

Bacterial Detectives: Investigating fabric presence and type on grave soil necrobiome dynamics



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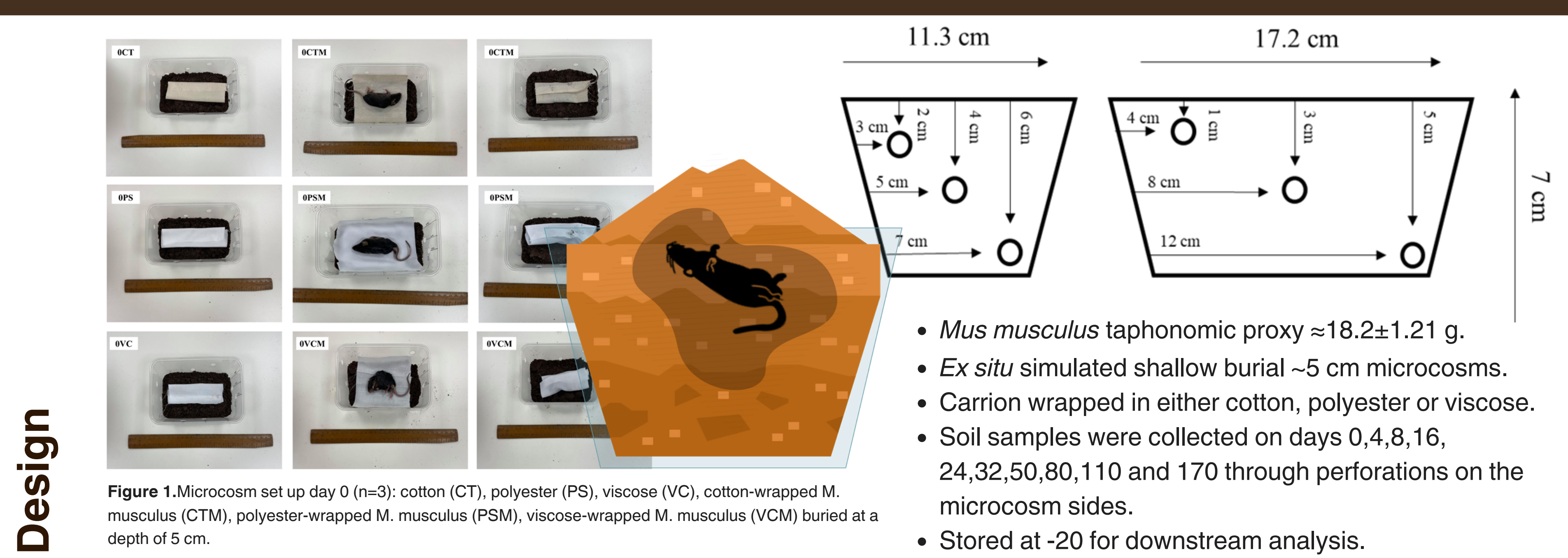
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Background

- Microbes have proven a valuable forensic tool, with successful applications in diverse fields such as geolocation, wildlife crime, sexual offenses, and determining cause of death.
- While the microbiome is inherently variable and individualized, after death, the carrion-associated microbial community and composition exhibits a non-stochastic succession known as the **thanatomicrobiome**.
- Terrestrial deposited remains interact with the local environment creating a unique landscape known as the **cadaver decomposition island (CDI)**.
- The soil **necrobiome** has proved effective in delineating grave locale and time since death or **post mortem interval (PMI)**.
- Like all methods of predicting PMI, abiotic and biotic variables can impact the accurate predictability of grave soil bacterial succession.
- Fabric is frequently encountered in forensic investigations and is well-documented for its ability to influence the rate and nature of decomposition as well as entomological activity, based on factors such as its presence, weight, and type.
- 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.

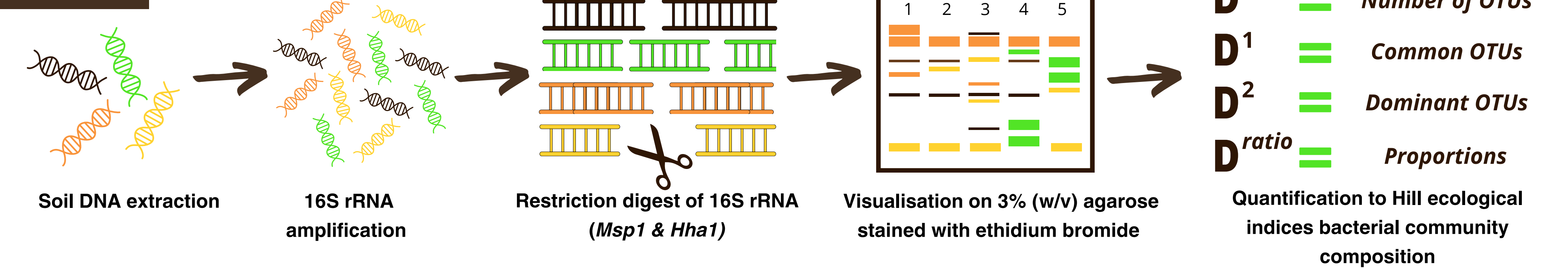


The aim of this research was to assess what impact, if any, does the presence of type of carrion-associated fabric have on measurable grave soil necrobiome community succession.



- Mus musculus* taphonomic proxy $\approx 18.2 \pm 1.21$ g.
- Ex situ* simulated shallow burial ~ 5 cm microcosms.
- Carrion wrapped in either cotton, polyester or viscose.
- Soil samples were collected on days 0, 4, 8, 16, 24, 32, 50, 80, 110 and 170 through perforations on the microcosm sides.
- Stored at -20°C for downstream analysis.

RFLP



NGS

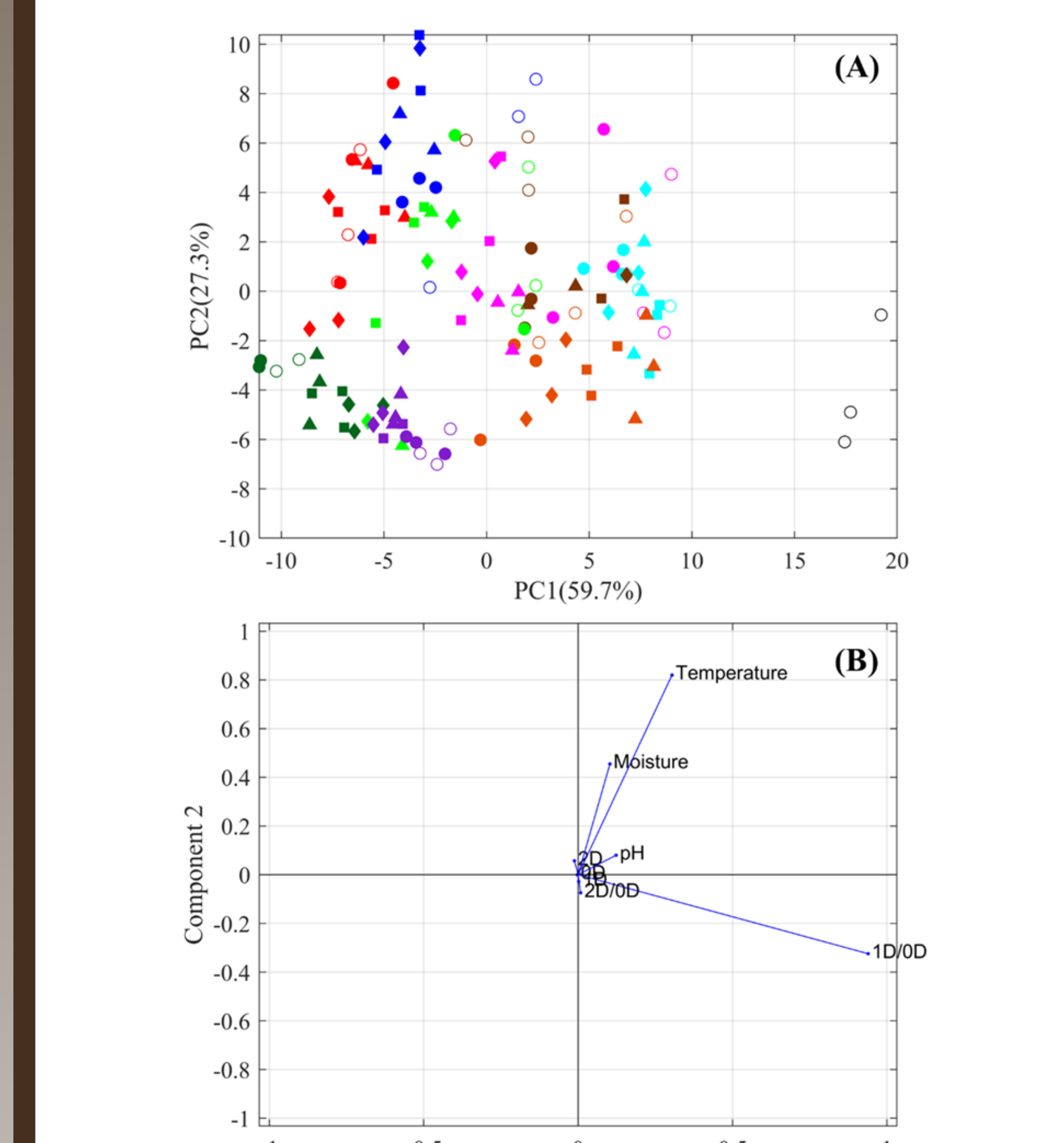
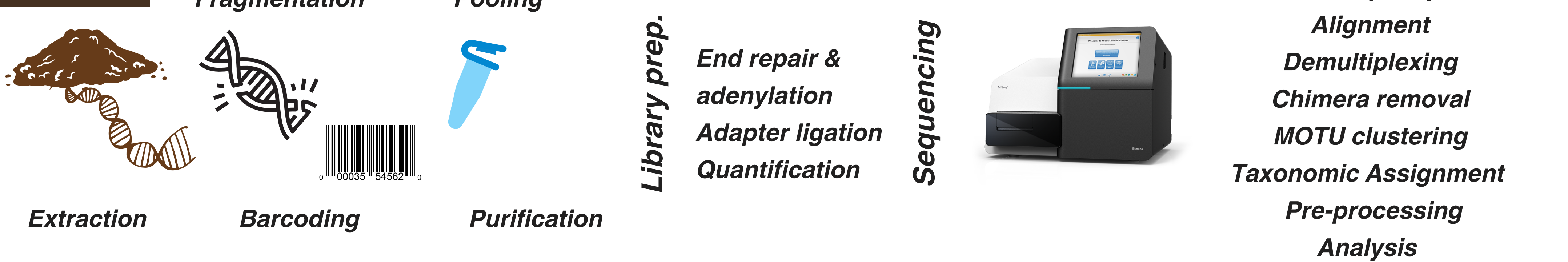


Figure 2. (A) PC scores plot on edaphic soil data and Hill ecological indices of experimental microcosms: soil-only (○), unwrapped *M. musculus* (●) and those wrapped in cotton (CTM; ▲), polyester (PSM; ■) and viscose (VCM; ◆) burial microcosm treatments over 170 days sampled on days 0, 4, 8, 16, 24, 32, 50, 80, 110 and 170. (B) PC loadings plot of soil microcosms.

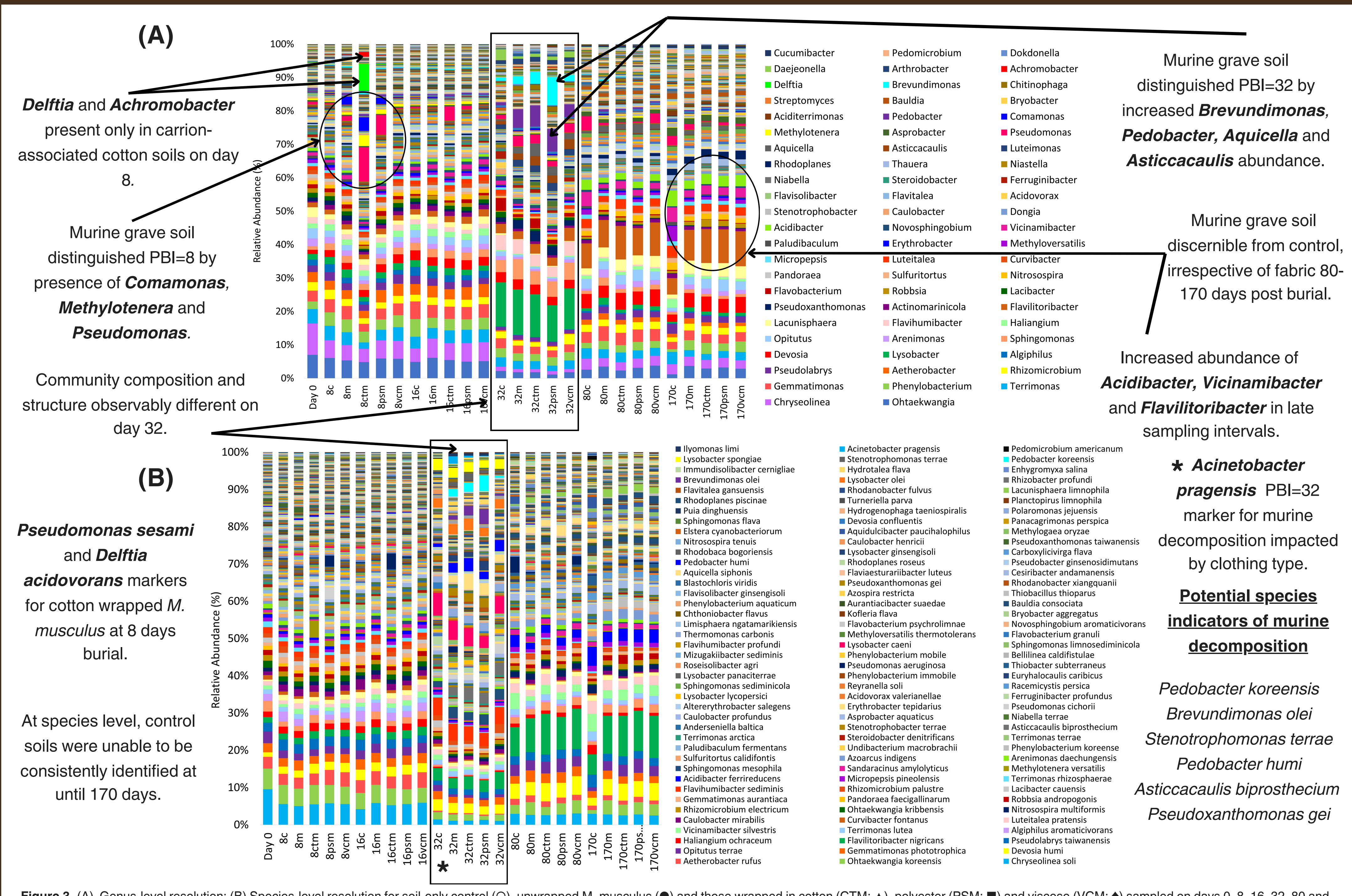


Figure 3. (A) Genus-level resolution; (B) Species-level resolution for soil-only control (○), unwrapped *M. musculus* (●) and those wrapped in cotton (CTM; ▲), polyester (PSM; ■) and viscose (VCM; ◆) sampled on days 0, 8, 16, 32, 80 and 170 post burial.

Discussion

- Two-way ANOVA with replication revealed significant temporal variation in bacterial community richness, diversity, and evenness, as evaluated through RFLP analysis ($p < .001$).
- Principal component analysis (PCA) showed temporal clustering of grave soil samples irrespective of clothing presence or type. Edaphic changes in the soil and the ratio of common species was highlighted in the loadings plot accounting for over 85% of the observed variation.
- Metagenomic 16S DNA sequencing revealed prominent bacterial indicators of both decomposition and clothing, discernible at both the genus and species levels.
- Presence of *Achromobacter*, *Pseudomonas (sesami)*, and *Delftia (acidovorans)* in carrion grave soil associated with cotton on day 8 post-burial simulation suggests potential environmental interactions, likely influenced by agricultural practices, soil composition, and microbial ecology of cotton fields.
- At the genus level, murine decomposition at PBI=8 featured *Comamonas*, *Methylotenera*, and *Pseudomonas*, irrespective of clothing presence or type. While not primary decomposers, their metabolic flexibility, especially in using single-carbon compounds as shown by *Methylotenera*, implies their role in organic matter degradation.
- PBI=32, notable presence of *Pedobacter koreensis* and *Stenotrophomonas terrae*, known for their metabolic versatility, likely aid in the degradation of necromass, facilitating nutrient release and ecosystem recycling where *Brevundimonas olei*'s presence may denote its involvement in hydrocarbon degradation, influencing VOC production (smell of death).
- Increased abundance of *Acidibacter*, *Vicinibacter*, and *Flavilitorbacter* in late sampling intervals during decomposition may be attributed to factors such as enhanced availability of organic matter and nutrients, microbial succession favouring late-stage decomposers, pH changes favouring acidophilic bacteria, and symbiotic interactions shaping population dynamics.
- The study's limitations, such as size, species, absence of natural settings, and lack of insect access, may impact both endogenous and exogenous microbiomes. Consequently, they could affect soil population dynamics, community composition, and microbial interactions.