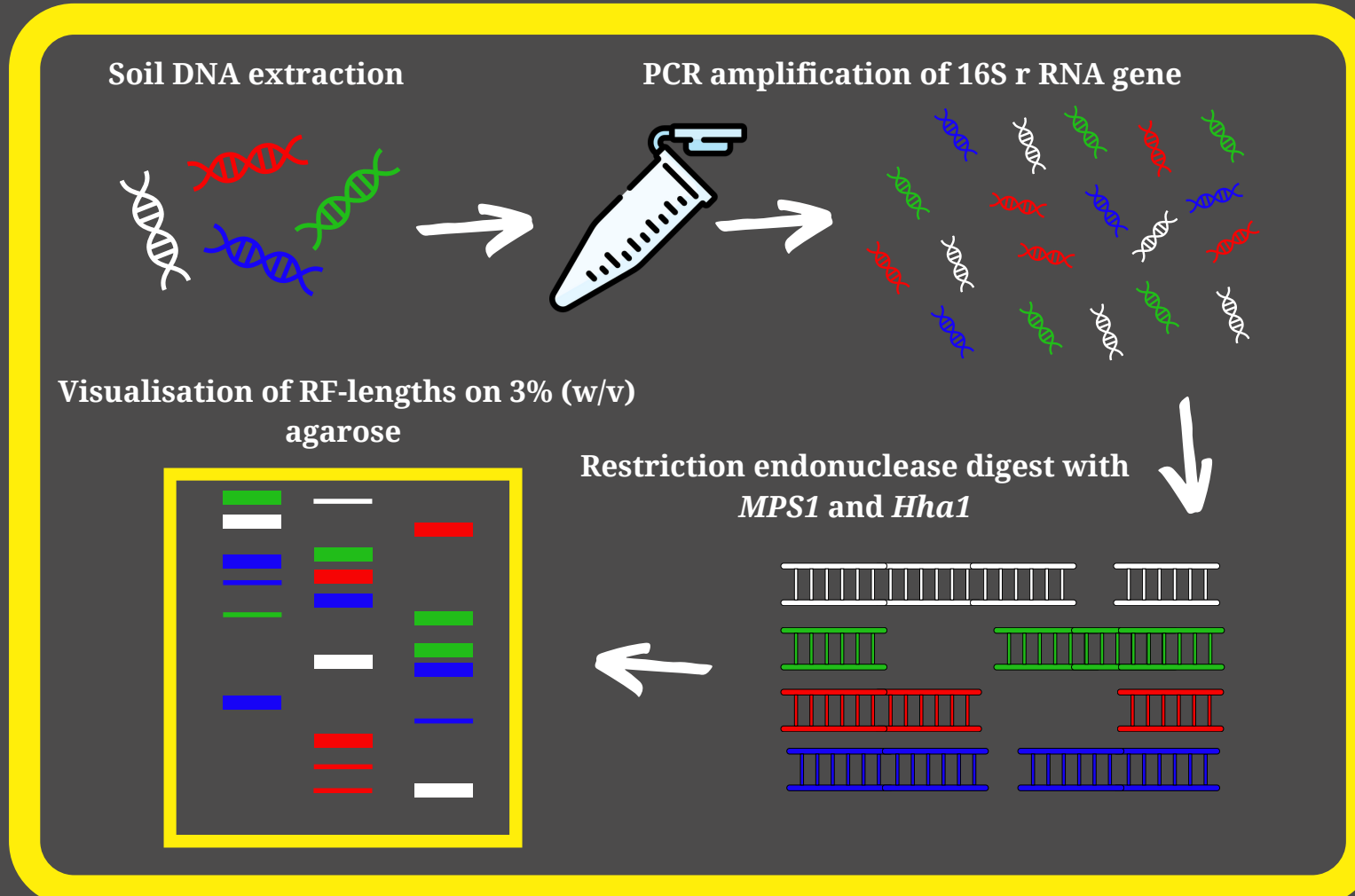
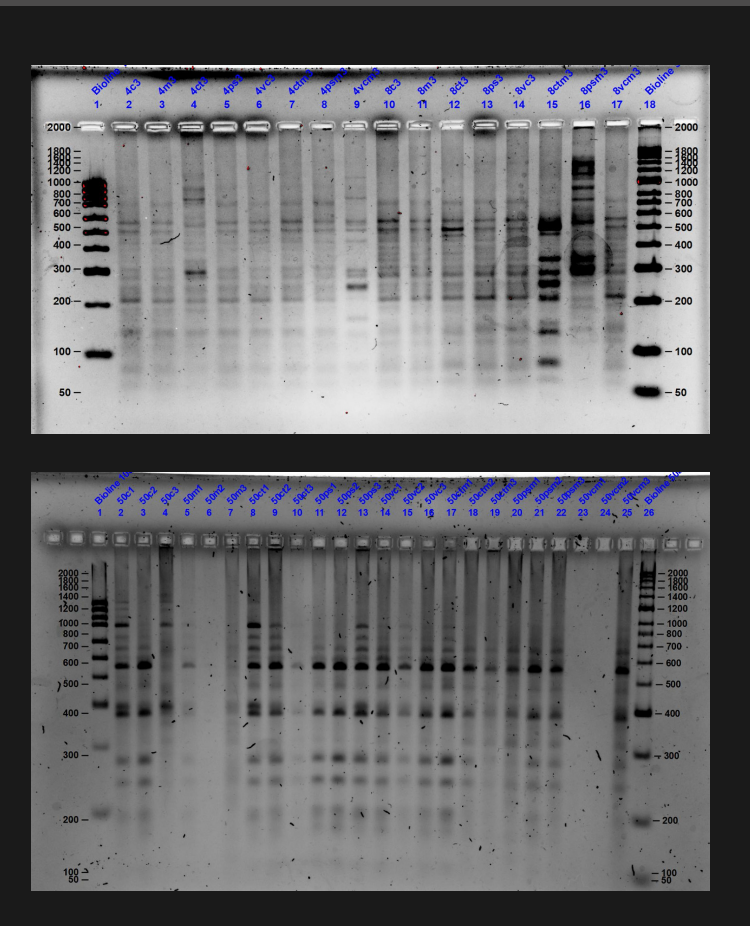
# The Study

To study the impact of clothing on the predictive succession of grave soil microbial structure, murine burial microcosms were maintained and sampled for 50 days at ambient temperature. Composite soil samples (~3g), collected over 8 sampling intervals, were assessed using the molecular technique restriction fragment length polymorphism (RFLP). The RF lengths were visualised with ethidium bromide under UV light. The gel derived data was used to calculate Hill ecological numbers representing: richness in taxa (<sup>0</sup>D), common taxa (<sup>1</sup>D) and dominant taxa (<sup>2</sup>D). From these numbers, the Hill ratio can be calculated to evaluate the proportions of common (<sup>1</sup>D/<sup>0</sup>D) and dominant (<sup>2</sup>D/<sup>0</sup>D) taxa in the soil microbial community.

Control Cotton Polyester Viscose

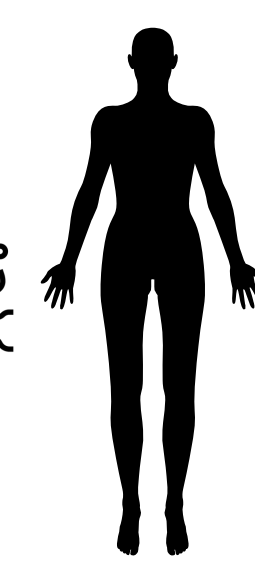


## Restriction Fragment Length Polymorphism

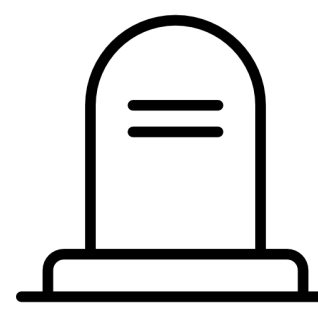


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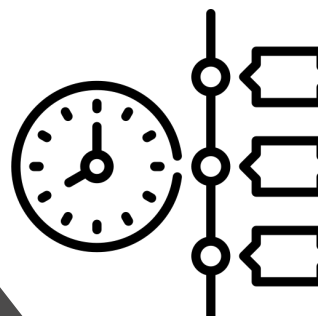
The creation of the Human Microbiome Project (HMP) has revealed that microbial cells outnumber somatic cells 10-fold!  
 There are significant divergences between individualistic microbiomes as a result of abiotic and biotic factors such as disease, lifestyle choices such as diet, and even urban living environments.<sup>1-4</sup>  
 Novel research has indicated that these personal signatures could be utilised in forensic identification of keyboard, phone surfaces and potentially weapons.<sup>5-6</sup>



The post-mortem microbiome begins to homogenise between individuals and becomes non-stochastic (predictable) with time.  
 Like entomology, the succession of bacteria can be predictive of post mortem interval (PMI) or time since death.<sup>7-10</sup>



In a clandestine burial enteric microbes leech into the surrounding soil.  
 Propagation of fluids, expelled by the cadaver permeates the soil influencing the endogenous microbial community.<sup>11</sup>  
 The succession of the grave soil community structure can be utilised as a non-destructive means of PMI.<sup>11-14</sup>



**Animal models**  
 One of the pivotal limitations that face human decomposition studies is the lack of Human Taphonomy Facilities and laws in the UK and many other regions globally.<sup>15</sup>  
**Porcine**  
*Sus scrofa domestica* are forensically relevant proxies in microbial succession studies due to size, anatomy, physiology, immunity and diet.<sup>16</sup>  
**Murine**  
 Mice are frequently utilised as analogues in research due to their comparable genomes with murine models accounting for 95% of all animal research into human disease and drug development. The size and cost of rodents are attractive to the forensic research discipline, allowing a volume of destructive replicates with size constraints.<sup>17,18</sup>



### Clothing and decomposition

- The presence and type of clothing can retard decomposition, in particular in the putrid decay stage.<sup>19,21-23</sup>
- Clothing can act as an entomological refuge from environmental conditions forming large localised larval masses.
- Decomposition associated textiles suggest interference with lateral fluid seepage in clandestine burials.<sup>24,26</sup>
- Similarly, clothing itself decomposes therefore, interpreting the ecological impact and interaction with both enteric and endogenous communities is crucial.<sup>24,28</sup>

Being an emerging forensic approach there remain gaps in the literature that are crucial to address prior to validation for in-field use and to improve the accuracy of the model.<sup>15</sup>

Previous works have identified influences of both abiotic and biotic factors such as mass, ambient temperature, scavenger availability, and moisture conditions on the grave soil microbial landscape.<sup>11,13, 16-18</sup>



# The Findings

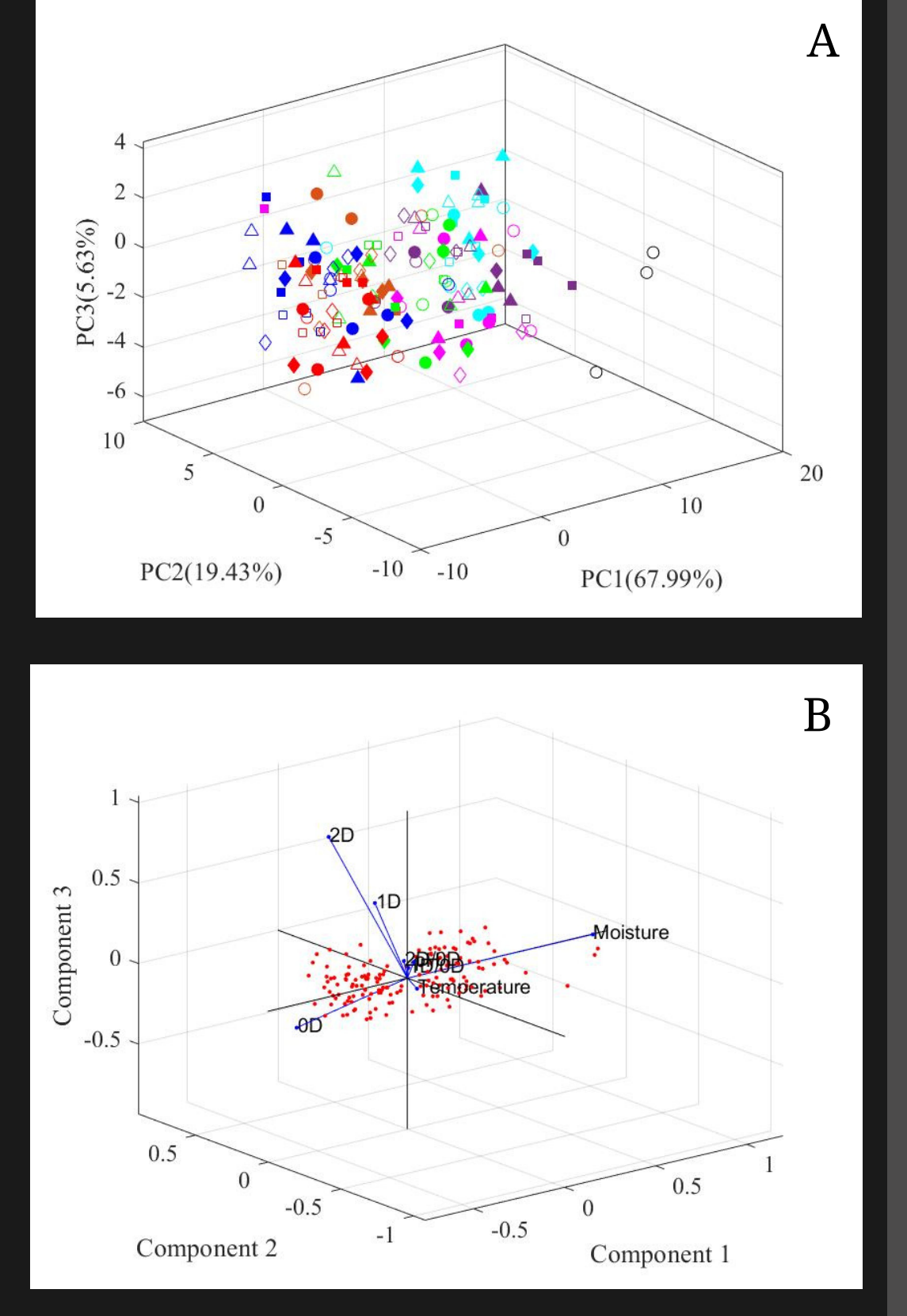


Figure 1. A) Principal component analysis (PCA) scores plot for clothing parameters based on edaphic data and Hill ecological indices (control (○), unwrapped mouse (●), cotton (△), polyester (▽), viscose (◇), cotton wrapped mouse (▲), polyester-wrapped mouse (◆) and viscose wrapped mouse (◆) burial microcosm treatments over 50 days; sampled on days 0, 4, 8, 16, 24, 32, 40 and 50. PCL1, PCL2 and PCL3 explained 87.25% of the total variance observed. B) PCA biplot of 16S rRNA bacterial community ecological indices (Hill number <sup>0</sup>D, <sup>1</sup>D and Hill ratios <sup>1</sup>D/<sup>0</sup>D and <sup>2</sup>D/<sup>0</sup>D).

- Two-way ANOVA with replication identified significant temporal differences in edaphic variables between the eight clothing parameters.
- Observable differences in bacterial community composition, both in the RFLP-derived gel data and graphical representation of ecological indices (Hill numbers) highlighted variation between the control, murine decomposition and those associated with cotton, polyester, and viscose (Figure 2).
- Two-way ANOVA with replication identified slight temporal differences in RFLP-derived ecological indices over the 50 day sampling period but did not identify significant differences between the control and experiential parameters.
- The principal component analysis (PCA) of the bacterial Hill ecological indices showed some temporal clustering of microcosms in the presence and absence of *M.musculus* (Figure 1).

# The Conclusions

- Although no statistical significance identified from the RFLP-derived indices was observed, visible changes to operational taxonomic units both temporally and in response to clothing fabric suggest compositional community shifts in grave soil.
- Moreover, high resolution metagenomic sequencing will be applied to add taxonomic detail to the associated grave soil samples.



Scan the QR code for further information on the figures and references used in this poster

Insect exclusion

Proxy size

Environmental conditions

RFLP vs DGGE

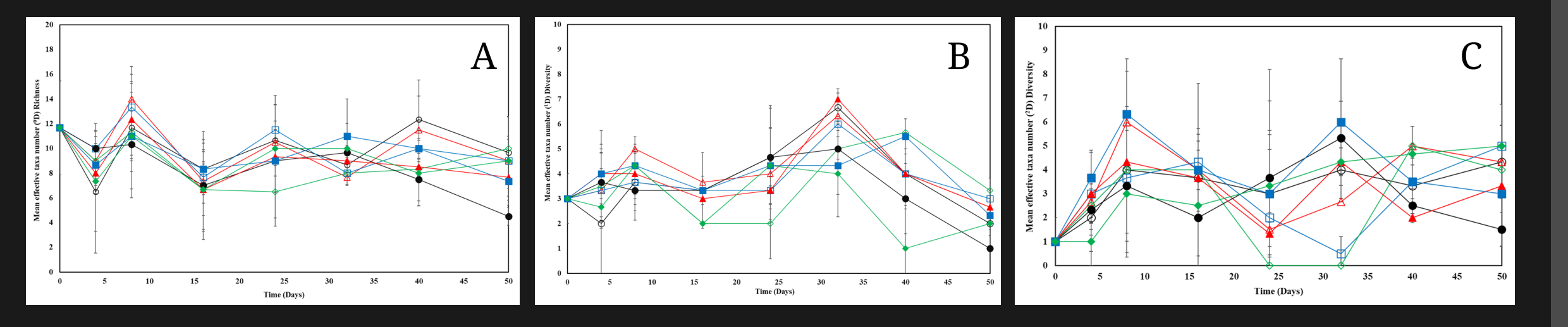


Figure 2. Changes in average (n=3) A) richness (D) B) diversity (D) C) diversity (D) of the control (○), unwrapped mouse (●), cotton (△), polyester (▽), viscose (◇), cotton wrapped mouse (▲), polyester-wrapped mouse (◆) and viscose wrapped mouse (◆) burial microcosm treatments over 50 days; sampled on days 0, 4, 8, 16, 24, 32, 40 and 50. Average ambient external humidity (%). Bars denote Standard Deviation.

# Assessing Gravesoil Bacterial Community Structure in the Presence of Different Fabrics Associated With a Murine Analogue

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