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The Functional and Structural Succession of Mesic-Grassland Soil Microbiomes Beneath Decomposing Large Herbivore Carcasses

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ABSTRACT

Plant detritus is abundant in grasslands but decomposes slowly and is relatively nutrient-poor, whereas animal carcasses are labile and nutrient-rich. Recent studies have demonstrated that labile nutrients from carcasses can significantly alter the long-term soil microbial function at an ecosystem scale. However, there is a paucity of knowledge on the functional and structural response and temporal scale of soil microbiomes beneath large herbivore carcasses. This study compared microbiome functions and structures of soil beneath *Connochaetes taurinus* (hereafter 'wildebeest') carcasses at various postmortem intervals of decomposition to matched control samples over 18 months. Microbial functions were compared by their community-level physiological profiles determined by sole-carbon substrate utilisation and structures by metagenomic sequences using 16S rRNA gene markers. Overall metabolism and metabolic diversity remained increased and functionally dissimilar to control soils throughout the experimental period, with successive sole-carbon substrate utilisation observed. Conversely, diversity was initially reduced and structurally dissimilar from the control soil but recovered within the experimental period. The study contributes to the knowledge of carcass decomposition by investigating the long-term soil microbiome dynamics resulting from large herbivore carcasses decomposing in a mesic grassland. Microbial functional succession and ecologically relevant bacterial biomarkers of soil beneath the decomposing carcasses were identified for various postmortem intervals.

1 | Introduction

Grasslands worldwide are rapidly degrading. They cover approximately 26% of the earth's ice-free land and contain approximately 20% of its soil organic matter (SOM), the foundation of soil nutrient availability, biological activity, environmental resilience and erosion control (Montanarella et al. 2015). Grasslands are particularly suitable for herds of large ungulate herbivores and are of great agricultural and economic importance (van As et al. 2012). When managed at low stocking rates, large herbivores increase grassland plant diversity, productivity and sustainability (Isbell et al. 2011). Grass, which is relatively nutrient-poor and slow to decompose, is nonetheless proficiently digested and absorbed by herbivores. The remaining nutrients are redistributed into relatively small patches when excreted as faeces and urine (Swift et al. 1979). Some nutrients are immediately available to plants, whereas others are returned to the soil (Owen-Smith 1999). Although the herbivore carcasses themselves are labile, rapidly decomposing and releasing nutrients, and are nutrient-rich, containing high concentrations of nitrogen, phosphorus and potassium, the nutrient losses from grasslands due to the removal of large herbivores for human consumption and other purposes before mortality have historically been deemed insignificant at the

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landscape ecosystem scale (Owen-Smith 1999; Parmenter and MacMahon 2009). However, it has been suggested that the structure of grassland soil microbiomes is driven by the quality of nutrient inputs over decades (Millard and Singh 2010; Strickland and Wickings 2015).

Labile nutrients are more efficiently assimilated into the microbial biomass and utilised in metabolic products (Yang and Janssen 2002; Carter, Yellowlees, and Tibbett 2007; Benninger, Carter, and Forbes 2008; Cotrufo et al. 2013; Macdonald et al. 2014; Strickland and Wickings 2015). An abundance of labile nutrients, such as from an ungulate carcass, can shift the soil microbial community beneath it towards a predominantly copiotrophic community specialising in decomposing labile nutrients rather than existing SOM. As the population of copiotrophs increases and then declines due to reduced labile nutrients, their biomass becomes the precursor for stable SOM, leading to an increase in SOM. In contrast, a small invertebrate carcass primarily primes the soil microbiome to produce more enzymes, resulting in the utilisation and potential reduction of existing SOM (Strickland and Wickings 2015). Therefore, large carcasses may significantly alter ecosystem processes by influencing long-term soil properties (Carter, Yellowlees, and Tibbett 2007). Carcass decomposition is rapid, requiring specific metabolic pathways from the soil microbiota that hydrolise the energy-rich, low molecular weight animal proteins, lipids, and nucleic acids into fatty acids, organic acids, organic nitrogen and phenols (Barton et al. 2013; Metcalf et al. 2013; Cobaugh, Schaeffer, and DeBruyn 2015). The labile nutrients create short-term accelerated microbial processes (hot moments) that result in soil microbiomes with faster process rates and intensive interaction (hotspots) that may exist for lengthy periods after the nutrients have dissipated (Kuzyakov and Blagodatskaya 2015). Recent studies have indicated that decomposing carcasses alter ecosystem scale processes by the longevity of the hotspots, demonstrating the ecological relevance of decomposing carcasses (Yang and Janssen 2002; Carter, Yellowlees, and Tibbett 2007; Benninger, Carter, and Forbes 2008; Strickland and Wickings 2015; Cotrufo et al. 2013; Macdonald et al. 2014). The microbial community functions of hotspots depend on the competing ecological groups, namely r-strategists that grow fast in abundant labile nutrients or K-strategists that can grow with minimal nutrients (Fontaine, Mariotti, and Abbadie 2003; Kuzyakov and Blagodatskaya 2015; van Elsas 2019). The competition for nutrients by these ecological groups can be observed through the dynamic succession of microbial taxa, which may answer fundamental ecological and forensic questions.

Microbial functions are the foundation of soil quality, stability and resilience (Allison and Martiny 2008; Habig and Swanepoel 2015). Changes in function are often inferred from changes in microbiome structure. The highly conserved 16S rRNA marker gene can accurately determine microbiome structure. Some of the taxa identified by these marker gene sequences are known to possess specific metabolic properties. Therefore, microbiome metabolic functions can be predicted based on the relative abundance of these marker genes within a soil microbiome (Metcalf et al. 2016). The accuracy of these inferences is questionable and spurious for complex microbiomes (Franzosa et al. 2018; Escalas et al. 2019). Furthermore, it excludes functions derived from transposable elements and plasmids, which often confers metabolic processes (Barton and Northup 2011; Escalas et al. 2019; Nielsen and van Elsas 2019). Therefore, methods that analyse the function directly, such as oxygen consumption assays (Gomez and Garland 2012), multiple substrate-induced respiration (Singh et al. 2018), enzyme activity assays (Scola et al. 2018) and sole-carbon substrate utilisation (Garland 1997; Pechal et al. 2013; Heo et al. 2021) are often utilised, although these methods only include the fraction of culturable microbiota (Amann, Ludwig, and Schleifer 1995).

Carcasses progress through six stages of decomposition (*fresh*, *bloat*, *active decay*, *advanced decay*, *dry* and *remains*) (Payne 1965). Their rate of decomposition and the microbiome functional and structural successions of the soil beneath are influenced by the carcass mass (Singh et al. 2018), the a priori microbial structures and functions of the soil (Lauber et al. 2014; Dalemberg and Jager 1981; Fontaine, Mariotti, and Abbadie 2003), temperatures, humidity, precipitation and ultraviolet (UV) radiation (Cotrufo et al. 2013; Singh et al. 2018; Tiao et al. 2012; Puissant et al. 2015). Silva et al. (2022) observed that increasing UV radiation and a 5°C–10°C rise in air temperature significantly enhanced the catabolism of polymers, carbohydrates, carboxylic acids, amines and amino acids in the soil microbiota. In addition, they noted changes in the relative abundance of alpha-, beta- and deltaproteobacteria, as well as Clostridia. Moreover, access to the carcass by necrophagous invertebrates and scavengers significantly influences the decomposition process and the surrounding soil microbiome (Benninger, Carter, and Forbes 2008; Aitkenhead-Peterson et al. 2012; Crippen, Benbow, and Pechal 2016). These aspects have predominantly been studied within forensic contexts, focusing on determining the circumstances surrounding death, indicating particulars such as the individual's height-to-weight ratio (Mason et al. 2022), and estimating the postmortem interval (PMI), using soil nutrients, pH and microbial biomarker succession as indicators. Most existing literature examines relatively small carcasses over short durations (less than 1 year) (Lauber et al. 2014; Cobaugh, Schaeffer, and DeBruyn 2015; Metcalf et al. 2016; Weiss et al. 2016; Singh et al. 2018; Heo et al. 2021; Mason, Taylor, and DeBruyn 2023), primarily utilising human corpses and human-like animal carcasses such as swine (*Sus scrofa*) and rodents due to their similarities in mass, decay rates and skin structure. A lack of research using carcasses exceeding 50 kg and further research using a variety of larger carcasses over extended periods in different ecosystems with varied environmental conditions has been recommended for forensic and ecological purposes (Barton et al. 2013; Weiss et al. 2016). To obtain the ecologically relevant biomarkers, metagenomic analysis that supports high-dimensional class comparisons and determines the magnitude of enrichments (biologically relevant taxa) is essential (Segata et al. 2011).

The grasslands of southern Africa are unique for their extraordinary number and diversity of large-bodied herbivores, including *Connochaetes taurinus* (wildebeest) (140–410 kg) and numerous predators and scavengers (Jones, Strauss, and Holekamp 2015). Besides their mass, wildebeest are herbivores with robust skin structures, properties rarely described in existing carcass decomposition literature (Towne 2000; Macdonald et al. 2014; Risch et al. 2020). In addition, up to 386 kg/km^2 of herbivore carcasses have been recorded annually in natural grassland and savannah woodlands in southern Africa (Jones, Strauss, and Holekamp 2015), and up to 6250 river drownings (Subalusky et al. 2017), shaping the respective microbiomes, ecosystem nutrient budgets and their decomposition dynamics. Considering the degradation of grasslands, their relevance to ecosystem maintenance as carbon sinks (Smith 2014) and their economic and agricultural importance, our understanding of the effects of decomposing carcasses on soil microbiomes is crucial. It was hypothesised that distinct changes in overall metabolism, substrate utilisation, and bacterial community composition would be observed at different PMIs, as characterised by Biolog EcoPlates and 16S rRNA gene sequencing. The study describes specific metabolic functions and bacterial biomarkers of ecological relevance during large carcass decomposition.

2 | Experimental Procedures

2.1 | Study Area and Sampling

The study was conducted at the Telperion Nature Reserve in South Africa (25.7039° S, 28.9814° E; 1300 ± 120 m ASL), which is within a mesic-grassland and bushveld biomes ecotone (Visser et al. 1961) and has a dry-winter subtropical highland climate (Mucina and Rutherford 2006). Ten wildebeest carcasses (209–247 kg) were obtained from the reserve's culling process (January 2019). The wildebeest were humanely euthanised with a single shot to the head using a small calibre hunting rifle. They were then secured to metal grids while fresh and placed within typical grassland vegetation on level ground, more than 900 m apart (Figure S1). Soil samples were aseptically collected (0–10 cm depth) within 1 month after the carcasses had ruptured and at 6-, 12- and 18-month PMIs. Three soil samples were collected below each carcass (head, thorax, hindquarters) and composited as a *Beneath* sample replicate, and three soil samples were collected at a 5-m radius from each carcass and composited as its *Control* sample replicate (Figure S2). The 80 samples were placed in Ziploc bags and kept at 4°C. Within 24 h, a portion of each sample was removed and used in the soil microbial function analysis, and the remainder was frozen at −80°C.

2.2 | Microbiome Function

2.2.1 | EcoPlate Preparation

Sole-carbon substrate utilisation analysis *vis* functional analysis was performed using Biolog EcoPlates as described by Habig and Swanepoel (2015), with some modifications. The soil samples were aseptically sifted through a 2-mm steel mesh to remove roots and pebbles. Aliquots were made by adding 10 g of soil to 90 mL of sterile ultra-pure water in a glass vessel. The vessels were placed in a shaking incubator for 1 h at 200 rpm (25°C) to liberate the soil microbiota and then kept stationary for 1 h to facilitate particle precipitation. The supernatant was diluted (1:3000) and inoculated (150 μL per well) into the 96-well EcoPlates containing a triplicate of 31 environmentally relevant sole-carbon substrates and a blank well. The plates were incubated at 25°C for 216 h, and the optical density (OD) of colour development, produced by tetrazolium salts in all the wells, was measured at 590 nm daily.

2.2.2 | Community-Level Physiological Profiling

The OD values of the substrate-containing wells were corrected by subtracting blank-well values and substituting negative values with 0. The phases of average well-colour development (AWCD) (lag, exponential or stationary), the number of unresponsive wells, colour development outside the linear OD range, and the variance between well-colour development were used as criteria (Weber and Legge 2010) to select a single incubation time point (144 incubation hours) to compare the microbial function. AWCD, which indicates the total metabolic function (Pechal et al. 2013), Simpson's diversity (Simpson 1949) and Pielou's evenness index values were compared between groups. The OD values were Wisconsin double-standardised (Legendre and Legendre 2012), and the Bray–Curtis dissimilarity index values (Bray and Curtis 1957) were calculated using the (*vegdist: vegan-package* version 2.6-4) (Oksanen et al. 2023). The dissimilarities were compared by rank order analysis of dissimilarity (ANOSIM) (*anosim: vegan-package*) (Clarke 1993), multivariate analysis of variance (PERMANOVA) (Anderson 2001) (*adonis2*) and beta dispersion (PERMDISP) (Anderson, Ellingsen and McArdle 2006) (*betadisper*) using 9999 random permutations. Non-metric multidimensional scaling (nMDS) (Kruskal 1964) was performed on the dissimilarity results and visualised using the *ggplot2* package (Wickham 2016). The 31 carbon substrates were grouped into six substrate guilds (Table S1), representing their chemical nature (Rutgers et al. 2016), and the similarity percentages (SIMPER) between the groups were calculated (Clarke 1993). The differences in mean substrate-guild utilisation were compared (Zak et al. 1994).

2.3 | Soil Nutrient Analysis

Soil nutrient contents of the *Control* and *Beneath* groups from sample Sites 2, 4, 5 and 8 ($n = 4$) (Figure S1) of each PMI were analysed by the Institute of Soil, Climate and Water of the Agricultural Research Council of South Africa. Ammonium and nitrate ion contents were measured using the potassium chloride extract method (ISO 2003), and sulphate was measured using a 1:10 soil water extraction method (ISO 1995). After filtration, the ion contents were measured using spectrophotometry. Phosphorus content was measured using the Bray 1 method (Bray and Kurtz 1945). A redundancy analysis (RDA) was performed using the forward selection approach (Blanchet, Legendre, and Borcard 2008) of the soil nutrients most likely to explain the dissimilarity in microbial function between the groups at the same PMI.

2.4 | Microbiome Structure

2.4.1 | Dataset Creation and Analysis

The environmental DNA of the soil samples from Sites 2, 4, 5 and 8 ($n = 4$) (Figure S1) of each PMI were recovered and purified using the Dneasy PowerSoil DNA isolation kit (Qiagen, Valencia, CA, USA). Omega Bioservices (Norcross, USA) amplified the V3-V4 region of the 16S rRNA genes using the 341F-785R primer pair. The amplicon DNA was sequenced with an Illumina MiSeq v3 platform using the KAPA HiFi

PCR kit (KAPA Biosystems 2024) and provided 300 base-pair (bp) forward and reverse sequence reads (adapters removed) in FASTQ files.

The divisive amplicon de-noising algorithm (DADA) was applied to the FASTQ files using the *dada2* package version 1.26 (Callahan et al. 2016) to remove sequence reads containing errors. The default pipeline was used for quality filtering apart from the maximum expected errors, which was set to two and three for forward and reverse reads with the *filerAndTrim* function. In addition, the first 25 and last 10 nucleotides were trimmed from each read, and only forward and reverse reads of more than 270 and 205 were included in the downstream analysis. The paired-end reads were merged with the *mergePairs* function using the default minimum overlap of 12 bp with no bp mismatches. De novo chimaeras were detected and removed (6.7%), and taxonomy was assigned to the remaining amplicon sequence variants (ASVs) from the SILVA rRNA reference database (release 138.1) (www.arb-silva.de/) (Quast et al. 2013).

Results from the DADA analysis were analysed using the *phyloseq* package version 1.41 (McMurdie and Holmes 2013). Rarefaction curves were created (Figure S7) to establish sample coverage, and the sampling depths were normalised by random subsampling (rarefying) the data to 41,094 subsamples (Lin and Peddada 2020) using the *rarefy_even_depth* function and reduced the gene inventory from 24,570 to 24,080 unique ASVs. Observed richness and inverse Simpson's diversity index values (Simpson 1949) were estimated using the *estimate_richness* function and statistically compared for the different groups at the same PMI and the same group at different PMIs. Principal coordinate analyses (PCoA) (Gower 1966) were performed using the *ordinate* function, PERMANOVA and PERMDISP was performed to establish if there were significant differences between the groups and the ordination results were illustrated using the *rgl* package version 1.3.1 and *ggplot2*.

A subset of the normalised data, including only bacteria and excluding unassigned ASVs, was processed through the linear discriminant analysis (LDA) effect size (LEfSe) algorithm. The LEfSe algorithm was applied through the *run_lefse* function of the *microbiomeMarker* package version 1.3.2 (Cao et al. 2022). The algorithm uses the Kruskal–Wallis rank-sum test to indicate significant differences between class abundances. Then, it uses the Wilcoxon rank-sum test to compare the subclasses of the identified classes to test for statistical differences in abundance ($p \leq 0.05$). LDA was used to estimate the effect size of the identified classes and subclasses (LDA score > 2.5), indicating them as biomarkers most likely to explain the differences between the groups and PMIs. The discovered biomarkers were illustrated in a cladogram using the *plot_cladogram* and *plot_ef_bar* functions as they were the most relevant biomarkers at each PMI (Segata et al. 2011; Lin and Peddada 2020).

All statistical analysis was performed in *R* version 4.3.3 (R Core Team 2023). The Wilcoxon signed-rank (Wilcoxon 1945) (*wilcox. test* function, paired = TRUE) and the Kruskal–Wallis rank-sum tests (Kruskal and Wallis 1952) (*kruskal.test*) were used to compare two and multiple independent groups for statistically significant differences. Significant results in the Kruskal–Wallis test were followed by the post hoc Dunn's test (Dunn 1964) (*DunnTest*; *DescTools*) for pairwise comparisons. Dunnet's test (Dunnett 1955) (*DunnettTest*; *DescTools*) was used to compare the *Beneath* groups at different PMIs to a composite control. Results were adjusted with the Benjamini–Hochberg (BH) adjustment for a false discovery rate (Benjamini and Hochberg 1995) and considered statistically significant at a $p < 0.05$.

3 | Results and Discussion

3.1 | Site Description and Carcass Decomposition

The mean ambient temperatures of the study area were between 5°C and 23°C, with an annual mean of 40 mm of rain during the dry-winter periods (May–September) and between 15°C and 28°C with 510 mm of rain during the wet summer period (November–March) (Figure S3) (SAWS 2021). The vegetation was heterogeneous, consisting of grassland and woodland communities. Common grassland species include *Fimbristylis hispidula*, *Cleome rubella*, *Eragrostis curvula*, *Phyllanthus parvulus*, *Cynodon dactylon* and the encroaching shrub *Seriphium plumosum* and *Kyllinga brevifolia* and *Perotis patens* in the degraded grassland areas (Graham, Barrett, and Brown 2020).

The stages of carcass decomposition were categorised as *fresh*, *bloated*, *active decay*, *advanced decay*, *dry* and *remains* by their maggot activity and appearance (Figure S4) (Payne 1965). Within 1 month, when the first soil samples were collected, some carcasses were in *active decay* while others were in *advanced decay* (Figure S5A,B). Some carcasses were intact but dry at 6 months, whilst others were scattered *remains* (Figure S5C). At 12 and 18 months, very little remained of the carcasses apart from hair and bones (Figure S5D,E). Small to medium-sized vertebrate scavengers intermittently disturbed the carcasses (Figure S5F), but large sections of the carcasses had not been removed. The wildebeest carcasses had entered the *bloat* stage within 24 h, and anaerobic microorganisms would have released enzymes, hydrolysing carbohydrates, proteins and lipids and producing organic and inorganic gasses (Benninger, Carter, and Forbes 2008; Forbes and Carter 2015). Internal pressure from the gas causes the bloating, which leads to the purging of fluids and eventual rupture of the carcasses. The difference in stages of decomposition observed at the first sampling time point may have resulted from variations in the extent of carcass rupture, exposure to the soil microbiota (Lauber et al. 2014), insect, mite and nematode activity (Weiss et al. 2016; Heo et al. 2021) and vertebrate scavagers (Figure S5F).

3.2 | Functional Succession

3.2.1 | Community-Level Physiological Profiles

After 1 month, during *active* to *advanced decay*, the average colour development of the EcoPlates *vis* overall metabolic function of the *Beneath* group was significantly different (Wilcoxon signed-rank: $V = 50$, $p < 0.002$) from the control soil, having a twofold increase in Ecoplate OD measurements (Table 1). The metabolic function remained significantly different throughout

TABLE 1 | Community-level physiological profiles compared after 144 incubation hours.

	Mean overall metabolism and functional diversity (±SEM)			
	Control	**Beneath**	**Control**	**Beneath**
	1 Month		6 Months	
AWCD	0.41 ± 0.05^{A}	1.06 ± 0.02^{B}	0.08 ± 0.02^{A}	0.30 ± 0.08^{B}
D^2	18.8 ± 1.08^{A}	24.6 ± 0.83^{B}	8.8 ± 1.14^{A}	13.7 ± 1.47^{B}
J'	0.91 ± 0.01^{A}	0.96 ± 0.01^{B}	0.75 ± 0.04^{A}	0.83 ± 0.02^{B}
	12 Months		18 Months	
AWCD	0.31 ± 0.05^{A}	0.42 ± 0.06^{B}	0.11 ± 0.02^{A}	0.18 ± 0.02^{B}
D^2	15.5 ± 1.30^{A}	20.2 ± 1.10^{B}	11.0 ± 1.26^{A}	17.8 ± 1.03^{B}
J'	0.87 ± 0.01^{A}	0.92 ± 0.01^{B}	0.80 ± 0.02^{A}	0.90 ± 0.01^{B}

Note: The average well-colour development (AWCD), inverse Simpson's (D^2) and Pielou's evenness (J') index values are indicated with the standard error of the mean (SEM). The letters indicate significant differences (Wilcoxon signed-rank: $p < 0.05$, $n = 10$) in the median of paired observations between the *Control* and *Beneath* groups of the same postmortem interval (the same letter indicates no significant difference).

the experimental period (Table S2). The results revealed that the increase in overall metabolic function is not analogous to the ephemeral nature of carcass decomposition, having not been reduced to control levels after the carcasses were completely decomposed. A similar increase in overall metabolic activities was observed by Cobaugh, Schaeffer, and DeBruyn (2015) beneath human cadavers during the *advanced decay* stage by measuring soil CO_2 production. However, their study ended before the *dry* stage of decomposition. Alpha diversity, measured by the inverse Simpson's diversity (D^2) and Pielou's evenness (J') index values, remained significantly elevated ($p < 0.05$) in the *Beneath* group compared to controls throughout the entire experimental period (Table 1), indicating the enduring impact of carcass decomposition on soil microbiome functional diversity.

Preliminary analysis identified seasonality as a confounding factor affecting beta diversity (functional dissimilarity), with *Control* group time points from different seasons (wet and dry) showing statistically significant dissimilarity ($p < 0.05$). Consequently, functional dissimilarity comparisons were limited to *Control* and *Beneath* samples collected at the same time points. Similar to alpha diversity, functional dissimilarity between the groups remained significantly different ($p < 0.05$) throughout the experimental period. nMDS ordination plots of Bray–Curtis dissimilarities (Figure 1a) illustrate that within 1 month, the *Beneath* group formed a small cluster separate from the *Control* group, which was more dispersed. Analysis of similarity indicated that the groups were significantly dissimilar, having only some similarities in substrate utilisation (ANOSIM: $R = 0.35$, $p < 0.001$). Although the difference remained statistically significant throughout the experimental period, dissimilarities steadily decreased. At 6 months, the groups were classified as different with some similarities (ANOSIM: $R = 0.26$, $p = 0.001$) and at 12 and 18 months, they were similar with some differences ($R < 0.25$). Within 1 month, the group dispersions around their geometric centres (Figure 1b) were significantly different (PERMDISP: $F_{1,18} = 20.88$, $p < 0.001$), and the group geometric centres were different (PERMANOVA: $F_{1,18} = 4.20$, $R^2 = 0.19$; $p < 0.001$). These parameters remained significantly different ($p < 0.05$) between the groups throughout the experimental period.

The functional diversity and dissimilarity results place the effect of large herbivore carcasses past 18 months and support observations (Strickland and Wickings 2015; Singh et al. 2018) that decomposing carcasses have a long-term influence on the soil microbiome processes of their immediate environment. Increased functional diversity advances ecosystem functioning (Carter, Yellowlees, and Tibbett 2007; Parmenter and MacMahon 2009; Strickland and Wickings 2015; Maron et al. 2018; Escalas et al. 2019). Although carcass decomposition has historically been deemed irrelevant because of its rapid decomposition, uneven distribution and minimal nutrient contribution to the total landscape (Owen-Smith 1999). The short-term availability of labile carcass nutrients created *hot moments* of accelerated microbial processes observed in the twofold increase in overall metabolism. The *hot moments* created long-term *hotspots* with greater overall metabolic potential and functional diversity that persisted for over 18 months. Intermittent *hot moments* may have an accumulative effect on the microbial dynamics at an ecosystem scale (Kuzyakov and Blagodatskaya 2015), which is linked to ecosystem functions (Escalas et al. 2019), improved soil fertility and climate regulation (Delgado-Baquerizo et al. 2016).

3.2.2 | Substrate-Guild Utilisation

Within 1 month, amino acid and amine utilisation were significantly increased (Wilcoxon: $V = 0$, $p = 0.002$; $V = 2$, $p = 0.006$) and polymer utilisation was significantly reduced ($V = 49$, $p = 0.027$) (Figure 2a) beneath the carcasses. The SIMPER analysis of substrate-guild utilisation indicates that amino acids, which consist of an amino and carboxylic acid group, and amine contributed 44% to the functional dissimilarity between the *Beneath* and *Control* groups. Polymers contributed 19.7% to dissimilarity (Table 2). Proteases from the bacteria degrade proteins from the carcasses into amino acids. Some amino acids are deaminated to carboxylic acids and ammonia or decarboxylated to amines and carbon dioxide (Forbes and Carter 2015). The biogenic amines, such as putrescine, can be deaminated and oxidised to carboxylic acids, such as γ-aminobutyric acid (Luengo and Olivera 2020). Arginine, putrescine and γ-aminobutyric acid are included as sole-carbon substrates in EcoPlates. The decreased

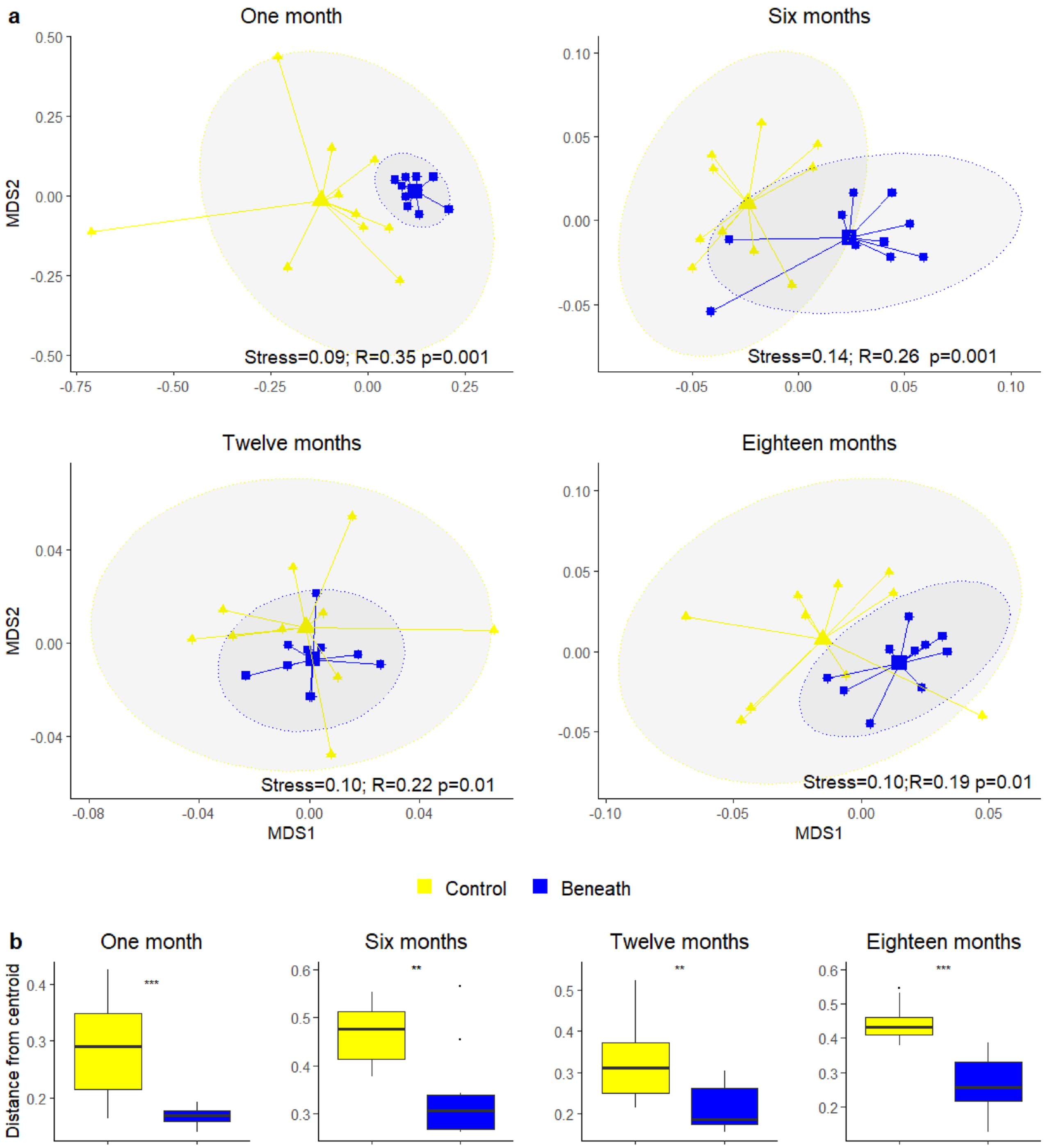

FIGURE 1 | Dissimilarity and dispersion of substrate utilisation for the four postmortem intervals. (a) Non-metric multidimensional scaling (nMDS) indicates stress values, analysis of similarities (R) and statistical significance (p). (b) Box plots illustrating β-dispersion around the group's geometric centre. Significant differences are indicated as; ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$.

polymer utilisation resulted from the increased availability of labile, low molecular weight, high-energy amino acids and amines (Fontaine, Mariotti, and Abbadie 2003), which are preferred as they rapidly incorporate into the citric acid cycle, glycolysis and the pentose phosphate pathways (Koner et al. 2021). Increased free amino acids and peptides in soil and their utilisation by soil microbiota have been reported up to six months postmortem beneath kangaroo carcasses (17–80 kg) (Macdonald et al. 2014). Our results show that amino acid utilisation was not significantly elevated after 6 months (Figure 4b,c).

Carboxylic acid utilisation was significantly increased ($V = 4$, $p = 0.014$) beneath the carcasses at 6 months (Figure 2b), accounting for 23% of the functional dissimilarity (Table 2). It remained significantly increased ($V = 8$, $p = 0.049$) after 12 months (Figure 2c). Polymer utilisation was also notably elevated at 12 months, although not statistically significant. Carboxylic acids with aliphatic chains (fatty acids) are produced by the hydrolysis of lipids and the fermentation of carbohydrates (Forbes and Carter 2015). The mounting pool of carboxylic acids stimulated the soil microbiomes' carboxylic acid utilisation potential

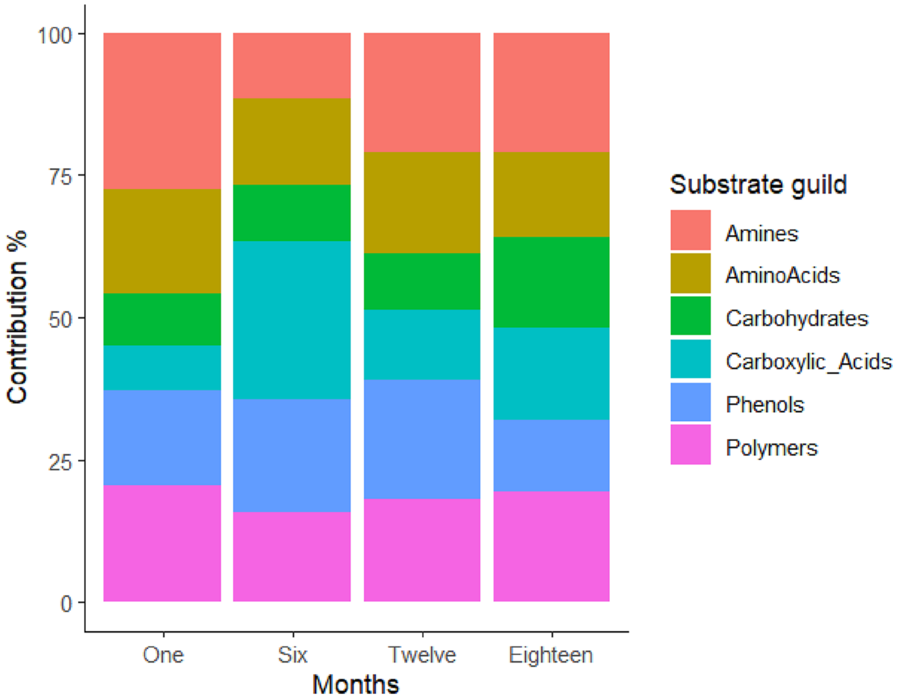

FIGURE 2 | Stacked bar chart of the SIMPER analysis dissimilarity contribution percentages of various substrate guilds, illustrating the relative impact of each substrate guild on the overall community dissimilarity at 1, 6, 12 and 18 months postmortem intervals.

TABLE 2 | Soil nutrient measurements.

	Soil nutrients (ppm ± SEM)			
	Control	**Beneath**	**Control**	**Beneath**
	1 Month		6 Months	
NH_4	$13.8 \pm 1.2^{A(a)}$	$314.3 \pm 43.2^{B(a)}$	$10.9 \pm 0.9^{A(a)}$	$352.8 \pm 65.8^{B(a)}$
NO_3	$1.3 \pm 0.3^{A(a)}$	$1.3 \pm 0.2^{A(a)}$	$2.4 \pm 0.3^{A(a)}$	$80.0 \pm 12.2^{B(b)}$
P	$3.2 \pm 1.4^{A(a)}$	$54.1 \pm 6.6^{A(a)}$	$5.8 \pm 1.9^{A(a)}$	$304.3 \pm 16.5^{B(b)}$
SO_4	$18.4 \pm 1.7^{A(a)}$	$155.3 \pm 35.1^{B(a)}$	$14.1 \pm 1.4^{A(a)}$	$152.2 \pm 49.3^{B(a)}$
	12 Months		18 Months	
NH_4	$11.8 \pm 1.3^{A(a)}$	$71.0 \pm 41.9^{A(a)}$	$14.0 \pm 2.3^{A(a)}$	$49.8 \pm 22.9^{A(a)}$
NO_3	$1.8 \pm 0.6^{A(a)}$	$42.9 \pm 28.1^{B(b)}$	$3.4 \pm 1.3^{A(a)}$	$13.2 \pm 8.9^{A(b)}$
P	$4.1 \pm 2.0^{A(a)}$	$242.6 \pm 44.3^{B(bc)}$	$5.5 \pm 2.4^{A(a)}$	$210.7 \pm 8.6^{B(c)}$
SO_4	$22.5 \pm 4.6^{A(a)}$	$36.7 \pm 22.0^{A(b)}$	$19.9 \pm 3.8^{A(a)}$	$28.4 \pm 13.1^{A(b)}$

Note: Significant differences (Dunn's: $N = 4$, $p < 0.05$) in the median ammonium (NH_4), nitrate (NO_3), available phosphorus (P) and sulphate (SO_4) content between the *Control* and *Beneath* groups of the same postmortem intervals are indicated by different capital letters and between the same group at different postmortem intervals are indicated in brackets (). Mean values in parts per million (ppm) ± standard error of the mean (SEM).

(Grayston et al. 2001), resulting in increased utilisation at 6 and 12 months.

After 18 months, significant increases in the polymer ($V = 11$, $p = 0.106$), carbohydrate ($V = 7$, $p = 0.037$) and amine ($V = 5$, $p = 0.019$) utilisation were observed (Figure 4d). As energy-rich low-molecular-weight substrates (amines and amino acids) are exhausted, high-molecular-weight lower-energy substrates, polymers and carbohydrates are utilised (Fontaine, Mariotti, and Abbadie 2003). Underlying microbial functions within EcoPlate substrate guilds have been observed by Rutgers et al. (2016), and we, similarly, address specific carbon substrates further. Increased utilisation of substrates, α-cyclodextrin and glycogen (Table S1) contributed most to the increase in polymer guild utilisation. These substrates contain glucose subunits, thus being a subcategory of carbohydrates. Carbohydrate and polymer utilisation contributed 37% to the observed dissimilarity (Figure 1). The increased utilisation of glucose-containing substrate corresponds with observations by Singh et al. (2018), although they estimated the increase in glucose utilisation to be between 8 and 12 months postmortem below human corpses.

Underlining functions were also observed within the amine guild. The increased utilisation of amines observed within the first months (Figure 3a) resulted from the significant ($p = 0.027$) increases in phenylethylamine utilisation (Table S1), whereas after 18 months (Figure 3d), it resulted from an increase in putrescine utilisation ($p = 0.049$). Amine utilisation contributed 21% to the observed functional dissimilarity after 18 months (Figure 1).

3.3 | Soil Nutrients

Significant differences in ammonium, sulphate, nitrate and phosphorus content could not be detected between the control samples of different PMIs (Kruskal–Wallis: $p > 0.05$) (Table 2), validating a 5-m radius from the carcasses (Figure S2) as a boundary beyond which control samples could be recovered. As the *Beneath* group PMIs had significant differences, Dunn's test was conducted to compare the *Control* and *Beneath* groups at the same and different PMIs. After a month of decomposition, ammonium increased 20-fold (Dunn's: $z = 2.68$, $p < 0.015$), and sulphate increased 8-fold ($z = 2.26$, $p = 0.042$) beneath the carcasses and remained significantly increased after 6 months (NH_4: $z = 3.51$, $p = 0.006$; SO_4: $z = 3.03$, $p = 0.017$). The increases in ammonium and sulphate may be attributed to the deamination of amino acids to carboxylic acid (Parmenter and MacMahon 2009; Forbes and Carter 2015). Although nitrification of ammonia ordinarily starts 3 days after its augmentation to soil (Webster et al. 2005), it was not evident beneath the carcasses after a month of decomposition despite the abundance of ammonium (Table 2). As Aitkenhead-Peterson et al. (2012) and Keenen et al. (2018) observed, undisturbed carcasses create an anaerobic environment beneath them, possibly preventing nitrification. Keenen et al. (2018) further indicated that the depletion of soil O_2 was accompanied by increased respiration without change to soil moisture, indicating that the stimulated heterotrophs were consuming the oxygen. They further suggested that soil oxygen availability was a chemical parameter to delineate vertebrate decomposition into three distinct biogeochemical phases. The heavy wildebeest carcasses were relatively undisturbed, releasing copious amounts of nutrients into the soil until they were lighter from desiccation and could be moved by the scavengers (Figure S5F). After 6 months, the mean nitrate levels had increased 33-fold beneath the carcasses ($z = 2.22$ $p < 0.036$) (Table 2) and were still elevated by 12 months ($z = 2.43$, $p = 0.026$).

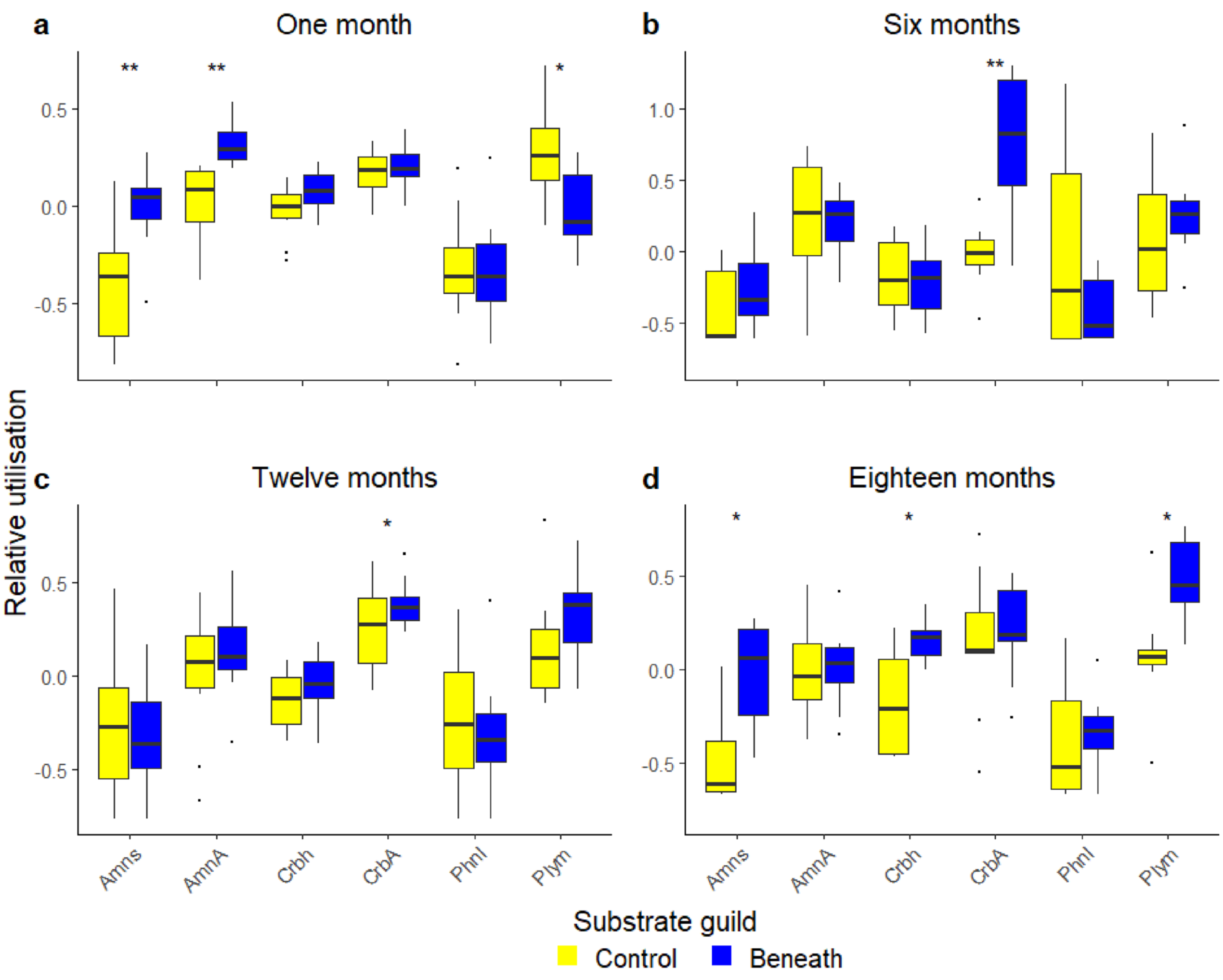

FIGURE 3 | Relative utilisation of the substrate guilds: Amines (Amns), amino acids (AmnA), carbohydrates (Crbh), carboxylic acids (CrbA), phenols (Phnl) and polymers (Plym) at 1, 6, 12 and 18 months after carcass placement. Values were compared at 144 h of incubation. Significant differences (Wilcoxon signed-rank test) between group means are indicated as ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$ ($n = 10$).

At 1 month, phosphorus beneath the carcasses was elevated but not statistically different from the control sample ($z = 1.77$, $p = 0.082$). At 6 months, it had increased 52-fold compared to controls ($z = 2.94$, $p < 0.012$) and remained significantly elevated throughout the experiment (Table 2). Benninger, Carter, and Forbes (2008) observed a rapid increase in phosphorous beneath swine (*S. scrofa*) carcasses, culminating at 15 days and decreasing through a sampling period of 100 days. Phosphorus content is proportionate to body weight and at different ratios to body weight in different species (Parmenter and MacMahon 2009). The amounts of phosphorus contained in wildebeest are more comparable to *Bos bison* (bison) (> 318 kg). Soil from beneath bison reportedly contained 200 ppm phosphorus after 12 months and remained significantly increased for 3 years (> 100 ppm) when measurements ceased (Towne 2000).

A RDA (Figure S6), performed to evaluate the association between soil nutrients and microbial function beneath the carcasses, indicated that ammonium was significantly ($p = 0.016$) linked to the variance in substrate-guild utilisation and accounted for 40% of the variance 1-month postmortem. At 6 months, nitrate and phosphorus significantly ($p = 0.031$) explained 46% of the observed variance, correlating with the *Beneath* group's carboxylic acid, polymer and amino acid utilisation. The soil nutrients were independent of the substrate utilisation at 12 months; however, after 18 months, phosphorus correlated significantly ($p < 0.037$) with polymer, carbohydrate and amino acids utilisation.

3.4 | Structural Succession

3.4.1 | Richness and Diversity

Seasonal climate variations have a weak but significant effect on the diversity of soil bacteria (Zhao, Shu, and Hao 2022). Therefore, the *Beneath* and *Control* samples of the same time point were compared, eliminating seasonal variation as a confounding factor. Significant reductions of the species richness (Observed) and inverse Simpson's diversity index values were measured beneath the carcasses after 1 month (Figure 4). The abundance of labile nutrient-rich proteins and lipids may be causing some taxa to dominate the microbial community. The richness and diversity recovered sigmoidally, and after 12 months, species richness and, at 18 months, diversity was no longer significantly ($p < 0.05$) different from the control soil. Reduced diversity has been observed beneath rodent and swine carcasses in short (< 2 months) and long-term studies (Metcalf et al. 2013; Weiss et al. 2016) and a long-term study (24 months) with human cadavers (Singh et al. 2018). Conversely, Cobaugh, Schaeffer, and DeBruyn (2015) indicated that richness and diversity remained similar to control soil during *active decay* and only increased during *advanced decay* (3–6 months) beneath human cadavers.

The *Control* group had significant differences in species richness between different PMIs (Kruskal–Wallis: $\chi^2 = 11.5$, df = 3, $p < 0.009$) as the 12th and 18th month's richnesses were significantly (Dunn's: $p < 0.05$) reduced compared to the sixth month (Figure 4). Singh et al. (2018) found a significant decrease ($p < 0.05$) in richness at 1 m, but not 5 m, from human cadavers (47–153 kg) and suggested that the effect of decomposition is not limited to the soil directly beneath the decomposing bodies. The reduction in the richness that we observed in the 5-m *Control* soil from substantially larger carcasses (209–247 kg) was not a result of nutrients (ammonia, nitrate, sulphate and phosphorus) seeping from the carcass to that radius, as indicated by the soil nutrient results (Table 2). The reduced richness may have resulted from the bioturbation of the surrounding soil (West and Whitman 2022) by animals that frequented the carcasses. However, species diversity was not significantly different in the *Control* soil.

3.4.2 | Compositional Dissimilarities

The *Control* group maintained compositional homogeneity and stability over 18 months with ordination centroids (PERMANOVA: $F_{3,11} = 0.62$, $R^2 = 0.14$; $p = 0.993$) and dispersion (PERMDISP: $F_{3,11} = 0.73$, $p = 0.572$) not being statistically different over time (Figure 5). The *Beneath* group formed distinct clusters with different centroids (PERMANOVA: $F_{3,12} = 8.12$, $R^2 = 0.67$, $p = 0.001$) and dispersions (PERMDISP: $F_{3,12} = 3.50$, $p = 0.039$) at the different PMIs. A pairwise comparison of *Beneath* group centroids revealed that the 1-month PMI differed significantly from the 6-month interval and that the 1- and 6-month intervals differed from 12- and 18-month intervals, suggesting that the microbiomes changed successively. The 12- and 18-month intervals did not differ (pairwise PERMANOVA: $F_{1,6} = 1.04$, $R^2 = 0.15$, adjusted $p = 0.374$); thus, the succession rate had been reduced. A pairwise comparison of dispersion revealed that the sample replicates at 1 and 6 months were less dispersed (adjusted $p < 0.05$) than the 12 and 18 months, which overlapped substantially (Figure 5), indicative of the subsample replicate differences increasing.

Significant dissimilarities (PERMANOVA: $p < 0.05$) between the *Beneath* and *Control* groups were observed (Figure S8) at the 1-, 6- and 12-month PMIs. After 18 months, the groups were no longer different (PERMANOVA: $F_{1,5} = 1.78$, $R^2 = 0.26$, $p = 0.143$) as the *Beneath* group returned to a structure similar to the *Control* group. Cobaugh, Schaeffer, and DeBruyn (2015) suggested a possible return to the original community structure of soil beneath human cadavers (50–77 kg), having monitored dissimilarities to a control group over 7 months; however, it was not observed in the experimental period. Our results suggest that soil beneath large herbivore carcasses (209–247 kg) had returned to a community structure similar to the *Control* soil after 18 months, as the null hypothesis of no significant difference in dissimilarity could not be rejected after 18 months.

3.4.3 | Bacterial Biomarkers

Over 350 taxa were significantly (Kruskal–Wallis and Wilcoxon: $p < 0.05$) enriched throughout the experimental period (Table S3), many of which have been observed in the existing literature. Only the ecologically relevant bacterial biomarkers (LEfSe: $p < 0.05$, LDA $\log_{10} > 2.5$) of various taxonomic levels, identified by comparing pooled *Control* to the *Beneath* groups at different PMIs, are illustrated in Figure 6 and discussed.

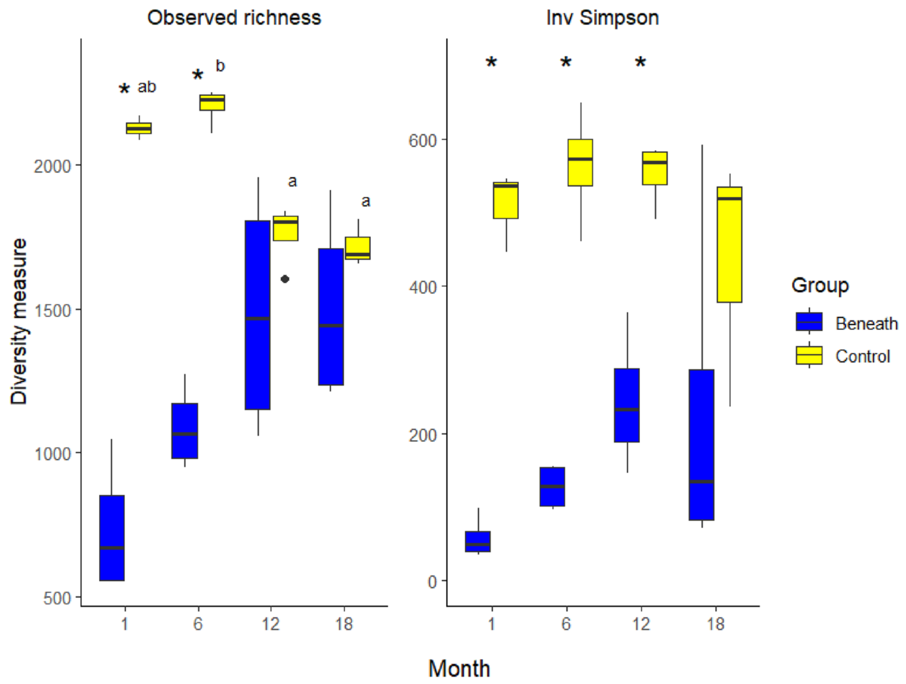

FIGURE 4 | Box plots illustrating the Observed richness and Inverse Simpson's diversity index values after 1, 6, 12 and 18 months. An asterisk (*) indicates a statistical difference between a Beneath and *Control* group of the same time point (Wilcoxon: $N = 4$, $p < 0.05$). *Control* group postmortem intervals with the same letter are not significantly different (Dunn's test: $N = 4$, $p < 0.05$).

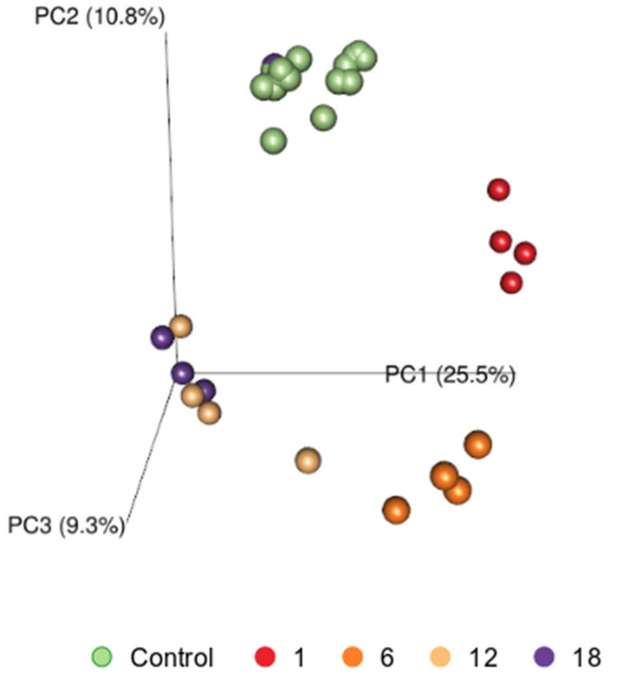

FIGURE 5 | Bray–Curtis dissimilarities-based principal coordinate analysis (PCoA) of the *Beneath* and *Control* groups at all time points (1, 6, 12 and 18 months).

Biomarkers identified by comparing only the *Control* and *Beneath* groups of the same PMIs are shown in Figures S9–S13.

Compared to the control group, Verrucomicrobia, Acidobacteriota and Chloroflexota exhibited a diminished presence beneath the carcasses. Verrucomicrobia, particularly Chthoniobacterales and Candidatus Xiphinematobacter, are oligotrophic K-strategists common in grassland soil microbiomes (Bergmann et al. 2011). This contrasts with Risch et al. (2020), who found positive correlations between Verrucomicrobia genera and soil beneath bison and elk. The reduction in Acidobacteria is likely due to pH elevation from ammonium produced during protein and lipid catabolism (Metcalf et al. 2013; van Elsas 2019). Chloroflexi, including phototrophic subgroups, may have diminished due to reduced solar radiation beneath the carcasses.

After 1 month, Bacillota (Firmicutes) were highly enriched beneath the carcasses, particularly Savagea and Sporosarcina (Planococcaceae), associated with faeces and urine degradation (Dworkin 2006; Patil et al. 2021), and Pseudogracilibacillus, linked to composting (Ma et al. 2022). Also enriched were *Tissierella* (Clostridiales), often found in gravesoil (Metcalf et al. 2013; Cobaugh, Schaeffer, and DeBruyn 2015; Lauber et al. 2014; Mason, Taylor, and DeBruyn 2023), and Erysipelotrichaceae (*Erysipelothrix*), known for lipid catabolism and containing ungulate pathogens (Mavrot et al. 2020). While Firmicutes increase during the bloat phase (Metcalf et al. 2013; Cobaugh, Schaeffer, and DeBruyn 2015), they decrease after rupture due to oxygen exposure. However, spore-forming anaerobes like Clostridiales remained enriched for 12 months and can dominate late-stage decomposition in graves (Kim et al. 2017).

Burkholderiaceae (genus *Paenalcaligenes*) and Wohlfahrtiimonadaceae (genus *Ignatzschineria*) from Class Gammaproteobacteria were also highly enriched beneath the carcasses. Gammaproteobacteria's enrichment has been documented in both short-term (Metcalf et al. 2013; Lauber et al. 2014; Cobaugh, Schaeffer, and DeBruyn 2015) and a long-term study by Singh et al. (2018) indicated a correlation in its abundance with carcass mass. Gammaproteobacteria are prevalent on mammal carcass skin and in the necrophagous dipteran

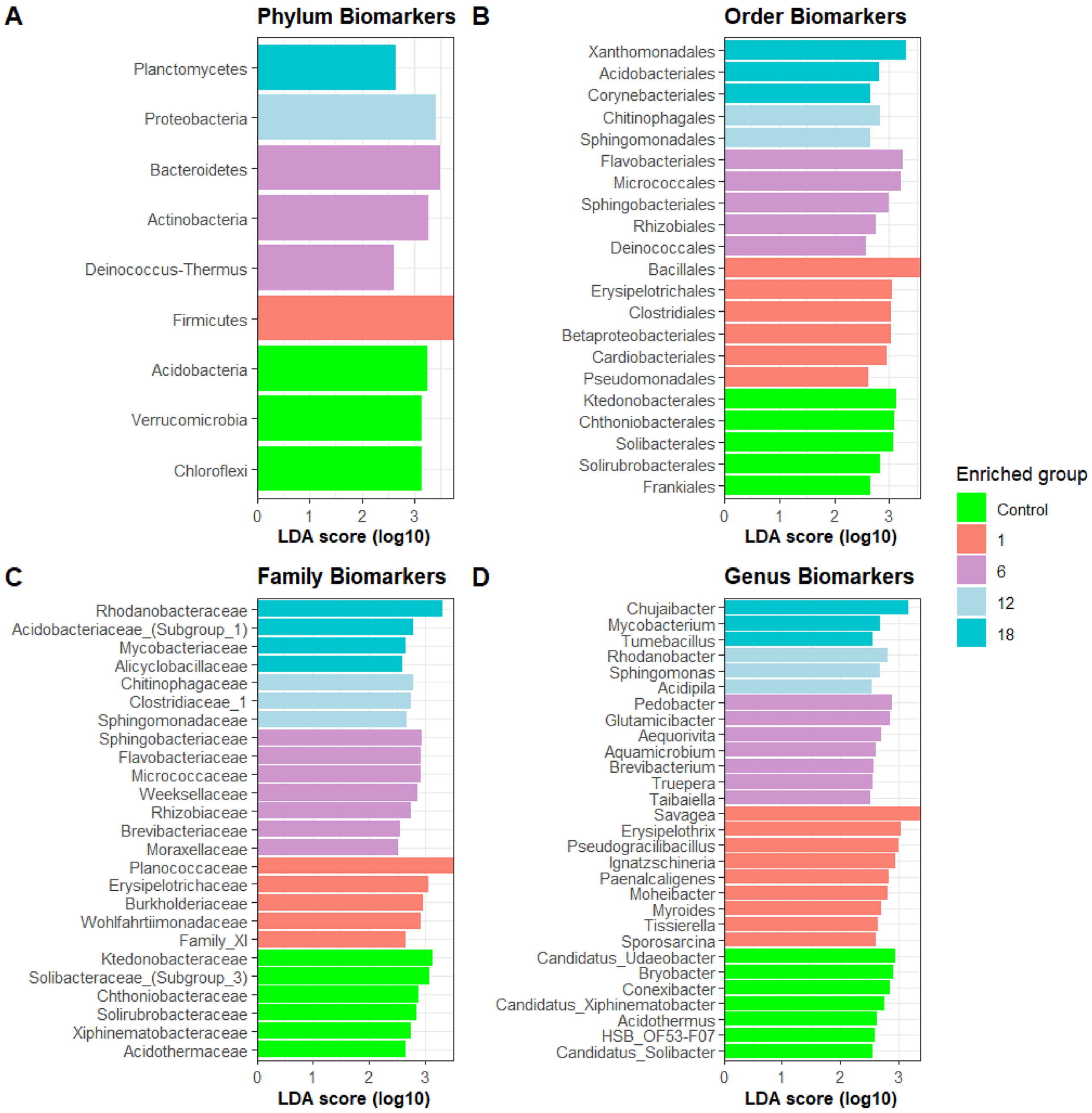

FIGURE 6 | Ecologically relevant bacterial biomarkers identified through LEfSe analysis comparing control soil to soil beneath *Connochaetes taurinus* (wildebeest) carcasses ($n = 4$) at 1, 6, 12 and 18 months postmortem. LDA plots indicate biomarkers at the phylum, order, family, and genus levels with LDA scores > 2.5 (Kruskal–Wallis and Wilcoxon rank-sum tests, $p < 0.05$).

larval microbiome (Pechal et al. 2014; Weatherbee, Pechal, and Eric Benbow 2017). Burkholderiaceae have been isolated from the gut of soldier fly larvae (Lee et al. 2013), and frequent carcasses during late *advanced decay* (Cobaugh, Schaeffer, and DeBruyn 2015) and are present in the study area (Picker, Griffiths, and Weaving 2012). Available phosphorus that accumulated in the soil after six months (Table 2) provided a competitive advantage to Burkholderiaceae, which can solubilise phosphate (Stephen et al. 2015).

Despite the ungulate rumen housing numerous non-spore-forming Bacteroidota (Bacteroidetes) (McCann, Wickersham, and Loor 2014) that increase during the bloat phase (Metcalf et al. 2013; Cobaugh, Schaeffer, and DeBruyn 2015), only the genera *Moheibacter* and *Myroides*, from families Weeksellaceae and Flavobacteriaceae, were highly enriched beneath the carcasses after a month. However, after 6 months, Bacteroidetes and the families Weeksellaceae and Flavobacteriaceae (genus *Aequorivita*), as well as Sphingobacteriaceae (*Pedobacter*), dominated the carcass soil microbiome (Figure 6). They are known to degrade complex molecules such as polysaccharides as well as proteins (Gavriilidou et al. 2020; Figueiredo et al. 2022), and the enriched genus *Aequorivita* produces esterases that break down fats and oils (Zhang et al. 2022). Cobaugh, Schaeffer, and DeBruyn (2015) also observed an increase in bacteroidetes after 6 months when their study ended. After 6 months, the phylum Actinomycetota (Actinobacteria) and its order Micrococcales, associated with mammalian skin and blood (Dastager et al. 2014), were highly enriched beneath the carcasses. Particularly, the genera *Glutamicibacter* and *Brevibacterium*, which are linked to carboxylic acid metabolism (Pham et al. 2017; Yastrebova, Malysheva, and Plotnikova 2022), showed significant enrichment. Their enrichment coincided with increased carboxylic acid utilisation observed from the EcoPlate results (Figure 3b). Conversely, the Actinobacteria orders Frankiales and Solirubrobacteriales were significantly reduced beneath the carcasses throughout the experiment (Figure 6B). Frankiales are known for their symbiotic nitrogen-fixing relationship with plants (Sen et al. 2014), notably absent beneath the carcasses and grids, likely explaining Frankiales' and Solirubrobacteriales' diminished presence.

The thermophilic phylum Deinococcus-Thermus (genus *Truepera*) was significantly enriched beneath the carcasses after 6 months during winter. *Truepera*, an extremophile with an optimal growth temperature of 50°C, can reduce nitrate under high-temperature conditions (Shi et al. 2022) and assimilate various sugars, carboxylic acids and amino acids (Albuquerque et al. 2005). Its enrichment coincided with a 33-fold increase in nitrate levels (Table 2) and increased utilisation of carboxylic acids (Figure 3b). The combination of elevated temperatures beneath the carcass remains and grids under the winter sun, along with high nitrate levels and carboxylic acids, likely resulted in the enrichment of Truepera. In addition, the Proteobacteria family Rhizobiaceae (genus *Aquamicrobium*), which can reduce nitrate to nitrite or nitrogen gas under oxic and anoxic conditions while utilising carboxylic acids as carbon sources (Bambauer et al. 1998), was also enriched. Moraxellaceae, another Proteobacteria family that includes species such as *Moraxella bovis*, which causes bovine keratoconjunctivitis (Loy and Brodersen 2014), was also enriched beneath the carcasses.

After 12 months, Proteobacteria emerged as the dominant phylum, with notable enrichment of the genus *Rhodanobacter* from the order Xanthomonadales, as well as the order Sphingomonadales and its genus *Sphingomonas* (Figure 6). However, after 12 months, the Bacteroidetes family, Chitinophagaceae, emerged as the dominant family-level biomarker. During *advanced decay*, the soil beneath the carcasses became littered with fly-pupal casings, primarily composed of chitin. Chitinophagaceae includes numerous genera that metabolise chitin (Beier and Bertilsson 2013); thus, its enrichment is well-justified.

After 18 months, when only scattered bones, dried skin and hair remained, Planctomycetota (hereafter Planctomycetes) was the dominant phylum-level biomarker. Although, order Xanthomonadales, a Proteobacteria, its family Rhodanobacteraceae and the genus *Chujaibacter* were the most enriched subordinate carcass soil biomarkers. These results substantiate observations by Cobaugh, Schaeffer, and DeBruyn (2015) that Xanthomonadales and Sphingomonadales are suitable long-term estimators of PMIs, although the specific genera we identified were different. In addition, the genus *Mycobacterium*, an Actinobacteria with pathogenic members such as *M. bovis* (Hines, Kreeger, and Herron 1995; Kuria 2019), whose environmental longevity has been questioned (Conteddu et al. 2024), as well as the Firmicutes genus *Tumebacillus*, a species that have been isolated from the gut of vultures (Sung et al. 2018), were identified as highly enriched after 18 months.

4 | Conclusions

The results from this study contribute to our understanding of the enduring effect that carcass decomposition has on the immediate soil microbial environment and may have utility in postmortem microbial forensics. Functional changes to the soil microbiome beneath the carcasses occurred successively well beyond the visible carcass decomposition. The overall soil microbial function and functional diversity increased during *active decay* and remained elevated compared to the control soil for the entire experimental period. The microbiome also remained functionally dissimilar from the control soil, although the dissimilarity was reduced over time. The sole-carbon substrate utilisation assay indicated that metabolism shifted from increased amino acid and amine utilisation during the first month postmortem to carboxylic acid utilisation between 6 and 12 months and polymer, carbohydrate and amine utilisation after 18 months. Variations of soil nutrients (ammonium, nitrates and phosphorus) significantly explained the variation in carbon-substrate utilisation.

The soil microbiomes beneath the carcasses underwent successive structural changes exceeding 18 months. Species richness and diversity decreased during *active decay*. Richness recovered within 12 months and diversity by 18 months. The microbiome structure beneath the carcasses was significantly dissimilar from the control group for up to 12 months and was successively dissimilar between PMIs for up to 6 months. Firmicutes families, especially those associated with necrophagous dipteral larvae, dominated the soil microbiomes beneath carcasses during *active decay*. Bacteroidetes and Proteobacteria families were enriched during *advanced decay*, and Planctomycetes remained enriched post-carcass decay. Some enriched genera included ungulate pathogens, which remained enriched in the soil post-carcass decomposition and may be specific to the animal species utilised in the study.

The soil microbiomes exposed to carcass decomposition exhibited significant functional and structural differences even beyond the experimental period, with biomarkers of carcass decomposition still detectable. These results underscore the substantial impact that the decomposition of large carcasses can have at an ecosystem scale. Given that the association between function and structure in these microbiomes is only loosely related, further research is necessary to fully understand its ecological implications.

Author Contributions

Jacques Fouché: conceptualization, methodology, formal analysis, project administration, visualization, writing – original draft, data curation, investigation. **Pedro H. Lebre:** writing – review and editing, validation, supervision. **Haemish A. Melville:** conceptualization, writing – review and editing, funding acquisition. **Don A. Cowan:** writing – review and editing, funding acquisition, supervision, validation.

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Ethics Statement

This manuscript does not report data collected from humans or animals. It only involves animals sacrificed as a byproduct of other research, and the University of Pretoria deemed this not to be classified as animal research. The animals were humanely euthanised under an approved protocol of the University of South Africa.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are openly available in Harvard Dataverse Network at https://doi.org/10.7910/DVN/OJ3IK8.

References

Aitkenhead-Peterson, J. A., C. G. Owings, M. B. Alexander, N. Larison, and J. A. Bytheway. 2012. "Mapping the Lateral Extent of Human Cadaver Decomposition With Soil Chemistry." *Forensic Science International* 216: 127–134. https://doi.org/10.1016/j.forsciint.2011.09.007.

Albuquerque, L., C. Simões, M. F. Nobre, et al. 2005. "*Truepera radiovictrix* gen. nov., sp. nov., a New Radiation Resistant Species and the Proposal of *Trueperaceae* fam. nov." *FEMS Microbiology Letters* 247: 161–169. https://doi.org/10.1016/j.femsle.2005.05.002.

Allison, S. D., and J. B. Martiny. 2008. "Resistance, Resilience, and Redundancy in Microbial Communities." *Proceedings of the National Academy of Sciences of the United States of America* 105: 11512–11519. https://doi.org/10.1073/pnas.0801925105.

Amann, R. I., W. Ludwig, and K. H. Schleifer. 1995. "Phylogenetic Identification and In Situ Detection of Individual Microbial Cells Without Cultivation." *Microbiological Reviews* 59: 143–169. https://doi.org/10.1128/mr.59.1.143-169.1995.

Anderson, M. J., 2001. "A New Method for Non-Parametric Multivariate Analysis of Variance." *Austral Ecology* 26: 32–46. https://doi.org/10.1111/j.1442-9993.2001.01070.pp.x.

Anderson, M. J., K. E. Ellingsen, and B. H. McArdle. 2006. "Multivariate Dispersion as a Measure of Beta Diversity." *Ecology Letters* 9: 683–693. https://doi.org/10.1111/j.1461-0248.2006.00926.x.

Bambauer, A., F. A. Rainey, E. Stackebrandt, and J. Winter. 1998. "Characterization of *Aquamicrobium defluvii* gen. nov. sp. nov., a Thiophene-2-Carboxylate-Metabolizing Bacterium from Activated Sludge." *Archives of Microbiology* 169: 293–302. https://doi.org/10.1007/s002030050575.

Barton, L. L., and D. E. Northup. 2011. *Microbial ecology*. Hoboken, NJ: John Wiley and Sons.

Barton, P. S., S. A. Cunningham, D. B. Lindenmayer, and A. D. Manning. 2013. "The Role of Carrion in Maintaining Biodiversity and Ecological Processes in Terrestrial Ecosystems." *Oecologia* 171: 761–772. https://doi.org/10.1007/s00442-012-2460-3.

Beier, S., and S. Bertilsson. 2013. "Bacterial Chitin Degradation—Mechanisms and Ecophysiological Strategies." *Frontiers in Microbiology* 4: 149. https://doi.org/10.3389/fmicb.2013.00149.

Benjamini, Y., and Y. Hochberg. 1995. "Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing." *Journal of the Royal Statistical Society: Series B* 57: 289–300. https://doi.org/10.1111/j.2517-6161.1995.tb02031.x.

Benninger, L. A., D. O. Carter, and S. L. Forbes. 2008. "The Biochemical Alteration of Soil Beneath a Decomposing Carcass." *Forensic Science International* 180: 70–75. https://doi.org/10.1016/j.forsciint.2008.07.001.

Bergmann, G. T., S. T. Bates, K. G. Eilers, et al. 2011. "The Under-Recognised Dominance of Verrucomicrobia in Soil Bacterial Communities." *Soil Biology and Biochemistry* 43: 1450–1455.

Blanchet, F. G., P. Legendre, and D. Borcard. 2008. "Forward Selection of Explanatory Variables." *Ecology* 89: 2623–2632. https://doi.org/10.1890/07-0986.1.

Bray, J. R., and J. T. Curtis. 1957. "An Ordination of the Upland Forest Communities of Southern Wisconsin." *Ecological Monographs* 27: 325–349.

Bray, R. H., and L. T. Kurtz. 1945. "Determination of Total, Organic and Available Forms of Phosphorus in Soils." *Soil Science* 59: 39–46.

Callahan, B. J., P. J. McMurdie, M. J. Rosen, A. W. Han, A. J. A. Johnson, and S. P. Holmes. 2016. "DADA2: High-Resolution Sample Inference From Illumina Amplicon Data." *Nature Methods* 13: 581–583. https://doi.org/10.1038/nmeth.3869.

Cao, Y., Q. Dong, D. Wang, P. Zhang, Y. Liu, and C. Niu. 2022. "microbiomeMarker: An R/Bioconductor Package for Microbiome Marker Identification and Visualization." *Bioinformatics* 38: 4027–4029. https://doi.org/10.1093/bioinformatics/btac438.

Carter, D. O., D. Yellowlees, and M. Tibbett. 2007. "Cadaver Decomposition in Terrestrial Ecosystems." *Naturwissenschaften* 94: 12–24. https://doi.org/10.1007/s00114-006-0159-1.

Clarke, K. R. 1993. "Non-Parametric Multivariate Analyses of Changes in Community Structure." *Austral Ecology* 18: 117–143.

Cobaugh, K. L., S. M. Schaeffer, and J. M. DeBruyn. 2015. "Functional and Structural Succession of Soil Microbial Communities Below Decomposing Human Cadavers." *PLoS One* 10: e0130201. https://doi.org/10.1371/journal.pone.0130201.

Conteddu, K., H. M. English, A. W. Byrne, et al. 2024. "A Scoping Review on Bovine Tuberculosis Highlights the Need for Novel Data Streams and Analytical Approaches to Curb Zoonotic Diseases." *Veterinary Research* 55: 64. https://doi.org/10.1186/s13567-024-01314-w.

Cotrufo, M. F., M. D. Wallenstein, C. M. Boot, K. Denef, and E. Paul. 2013. "The Microbial Efficiency-Matrix Stabilisation (MEMS) Framework Integrates Plant Litter Decomposition With Soil Organic Matter Stabilization: Do Labile Plant Inputs Form Stable Soil Organic Matter?" *Global Change Biology* 19: 988–995. https://doi.org/10.1111/gcb.12113.

Crippen, T. L., M. E. Benbow, and J. L. Pechal. 2016. "Microbial Interactions During Carrion Decomposition." In *Carrion Ecology, Evolution, and Their Applications*, edited by M. E. Benbow, J. K. Tomberlin, and A. M. Tarone, 31–64. Boca Raton: CRC Press.

Dalemberg, J. W., and G. Jager. 1981. "Priming Effect of Small Glucose Additions to ^{14}C-Labeled Soil." *Soil Biology and Biochemistry* 13: 219–223. https://doi.org/10.1016/0038-0717(81)90024-9.

Dastager, S. G., S. Krishnamurthi, N. Rameshkumar, and M. Dharne. 2014. "The Family Micrococcaceae." In *The Prokaryotes*, edited by E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt, and F. Thompson, 455–498. Berlin, Heidelberg: Springer. https://doi.org/10.1007/978-3-642-30138-4_168.

Delgado-Baquerizo, M., F. T. Maestre, P. B. Reich, et al. 2016. "Microbial Diversity Drives Multifunctionality in Terrestrial Ecosystems." *Nature Communications* 7: 10541. https://doi.org/10.1038/ncomms10541.

Dunn, O. J. 1964. "Multiple Comparisons Using Rank Sums." *Technometrics* 6: 241–252.

Dunnett, C. W. 1955. "A Multiple Comparison Procedure for Comparing Several Treatments With a Control." *Journal of the American Statistical Association* 50: 1096–1121.

Dworkin, M. 2006. *The Prokaryotes: Vol. 4: Bacteria: Firmicutes, Cyanobacteria*. New York, NY: Springer.

Escalas, L. H., J. W. Voordeckers, Y. Yang, M. K. Firestone, L. Alvarez-Cohen, and J. Zhou. 2019. "Microbial Functional Diversity: From Concepts to Applications." *Ecology and Evolution* 9: 12000–12016. https://doi.org/10.1002/ece3.5670.

Figueiredo, G., M. Gomes, C. Covas, S. Mendo, and T. Caetano. 2022. "The Unexplored Wealth of Microbial Secondary Metabolites: The Sphingobacteriaceae Case Study." *Microbial Ecology* 83: 470–481.

Fontaine, S., A. Mariotti, and L. Abbadie. 2003. "The Priming Effect of Organic Matter: A Question of Microbial Competition?" *Soil Biology and Biochemistry* 35: 837–843. https://doi.org/10.1016/S0038-0717(03)00123-8.

Forbes, S. L., and D. O. Carter. 2015. "Processes and Mechanisms of Death and Decomposition of Vertebrate Carrion." In *Carrion Ecology, Evolution, and Their Applications*, edited by M. E. Benbow, J. K. Tomberlin, and A. M. Tarone, 13–30. Boca Raton: CRC Press.

Franzosa, E. A., L. J. McIver, G. Rahnavard, et al. 2018. "Species-Level Functional Profiling of Metagenomes and Metatranscriptomes." *Nature Methods* 15: 962–968. https://doi.org/10.1038/s41592-018-0176-y.

Garland, J. L. 1997. "Analysis and Interpretation of Community-Level Physiological Profiles in Microbial Ecology." *FEMS Microbiology Ecology* 24: 289–300. https://doi.org/10.1111/j.1574-6941.1997.tb00446.x.

Gavriilidou, A., J. Gutleben, D. Versluis, et al. 2020. "Comparative Genomic Analysis of *Flavobacteriaceae*: Insights Into Carbohydrate Metabolism, Gliding Motility and Secondary Metabolite Biosynthesis." *BioMed Central Genomics* 21: 569. https://doi.org/10.1186/s12864-020-06971-7.

Gomez, E., and J. L. Garland. 2012. "Effects of Tillage and Fertilisation on Physiological Profiles of Soil Microbial Communities." *Applied Soil Ecology* 61: 327–332. https://doi.org/10.1016/j.apsoil.2011.10.008.

Gower, J. C. 1966. "Some Distance Properties of Latent Root and Vector Methods Used in Multivariate Analysis." *Biometrika* 53: 325–338. https://doi.org/10.1093/biomet/53.3-4.325.

Graham, S. C., A. S. Barrett, and L. R. Brown. 2020. "Impact of *Seriphium plumosum* Densification on Mesic Highveld Grassland Biodiversity in South Africa." *Royal Society Open Science* 7: 192025. https://doi.org/10.1098/rsos.192025.

Grayston, S. J., G. S. Griffith, J. L. Mawdsley, C. D. Campbell, and R. D. Bardgett. 2001. "Accounting for Variability in Soil Microbial Communities of Temperate Upland Grassland Ecosystems." *Soil Biology and Biochemistry* 33: 533–551. https://doi.org/10.1016/S0038-0717(00)00194-2.

Habig, J., and C. Swanepoel. 2015. "Effects of Conservation Agriculture and Fertilisation on Soil Microbial Diversity and Activity." *Environments* 2: 358–384. https://doi.org/10.3390/environments2030358.

Heo, C. C., T. L. Crippen, S. N. Thornton, and J. K. Tomberlin. 2021. "Differential Carbon Utilisation by Bacteria in the Soil Surrounding and on Swine Carcasses With Dipteran Access Delayed." *Pure and Applied Geophysics* 178: 717–734. https://doi.org/10.1007/s00024-020-02608-8.

Hines, M. E., J. M. Kreeger, and A. J. Herron. 1995. "Special Topic Overview Mycobacterial Infections of Animals: Pathology and Pathogenesis." *Laboratory Animal Science* 45: 334–351.

Isbell, F., V. Calcagno, A. Hector, et al. 2011. "High Plant Diversity Is Needed to Maintain Ecosystem Services." *Nature* 477: 199–202.

ISO. 1995. *ISO 11048:1995. Soil Quality—Determination of Water-Soluble and Acid-Soluble Sulfate*. International Organization for Standardization. Accessed October 7, 2024. https://www.iso.org/standard/19029.html.

ISO. 2003. ISO/TS 14256-1:2003. Soil Quality—Determination of Nitrate, Nitrite and Ammonium in Field-Moist Soils by Extraction With Potassium Chloride Solution—Part 1: Manual Method. International Organization for Standardization. https://www.iso.org/standard/36706.html.

Jones, S. C., E. D. Strauss, and K. E. Holekamp. 2015. "Ecology of African Carrion." In *Carrion Ecology, Evolution, and Their Applications*, edited by M. E. Benbow, J. K. Tomberlin, and A. M. Tarone, 461–491. Boca Raton: CRC Press.

KAPA Biosystems. 2024. KAPA HiFi PCR Kit. https://www.kapabiosystems.com/product-applications/products/pcr/qpcr/kapa-hifi-qpcr-kits.

Keenen, S. W., S. M. Schaeffer, V. L. Jin, and J. M. DeBruyn. 2018. "Mortality Hotspots: Nitrogen Cycling in Forest Soils During Vertebrate Decomposition." *Soil Biology and Biochemistry* 212: 165–176.

Kim, H. Y., J. Seo, T. H. Kim, B. Shim, S. M. Cha, and S. Yu. 2017. "Pyrosequencing-Based Assessment of Microbial Community Shifts in Leachate From Animal Carcass Burial Lysimeter." *Science of the Total Environment* 587: 232–239.

Koner, S., J. S. Chen, B. M. Hsu, et al. 2021. "Assessment of Carbon Substrate Catabolism Pattern and Functional Metabolic Pathway for Microbiota of Limestone Caves." *Microorganisms* 9: 1789. https://doi.org/10.3390/microorganisms9081789.

Kruskal, J. B. 1964. "Non-Metric Multidimensional Scaling: A Numerical Method." *Psychometrika* 29: 115–129.

Kruskal, W. H., and W. A. Wallis. 1952. "Use of Ranks in One-Criterion Variance Analysis." *Journal of the American Statistical Association* 47: 583–621.

Kuria, J. K. N. 2019. "Diseases Caused by Bacteria in Cattle: Tuberculosis." In *Bacterial Cattle Diseases*, edited by H. A. E. Kaoud. London, UK: IntechOpen. https://doi.org/10.5772/intechopen.82051.

Kuzyakov, Y., and E. Blagodatskaya. 2015. "Microbial Hotspots and Hot Moments in Soil: Concept and Review." *Soil Biology and Biochemistry* 83: 184–199.

Lauber, C. L., J. L. Metcalf, K. Keepers, G. Ackermann, D. O. Carter, and R. Knight. 2014. "Vertebrate Decomposition Is Accelerated by Soil Microbes." *Applied and Environmental Microbiology* 80: 4920–4929. https://doi.org/10.1128/AEM.00957-14.

Lee, Y. Y., J. K. Lee, K. H. Park, et al. 2013. "*Paenalcaligenes hermetiae* sp. nov., Isolated From the Larval Gut of *Hermetia illucens* (Diptera: Stratiomyidae), and Emended Description of the Genus *Paenalcaligenes*." *International Journal of Systematic and Evolutionary Microbiology* 63: 4224–4229.

Legendre, P., and L. Legendre. 2012. *Numerical Ecology*. 3rd ed. Amsterdam: Elsevier.

Lin, H., and S. D. Peddada. 2020. "Analysis of Compositions of Microbiomes With Bias Correction." *Nature Communications* 11: 3514. https://doi.org/10.1038/s41467-020-17041-7.

Loy, J. D., and B. W. Brodersen. 2014. "*Moraxella* spp. Isolated From Field Outbreaks of Infectious Bovine Keratoconjunctivitis: A Retrospective Study of Case Submissions From 2010 to 2013." *Journal of Veterinary Diagnostic Investigation* 26: 761–768. https://doi.org/10.1177/1040638714551403.

Luengo, J. M., and E. R. Olivera. 2020. "Catabolism of Biogenic Amines in *Pseudomonas* Species." *Environmental Microbiology* 22: 1174–1192. https://doi.org/10.1111/1462-2920.14912.

Ma, Q., Y. Li, J. Xue, D. Cheng, and Z. Li. 2022. "Effects of Turning Frequency on Ammonia Emission During the Composting of Chicken Manure and Soybean Straw." *Molecules* 27: 472.

Macdonald, B. C., M. Farrell, S. Tuomi, P. S. Barton, S. A. Cunningham, and A. D. Manning. 2014. "Carrion Decomposition Causes Large and Lasting Effects on Soil Amino Acid and Peptide Flux." *Soil Biology and Biochemistry* 69: 132–140. https://doi.org/10.1016/j.soilbio.2013.10.042.

Maron, P. A., A. Sarr, A. Kaisermann, et al. 2018. "High Microbial Diversity Promotes Soil Ecosystem Functioning." *Applied and Environmental Microbiology* 84: e02738-17.

Mason, A. R., H. S. McKee-Zech, K. M. Hoeland, et al. 2022. "Body Mass Index (BMI) Impacts Soil Chemical and Microbial Response to Human Decomposition." *mSphere* 7: e00325-22. https://doi.org/10.1128/msphere.00325-22.

Mason, A. R., L. S. Taylor, and J. M. DeBruyn. 2023. "Microbial Ecology of Vertebrate Decomposition in Terrestrial Ecosystems." *FEMS Microbiology Ecology* 99: fiad006. https://doi.org/10.1093/femsec/fiad006.

Mavrot, F., K. Orsel, W. Hutchins, et al. 2020. "Novel Insights Into Serodiagnosis and Epidemiology of *Erysipelothrix rhusiopathiae*, a Newly Recognised Pathogen in Muskoxen (*Ovibos moschatus*)." *PLoS One* 15: e0231724.

McCann, J. C., T. A. Wickersham, and J. J. Loor. 2014. "High-Throughput Methods Redefine the Rumen Microbiome and Its Relationship With Nutrition and Metabolism." *Bioinformatics and Biology Insights* 8: 109–125.

McMurdie, P. J., and S. Holmes. 2013. "Phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data." *PLoS One* 8: e61217.

Metcalf, J. L., L. Wegener Parfrey, A. Gonzalez, et al. 2013. "A Microbial Clock Provides an Accurate Estimate of the Postmortem Interval in a Mouse Model System." *eLife* 2: e01104.

Metcalf, J. L., Z. Z. Xu, S. Weiss, et al. 2016. "Microbial Community Assembly and Metabolic Function During Mammalian Corpse Decomposition." *Science* 351: 158–162. https://doi.org/10.1126/science.aad2646.

Millard, P., and B. K. Singh. 2010. "Does Grassland Vegetation Drive Soil Microbial Diversity?" *Nutrient Cycling in Agroecosystems* 88: 147–158.

Montanarella, L., D. Pennock, N. McKenzie, et al. 2015. The Status of the World's Soil Resources Main Report.

Mucina, L., and M. C. Rutherford. 2006. *The Vegetation of South Africa, Lesotho and Swaziland. Strelitzia 19*. Pretoria: South African National Biodiversity Institute.

Nielsen, K. M., and J. D. van Elsas. 2019. "Horizontal Gene Transfer and Microevolution in Soil." In *Modern Soil Microbiology*, edited by J. D. van Elsas, J. T. Trevors, A. S. Soares, and P. Nannipieri, 105–123. Boca Raton: CRC press.

Oksanen, J., G. Simpson, F. Blanchet, et al. 2023. vegan: Community Ecology Package. R package version 2.6-4. https://CRAN.R-project.org/package=vegan.

Owen-Smith, N. 1999. "The Animal Factor on Veld Management." In *Veld Management in South Africa*, edited by N. Tainton, 117–137. Pietersburg: University of Natal Press.

Parmenter, R. R., and J. A. MacMahon. 2009. "Carrion Decomposition and Nutrient Cycling in a Semiarid Shrub–Steppe Ecosystem." *Ecological Monographs* 79: 637–661. https://doi.org/10.1890/08-0972.1.

Patil, V. S., Y. Lugani, R. D. Chaudhari, et al. 2021. "Description and Genomic Insights Into a Multidrug Resistant Novel Bacterium *Savagea serpentis* sp. nov., Isolated From the Scats of a Vine Snake (*Ahaetulla nasuta*)." *Antonie Van Leeuwenhoek* 114: 687–696.

Payne, J. A. 1965. "A Summer Carrion Study of the Baby Pig *Sus scrofa* Linnaeus." *Ecology* 46: 592–602.

Pechal, J. L., T. L. Crippen, M. E. Benbow, A. M. Tarone, S. Dowd, and J. K. Tomberlin. 2014. "The Potential Use of Bacterial Community Succession in Forensics as Described by High Throughput Metagenomic Sequencing." *International Journal of Legal Medicine* 128: 193–205.

Pechal, J. L., T. L. Crippen, A. M. Tarone, A. J. Lewis, J. K. Tomberlin, and M. E. Benbow. 2013. "Microbial Community Functional Change During Vertebrate Carrion Decomposition." *PLoS One* 8: e79035.

Pham, N.-P., S. Layec, E. Dugat-Bony, M. Vidal, F. Irlinger, and C. Monnet. 2017. "Comparative Genomic Analysis of *Brevibacterium* Strains: Insights Into Key Genetic Determinants Involved in Adaptation to the Cheese Habitat." *BMC Genomics* 18: 955. https://doi.org/10.1186/s12864-017-4322-1.

Picker, M., C. Griffiths, and A. Weaving. 2012. *Field Guide to Insects of South Africa*. Cape Town: Struik Publishers.

Puissant, J., L. Cécillon, R. T. Mills, et al. 2015. "Seasonal Influence of Climate Manipulation on Microbial Community Structure and Function in Mountain Soils." *Soil Biology and Biochemistry* 80: 296–305.

Quast, C., E. Pruesse, P. Yilmaz, et al. 2013. "The SILVA Ribosomal RNA Gene Database Project: Improved Data Processing and Web-Based Tools." *Nucleic Acids Research* 41: D590–D596.

R Core Team. 2023. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing. https://www.R-project.org/.

Risch, A. C., A. Frossard, M. Schütz, B. Frey, A. W. Morris, and J. K. Bump. 2020. "Effects of Elk and Bison Carcasses on Soil Microbial Communities and Ecosystem Functions in Yellowstone, USA." *Functional Ecology* 34: 1933–1944.

Rutgers, M., M. Wouterse, S. M. Drost, et al. 2016. "Monitoring Soil Bacteria With Community-Level Physiological Profiles Using Biolog™ ECO-Plates in The Netherlands and Europe." *Applied Soil Ecology* 97: 23–35.

Scola, V., J. B. Ramond, A. Frossard, et al. 2018. "Namib Desert Soil Microbial Community Diversity, Assembly, and Function Along a Natural Xeric Gradient." *Microbial Ecology* 75: 193–203. https://doi.org/10.1007/s00248-017-1009-8.

Segata, N., J. Izard, L. Waldron, et al. 2011. "Metagenomic Biomarker Discovery and Explanation." *Genome Biology* 12: R60.

Sen, A., V. Daubin, D. Abrouk, I. Gifford, A. M. Berry, and P. Normand. 2014. "Phylogeny of the Class *Actinobacteria* Revisited in the Light of Complete Genomes. The Orders '*Frankiales*' and *Micrococcales* Should Be Split Into Coherent Entities: Proposal of *Frankiales* ord. nov., *Geodermatophilales* ord. nov., *Acidothermales* ord. nov. and *Nakamurellales* ord. nov." *International Journal of Systematic and Evolutionary Microbiology* 64: 3821–3832.

Shi, S., Z. Lin, J. Zhou, X. Fan, Y. Huang, and J. Zhou. 2022. "Enhanced Thermophilic Denitrification Performance and Potential Microbial Mechanism in Denitrifying Granular Sludge System." *Bioresource Technology* 344: 126190.

Silva, I., M. Alves, C. Malheiro, et al. 2022. "Short-Term Responses of Soil Microbial Communities to Changes in Air Temperature, Soil Moisture and UV Radiation." *Genes* 13: 850.

Simpson, E. H. 1949. "Measurement of Diversity." *Nature* 163: 688.

Singh, B., K. J. Minick, M. S. Strickland, et al. 2018. "Temporal and Spatial Impact of Human Cadaver Decomposition on Soil Bacterial and Arthropod Community Structure and Function." *Frontiers in Microbiology* 8: 2616. https://doi.org/10.3389/fmicb.2017.02616.

Smith, P. 2014. "Do Grasslands Act as a Perpetual Sink for Carbon?" *Global Change Biology* 20: 2708–2711.

South African Weather Services (SAWS). 2021. Precipitation, Maximum and Minimum Temperatures for Bronkhorstspruit Station 0514408AX for Witbank Station 05153208 From 2016 to 2020. http://rsmc.weathersa.co.za.

Stephen, J., S. Shabanamol, K. S. Rishad, and M. S. Jisha. 2015. "Growth Enhancement of Rice (*Oryza sativa*) by Phosphate Solubilising *Gluconacetobacter* sp. (MTCC 8368) and *Burkholderia* sp. (MTCC 8369) Under Greenhouse Conditions." *3 Biotech* 5: 831–837.

Strickland, M. S., and K. Wickings. 2015. "Carrion Effects on Belowground Communities and Consequences for Soil Processes." In

Carrion Ecology, Evolution, and Their Applications, edited by M. E. Benbow, J. K. Tomberlin, and A. M. Tarone, 93–106. Boca Raton: CRC Press.

Subalusky, A. L., C. L. Dutton, E. J. Rosi, and D. M. Post. 2017. “Annual Mass Drownings of the Serengeti Wildebeest Migration Influence Nutrient Cycling and Storage in the Mara River.” *Proceedings of the National Academy of Sciences of the United States of America* 114: 7647–7652. https://doi.org/10.1073/pnas.1614778114.

Sung, J., H. S. Kim, J. Y. Lee, et al. 2018. “*Tumebacillus avium* sp. nov., Isolated From the Gut of a Cinereous Vulture, *Aegypius monachus*.” *International Journal of Systematic and Evolutionary Microbiology* 68: 1659–1664. https://doi.org/10.1099/ijsem.0.002725.

Swift, M. J., O. W. Heal, J. M. Anderson, and J. M. Anderson. 1979. *Decomposition in Terrestrial Ecosystems*. Vol. 5. Oakland, CA: University of California Press.

Tiao, G., C. K. Lee, I. R. McDonald, D. A. Cowan, and S. C. Cary. 2012. “Rapid Microbial Response to the Presence of an Ancient Relic in the Antarctic Dry Valleys.” *Nature Communications* 3: 660.

Towne, E. G. 2000. “Prairie Vegetation and Soil Nutrient Responses to Ungulate Carcasses.” *Oecologia* 122: 232–239.

van As, J., J. du Preez, J. Brown, and N. Smit. 2012. *The Story of Life and the Environment; an African Perspective*. South Africa: Penguin.

van Elsas, J. D. 2019. “The Soil Environment.” In *Modern Soil Microbiology*, edited by J. D. van Elsas, J. T. Trevors, A. S. Soares, and P. Nannipieri, 179–192. Boca Raton: CRC press.

Visser, H. N., J. J. Spies, C. P. Fourie, J. J. Viljoen, A. P. G. Söhnge, and F. A. Venter. 1961. *Die geologie Van Die Gebied Tussen Middelburg en Cullina, Transvaal*. Pretoria: Government Printer.

Weatherbee, C. R., J. L. Pechal, and M. Eric Benbow. 2017. “The Dynamic Maggot Mass Microbiome.” *Annals of the Entomological Society of America* 110: 45–53.

Weber, K. P., and R. L. Legge. 2010. “Community-Level Physiological Profiling.” In *Bioremediation. Methods in Molecular Biology*, edited by S. Cummings, vol. 599, 263–281. New York: Humana Press.

Webster, G., T. M. Embley, T. E. Freitag, Z. Smith, and J. I. Prosser. 2005. “Links Between Ammonia Oxidiser Species Composition, Functional Diversity and Nitrification Kinetics in Grassland Soils.” *Environmental Microbiology* 7: 676–684.

Weiss, S., D. O. Carter, J. L. Metcalf, and R. Knight. 2016. “Carcass Mass Has Little Influence on the Structure of Gravesoil Microbial Communities.” *International Journal of Legal Medicine* 130: 253–263.

West, J. R., and T. Whitman. 2022. “Disturbance by Soil Mixing Decreases Microbial Richness and Supports Homogenising Community Assembly Processes.” *FEMS Microbiology Ecology* 98: e12345.

Wickham, H. 2016. *ggplot2: Elegant Graphics for Data Analysis*. New York: Springer-Verlag.

Wilcoxon, F. 1945. “Individual Comparisons by Ranking Methods.” *Biometrics Bulletin* 1: 80–83.

Yang, H. S., and B. H. Janssen. 2002. “Relationship Between Substrate Initial Reactivity and Residues Ageing Speed in Carbon Mineralization.” *Plant and Soil* 239: 215–224.

Yastrebova, O. V., A. A. Malysheva, and E. G. Plotnikova. 2022. “Halotolerant Terephthalic Acid-Degrading Bacteria of the Genus *Glutamicibacter*.” *Applied Biochemistry and Microbiology* 58: 590–597.

Zak, J. C., M. R. Willig, D. L. Moorhead, and H. G. Wildman. 1994. “Functional Diversity of Microbial Communities: A Quantitative Approach.” *Soil Biology and Biochemistry* 26: 1101–1108.

Zhang, H., P. Perez-Garcia, R. F. Dierkes, et al. 2022. “The Bacteroidetes *Aequorivita* sp. and *Kaistella jeonii* Produce Promiscuous Esterases With PET-Hydrolyzing Activity.” *Frontiers in Microbiology* 12: 803896. https://doi.org/10.3389/fmicb.2021.803896.

Zhao, X. F., W. S. Shu, and Y. Q. Hao. 2022. “Seasonal Climate Variations Promote Bacterial α-Diversity in Soil.” *Microbial Ecology* 83: 513–517.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.