EFFECT OF LARGE HERBIVORE DECOMPOSITION ON THE SUCCESSION OF MESIC-GRASSLAND SOIL MICROBIOMES

By

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Declaration

I declare that the dissertation, which I hereby submit for the degree PhD in Microbiology at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

_____ January 2024

‘A grain in the balance will determine which individual shall live and which shall die - which variety or species shall increase in number, and which shall decrease, or finally become extinct.’

— Charles Darwin, The Origin of Species

Ter nagedagtenis van Johann

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Abstract

Plant detritus is abundant in grassland but decomposes slowly and is relatively nutrient-poor, whereas animal carcasses are labile and nutrient-rich. Although nutrients from carcasses are highly sought-after, historically, they have been considered insignificant due to their brief decomposition period and minor contribution to the overall landscape nutrition. Recent studies have demonstrated that carcasses significantly alter long-term soil properties at an ecosystem scale. There is a paucity of empirical evidence of the temporal scale of functional and structural succession of the soil microbiome during and after carcass decomposition. Over a period of eighteen months, this study evaluated the functional and structural succession of the soil microbiomes beneath ten *Connochaetes taurinus* (wildebeest) carcasses. Functional succession was measured by the utilisation of 31 ecologically relevant carbon substrates using Biolog™ EcoPlates™. Metagenomic analysis of 16S rRNA genes evaluated the bacterial community structural succession. Functional analysis results indicated that most soil microbial processes beneath the carcasses were accelerated for a limited period but resulted in an enduring increase in functional diversity. Substrate utilisation shifted successively and remained evident throughout the study period. Conversely, bacterial diversity was significantly reduced and dissimilar to control soil, although it recovered incrementally to the control soil levels within eighteen months. Biomarkers at different taxonomic levels were identified at various postmortem intervals up to eighteen months.

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List of Abbreviations

AmnA.....amino acids

Amns..... amines

ANOSIM..... Analysis of Similarity

AWCD .....average well colour development

ASV.....amplicon sequence variants

BH..... benjamini and hochberg

CLPP..... community-level physiological profile

CrbA.....carboxylic acids

Crbh..... carbohydrates

D^2 .....inverse Sympson's diversity index

$J^{'}$ ..... Pielou's evenness index

LEfSe ..... Linear Discriminant Analysis of Effect Size

LDA .....linear discriminant analysis

nMDS.....non-metric multidimensional scaling

OD..... optical density

PERMANOVA .....Permutational Multivariate Analysis of Variance

PCoA.....Principal Coordinate Analysis

PERMDISP..... Permutational Distribution

Phnl ..... phenols

Plym.....polymers

PMI ..... postmortem interval

RDA ..... redundancy analysis

Chapter 1
General introduction

1.1. Background

Soil sustains life by providing a stable structure, retaining and transmitting moisture and gas, suppressing pathogens and retaining and cycling nutrients (Doran and Zeiss, 2000; Leff et al., 2015; Swanepoel et al., 2015; Lal, 2016). The life it supports consists of plants, whilst microbiota contribute 15%, and animals less than 1% of the total biomass (Barton et al., 2019). Nutrient cycling within the soil is performed primarily by the microbiota (Leff et al., 2015). Due to the biomass ratios, the slow-decomposing plant detritus, which are relatively nutrient- and energy-poor (Swift et al., 1979), contribute most to the total soil nutrients. Animals contribute to soil nutrients through their urine, faeces and, once expired, their carcasses. At the ecosystem scale, the nutrient contribution from carcasses has historically been considered ephemeral and trivial compared to plant contributions (Putman, 1978; Owen-Smith, 1999; Benbow et al., 2015). Veld (field) management textbooks state that “The loss of nutrients through the export of animal products is not considered to be important in extensive systems” (Owen-Smith, 1999, p. 136). Parmenter and MacMahon (2009) evaluated the overall contribution of carcasses to the semi-arid shrub-steppe and indicated that decomposing carcasses contribute very little to ecosystem-level (landscape scale) nutrient cycling. Nevertheless, carcasses are nutrient- and energy-rich, significantly contributing to local soil nutrient turnover (Parmenter and MacMahon, 2009; Mason and DeBruyn, 2023). Notably, a significant increase in phosphorus levels has been measured up to five years postmortem (Barton et al., 2016). In assessing the impact of long-term carbon inputs, Millard and Singh (2010) suggested that the quality and composition of external organic matter significantly influence the structure and function of grassland-soil microbiomes, particularly bacteria, over decades.

Therefore, carcass decomposition has ecological aspects that require our attention. The soil quality of grasslands is vital to ecosystem conservation and agriculture because grasslands support herbivores. High herbivore stocking rates reduce grassland plant diversity, productivity and sustainability (Bakker et al., 2006; Naeem et al., 1994; Tilman et al., 1996; Isbell et al.,

2011). Grasslands on every continent are rapidly degrading (Montanarella et al., 2015), emphasising the need to investigate carcass decomposition's effects on grassland soil microbial function and structure.

Because soil microbiota have decomposed carcasses for over 400 million years, many soil microorganisms have evolved metabolic pathways that rapidly degrade nutrient- and energy-rich proteins and lipids from carcasses (Howard et al., 2010; Metcalf et al., 2016). The decomposing carcasses change the soil pH, salinity, and soil nutrients (Macdonald et al., 2014) and the function and structure of soil microbiomes in its immediate environment (Howard et al., 2010; Metcalf et al., 2013; Cobaugh et al., 2015; Weiss et al.,2016; Singh et al., 2018). These changes are evident from the soil during and after carcass decomposition; therefore, research on carcass decomposition has forensic utility. The soil beneath and surrounding human and animal carcasses have been studied during their decomposition to discover patterns of microbial succession and identify microbial biomarkers that could answer forensic questions such as the time since death, referred to as postmortem interval (PMI), location of death and decomposition and cause of death (Metcalf et al., 2013; Cobaugh et al., 2015; Weiss et al.,2016; Singh et al., 2018).

Research on carcass decomposition has shown that grave-soil microbial succession is predictable (Weiss et al., 2016) but strongly influenced by biotic and abiotic factors. These factors can affect the rate at which carcasses progress through decomposition phases (Payne, 1965) and change the necrobiome and soil microbiome’s function and structure. The biotic factors include carcass mass (Spicka et al., 2011; Singh et al., 2018), the carcasses’ microbiome (Forbes and Carter, 2015), the microbiomes of its immediate environment (Lauber et al., 2014) and its exposure to necrophagous invertebrates and scavengers (Pechal et al., 2013; Crippen et al., 2016). The abiotic factors include temperature, seasonality (Meyer et al., 2013) and precipitation (Singh et al., 2018).

From a forensic and ecological perspective, there is a paucity of research on the structural and especially the functional succession of soil microbial communities exposed to decomposition. As carcass decomposition studies are mainly forensically focused, most studies have utilised carcasses that resemble humans (rodents and swine) weighing less than 50 kg. Studies using larger carcasses have been recommended (Weiss et al., 2016). Few studies have investigated the

decomposition of large carcasses. Towne (2000) investigated the soil nutrient and vegetation responses to decomposing bison (*Bos bison*) and cattle (*B. taurus*) that are over 200 kg, as well as deer (*Odocoileus virginianus*) carcasses. Bump et al. (2009) examined the effect of white-tailed deer (*Odocoileus virginianus*), typically 68 to 136 kg, carcasses on the herbaceous layers and soil macronutrients in a northern hardwood forest. At the time of the literature review, structural and functional succession of soil microbial communities beneath carcasses greater than 200 kg have not been studied. The cost of acquiring carcasses has limited the number of sample replicates used in decomposition studies, and more sample replicates have been recommended to compensate for variances between sample replicates (Weiss et al., 2016). More sampling time points during decomposition are required to elucidate succession dynamics (Weiss et al., 2016). As indicated by Singh et al. (2018), "more comprehensive analysis of soil biota is still lacking (e.g., long-term impact on microarthropods and soil microorganisms)", especially studies that continue past carcass decomposition. Singh et al. (2018) assessed soil bacteria and arthropod communities of decomposing human cadavers over 732 days, the longest study reviewed. Cobaugh et al. (2015) examined the microbial succession under cadavers for up to 198 days. Differences in the structural and functional succession of the soil microbiota beneath species other than rodents, swine and humans, as well as the microbial biomarkers unique to those species present in the soil, require further study. Literature on the functional succession of grave-soil communities is minimal. Studies have inferred function based on structural succession (Metcalf et al., 2013), measuring total microbial respiration (Cobaugh et al., 2015), substrate-induced respiration with minimal substrates (Singh et al., 2018) and sole-carbon substrate utilisation, mainly from rodents and swine weighing less than 50 kg and humans.

Southern African grasslands contain numerous large (>200 kg) herbivores such as *Connochaetes .spp* (wildebeest). Wildebeest have naturally high natality and mortality rates (Owen-Smith, 1999; Jones et al., 2015) and are ideal for studying large herbivore carcass decomposition in grassland and savanna environments. Culling of large herbivores may be required under conditions such as drought and overpopulation (over-stocking) (Walker et al., 2001), and their carcasses are ordinarily left in the field (veld). These conditions create an opportunity to study the decomposition of large herbivores and the structural and functional succession of the soil microbiomes beneath them under semi-controlled conditions using numerous sample replicates simultaneously over an extensive period.

1.2. Aim and objectives

The current body of research predominantly focuses on the short-term temporal scale and succession of soil microbial communities and their inferred functions beneath relatively small decomposing animals. However, a significant research gap remains in understanding the long-term structural and functional succession beneath large animals across diverse environments. There is a critical need to elucidate the duration of succession, identify biologically meaningful taxa within these environments, and characterise the functional traits of the successive microbial communities. Addressing this gap is essential for enhancing our understanding of the long-term ecological impacts of carcass decomposition in mesic grassland ecosystems. This study aimed to characterise soil microbial functional and structural succession beneath large herbivores (*Connochaetes taurinus*) decomposing in a typical South African mesic grassland. The specific objectives were to:

1. Review the existing literature on functional and structural changes observed from soil microbiomes exposed to animal decomposition (Chapter 2).
2. Evaluate the function of the soil microbial communities from its substrate utilisation by testing the null hypothesis of no significant difference between soil from beneath the carcasses and control soil at one, six, twelve and eighteen-month PMIs. The aspects of substrate utilisation that were compared included:
    a. the overall metabolic activity differences of the soil microbial communities of each PMI,
    b. the functional and beta diversity (dissimilarity),
    c. differences in the specific substrate guilds being utilised and
    d. differences in the soil nutrients available at each PMI (Chapter 3).
3. Compare the soil microbial community structures at the PMIs by testing null hypotheses of no significant differences in
    a. structural alpha diversity and dissimilarity,

b. phylum-level taxonomic abundances

c. the biologically relevant microbial biomarkers and

d. differences in functional genes inferred from the taxonomic abundance (Chapter 4).

1.3. Ethical considerations

Ethical approval for this study was obtained from the Centre of Microbial Ecology and Genomics of the University of Pretoria.

The euthanisation of ten *Connochaetes taurinus* was performed by the Telperion Game Reserve (https://ogresearchconservation.org/telperion-nature-reserve/) in the Mpumalanga Province of South Africa for conservation purposes. The described research only involved the collection of soil samples and the culturing and extracting of DNA from the naturally occurring prokaryotes beneath the culled *Connochaetes taurinus*.

Personal risks of injury associated with the fieldwork in the wildlife conservation area included sunburn, snakebites, insect and arachnid bites and scratches from thorns. However, all possible precautions were taken to avoid injuries, such as wearing clothing suitable for fieldwork, gloves, hats, and sun protection. No equipment was disposed of or discarded in the natural environment.

Laboratories at the University of South Africa and the University of Pretoria were used following the prescribed laboratory safety practices. The researcher was not directly exposed to chemicals by consumption, inhalation or absorption, as the proper laboratory procedures were applied. Volatile substances were handled under a fume hood, and protective laboratory coats and nitrile gloves were worn. Biohazard boxes and liquid containers provided by the laboratories were utilised to dispose of laboratory consumables.

1.4. Thesis outline

- Chapter 1 briefly describes the study background and motivation, the paucity of collective knowledge, the justification of the study, and the study's aim and objectives. It indicates the ethical considerations and provides an outline of the thesis.
- Chapter 2 contains a literature review of recent studies with similar aims and describes the best practices of current carcass decomposition study designs and the methods applied within this study.
- Chapter 3 tests the null hypotheses of no difference in soil nutrients, overall microbial metabolism, functional alpha diversity, functional dissimilarity, and substrate utilisation between soil from beneath the carcasses and the control soil at the four PMIs.
- Chapter 4 tests the null hypotheses of no difference in microbial alpha diversity and dissimilarity of the microbiomes beneath the carcasses and the control soil of the same PMI. It also indicated each PMI's biologically relevant microbial biomarkers and compared sole-carbon substrate utilisation to inferred functional gene abundance.
- Chapter 5 provides the study's overall conclusions and recommendations for further studies.

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Chapter 2
Literature review

Soil, an essential component of terrestrial ecosystems, provides vital services such as nutrient cycling, water retention, and plant support. The quality of soil is pivotal in maintaining these services and supporting life. The soil microbiome plays a critical role in driving these processes. Grasslands, which cover a significant portion of the earth's land surface, depend heavily on their soil microbiomes for productivity and sustainability. This chapter highlights some of the relationships between soil microbiomes and grasslands, focusing on how the decomposition of large herbivore carcasses influences the structure and function of these microbial communities. The chapter begins by examining the general characteristics and importance of soil microbiomes within grassland ecosystems. It then transitions into an in-depth discussion of carcass decomposition, detailing the stages involved and the role of soil microbes in this process. Subsequently, the chapter provides a critical analysis of existing research on the impact of carcass decomposition on soil microbial communities, including discussions of experimental designs and analytical approaches supported by examples from the literature. Finally, the chapter identifies the key gaps in the current understanding of this topic addressed in the research chapters and outlines the research questions this thesis aims to address.

2.1. Soil microbiomes and grasslands

Soil is a combination of degraded rock, minerals, and organic matter that has developed over centuries (Ashman and Puri, 2008). The soil environment is dynamic as its structure, moisture content, inhabitants, soil organic matter (SOM), and ecosystem functions consistently change. Soil quality is defined as its capacity to sustain ecological productivity, environmental quality, and plant and animal life (Doran and Parkin 1994). Good quality soil, therefore, has to perform functions such as carbon dioxide sequestration and nitrogen-fixing, decomposition of plant litter, organic waste and carrion, the degradation of pollutants, the sequestration of metals (Silver and Phung, 2005), the suppression of pathogens (Cha et al., 2016; Weller et al., 2002), water retention and transmission, and the formation and transformation of compounds (Lal, 2016). To evaluate the sustainable utilisation of soil, its health and quality should consistently be evaluated (Doran and Zeiss, 2000).

Soil microorganisms, especially bacteria, are central to soil quality and ecosystem functions. Soil bacteria contain the largest of the known bacterial genomes with a substantial amount of open reading frames, resulting in exceptionally diverse functions, many of which are unknown. These diverse functions may have resulted from selection pressures due to nutrient and environmental inconsistencies. The functional diversity of soil bacteria affords rapid adaptation to environmental changes. (van Elsas et al., 2019). In addition, bacterial genomes theoretically consist of core genes present in all the species members and accessory genes present only in some members. The accessory genes can be transferred by horizontal gene transfer, even to distantly related species. The donated DNA may afford the receiver a niche-specific metabolic function. Horizontal gene transfer is prevalent in soil, especially in the rhizosphere (Nielsen and van Elsas, 2019).

Subclasses α, β, γ, δ and ε of the phylum Pseudomonadota (Proteobacteria), Bacillota (Firmicutes), Actinomycetota (Actinobacteria) and Bacteroidota (Bacteroidetes), are commonly isolated from soil using culture-dependent methods. Subclasses α, β and γ-Proteobacteria contain many r-strategists. Some are plant pathogens; others form symbiotic relationships with plants, assisting in nitrogen fixation, whereas some degrade xenobiotics. δ-Proteobacteria contain many sulfate-reducing bacteria. Firmicutes, another fast-growing r-strategist, can be abundant when labile carbon sources are available. These include *Bacillus* and *Clostridium,* which form endospores, allowing them to survive prolonged periods of low nutrients. They are also found in the digestive tracts of animals, some of which are pathogens. Most Actinobacteria are K-strategists and include species such as *Micrococcus* that can accumulate carbon polymers during periods of abundance and utilise them during periods of deprivation. Many Actinobacteria can degrade anthropogenic compounds. Some, such as *Frankia* spp, are nitrogen-fixing and closely associated with plants (Willey et al., 2014; van Elsas., 2019). Culture-independent methods, mainly using 16S rDNA libraries, have indicated that Acidobacteriota (Acodobacteria), Planctomycetota (Planctomycetes), Chloroflexota (Chloroflexi) and Verrucomicrobiota (Verrucomicrobia) are also prevalent in most soils. Acidobacteria and Verrucomicrobia have been detected in almost all soil samples, although very few species of Verrucomicrobia have been isolated (van Elsas et al., 2019). Verrucomicrobia are particularly dominant in grassland soil (Bergmann et al., 2011) but recalcitrant to culture (da Rocha, 2019).

Plants contribute 450 billion tonnes of the earth's biomass (Barton et al., 2019), 100 billion of which are produced annually (Gessner et al., 2010). The soil environment is nutrient-poor, mainly containing oligotrophic microbiota (K-strategists) that rely on plant-derived detritus, mainly cellulose and lignin, for nutrients. The rhizosphere, a narrow area surrounding plant roots, is a *hotspot* for soil-microbiome plant interactions as it is relatively nutrient-rich and contains copiotrophic (r-strategist) microbiota. Complete degradation of plant detritus can take several days to many years. Lignin and cellulose contribute little to the persistence of SOM, and it has been proposed that relatively labile compounds that cause higher microbial growth for a short period of time contribute more to the stable mineral-associated SOM pool. (Montanarella et al., 2015).

Grasslands are ecosystems where grasses dominate the vegetation and may include patchily distributed forbs and shrubs (Dixon et al., 2014). Grasslands are especially suitable for large herds of herbivores, with different herbivore species occupying the same ecological niche in different geographical regions (van As et al., 2012). Its ability to support large herds gives it agricultural application. Therefore, the term grassland includes natural grassland, rangelands, pastureland, meadows and grass sown with pasture and fodder crops. Grasslands cover approximately 26% of the world's ice-free land and contain approximately 20% of the world's SOM. Eight per cent of grasslands have been degraded by overgrazing, directly impacting grassland SOM content (Montanarella et al., 2015). When managed at low stocking rates, herbivores increase grassland plant diversity (Olff and Ritchie, 1998; Bakker et al., 2006), which, in turn, increases grassland productivity and sustainability (Naeem et al., 1994; Tilman et al., 1996; Isbell et al., 2011). Grasslands support most of the world's wild and domestic ungulate herbivores. Angulate herbivores utilise their gut bacteria to digest nutrient-poor cellulose by consuming the grass. After digestion, the remaining nutrients are redistributed to the soil as faeces and urine. The overall effect is the collection of relatively nutrient-poor nutrients from a large area being concentrated into small labile patches of nutrients, which insects further distribute over the area, returning about 20% of the nitrogen to the soil (Owen-Smith, 1999). Grass detritus and faeces are relatively nutrient-poor compared to decomposing carcasses (Metcalf et al., 2013; Cobaugh et al., 2015; Barton et al., 2019). The amount of nutrients obtained from carcass decomposition has previously been considered irrelevant to large ecosystems, irrespective of being nutrient-rich and labile (Putman, 1978; Owen-Smith, 1999;

Parmenter and MacMahon, 2009). Plant detritus are notoriously recalcitrant and consist mainly of high molecular weight, low-energy complex carbohydrates such as cellulose and lignin. Four hundred million years of intermittent inputs of labile nutrients, low molecular weight, high-energy proteins and lipids obtained from carcasses have enabled rapid carcass decomposition by soil microbiota. Microbiota possessing metabolic pathways that metabolise labile nutrients more rapidly than others but remain viable for extended periods without labile nutrients have been evolutionarily favoured. The rapid degradation of animals compared to plants detritus may indicate the disproportionate significance of labile nutrients to the ecosystem (Swift et al., 1979; Carter et al., 2007; Parmenter and MacMahon, 2009; Benbow et al., 2016; Metcalf et al., 2016).

Decomposing labile nutrients, such as carcasses, creates ‘hot moments’ and ‘hotspots’. As defined by Kuzyakov and Blagodatskaya (2015), a hot moment is a short-term event that accelerates microbial processes in a limited area, for example, when a limiting environmental factor, such as a low-molecular-weight energy-rich carbon source, becomes available for a limited period. If the amount of labile nutrients is extensive enough, not only will the metabolic processes of the existing microbiota be accelerated, but the microbial structure of the community will be altered, increasing the number of microbiota that benefit. In such case, a hot moment may create a ‘hotspot’, a small volume of soil containing microbiota with accelerated rates of particular metabolic pathways and interaction. The accelerated process rates (functions) may continue for lengthy periods after the labile nutrients have been utilised. The longevity of hotspots depends on the rate at which the ratio of r-strategists, growing fast in nutrient abundance but poor interspecific competitors, and K-strategists, growing with minimal nutrients but good interspecific competitors (van Elsas et al., 2019), return to pre-exposure levels (Fontaine et al., 2003; Allison and Martiny, 2008; Kuzyakov and Blagodatskaya, 2015). Competition between the ecological groups for labile nutrients and associated environmental factors can be observed through the succession of the microbial community taxonomic abundances (Cobaugh et al., 2015; Metcalf. et al., 2016;) and the rate of different metabolic processes (Metcalf. et al., 2016; Heo et al., 2021). Prolonged succession initiated by carcass decomposition may have relevance at the landscape and ecosystem scale if hotspots persist for a substantial time, altering landscape and ecosystem processes (Yang and Janssen, 2002; Carter et al., 2007; Benninger et al., 2008; Bradford et al., 2008; Strickland et al., 2009; Cotrufo et al., 2013; Macdonald et al., 2014). For example, Bump et al. (2009) mapped ungulate carcass locations between 1958 and 2006 and

performed carcass isotope, leaf tissue and soil microbial phospholipid fatty acid analysis. Their study indicated that wolves (*Canis lupus*) modulate soil nutrient heterogeneity and plant nitrogen and also increase and alter the fungal and bacterial ratios of the landscape by configuring the distribution of ungulate carcasses.

2.2. Carcass decomposition

Payne (1965) divided carcass decomposition in natural settings into six decomposition stages: 'fresh', 'bloated', 'active decay', 'advanced decay', 'dry', and 'remains'. Subsequent studies refer to these or similar stages when describing carcass decomposition chronologically (Carter et al., 2007; Cobaugh et al., 2015; Metcalf. et al., 2016; Heo et al., 2021). The fresh stage starts once the animal dies until the abdomen becomes bloated due to the gas production from putrefaction (Figure 1A). The 'bloated' stage ends when fluid and nutrients escape from the carcasses, organisms such as Diptera (flies) enter the carcasses, and the innards are exposed to air, which starts the 'active decay' stage (Figure 1B). During 'active decay', carcasses lose most of their mass by liquids and nutrients seeping into the soil, as well as being consumed by dipteran larvae (maggots) (Figure 1B), carrion beetles, and other invertebrate and vertebrate scavengers. Once the fluid and most nutrients have been removed, identified by the absence of maggots (Figure 1C), the 'advanced decay' stage starts. The 'dry' stage is depicted in Figures 1D and E, and the 'remains' stage in Figure F. Carcass decomposition stages have been subdivided into 'bloat-active', 'active-advanced', and different levels of advanced (Cobaugh et al., 2015). A single carcass can also be in different stages of decomposition at a single point in time (Matuszewski et al., 2010).

Figure 1: Stages of carcass decomposition. Diptera enters the bloated carcass through an orifice during the 'bloated' stage of decomposition (A). Dipteran larvae extruded from the carcass during 'active decay' (B). The absence of maggots indicates the 'advanced decay' stage of decomposition (C). The 'dry' stage starts when most of the nutrients have been removed, and the carcass becomes desiccated (D and E). The 'remains' stages (F).

Climate-related factors influence carcass decomposition rates (Megyesi et al., 2005; Carter et al., 2007; Carter et al., 2010). Most carcass decomposition studies are forensically related, although non-human, vertebrate subjects are frequently used (Metcalf. et al., 2013; Weiss et al., 2016; Heo et al., 2021). To compensate for different temperatures in different geographical areas and fluctuations of temperature at the same location, forensic scientists use Accumulated Degree-Days (ADD) (Equation 1) and Accumulated Degree-Hours (ADH) as a more accurate method of estimating the time since death, referred to as postmortem interval (PMI) (Megyesi et al., 2005). Soil moisture, especially in combination with soil type, also influences the rate of carcass decomposition (Carter et al., 2007). Biotic factors that influence the rate of decomposition

include carcass mass (Hewadikaram and Goff, 1991; Vass et al., 1992; Spicka et al., 2011), the presence of vertebrate scavenges (Benninger et al., 2008), and the presence and the extent of infestation by arthropods such as dipteran families Calliphoridae (blow flies) and Sarcophagidae (flesh flies), and coleopterans families such as Silphidae, Dermestidae, Trogidae, Histeridae, and Staphylinidae (Merritt and De Jong, 2019).

$$ADD\ i\ =\ [\{T\min + T\ max\} \div 2] - i$$

Equation 1: Calculation of Accumulated Degree-Days. *T min* is the minimum temperature, and *T max* is the maximum temperature for the day. *i* represents the minimum threshold over which the accumulation is considered (Michaud and Moreau, 2009).

Bacteria that facilitate carcass decomposition have various origins. Initially, most originate from the intestinal tract of the carcass and rapidly increase when macromolecules of the carcass (carbohydrates, proteins, lipids, and nucleic acids) are transformed into simpler molecules. As putrefaction intensifies and gas production increases, the skin ruptures, giving insects access inside the carcass and exposing the anaerobic bacteria to oxygen (Carter et al., 2007). The increase in oxygen alters the necrobiome, reducing anaerobic bacteria and increasing aerobic bacteria. The insects that gain access to the carcass introduce mites, nematodes and bacteria (Guo et al., 2016; Weiss et al., 2016; Iancu et al., 2020; Heo et al., 2021). The soil microbiome also contributes to carcass decomposition. A study by Lauber et al. (2014) indicated that the mice carcasses placed on soil with intact microbiota decomposed 2 to 3 times faster than mice placed on sterile soil.

2.3. Experimental designs and analysis

Randomised experimental designs with adequate, independent replicates and control samples are required to minimise bias and limit the effects of sampling error. Field experiments have lower precision than laboratory experiments because they vary more between samples, resulting from numerous differences in an uncontrolled environment. The lower precision of field experiments necessitates additional replication to reduce type II error (except the null hypothesis in error) during hypothesis testing (Schoenly et al., 2015). A replicate is the smallest unit of a sample in an experiment to which the same treatment is applied. In a laboratory experiment by Metcalf et al. (2013), the microbial communities of 40 mice (n = 40) were evaluated during their

decomposition. The relatively high amount of samples for a laboratory experiment produced low variation; thus, high inference strength between samples and *p-values* of < 0.001 were reported. A slight variation could be observed from the depicted error bars in the study. The cost of obtaining mice for experiments is low relative to large vertebrates. Weiss et al. (2016) investigated similar microbiomes of swine and used twelve carcasses in four different size categories (n = 3) and, in a study by Cobaugh et al. (2015), four human cadavers that were similar in weight were utilised (n = 4). To prevent pseudoreplication, replicates of the same treatment should not be pooled (*sacrificial pseudoreplication*). Replicates should also be statistically independent in space and time (*spatial* and *temporal pseudoreplication*) (Schoenly et al., 2015).

Control samples should ideally be collected at the same PMI as treatment samples, as Zhao et al. (2022) indicated that seasonal variations in precipitation and temperature had weak but significant effects ($p < 0.05$) on the alpha-diversity (α-diversity) of soil microbial communities *in situ*. In the study by Cobaugh et al. (2015), control samples were collected two meters from the cadavers, whereas Weiss et al. (2016) collected control samples five meters from the swine carcasses. Singh et al. (2018) indicated that changes to soil microbial communities are not limited to soil beneath cadavers. Multivariate analysis indicated that microbial communities beneath and one meter from decomposing cadavers had different structural variabilities and less α-diversity than communities five meters from the cadavers.

For observations to be independent, the carcasses should be spaced so that organisms cannot be dispersed between carcasses. Although microorganisms are relatively immobile, insect vectors such as flies disperse them. If carcasses were placed simultaneously and their metacommunities were linked by dispersion, unrealistic synchronisation of metacommunity succession may be observed. Conversely, carcasses placed at different time points may show indistinct successional patterns (Schoenly et al., 2015). In the study by Weiss et al. (2016), the carcasses were placed simultaneously, and the distances between carcasses were not reported. In the study by Cobaugh et al. (2015), initial soil samples were collected from the exact area where the cadavers were placed, and the collection of control samples (two meters from the cadavers) were collected simultaneously with treatment samples (beneath the cadavers). To the author's knowledge, no recent vertebrate decomposition occurred in the area where the cadavers were placed. The study

did not indicate the distance between cadavers. The cadavers were placed simultaneously, and dispersion of microbiota by insect vectors may have occurred.

Cobaugh et al. (2015) indicated that the soil microbial community of one of the four cadavers had become distinct from the others as decomposition progressed. It was noted that the cadaver was placed on a slope during a comparatively warmer period. Attention should be given to the additional factors of the immediate environment to reduce variation between samples in field experiments. For example, Weiss et al. (2016) ensured that the swine carcasses were placed on similar soil types in a grassland. The decomposition sites were flat because sloped sites may affect the direction in which leachate moves once seeped from the carcasses. Care was taken to reduce scavenging by vertebrates whilst not impairing insect activity.

Griffiths et al. (2003) evaluated the spatiotemporal variation of grassland soil bacterial communities for different seasons over two years. The metabolic profiles were compared using Biolog™ Gram-negative microplates, and the molecular profiles were compared using 16S rRNA and 16S rDNA gene fragments denaturing gradient gel electrophoresis (DGGE) analysis. Core samples were collected at a 0¬20 cm depth, of which the 0¬5 cm was in the organic horizons, and 15¬20 cm was within the mineral horizon. Separating soil core samples at 5 cm intervals indicated that metabolic activity was at its lowest at 15¬20 cm, and the metabolic activity could be linked to changes in the DNA and RNA DGGE profiles. A seasonal variation could be distinguished at 5¬15 cm, whilst the 15¬20 cm depths were relatively stable. Fierer et al. (2003) observed that deeper soil horizons had a two- to three-fold reduction in microbial densities and that most of the microbial biomass of a 2 m sample would be found within the first 25 cm. Phospholipid fatty-acid (PLFA) analysis indicated that Gram-positive bacteria increased with depth, and Gram-negative bacteria were most abundant at the surface, reducing with depth. After analysing differences in microbial communities at different depths, Rong et al. (2023) recommended adopting specific standards to achieve reliable results when investigating community assemblies in soil, recommending a sampling to a depth of 15 cm.

Various sample depths have been used to study changes in soil microbial communities beneath carcasses. Investigating the biochemical changes to the soil, Benninger et al. (2008) collected samples from 0¬5 cm below the carcasses. Cobaugh et al. (2015) collected samples from the top 0¬3 cm of soil beneath the cadavers using a 0.8 cm corer, whereas Singh et al. (2018) aseptically

collected samples from 0 to 10 cm under cadavers using a stainless steel soil corer. Heo et al. (2021) used a shovel and a tin disinfected with Lysol before sampling.

2.4. Functional succession

A degree of functional redundancy has been observed in soil microbial communities. Disturbances that change the microbial community structure do not always change the microbial functions (Allison and Martiny 2008). Moreover, microbial communities that are geographically isolated but are exposed to similar environmental conditions may be structurally different but perform similar microbial functions. (Garland and Mills, 1991; Lozupone and Knight, 2005). Therefore, performing taxonomic studies in isolation may not describe ecological functions accurately (Garland and Mills, 1991; Nannipieri et al., 2003; Stefanowicz, 2006; Escalas et al., 2019).

Carbon sequestration, carbon cycling, SOM formation, and the relationships between biodiversity and function are leading microbial ecological research topics. Although these topics should form part of carcass decomposition study goals, empirical studies of functional shifts of soil microbiomes resulting from decomposition are limited (Carter et al., 2007).

Pechal et al. (2013) compared the function succession of carrion necrobiomes exposed to necrophagous to unexposed carrion. Their findings indicated that seasonal changes influence microbial functional activity and that annual inconsistencies were observed in the microbial function, highlighting the need for a deeper understanding of these processes. Using sodium-hydroxide carbon dioxide trap and titration to assess soil microbial respiration, Cobaugh et al. (2015) evaluated the functional succession of soil microbial communities below human cadavers. They observed distinct changes in respiration and biomass production that corresponded to changes in microbial structure.

Next-generation sequencing (NGS) approaches created new opportunities to explore the relationships and general mechanisms of ecosystem functions (Crippen et al., 2016). A study by Metcalf et al. (2016) used putative functional prediction software, Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt), to indicate functional diversity and enzyme gene abundances of bacteria in the abdomens of mice during decomposition. Distinct differences in functional beta-diversity (β-diversity) were observed

between the early and late decomposition stages, and marked increases were observed in carrion decomposition-related enzymes.

Singh et al. (2018) compared the soil microbial function beneath decomposing human cadavers to the surrounding soil by substrate-induced respiration of seven substrates (glycine, cellulose sucrose, oxalic acid chitin glucose and citric acid). The respiration at one and five meters was similar, whereas respiration beneath the cadavers was significantly different. Heo et al. (2021) compared the 'microbial metabolic community profiles' *vis* community-level metabolic profiles of swine carcasses exposed with immediate and seven- and fourteen-day delays to necrophagous insects. They examined buccal, skin, and anal swabs as well as soil samples taken from beneath the decomposing swine and cultured the samples in sole-carbon substrates using Biolog™ EcoPlates™, following the methods described by Pechal et al. (2013) and Weber and Legge (2010). The results from Heo et al. (2021) demonstrated that the exclusion of insects during decomposition could be detected using EcoPlate™ cultures for both animal swabs and soil samples, with statistically significant differences ($p < 0.05$) observed in the comparisons.

2.4.1 Biolog™ EcoPlate™ method of determining soil function.

The term community-level physiological profiling (CLPP) (Lehman et al., 1995) is mainly used to describe microbial communities based on their utilisation of sole-carbon substrates measured during culturing in 96-well Biolog™ microplates (Biolog™ Inc., Hayward, CA, USA). Gram-negative microplates, Gram-positive microplates and EcoPlates™ are frequently used to describe microbial communities. Characterising microbial communities entirely by their Biolog™ community profiles may lead to inaccurate conclusions if certain factors are not considered (Preston-Mafham et al., 2002). Most bacteria are not culturable (Amann et al., 1995). Co-culture-dependent and fastidious microbial groups are excluded (Smith et al., 2006), and many of the microbiota cannot be cultured in single-carbon substrates and within the artificial microplate environment (Degens and Harris, 1997). In addition, if a comparison is made of microbial communities too early during incubation, there is a bias towards rapidly growing bacteria (Preston-Mafham et al., 2002). In contrast, late comparison may be influenced by growth inhibition (allelopathy) or secondary metabolites (Barton and Northup, 2011).

Furthermore, inoculation of microplates should occur soon after sample collection to reduce shifts in microbial function resulting from the sample collection and storage-induced changes to the microbiota (Shishido and Chanway, 1998). High inoculation densities may result in a slight variance in colour development between wells, and serial dilutions are recommended. Low inoculation density may result in no colour development as formazan production does not occur at cell densities less than 10^5 cells mL^{-1}, although colour development is also dependent on the species present as well as their metabolic activity (Konopka et al., 1998; Preston-Mafham et al., 2002; Weber and Legge, 2010). If the research question requires a comparison of actual microbial community function for different treatments, inoculum density is not a concern should the same sample dry weight be used for plate inoculation (Preston-Mafham et al., 2002). Similar inoculation densities may be required to compensate for confounding factors such as climate variations during sampling (Rutgers et al., 2016). Despite the limitation of CLPP by sole-carbon substrate utilisation, comparisons of communities using Biolog™ microplates provide insight into the functional ability of the community, making differences apparent even if only from a fraction of the total microbial community (Preston-Mafham et al., 2002).

Figure 2 is a diagrammatic illustration of an EcoPlates™ containing a triplicate of 31 carbon substrates (Biolog, 2024). The carbon substrates in EcoPlates™ are selected explicitly for environmental applications as they compensate for the microbial cultures being overwhelmed by r-strategist (Campbell et al., 1997). The substrate utilisation of a single sample is measured in triplicate to increase statistical confidence (Weber and Legge, 2010). Each of the wells, including three blank wells, contains a chromogenic such as tetrazolium salt. When cells from an environmental sample metabolise either a sole-carbon substrate from a well or substrates introduced to the well from the inoculants, the tetrazolium is reduced to formazan by nicotinamide adenine dinucleotide + hydrogen (NADH) formed during cellular respiration. Colour development from formazan production is accurately measured by colourimetry at an optical density (OD) of 590 nm (Garland and Mills, 1991; Preston-Mafham et al., 2002; Weber and Legge, 2010). To exclude colour that resulted from substrates present in the inoculants, colour development from the blank wells is deducted from the colour development of the sole-carbon substrate-containing wells, a process called standardisation. Because standardisation may produce negative values, negative values are converted to 0 (Haack et al., 1995). Differences in

inoculation density can be compensated for by dividing the OD density values of each well by the average well-colour development AWCD of the EcoPlate (Garland, 1997).

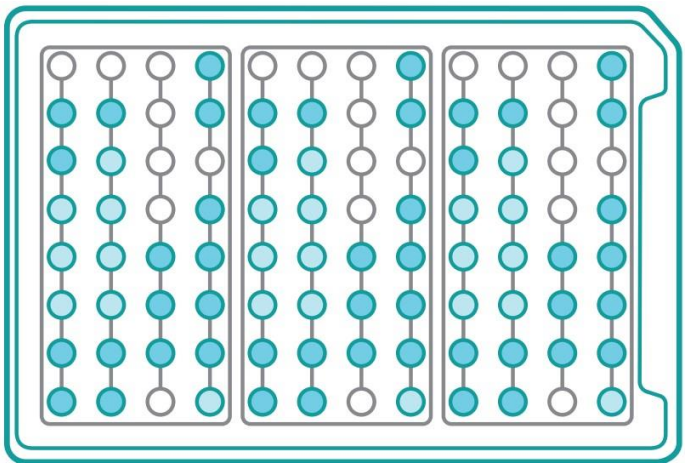

Figure 2: A schematic representation of an EcoPlate™ used to analyse soil microbial community function—source: Biolog (2024). An EcoPlate™ contains 96 wells arranged in an 8x12 grid, consisting of triplicates of 31 sole-carbon-substrate wells and a blank well. All wells contain tetrazolium salts, which are responsible for colour development during substrate utilisation.

Three main methods are used to compare CLPP: (i) Comparing OD values for the wells of all the microplates after a specific incubation time. (ii) Comparing OD value for each plate when a specific average well-colour development (AWCD) has been reached irrespective of the incubation time. (iii) Kinetic analysis of the OD values. Each method has advantages and disadvantages that should be considered along with the research question and the preliminary results obtained (Weber and Legge, 2010). When selecting a specific incubation time to compare community profiles, the variance between microplate well ODs should be as high as possible without any wells exceeding a value of two, which falls outside the linear absorbance range (Weber and Legge, 2010). The average well-colour development of the microplates should be within the same phase of colour development and past the lag phase. Using OD values of the later stages of incubation can provide more accurate information because the dispersion of OD values increases as incubation proceeds (Preston-Mafham et al., 2002; Weber and Legge, 2010).

Different methods have been used to prepare the samples for microplate inoculation. Some researchers remove larger roots and pebbles aseptically using various methods before suspending samples in sterile water of a buffer solution. Aliquots are suspended in glass vessels either by shaking the vessels or by agitated incubation, which liberates soil microorganisms. Aliquots may

be serially diluted based on preliminary testing of inoculation densities and the specific research question. Aliquots are afforded time for the precipitation of debris or centrifuged. Microplates are inoculated with 150 µL of supernatant per well (van Eekeren et al., 2008; Habig and Swanepoel, 2015; Koner et al., 2021).

Community-level physiological profiles are usually compared by their average well-colour development, differences in α-diversity, Shannon (Shannon and Weaver, 1949) and Simpsons (Simpson, 1949) indexes, and β-diversity, also called dissimilarity (Whittker, 1960) using carbon substrates. Multivariate analysis of variance usually involves the transformation of data to improve data normality and homogeneity of variance (Anderson, 2001; Legendre and Legendre, 2012; Weber et al., 2007). Results are depicted by ordination plots (van Eekeren et al., 2008; Rurgers et al., 2016) or hierarchical cluster analysis (Habig and Swanepoel, 2015). Depending on the research question, the utilisation of specific substrates is evaluated (Rurgets et al., 2016), or the substrates may be placed in functional groups (guilds), and the mean or total guild utilisation compared (Preston-Mafham et al., 2002; Swanepoel et al., 2014).

2.5 Structural succession

In an *in vitro* experiment with controlled conditions, Metcalf et al. (2013) recorded a reduction of bacterial diversity on the skin, in the abdomen and the ‘gravesoil’ of mice over 48 days. In addition, they observed a significant difference in β-diversity (dissimilarity) between the bacterial communities from control soil and soil from beneath carcasses. Weiss et al. (2016) observed that, *in situ*, the α-diversity of the soil beneath swine (*Sus scrofa domesticus*) (150 kg) was reduced over 50 days of decomposition, also observing a significant dissimilarity in community composition. In addition, they reported that bacterial communities beneath carcasses of different masses were not significantly dissimilar ($p < 0.01$). Singh et al. (2018) confirmed an overall decrease in α-diversity beneath human cadavers (47 ¬ 153 Kg) during a long-term study in which samples were collected from 3 and 732 days after carcass placement and indicated that there were structural differences based on carcass size, conflicting with the results of Weiss et al. (2016). Neither of these studies reported that diversity and dissimilarity returned to pre-carcass or control soil structural composition.

Cobaugh et al. (2015) observed the richness (Chao1) and inverse Simpson diversity of soil microbial communities below decomposing cadavers. (5677 kg) remained similar to control soil during active decay and increased significantly during the later advanced decay stage, 'Advanced III' (36 months). Bray Curtis dissimilarities between the relative abundance of bacterial communities shifted during decomposition. The communities from beneath the cadavers were similar to the initial (control) samples during the 'bloated' and 'bloat-active' phases but diverged during the 'active' stage when decomposition fluids seeped into the soil. From a non-metric multidimensional scaling (NMDS) ordination plot, Cobaugh et al. (2015) suggested that the bacterial community structure was returning to the initial structure, although it remained structurally dissimilar ($p < 0.05$) during their experimental period.

During the initial stages of decomposition, most bacteria that degrade macromolecules (carbohydrates, lipids, proteins and nucleic acids) into simpler molecules stem from the animal's gut (Forbes and Carter, 2015). During the 'bloated' stage, as the oxygen is depleted, the fermentative anaerobes, including *Bacteroides* (Bacteroidetes), *Bifidobacterium* (Actinobacteria), *Enterobacter* (Gammaproteobacteria) and *Clostridium* (Firmicutes) supplant the aerobic bacteria, predominantly from the phyla Bacteroidetes and Firmicutes. (Metcalf et al., 2013; Cobaugh et al., 2015; Forbes and Carter, 2015). Metcalf et al. (2013) observed increased Rhizobiales (Alphaproteobacteria), which became the most abundant soil bacterial family in the soil beneath decomposing mice. They observed a decrease in Acidobacteria, known to be inversely proportionate to soil pH and for being oligotrophic K-strategists (Metcalf et al., 2013). Sphingobacteriaceae (Bacteroidetes) and Alcaligenaceae (Betaproteobacteria) increased during advanced decay. At a family level, Weiss et al. (2016) reported that *Candidatus* Chthoniobacteraceae from the phylum Verrucomicrobia reduced as carcass decomposition progressed as well as confirming the reduction of Acidobacteria described by Metcalf et al. (2013). Weiss et al. (2016) also reported an increase in Planococcaceae, Sporosarcina *sp*., Ignatzschineria *sp*., and Chitinophagaceae. Cabough et al. (2015) indicated that soil microbial communities beneath carcasses mainly consisted of the phyla Proteobacteria > Actinobacteria > Firmicutes in order of abundance and that Proteobacteria as a phylum remained constant throughout the study. However, the abundance of its subordinate taxa varied across the decomposition period. Alphaproteobacteria and Gammaproteobacteria, orders Enterobacteriales, Rhizobiales, Xanthomonadales and Sphingomonadales, increased throughout decomposition.

Betaproteobacteria orders Shinella, Paenalcaligenes and Pseudorhodoferax increased whilst orders Naxibacter and Massilia decreased. Phyla Acidobacteria, Armatimonadetes, Nitrospira and Verrucomicrobia also decreased as decomposition progressed. In addition, Cabough et al. (2015) indicated that taxa that were initially reduced during decomposition increased during the latter part of advanced decay. Bacteroidetes reached their peak relative abundances during the 'bloated' stage as carcasses ruptured. Actinobacteria increased throughout decomposition. Planctomycetes initially increased but returned to their original relative abundance during the late advanced stage, whereas Firmicutes initially increased but decreased as decomposition progressed.

Singh et al., 2018 confirmed, at the class level, that Actinobacteria, Gammaproteobacteria, and Bacilli (Firmicutes) increased in relative abundance under human cadavers and that Acidobacteria and Verrucomicrobia were reduced. In addition, they concluded that environmental conditions and carcass mass might affect the soil microbial community structure during decomposition as increased carcass mass correlated significantly with the relative abundance of Gammaproteobacteria, and the relative abundance of Actinobacteria correlated positively with cumulative precipitation.

2.5.1. Soil Metagenomics

Most microbiota obtained directly from the soil are unculturable due to our limited knowledge of their biological niches and inability to create the required media and culture conditions (Amann et al., 1995; Hongoh et al., 2003). Therefore, most soil microbiome research relies on metagenomics. Metagenomics is the analysis of the collective genomes present in soil microbiomes (Sjöling et al., 2019). Soil metagenomics depends on culture-independent methods such as increasing (amplifying) the number of DNA copies of the microbiota in the soil using the polymerase chain reaction (PCR) and then determining the DNA sequences (gene sequencing). Shotgun metagenomics shears the entire community's genomes in short 100¬150 base-pair (bp) reads, which are then sequenced and analysed through numerous bioinformatics steps to describe and compare the microbial community (Quince et al., 2017). For amplicon metagenomics, a marker gene is first amplified by PCR and then sequenced to describe the microbial community. The microbial community must have a common marker gene containing sufficiently conserved and variable sections for taxonomic identification.

The DNA transcribed to the small-subunit ribosomal RNA (ssu-rRNA gene) has been the main focus of marker-gene amplicon sequencing. Because of the crucial role of the ribosome, its relatively small gene size (1550 bp) and the highly conserved and variable regions it contains, the ssu-rRNA gene is an ideal marker gene. Utilising the bacterial ssu-rRNA (16S rRNA) gene as a marker gene has dramatically improved our ability to compare bacterial communities according to their composition and diversity (Sjöling et al., 2019). Currently, most systems and methods used for sequence analysis cannot read the entire length of the 16S rRNA gene, and it is common practice to use only a section of the whole sequence. The 16S rRNA gene has been divided into nine hypervariable regions (V1¬V9) from which taxonomy can be distinguished. Using standard V4 primers, Cobaugh et al. (2015) effectively conducted microbial profiling to characterize the soil microbial community structure beneath human cadavers, successfully identifying taxa up to the genus level.

After sample collection, the first step is extracting DNA, which can either be performed directly from the sample or indirectly. Indirect DNA extraction involves the liberation of the microbiota from the sample, after which their cell membranes are broken down (lysed) to liberate the DNA, or by direct extraction, where the cells are lysed within the colloidal suspension and the DNA extracted. Lysing can be achieved by enzymatic, chemical and physical disruption, and the three methods are frequently combined. The sample's origin needs consideration during extraction. Soil samples contain large amounts of humic and fulvic acid that can inhibit the PCR and should be removed beforehand (Bonnichsen et al., 2019). There are several protocols for directly extracting and purifying DNA from soil, many of which are incorporated into kits (Kamble et al., 2020). The Qiagen DNeasy PowerSoil® (Qiagen, Valencia, CA, USA) kit is one of many kits developed to extract high-quality DNA from soil containing elevated levels of humic acid (Lear et al., 2018). Once the DNA has been extracted and the marker gene amplified and sequenced, the gene sequences must be analysed to obtain meaningful results. The raw sequence data, usually stored in the *de facto* FASTQ format, are complex and extensive, and their analysis requires biological, computational and statistical techniques (bioinformatics).

Several propriety and open-source bioinformatics software tools have been created, of which the open-source Mothur (Schloss et al., 2009) and QIIME (Caporaso et al., 2010) software packages are the most popular and have many commonalities. Numerous sequential intermediary steps (a

pipeline) are required for the analysis. Pipelines have variable parameters that may alter the research outcome. These parameters include the removal of primer sequences that flank the marker gene sequences, the removal of low-quality sequences, and allocating the sequences to their respective samples according to their index sequences (demultiplexing). The sequences are clustered (binned) according to their similarity, utilising different or a combination of algorithms. Sequences with a similarity equal to or greater than 97% are referred to as operational taxonomic units (OTUs). Taxonomy can be assigned to the OTU sequences utilising reference databases such as GreenGenes (http://greengenes.lbl.gov/) and SILVA (www.arb-silva.de). The pipelines and algorithms utilised in bioinformatics are not standardised, and recent algorithms provide higher taxonomic resolution (Callahan et al., 2016). For example, the Divisive Amplicon Denoising Algorithm (DADA) (Figure 3), which adjusts for amplicon errors, can accurately produce data sets of Amplicon Sequence Variants (ASVs) that differ by a single nucleotide by implementing a quality-aware model of amplicon errors (Callahan et al., 2016; Callahan et al., 2017).

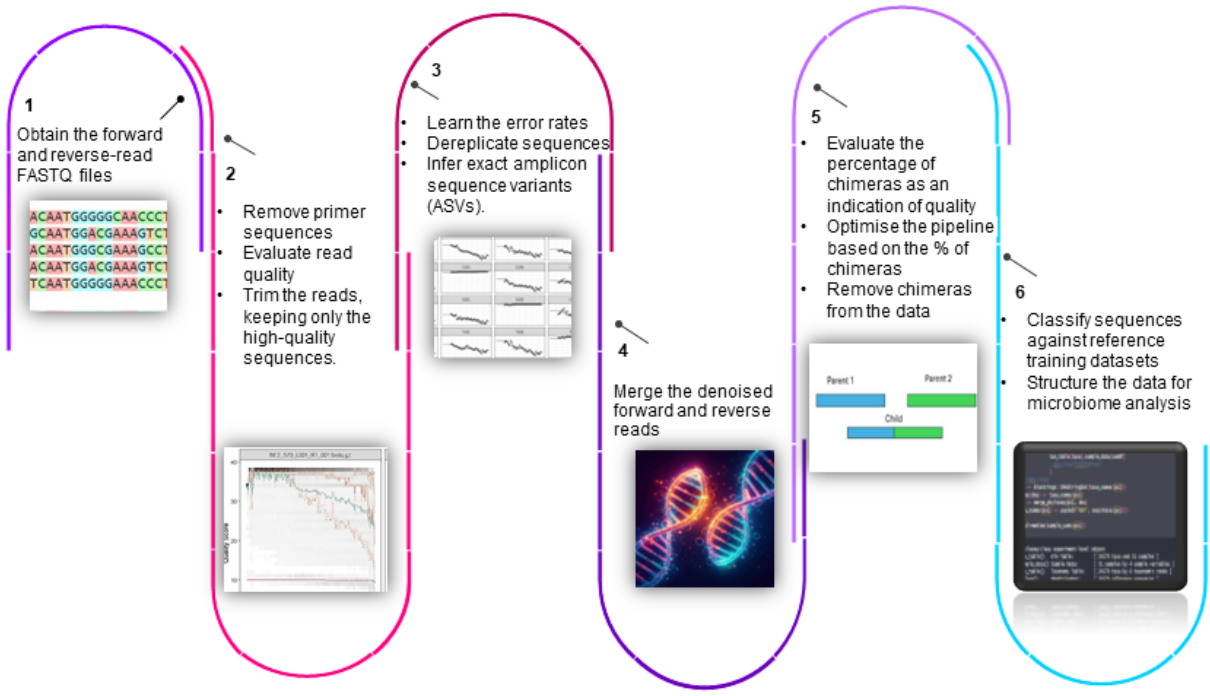

Figure 3: The divisive amplicon denoising algorithm pipeline, which accurately produces data sets of amplicon sequence variants.

Metagenomic studies typically include α- and β-diversity analysis, differential abundance analysis at various taxonomic levels and hypothesis testing (Metcalf et al., 2013; Pechal et al., 2013; Weiss et al., 2016; Singh et al., 2018). When statistically analysing metagenomic data sets, the fraction of the metagenome represented by the data set (coverage) is critically important to prevent type I and II statistical errors. Rarefaction is a qualitative method to assess the coverage (Rodriguez-R and Konstantinidis, 2014) and involves the subsampling of samples at an increasing amount of sequences (increasing sampling depths) until the entire sample's sequences are included in the subsample (Schloss and Handelsman, 2005; Schloss et al., 2009). A collectors curve (rarefaction curve) is created by plotting the number of unique sequences (species) observed from each subsample as a function of the number of sequences in the subsample (Figure 27, Appendix C). As the species richness becomes saturated, the slope of the curve approaches zero (plateau), indicating full coverage (Rodriguez-R and Konstantinidis, 2014; Lin and Peddada, 2020). To prevent statistical errors, the samples that do not plateau should be excluded from hypothesis testing against samples that did (Rodriguez-R and Konstantinidis, 2014; Weiss et al., 2017; Lin and Peddada, 2020).

Even saturated samples can differ considerably in sequence depth, and normalisation of the data set is usually required (Schloss and Handelsman, 2005; McMurdie and Holmes, 2014; Cameron et al., 2021). Most frequently, the library sizes are rarefied to the same sampling depth (Weiss et al., 2017; Lin and Peddada, 2020), such as the smallest sample size (Cobaugh et al., 2015). Rarefying library sizes has negative aspects. A study by Cameron et al. (2021) indicated that ordination patterns remained well preserved with substantially rarified data, whereas α-diversity index values were reduced and became more variable. Rarefying samples for normalisation has been criticised for potentially reducing statistical power and excluding valid data (McMurdie and Holmes, 2014; Weiss et al., 2017; Lin and Peddada, 2020; Cameron et al., 2021). Alternative methods of normalisation such as total-sum scaling (TSS), Cumulative-Sum Scaling (CSS) and Trimmed Mean of M-values (TMM) have been utilised, each with its assumptions, advantages and disadvantages (Weiss et al., 2017; Lin and Peddada, 2020).

An essential aspect of characterising microbial succession is an examination of shifts in the relative abundance of taxa at various taxonomic levels (Metcalf et al., 2013; Lauber et al., 2014; Cobaugh et al., 2015; Weiss et al., 2016; Singh et al., 2018). Estimating the relative abundance of carcass-associated soil microbiomes has primarily involved normalising data followed by parametric or non-parametric hypothesis testing of the differences between treatments. Relative abundance analysis has mainly been performed using software packages such as QIIME (qiime.org/) and Mothur (Schloss et al., 2009). The need for statistical methods to assess quantitative differences of taxa across groups of samples has resulted in numerous algorithms, methods and software packages being developed for differential analysis (DA). The methods and algorithms need to account for non-normal distributions of data, variance of the mean, and small samples (n = 2¬3). The differential expression analysis for sequence count data applied by the DESeq2 package (Anders and Huber, 2010), initially developed for RNA sequence analysis, and the analysis of the composition of microbiomes (ANCOM) (Mandal et al., 2015) are examples of such methods. Each method has statistical assumptions, advantages and disadvantages, and some are considered better than others in reducing type I and II statistical errors (Love et al., 2014; Weiss et al., 2017; Lin and Peddada, 2020).

Some of the taxa identified as differentially abundant may be considered microbial biomarkers if they are indicative of biological processes. Microbial biomarkers can determine and predict

outcomes of ecological events and processes such as contamination (Karadžić et al., 2021), prevailing environmental conditions (Millard and Singh, 2010) and the presence of higher species (Iancu et al., 2020), and are helpful in ecotoxicology, conservation and agriculture as well as forensics. Identifying potential biomarkers may explain how biological samples differ and is the first step towards understanding the underlying mechanisms of microbial interactions. However, an analysis of why biological samples differ, referred to as a class comparison, should be consistent, provide statistically significant results, indicate the effect size of the biomarker, and be suitable for biological explanations. The linear discriminant analysis of effect size (LEfSe) method provides high-dimensional class comparisons of metagenomic data, determining the taxa most likely to explain differences between treatments. LEfSe utilises non-parametric tests to identify statistically significant ASVs between treatment groups. Additional tests are incorporated to ensure the differences are consistent across subclasses within each class, confirming their biological relevance. LEfSe then uses linear discriminant analysis (LDA) to estimate the effect size of each ASV, quantifying the magnitude of the differences and affording the prioritisation of each ASVs relative impact (Segata et al., 2011).

2.5. List of References

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Chapter 3
The functional response of mesic-grassland soil microbiomes beneath decomposing large herbivore carcasses

3.1. Introduction

The degradation of grasslands is occurring on every continent at an alarming rate. Grasslands cover approximately 26% of the world's ice-free land, and their soil contains approximately 20% of the world's soil organic matter (SOM) (Montanarella et al., 2015). They are perpetual carbon sinks (Smith, 2014) that may be more reliable than forests (Dass et al., 2018) if managed sustainably (Chang et al., 2021). Grasslands are particularly suitable for large herds of herbivores, with different herbivore species occupying the same ecological niche in different geographical regions (Van As et al., 2012). Its ability to support large herds gives it agricultural application. When managed at low stocking rates, herbivores increase grassland plant diversity (Olff and Ritchie, 1998; Bakker et al., 2006), which, in turn, increases grassland productivity and sustainability (Naeem et al., 1994; Tilman et al., 1996; Isbell et al., 2011). Although ample in grasslands, grass detritus decomposes slowly and is relatively nutrient-poor (Swift et al., 1979). Large herbivores, such as *Connochaetes taurinus* (wildebeest), are proficient in digesting grass. Although not as selective as smaller herbivores, large herbivores prefer large grass patches with the most nutrients, which they distribute to relatively small areas by faeces and urine. Some nutrients are immediately available to plants, whereas about 20% return to the soil (Owen-Smith, 1999). Despite being labile and nutrient-rich, nutrient losses by the exporting animals from the grasslands are considered unimportant in large ecosystems (Putman, 1978; Owen-Smith, 1999; Parmenter and MacMahon, 2009).

Soil and plant tissue near carcasses have increased nitrogen levels, increased plant growth, and are presumed to moderate microbial activity (Parmenter and Macmahon, 2009; Barton et al., 2013). Recent empirical studies have demonstrated that carcasses significantly alter ecosystem processes by influencing long-term soil properties, depending on what is considered long-term (Yang and Janssen, 2002; Carter et al., 2007; Benninger et al., 2008; Strickland et al., 2009; Bradford et al., 2008; Cotrufo et al., 2013; Macdonald et al., 2014; Benbow et al., 2016). Labile nutrients, for example, are more efficiently assimilated into microbial biomass and used in their

metabolic products (Yang and Janssen, 2002; Cotrufo et al., 2013). Extensive labile nutrient inputs, such as a wildebeest carcass, may change a microbiome's preferential substrate utilisation (Wu et al., 1993; Fontaine and Barot, 2005) because the microbial community structure shifts towards copiotrophs, potentially increasing SOM formation. In contrast, priming (Dalemberg and Jager, 1981) occurs when small amounts of labile nutrients augment the soil and the microbial activity is accelerated, but oligotrophic species remain dominant at a loss of SOM (Strickland and Wickings, 2015). Nevertheless, conditions that may increase SOM in an agricultural setting do not necessarily increase SOM in non-agricultural grasslands (Bradford et al., 2008).

Soil quality is defined by its functions, which include providing a stable structure, gaseous exchange, water retention and transmission, and nutrient cycling and retention. Thereby, soil sustains ecosystem productivity and supports plant and animal health and habitation (Swanepoel et al., 2015; Lal, 2016). Soil microbial functions are the foundation of soil quality, stability and resilience (Allison and Martiny, 2008; Habig and Swanepoel, 2015). A diverse group of functions affords good soil quality. The functions are usually performed by a diverse group of microorganisms that produce the variety of extracellular enzymes required to degrade and cycle the nutrients (Hirsch, 2019). Thus, functional diversity, related to genetic diversity, is affected by inter-specific interactions and abiotic and biotic environmental conditions, which can characterise and distinguish microbiomes. Microbial functional diversity can be defined as the number, types and rates at which a microbial community utilises various nutrients (Zak et al., 1994). Functional diversity links microbial structure and interactions, biogeochemical cycles, ecosystem functioning and responses to environmental change (Krause et al., 2014; Escalas et al., 2019). Microbial function, functional diversity and succession are often *inferred* from taxa, their phylogenetic relatedness, and their associated functional genes (Weiss et al., 2016; Metcalf et al., 2016).

The accuracy of inferring a wide range of microbial functions by a single highly conserved marker gene, such as 16S rDNA, is spurious and debatable for complex microbiomes (Iwai et al., 2016; Franzosa et al., 2018; Escalas et al., 2019), especially since it does not account for transposable elements and plasmids, which often confers catabolic processes (Barton and Northup, 2011, Escalas et al., 2019). Moreover, only partial and inaccurate function predictions

will be possible with limited knowledge of the associated functional genes. Therefore, researchers often employ other methods that analyse the *actual* function and functional diversity, such as Community-level Physiological Profiling (CLPP) by sole-carbon-substrate utilisation (Garland, 1997; Pechal et al., 2013), multiple substrate-induced respiration (Singh et al., 2018), multi-enzyme assays (Scola et al., 2018), oxygen consumption assays (Gomez and Garland, 2012), by itself or in combination. These methods have seldom been used in the functional analysis of necro-microbiome studies. (Pechal et al., 2013; Singh et al., 2018). Pechal et al. (2013) successfully used Biolog™ EcoPlates™ (Biolog Inc., CA, USA) to characterise microbial community functional change on the skin and within carcasses' cavities. Singh et al. (2018) effectively used limited substrates to investigate functional spatial and temporal dynamics during human cadaver decomposition. Our study used Biolog™ EcoPlates™ to measure the sole-carbon-substrate utilisation of necro-associated soil microbiomes to construct CLPP. Although the method has demonstrated repeatability, discriminating power, and sensitivity to environmental factors and reflects aspects of the indigenous bacterial community catabolism, it is not without biases, limitations and methodological difficulties (Haack *et* al., 1995; Preston-Mafham et al., 2002; Rutgers et al., 2016). The use of sole-carbon substrates excludes many syntrophic bacteria (van Elsas et al., 2019); not all bacteria and fungi can reduce tetrazolium, which is used as an indicator in EcoPlates (Preston-Mafham et al., 2002). Particular caution must be taken when collecting, treating, and inoculating samples. Moreover, CLPP must be constructed utilising a metric appropriate to the experimental design and research question (Weber and Legge, 2010).

Microbial community structure and, by extension, function changes during carcass decomposition and remain changed for a substantial time. However, there is a paucity of empirical evidence of the temporal scale and succession of the change (Finley et al., 2015; Guo et al., 2016; Metcalf et al., 2016; Strickland and Wickings, 2015; Wang et al., 2023). The extent of the change and the temporal scale and succession are influenced by unpredictable abiotic factors such as temperature, humidity, precipitation, and ultraviolet radiation (Carter et al., 2007; Tiao et al., 2012; Singh et al., 2018), as well as biotic factors such as former microbial structure and function, existing preferential substrate utilisation or priming (Dalemberg and Jager, 1981; Fontaine and Barot, 2005), carcass mass (Weiss et al., 2016; Singh et al., 2018), seasonality (Puissant et al., 2015) and scavenging (Benninger et al., 2008; Aitkenhead-Peterson et al., 2012;

Hyde et al., 2013). Most soil microbiome and nutrient studies related to carcass decomposition are forensically focused, investigating changes in microbial community structure to improve estimations of the time since death, called postmortem interval (PMI) of humans (Hopkins et al., 2000; Aitkenhead-Peterson et al., 2012; Barton et al., 2013; Benninger et al., 2008; Cobaugh et al., 2015; Lauber et al., 2014; Metcalf et al., 2016). As a consequence, primarily short time scales (<12 months) and small to medium-sized carcasses such as rodents, swine (~25 kg), kangaroo (~31 kg), and human cadavers (47 ¬153 kg) have been used. The grasslands of southern Africa are unique because of the extraordinary number and diversity of large-bodied herbivores (>200 kg, which include buffalo (*Syncerus caffer*), kudu (*Tragelaphus strepsiceros)* and wildebeest (*Connochaetes .spp*) (Owen-Smith 1999; Jones et al., 2015). Research on carcass decomposition with comparable sizes is limited (Towne, 2000). Up to 386 kg/km^2 of herbivore carcasses have been recorded annually in natural grassland and savannah woodlands in southern Africa, although the figure is likely underestimated (Jones et al., 2015). The grasslands are subjected to a significant difference in precipitation and temperatures between seasons, with mesic grasslands being more productive than arid grasslands. Southern Africa encompasses a diversity of large vertebrate predators and scavengers (Jones et al., 2015), influencing carcass decomposition dynamics and impacting soil microbial communities.

This study reports on the temporal scale and succession of soil microbial community function beneath large herbivore *Connochaetes taurinus* (wildebeest) carcasses within a mesic grassland of southern Africa over eighteen months. Overall catabolism, functional alpha-diversity (α-diversity), functional dissimilarity, preferred substrate-guild utilisation, and soil nutrients were compared at six-month intervals.

3.2. Materials and Methods

3.2.1. Study area

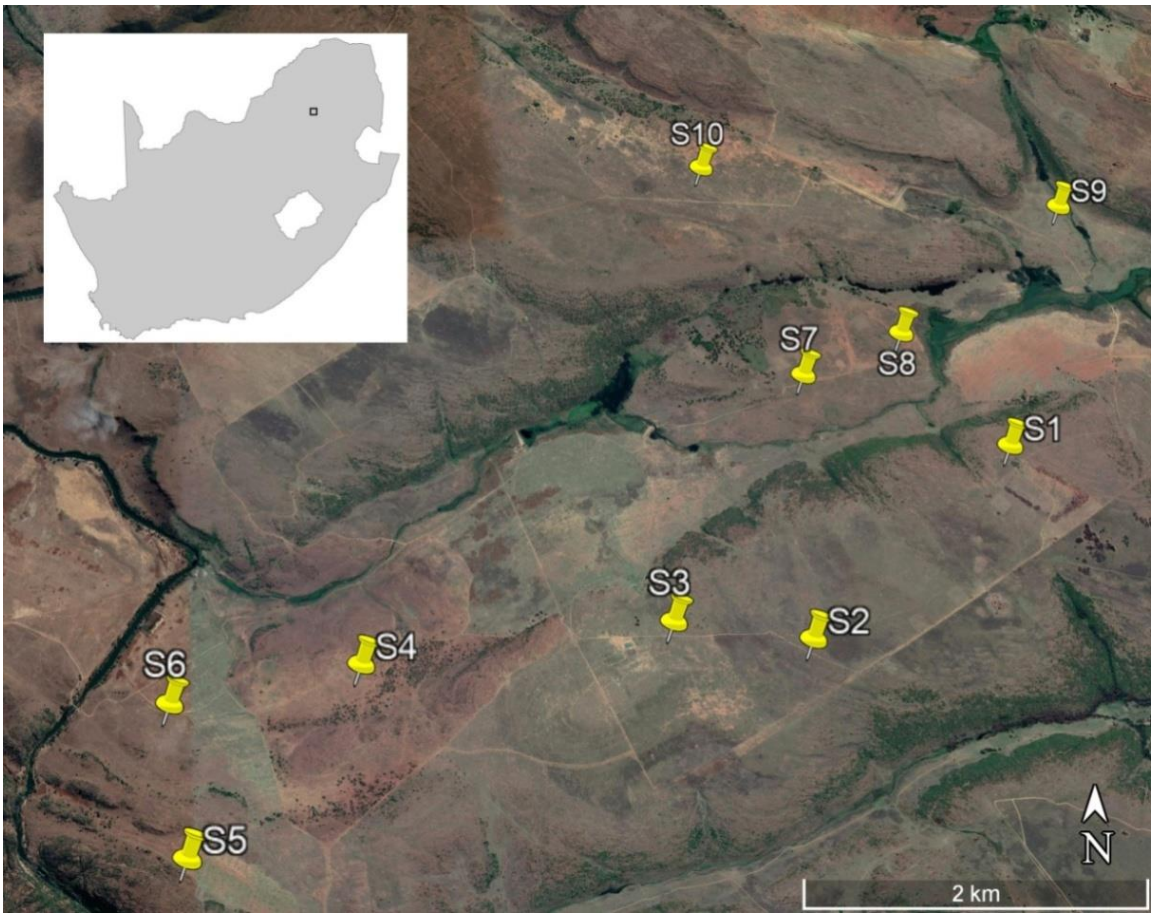

Figure 4: Modified map from Google Earth Pro (Google Earth Pro, 2020) indicating the sampling sites within the Telperion Nature Reserve, South Africa. The outline map of South Africa was obtained from the Ginko Maps Project (2018).

Figure 5: Visual contrast between winter and summer vegetation (J. Fouché).

The Telperion Nature Reserve, situated on the Mpumalanga and Gauteng provincial border in South Africa (25.7039° S, 28.9814° E), was selected as the study area (Figure 4). The study area forms part of the Mesic Highveld Grassland and Loskop Mountain Bushveld biomes ecotone and has a dry-winter subtropical highland climate (Mucina and Rutherford, 2006), significantly contrasting winter and summer vegetation (Figure 5).

Ten unspoiled and intact *Connochaetes taurinus* (Wildebeest) carcasses were obtained from the Nature Reserve's culling process on the 21st and 28th of January 2019. The carcasses were secured to metal grids (Figure 7) that prevented scavengers from removing large sections, after which the carcasses were placed in typical grassland vegetation and on level ground to reduce run-off during precipitation. The carcasses were placed more than 900 meters apart to encompass a variety of grassland conditions (Figure 4). The exact carcass locations were marked with dropper poles, and their coordinates were electronically tagged.

3.2.2. Experimental design and sampling

The experimental design is summarised in Figure 6. The first soil samples were collected during the wet season (November to March), one month postmortem, when the carcasses had opened naturally from decomposition, and its nutrients had seeped into the soil. Each soil sample consisted of three increments collected below the head, abdomen and hindquarters and were combined to represent the 'Beneath' sample of the carcass. In addition, three equidistant subsamples were collected at a five-meter radius from a carcass representing the 'Control' sample for a site (Figure 7). The samples were collected aseptically at a 0¬10 cm depth using a soil core-sampling tool with a 2 cm diameter. The corer was washed with water, dried with a paper towel, sprayed with 70% ethanol and flame-dried before taking a sample. Each composite sample was placed in a Ziploc™ bag and kept at four °C for 24-h before processing. Sampling also took place six, twelve, and eighteen months after the carcasses were placed (postmortem intervals), during which the exact location of the previous sampling was avoided.

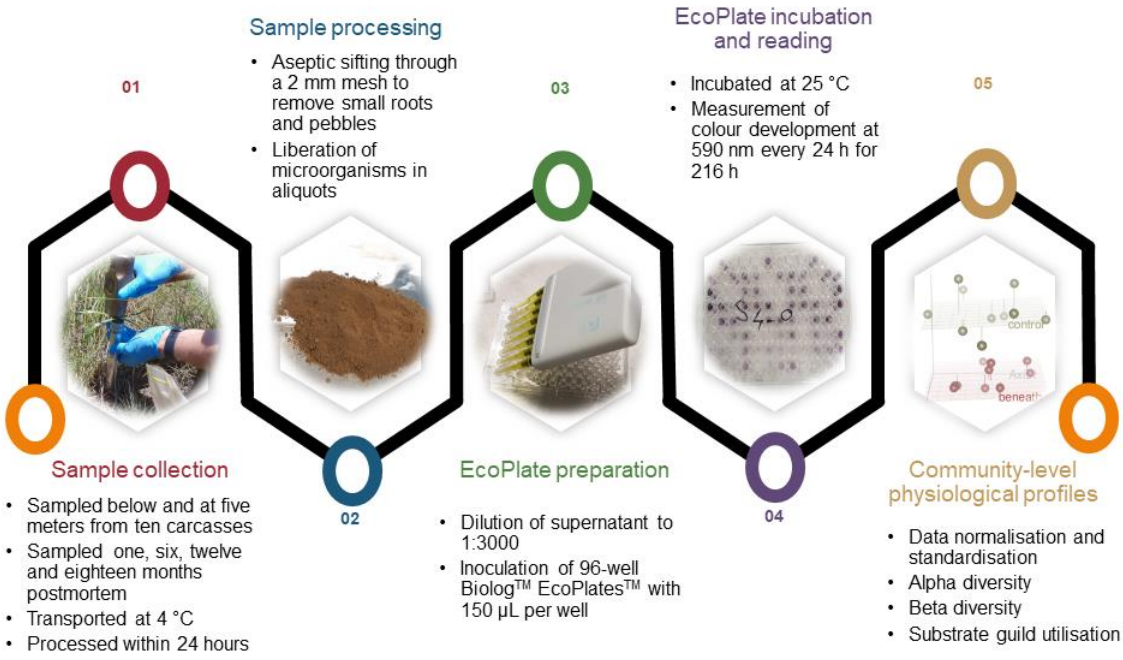

Figure 6: EcoPlate™ experimental design consisted of sample collection and processing, plate preparation, incubation and reading, and constructing community-level physiological profiles.

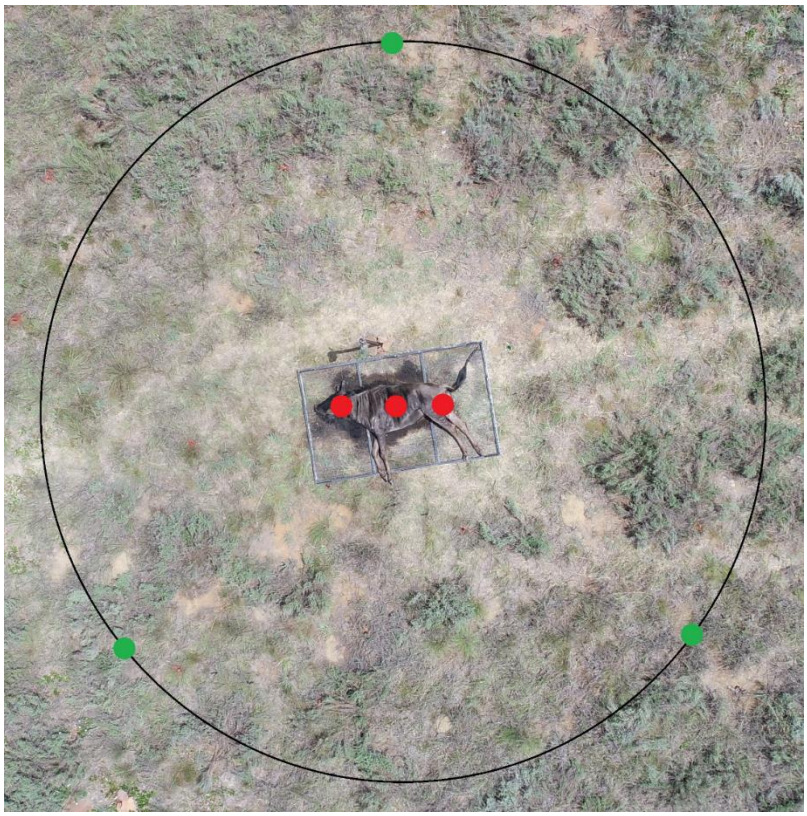

Figure 7: *Connochaetes taurinus* (Wildebeest) carcass positioned on a grid within typical grassland vegetation. Sampling increments were collected from beneath the head, abdomen, and hindquarters, as indicated in red, and combined to form the carcass's 'Beneath' sample. The black circle represents a five-meter radius (not to exact scale). Control samples, shown in green, were taken along the same radius at equal intervals from the centre, ensuring each sample was equidistant from the centre and from each other.

3.2.3. EcoPlate ™ preparation and standardisation

The soil samples and aliquots were prepared as described by Habig and Swanepoel (2015) with slight modifications. The soil samples were aseptically sifted through a 2 mm steel mesh to remove roots and pebbles. Ten-gram sample aliquots were added to glass vessels containing 90 mL of sterile ultra-pure water. The vessels were shaken for an hour at 200 rpm in an incubator at 25 °C to liberate soil microorganisms. After that, the vessels were kept stationary for an hour to facilitate particle precipitation. Based on preliminary test results of inoculation density, the supernatant was diluted 3000 times and used to inoculate 96-well Biolog™ EcoPlates™ with 150 µL per well. The remaining soil from the samples was frozen at -80 °C for subsequent metagenomic analysis.

Each EcoPlate™ has three control wells without substrate and a triplicate of 31 wells containing a single carbon substrate. All the wells contain tetrazolium dye, which produces formazan during carbon-substrate utilisation, resulting in colour development (Stefanowicz, 2006). The colour development was measured by its optical density (OD) at 590 nm directly after inoculation and every subsequent 24-hour time point up to 216-h, whilst the plates were incubated at 25 °C (Sofo and Ricciuti, 2019). The plates' mean control-well OD (OD0) was subtracted from the mean substrate-well OD (OD_k), and differences of less than 0 were converted to 0 to correct for colour development from substrates in the inoculum.

Data normalisation and transformation

Weber and Legge (2010) recommend selecting an appropriate metric of a single incubation time point, similar OD values, or a logistic curve fitting value for comparing carbon substrate utilisation. Control-well corrected (OD_k - OD_0) values were used to calculate EcoPlate™. Average well-colour development (AWCD) was monitored throughout the incubation period of each sample. The AWCD phase (lag, exponential or static), the number of unresponsive wells (OD_k <0.25) per group, wells outside the linear OD range (OD_k > 2), and the variance between well optical densities were considered as criteria in selecting an appropriate metric for comparison (Weber and Legge, 2010). Because similar AWCD occurred during different developmental phases for the Beneath and Control groups, a single time-point metric that met the before-mentioned criteria was selected for comparison. Comparing community profiles at

different AWCDs may incorrectly imply functional differences; hence, the control-well corrected OD values (OD_k - OD_0) were first normalised by the plate's AWCD before further analysis (Preston-Mafham et al., 2002; Weber and Legge, 2010) and calculated as;

$$\bar{OD}_k = \frac{(OD_k - OD_0)}{\frac{1}{31}\sum_{i=1}^{31}(OD_i - OD_0)}$$

Equation 2: Equation to normalise optical density (OD) values by the plate's AWCD The denominator represents AWCD.

Before hypothesis testing, a binary logarithmic transformation was implemented, improving the overall normality and homogeneity of variance as confirmed by the Shapiro-Wilk and Levene's tests (Legendre and Legendre, 2012; Weber et al., 2007).

3.2.4. Community-level Physiological Profiling and Statistical Analysis

All statistical analysis was performed in *R* version 4.2.0 (R Core Team 2022). The *stats* (ver. 4.2.0) and *car* (ver. 3.0-11) packages were used for univariate analysis, and the *vegan* community ecology package version 2.5-7 (Oksanen et al., 2017) was used for multivariate analysis. Two-dimensional visualisations were created with the *ggplot2* (ver. 3.3.5), and three-dimensional visualisations were created on *rgl (*ver. 0.107.14). Assumptions of normality and homoscedasticity were tested and not met. Therefore, non-parametric analysis and *post hoc* tests were performed with the Benjamini-Hochberg (BH) *p*-value adjustment for a false discovery rate (Benjamini and Hochberg, 1995). Because the control samples were collected at a radius of five meters from each carcass (Figure 4), univariate statistical tests were performed using the two-tailed Wilcoxon signed-rank test for paired data to reduce the effect of confounding factors. All multivariate statistical tests were performed with 9999 random permutations.

Average well-colour development

Average Well Colour Development (AWCD) can be used as an indication of overall catabolism (microbial function) (Pechal et al., 2013). The AWCD was compared at 144-h of EcoPlate incubation as it met the single time-point metric criteria (Preston-Mafham et al., 2002). The raw standardised AWCD results were compared between treatment groups of the same sampling month and within treatment groups at different sampling months. The loess smooth function in

the R package *ggplot2* (ver. 3.3.5) was used to fit smooth curves over the AWCD over time plots (Metcalf et al., 2016). The non-parametric Kruskal-Wallis and Dunn's pairwise *z*-score statistic was used to determine significant differences between the categories.

Alpha-diversity

Alpha-diversity (α-diversity), defined as the extent and number of substrates being utilised, was measured with diversity indexes. The inverse Simpson's diversity index (D_2) was selected for α-diversity measurement for its sensitivity to higher values (Morris et al., 2014) because low OD values are not indicative of substrate utilisation (Preston-Mafham et al., 2002). Pielou's evenness index (*J'*) measured the evenness of substrate utilisation. The Wilcoxon paired difference test was used to determine if there was a significant difference in α-diversity and evenness.

Beta-diversity and dispersion

To determine significant differences in functional dissimilarity/beta-diversity (β-diversity) between groups, substrate-utilisation data matrices were first Wisconsin double-standardised, after which Bray-Curtis dissimilarity index values were computed (Bray and Curtis, 1957; Legendre and Legendre, 2012). After that, non-metric multidimensional scaling (nMDS) was performed using the *metaMDS* function (Kruskal, 1964). Finally, an analysis of similarity (ANOSIM) was performed using the *anosim* function, which would indicate statistical significance in the rank order of dissimilarity (Clarke, 1993). The nMDS was visualised three-dimensionally with the *scatter3d* function. The ANOSIM statistic (*R*) and *p* values were added to the nMDS visualisations. The *betadisper* function was used to measure the dispersion around the geometric centre (PERMDISP) for each group (Anderson et al., 2006), and statistical differences were indicated by the permutation test using the *permutes* function. The *adonis* function was used to perform a permutational multivariate analysis of variance (PERMANOVA) and statistically confirm if the geometric centres of the groups were similar (Anderson, 2001).

Substrate-guild utilisation

Table 1: The 31 carbon substrates of the Biolog™ EcoPlate™ (Biolog, 2024), grouped into six chemical guilds.

Amines (Amns)	**Carbohydrates (Crbh)**	**Carboxylic acids (CrbA)**	**Phenols (Phnl)**
Phenylethylamine	β-Methyl-D-Glucoside	D-Galacturonic Acid	2-Hydroxy Benzoic acid
Putrescine	D-Xylose	Pyruvic Acid Methyl Ester	4-Hydroxy Benzoic acid
Amino acids (AmnA)	D-Galactonic Acid γ-Lactone	γ-Aminobutyric acid	**Polymers (Plym)**
L-Arginine	i-Erythritol	D-Glucosaminic Acid	Polysorbate 40
L-Asparagine	D-Mannitol	Itaconic Acid	Polysorbate 80
L-Phenylalanine	N-Acetyl-D-Glucosamine	α-Keto Butyric Acid	α-Cyclodextrin
L-Serine	D-Cellobiose	D-Malic Acid	Glycogen
L-Threonine	Glucose-1-Phosphate		
Glycyl-L-Glutamic Acid	D, L-α-Glycerol Phosphate		
	α-D-Lactose		
	D-Glucosaminic Acid		

Following Rutgers et al. (2016), the 31 carbon substrates were grouped into six substrate guilds representing their chemical nature (Table 1). The percentage that each substrate guild contributed towards describing β-diversity (SIMPER) was evaluated with the *simper* function (Clarke 1993). The differences in mean substrate-guild utilisation between treatment groups for each sampling month provided an additional method for comparing community profiles between groups (Zak et al., 1994).

Soil nutrient analysis

Soil samples of months one, six, twelve and eighteen from sites two, four, five and eight (Figure 4) from the Beneath and Control groups (Figure 7) were analysed by the Institute of Soil, Climate and Water of the Agricultural Research Council of South Africa. The potassium chloride extract method measured ammonium (NH_4) and nitrate (NO_3) ion contents. Phosphorus (P) content was measured using the Bray 1 method (Bray and Kurtz, 1945), and sulphate (SO_4) was measured using a 1:10 soil water extraction method.

Redundancy analysis

Substrate-guild utilisation data from sites two, four, five and eight were merged with their nutrient data to perform a Redundancy Analysis (RDA) and identify the nutrient(s) that significantly correlated with the observed clustering. The *ordistep* function of *vegan*, which uses a stepwise model-building approach, was used to identify models with statistical significance ($p < 0.05$). Model significance was evaluated through permutation tests (Borcard et al., 2011).

3.3. Results and Discussion

3.3.1. Site description

The study area lies at 1300 ± 120 m above sea level. Rocks from the Waterberg Group, consisting of conglomerate, grit sandstone and quartzite with subordinate shale (Visser et al., 1961), underlie the reserve. Small tributary streams and wetlands that join the Wilge River, which flows through the reserve, provide water throughout the year and allow for ridges, rocky cliffs, and valleys. Soil for the ten sites shared a similar water holding capacity (M = 29.8%, SD 4.41). The area is subjected to a dry-winter subtropical highland climate. In the two-year sampling period, the mean ambient minimum and maximum temperatures during the dry period (May to September) were 5 and 23 °C, respectively, during which an annual mean of 40 mm of precipitation occurred. During the wet period (November to March), the mean minimum and maximum temperatures were 15 and 28°C with 510 mm precipitation (SAWS, 2021) (Appendix A). The difference in precipitation results in a big contrast in vegetational coverage (Figure 4). The vegetation is heterogeneous, consisting of grassland and woodland communities maintained by microclimatic conditions (Bredenkamp and Brown, 2003). Common grassland species include *Fimbristylis hispidula*, *Cleome rubella*, *Eragrostis curvula*, *Phyllanthus parvulus* and *Cynodon dactylon*. The encroaching shrub *Seriphium plumosum* is present with *Kyllinga brevifolia* and *Perotis patens* in the more degraded grassland areas (Graham et al., 2020).

3.3.2. Carcass decomposition

Upon death, respiration ceases, which ends oxidative phosphorylation and initiates autolysis. Autolysis represents the first stage of decomposition, during which carbohydrates, proteins and lipids are liberated from the animal cells (Carter et al., 2007). As proposed by Payne (1965), carcass decomposition starts with the ‘bloated’ stage, during which time enzymes released by anaerobic microorganisms hydrolyses the carbohydrates, proteins and lipids within the carcasses, producing organic and inorganic gasses (Carter et al., 2007; Paczkowski and Schutz, 2011; Forbes and Carter, 2015). The accumulation of gases may cause the purging of fluids from orifices. Increased pressure within the carcass can cause the hide to tear and rupture, as observed in Figure 8B. The opened carcass allows aerobic microbial activity and seeping water, carbohydrates, proteins, lipids, and their derivatives into the soil (Paczkowski and Schutz, 2011;

Barton, 2015; Forbes and Carter, 2015). A single carcass can simultaneously exhibit different stages of decomposition (Matuszewski et al., 2010), and larger carcasses may take longer to decompose (Vass et al., 1992; Spicka et al., 2011). Soil microbiota accelerates carcass decomposition (Lauber et al., 2014), and the extent of acceleration may be affected by microbial community function and functional diversity (Maron et al., 2018).

In this study, the first samples were collected one month postmortem during the 'active stage', characterised by the opened carcasses and maggot activity reaching its peak, and the 'advanced stage' of decay (Figure 8A and B), recognised by an absence of maggots and partial desiccation described by Payne (1965). After six months, the carcasses were within the 'dry' and 'remains' stages (Figure 8C). After twelve and eighteen months, very little of the carcasses remained apart from hair and a few bones (Figures 8D and E). Throughout the experimental period, opportunistic scavengers such as *Phacochoerus africanus* (warthog) (Figure 8F) and *Lupulella mesomelas* (black-backed jackal) visited the carcass, disturbing them slightly. However, large pieces of carcass could not be removed by the scavengers.

Figure 8: Decomposition stages of *Connochaetes taurinus* (wildebeest) carcasses during sampling. (A) After one month, decomposition ranged between 'active' (A) and 'advanced decay' (B). After six months, the carcasses were at the 'dry' and 'scattered remains' stages (C), with the remains decreasing by twelve (D) and eighteen (E) months. Throughout the experimental period, scavengers such as *Phacochoerus africanus* (warthog) visited the carcass, which slightly disturbed the carcass (F).

3.3.3. Community-level profiles

Overall function

Average well-colour development results should be considered in relation to temperature and rainfall results (Appendix A). Dunn's pairwise comparison indicated that the AWCD of the Control group during the wet seasons (months one and twelve) was significantly different compared to the dry seasons (months six and eighteen). Differences in soil and atmospheric temperature and precipitation affect carcass decomposition and desiccation rates (Carter et al., 2007), as well as microbial function and assembly on and below carcasses (Pechal et al., 2013; Singh et al., 2018). Because seasonality was a confounding factor, community-level physiological profile (CLPP) comparisons were only made between the Beneath and Control groups of the same sampling month.

After a month of decomposition, the AWCD of the Beneath group increased considerably compared to Control, reaching the exponential growth phase after 72 incubation hours (Figure 9). Average well-colour development is indicative of microbial function (Pechal et al., 2013) and was significantly (p <0.05) different from the Control group after 144 incubation hours (Table 2). The microbial function was still significantly increased at six months (H (7) = 56.32, p <0.01;), but not at twelve and eighteen months. An unimodal functional increase resulting from a presumed structural shift of oligotrophs to copiotrophs (Metcalf et al., 2013) thus peaked and declined within a year, analogous to the carcasses' ephemeral nature (Carter et al., 2007; Crippen et al., 2016). Overall metabolic function, measured by AWCD, failed to reject the null hypothesis of a short-term increase (<18 months) in overall microbial function. Cobaugh et al. (2015) and Singh et al. (2018) studied overall microbial function from the soil beneath human corpses by measuring soil microbial respiration. In Cobaugh's study, respiration was measured by sodium-hydroxide carbon dioxide trap and titration. Respiration remained significantly (n = 4, p <0.05) elevated from the 'bloated' to the late 'advanced decay' decomposition phase when the last samples were collected (~ 4 months). Singh et al. (2018) measured microbial functional response by adding individual carbon substrates (glucose, glycine, sucrose, oxalic acid, chitin, and cellulose) to all soil samples. Respiration was measured directly from the slurries during incubation using infrared gas analysis. Elevated respiration was observed from soil beneath the corpses for up to 24 months after decomposition started.

Using the EcoPlate™ method, the microbiota is first liberated from the soil, thus excluding nutrients in the soil. However, the EcoPlate™ method relies on the extractability and culturability of the microbiota on sole-carbon substrates, which may explain the difference in experimental results.

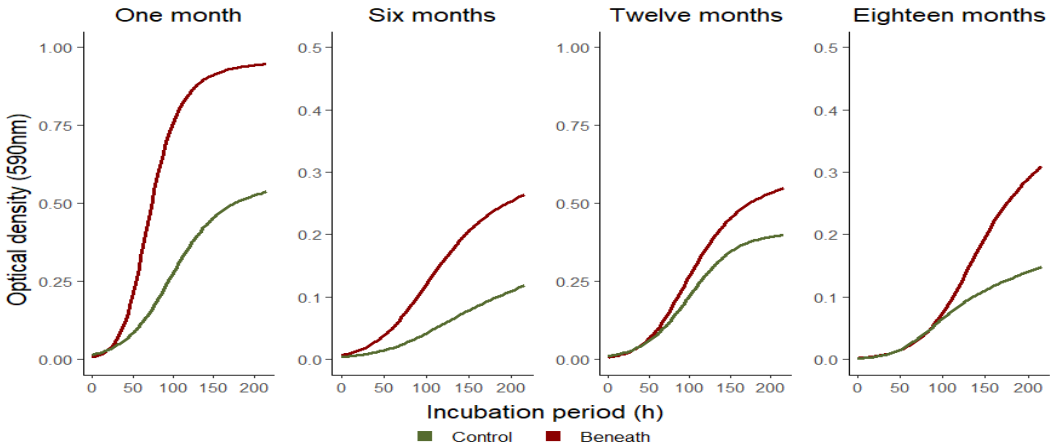

Figure 9: Average well colour development (AWCD) throughout the incubation period from soil collected five meters from (Control) and beneath the carcasses; one, six, twelve and eighteen months postmortem. The curves, created by LOESS (Locally Estimated Scatterplot Smoothing) represent the mean AWCD during incubation.

Functional diversity and dissimilarity

Wet months, one and twelve, had greater diversity and evenness than the dry months (six and eighteen) for the Beneath and the Control groups. The inverse Simpson's diversity (D_2) and Pielou's evenness (J') index values of the Beneath group remained elevated (Table 2: D_2) and significantly different (n = 10; p <0.05) from the Control group for the entire experimental period.

The three-dimensional ordination of Bray-Curtis-based nMDS (Figure 10A) illustrates the microbial community functional β-diversity (dissimilarity). After one month, the Beneath and Control groups were visually differentiated. The Beneath group formed a confined cluster on one side of the ordination plot, and the Control group formed a more expansive cluster on the opposite side. The observed pattern was confirmed by the ANOSIM results, which showed a significant dissimilarity between the treatment groups with some similarities in substrate utilisation (ANOSIM: R = 0.35, p <0.001; Figure 10A). The observed difference in the

dispersion of the groups around its geometric centre (beta-dispersion) was confirmed by a permutational test (PERMDISP: $F_{1,18}$ = 20.88, P <0.001; Figure 10B). Permutational multivariate variance analysis confirmed that the two groups' geometric centres differed statistically (PERMANOVA: $F_{1,18}$ = 4.20, R^2 = 0.19; p <0.001) after a month. The two groups were statistically different at every postmortem interval (ANOSIM: p <0.01), although the dissimilarity steadily decreased over time. At six months, the two groups could still be classified as different with some similarities (ANOSIM: R = 0.26), but at twelve and eighteen months, the groups were similar with some differences (ANOSIM: R <0.25) in substrate utilisation (Clarke, 1993). Beta-dispersion remained statistically different (p <0.01), with the Beneath groups being less dispersed from the centroid (Figure 10B). The Beneath and Control groups did not share the same centroid for up to eighteen months (PERMANOVA: $F_{1,18}$ = 2.26, R^2 = 0.11; p = 0.002).

Given that functional α-diversity and dissimilarity remained statistically different between the Control and Beneath groups throughout the experimental period, the null hypothesis of a short-term (<18 months) change in microbiome functional diversity is rejected. A paucity of soil functional diversity studies on carcass decomposition may result from carcass decomposition studies focusing on forensic taphonomy (Pechal et al., 2013; Finley et al., 2015; Metcalf et al., 2016). Utilising metagenomics and predictive function software, Metcalf et al. (2016) inferred an increase in functional traits associated with corpse decomposition based on the taxonomic-associated functional gene abundances. This approach does not account for functional traits obtained by horizontal gene transfer (Escalas et al., 2019), which is prevalent in nutritionally rich hotspots (Nielsen and van Elsas, 2019), nor does it evaluate the expression of the associated functional genes. Singh et al. (2018) measured substrate-induced respiration of soil slurries from beneath human corpses and observed significant (p <0.01, n = 19) differences in β-diversity and dispersion compared to peripheral soil (1 and 5 m radius) and over time (over 732 days). Substrate-induced respiration of their 1 and 5 m groups did not differ. In contrast to our result, Singh indicated that samples from beneath the corpses were more dissimilar (dispersed), and samples from 1 and 5 meters were less dispersed (Figure 10A).

Table 2: Community-level physiological profiles (CLPPs) compared after 144-h incubation. Differences in Average Well Colour Development (AWCD) were determined by *post hoc* Dunn's test with BH-adjusted p values, and inverse Simpson's (D_2) and Pielou's evenness (J') index values were compared by the two-tailed Wilcoxon paired difference test. Statistical differences (p <0.05) between the 'Beneath' and 'Control' groups of the same month are indicated by different capital letters and, within the same treatment group at different months, by different letters in brackets ( ). Mean values ± standard error.

	CLPP							
	One month		Six months		Twelve months		Eighteen months	
	Control	Beneath	Control	Beneath	Control	Beneath	Control	Beneath
AWCD	$0.41 \pm 0.05^{A(a)}$	$1.06 \pm 0.09^{B(a)}$	$0.08 \pm 0.02^{A(b)}$	$0.30 \pm 0.08^{B(bc)}$	$0.31 \pm 0.05^{A(a)}$	$0.42 \pm 0.06^{A(c)}$	$0.11 \pm 0.02^{A(b)}$	$0.18 \pm 0.02^{A(b)}$
D_2	18.8 ± 1.08^{A}	24.6 ± 0.83^{B}	8.8 ± 1.14^{A}	13.7 ± 1.47^{B}	15.5 ± 1.30^{A}	20.2 ± 1.10^{B}	11.0 ± 1.26^{A}	17.8 ± 1.03^{B}
J'	0.91 ± 0.01^{A}	0.96 ± 0.01^{B}	0.75 ± 0.04^{A}	0.83 ± 0.02^{B}	0.87 ± 0.01^{A}	0.92 ± 0.01^{B}	0.80 ± 0.02^{A}	0.90 ± 0.01^{B}

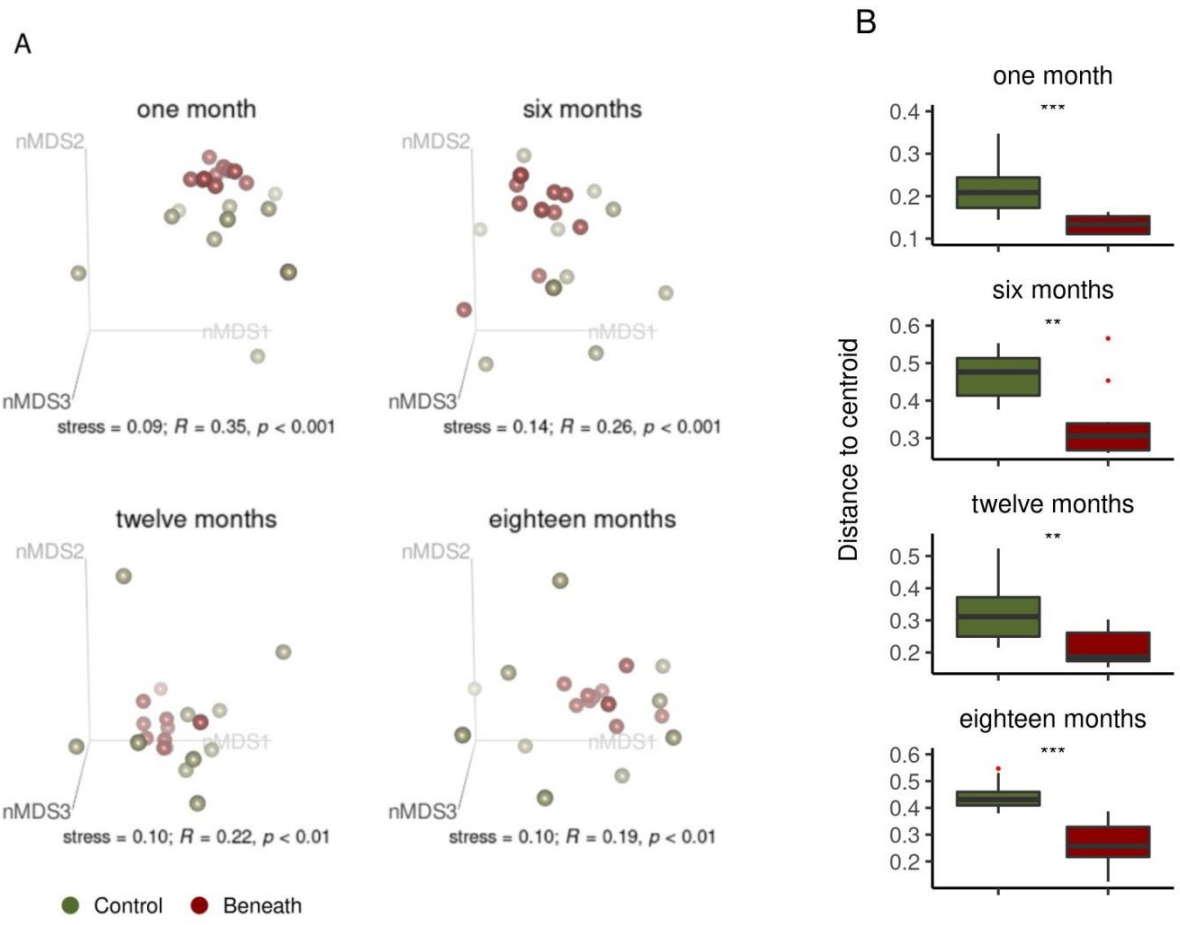

Figure 10: Dissimilarity and dispersion of substrate utilisation after 144-h incubation for the four sampling periods. (A) Non-metric multidimensional scaling (nMDS) indicates stress values, analysis of similarities (R) and statistical significance (p). (B) Box plots illustrating beta-dispersion around the group's geometric centre. Significant differences are indicated as; *** p <0.001, ** <0.01, * <0.05.

Although nutrients from animal carcasses have been deemed insignificant in extensive systems due to rapid decomposition, uneven distribution in space and time and minimal nutrient contribution to the total landscape (Owen-Smith 1999; Parmenter and MacMahon 2009;), the functional diversity results presented here support observations that large decomposing carcasses have a long-term influence on the soil processes of the immediate environment (Cotrufo et al., 2013; Macdonald et al., 2014; Strickland and Wickings, 2015; Benbow et al., 2016; Singh et al., 2018). Increased functional diversity advances ecosystem functioning (Maron et al., 2018; Escalas et al., 2019). As Kuzyakov and Blagodatskaya (2015) described, the short-term availability of labile nutrients, such as a decomposing carcass, creates a *hot moment* at a mesoscale with accelerated microbial processes. The *hot moment* creates a small soil volume with faster and more intensive interaction (a *hotspot*), which, in our study, had greater functional diversity that persisted for more than 18 months. Regular hot moments from similar nutrients will have an accumulative effect on the microbial dynamics at an ecosystem scale (Kuzyakov and Blagodatskaya, 2015). With soil microbiome functional diversity being linked to ecosystem functions (Louca et al., 2016; Escalas et al., 2019), an increase in function will likely have a positive impact on ecosystem services such as soil fertility, food and fibre production and, climate regulation (Delgado-Baquerizo et al., 2015).

Substrate-guild utilisation

The 31 sole-carbon substrates were grouped into six substrate guilds for direct comparison (Table 1). SIMPER analysis of EcoPlate results illustrates that dissimilarity between treatments was mainly driven by amines (Table 3), contributing 26% to β-diversity (Figure 10) at one month. The utilisation of both amine and the amino acid guild was significantly (n = 10, p <0.01) increased beneath the carcasses (Figure 11). Together, amines and amino acids, which consist of an amine and a carboxylic acid functional group, accounted for 44% of β-diversity. Similar to our results, Macdonald et al. (2014) indicated that soil microbiota beneath kangaroo carcasses significantly increased the turnover rate of the peptide (L-tri alanine) at three months and amino acid (L-alanine) at six months. Metcalf et al. (2016) predicted increased amino acid and amine degradation, utilising taxonomic abundance shifts with functional gene prediction software. Enzyme-level genes of lysine and arginine (amino acids) to produce cadaverine and putrescine (amines) were found to increase at later stages of decomposition, especially in the

desert and short grass landscapes. In our results, amines were significantly (p <0.05) increased at one month due to phenylethylamine (Table 2) (V = 6, p = 0.027) but not putrescine (V = 12, p = 0.131). At six months, phenylethylamine was still increased (V = 3, p = 0.010), although the mean of amines was not (p > 0.05). Phenylethylamine is a biogenic amine generated by the decarboxylation of L-phenylalanine. It has antimicrobial properties to *Escherichia coli* (Yeh et al., 2020), which could further affect microbial structure and function. After a month of decomposition, our results also show that the utilisation of polymers significantly decreased beneath carcasses (Figure 11). The decrease in polymer utilisation results from the increased availability of labile nutrients, which are low in molecular weight and high in energy (amino acids and amines) (Fontaine et al., 2003). These molecules are preferred because they rapidly incorporate into the citric acid cycle, glycolysis, and pentose phosphate pathways (Koner et al., 2021). Although polymer utilisation was significantly reduced (n = 10, V = 49, p = 0.027) beneath the carcasses, the increase in amino acid and amine utilisation still led to an overall catabolic increase (Table 2).

At six months, the Beneath group had a significant increase (n =10, V = 4, p = 0.014) in carboxylic acid utilisation (Figure 11). The difference in carboxylic acid utilisation accounted for most (23%) of the total functional variation between groups, having contributed the least (7%) during the first month (Table 3). Utilisations of the other substrate guilds were not statistically different from the Control group. Carboxylic acid utilisation remained elevated beneath the carcasses at twelve months and was the only significantly different (n =10, V = 8, p = 0.049) guild at this time (Figure 11), although its influence on β-diversity was reduced by 11% (Table 2). Some microbiota excrete proteases during carcass decomposition, degrading proteins into amino acids. Some amino acids are deaminated to carboxylic acids, ammonia, or decarboxylated to biogenic amines and carbon dioxide (Forbes and Carter, 2015). The biogenic amines, such as putrescine, can then be deaminated and oxidised to carboxylic acids, such as γ-aminobutyric acid (Luengo and Olivera, 2020). Arginine, putrescine and γ-aminobutyric acid are included in the assortment of sole-carbon substrates of EcoPlates™ and were grouped according to their substrate guilds (Table 1). More frequent sampling during the initial stages of decomposition may have provided the distinction between amino acid and amine utilisation. The pool of carboxylic acids is further increased by the hydrolysis of lipids, producing glycerol and fatty acids (carboxylic acids with aliphatic chains), as well as the fermentation of carbohydrates,

which produces carboxylic acids (Paczkowski and Schutz, 2011; Forbes and Carter, 2015). The microbiome beneath the carcasses had been stimulated by carboxylic acid, improving its potential to utilise this substrate guild (Grayston et al., 2001). Polymer utilisation was elevated beneath the carcasses at twelve months, although the difference was not statistically different (n = 10, V = 11, p = 0.106) (Figure 11).

By eighteen months, the carcasses had been degraded to scattered dry remains (Figure 5D). Amine utilisation significantly increased (n = *10*, V = 5, p = 0.019) in the Beneath samples, similar to the first month, contributing 21% to functional β-diversity (Table 2). The utilisation of putrescine significantly increased (V = 8, p = 0.049) at this late stage, correlating with the late-stage increases of putrescine degradation prediction of Metcalf et al. (2016). Rutgers et al. (2016) indicated that underlying structures beyond the substrate-guild grouping might be demonstrated in substrate-guild utilisation. Carbohydrate (n = *10*, V = 7, p = 0.037) and polymer (n = *10*, V = 6, p = 0.027) utilisation also significant increased at eighteen months. The polymers contributing to the increase, α-Cyclodextrin and glycogen, contain glucose subunits. Carbohydrates and polymer utilisation contributed 37% to functional β-diversity. As the energy-rich carbon substrates had been exhausted, the higher molecular weight lower energy substrates, which persist longer in soils, were being utilised (Fontaine et al., 2003). The increased utilisation of glucose-containing substrates corresponds with the findings of Singh et al. (2018), who observed an increase in glucose respiration from eleven human corpses (48¬153 kg) on grassland soil (Texas, USA). Singh, however, estimated the increase in glucose to be between eight and twelve months. As the corpses (n =11) were placed at different times of the year over two years, they had to compensate for precipitation and temperature differences, and the difference in increased glucose respiration timing may be a result of size (200¬250 kg), temperature and precipitation differences (Weiss et al., 2016).

Table 3: SIMPER analysis indicating the percentage contribution of each substrate guild in the observed β-diversity at different sampling times. The substrate guilds are ordered according to the overall rank of guild contribution.

	Contribution (%)			
Substrate guild	**One month**	**Six months**	**Twelve months**	**Eighteen months**
Amines	26.43	12.2	20.96	21.48
Polymers	19.74	16.7	18.26	19.89
Phenols	16.29	21.15	20.82	12.68
Amino Acids	17.8	16.15	17.8	15.14
Carboxylic Acids	9.24	23.28	11.29	13.48
Carbohydrates	10.5	10.52	10.87	17.33

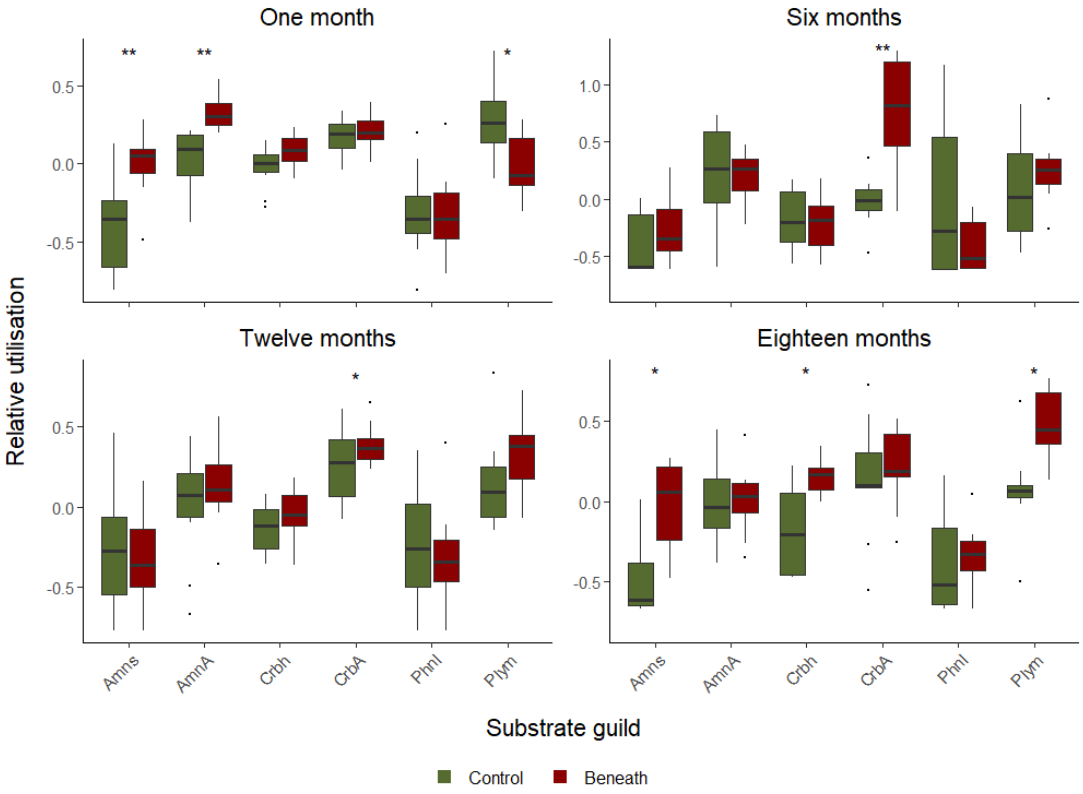

Figure 11: Relative utilisation of the six substrate guilds: amines (Amns), amino acids (AmnA), carbohydrates (Crbh), carboxylic acids (CrbA), phenols (Phnl) and polymers (Plym) at one, six, twelve and eighteen months after carcass placement. Values were compared at 144-h of incubation. Significant differences between group means are indicated as *** p <0.001, ** p <0.01, * p <0.05.

Soil nutrients

Control sample measurements of ammonium (NH_4), nitrate (NO_3), phosphorus (P) and sulphate (SO_4) taken at the different sampling time points were not statistically different ($n = 4$, $p > 0.05$) (Table 4). The similarity in Control samples validates a radius of five meters as a Control, as it indicates that nutrients from the carcasses did not spread to that extent during the experiment. At one month, the described deamination of amino acids to carboxylic acid and ammonia is reflected in the 20-fold increase of ammonium (NH_4: $\chi^2 = 27$, $df = 7$, $p < 0.001$). The deamination of sulphur containing amino acids and enzymes, found at high concentrations in animal tissue (Parmenter and MacMahon, 2009; Forbes and Carter, 2015), caused an 8-fold increase in sulphate (SO_4: $\chi^2 = 18.3$, $df = 7$ $p < 0.01$) during this period. Increases in ammonium and sulphate were still significant at six months but not at twelve and eighteen months. Webster et al. (2005) showed that the nitrifying bacteria in grassland soil start oxidising augmented ammonia within three days. Nitrification of ammonium was not evident beneath the wildebeest carcasses within the first month despite an abundance of ammonium at one month. The heavy wildebeest carcasses were relatively undisturbed until the 'advanced decay' stage when they were lighter from desiccation and easily moved by scavengers (Figure 5C). The anaerobic environment created beneath the carcasses may have prevented nitrification. Aitkenhead-Peterson et al. (2012) observed similar high-ammonium and low-nitrate levels beneath undisturbed corpses. At six months (dry and remains stage), nitrate levels beneath the wildebeest carcasses were increased 33-fold (NO_3: $\chi^2 = 24.6$, $df = 7$, $p < 0.001$) (Table 4).

At one month, phosphorus levels beneath the carcasses were elevated but not statistically different from the Control samples. At six months, it had increased 52-fold (243 ppm) compared to the Control group (P: $\chi^2 = 26.5$, $df = 7$ $p < 0.001$) and remained significantly elevated (Table 4) throughout the experiment. From measuring soil-extracted phosphorus and lipid-phosphorous beneath pig (*Sus scrofa*) carcasses, Benninger et al. (2008) observed a rapid increase in phosphorous, culminating in nearly 15 days and decreasing during the sampling period of 100 days. Phosphorus in soft tissue and bones is lost from carcasses to the soil at slower rates than nitrogen and sulphur. Moreover, it is contained at different ratios to body weight in different species (Parmenter and MacMahon, 2009) and proportionate to body weight. The amounts of phosphorus contained in wildebeest are more comparable to *Bos bison* (bison) (>318 kg) studied

by Towne (2000). Towne, who reported the first measurement at twelve months postmortem, indicated that phosphorus levels beneath the bison were 200 ppm and remained significantly increased for three years (100 ppm) when the measurement ceased.

Table 4: Inorganic nutrient differences between the 'Control' and 'Beneath' groups. Statistically significant differences (p <0.05) between group measurements of ammonium (NH_4), nitrate (NO_3), phosphorous (P) and Sulphate (SO_4) are indicated by different capital letters and within the same treatment group by different letters in brackets ( ). Nutrient mean (n = 4) differences were determined by Kruskal–Wallis and post hoc Dunn's test for pairwise comparison. (Mean values ± standard error)

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

Redundancy analysis (RDA) of the measured inorganic nutrients indicates that only ammonium (NH_4) could significantly (p = 0.016) be linked to the variance in substrate-guild utilisation after one month. The vertical axis of the tri-plot (Figure 12A) separates the Beneath and Control groups with amine and amino acid utilisation (blue arrows) correlating with the Beneath samples and ammonium. Ammonium accounted for 40% of the substrate utilisation variance. At six months, nitrate (NO_3) and phosphorus (P) significantly (p = 0.031) explained 46% of the observed variance, correlating with carboxylic acid, polymer and amino acid utilisation of the Beneath group (Figure 12B). The measured inorganic nutrients were independent of the substrate utilisation at twelve months (Figure 12C). At eighteen months, phosphorus was significant (p <0.037) to substrate utilisation, correlating with polymer, carbohydrate and amino acids utilisation (Figure 12D).

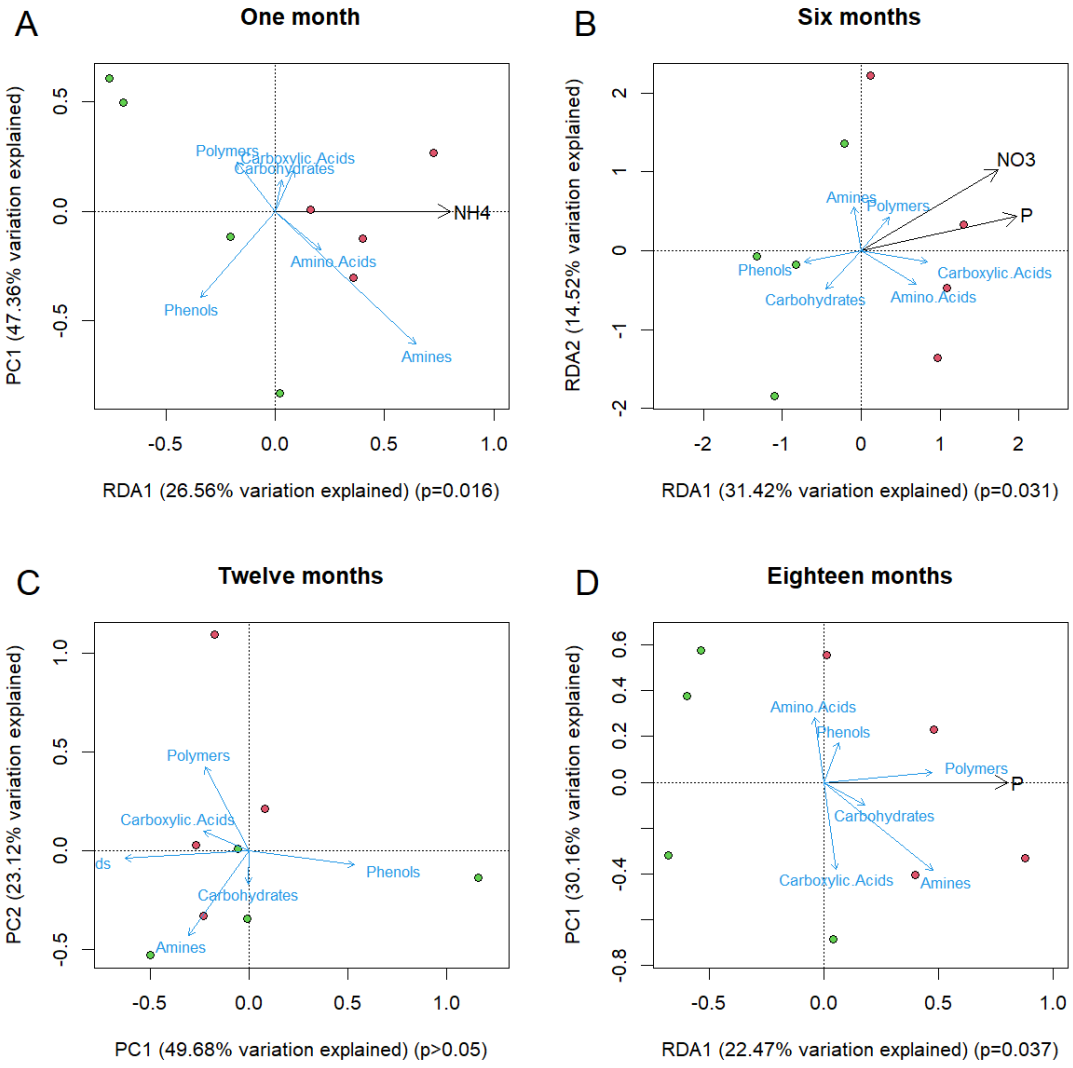

Figure 12: Redundancy Analysis (RDA) tri-plot illustrating the relationships between response variables (inorganic nutrients), explanatory variables (substrate-guild utilisation), and sample sites. Black arrows represent the response variables (NH4, ammonium; NO3, nitrate, and P, phosphorus while blue arrows represent the explanatory variables (Amines, Amino Acids, Carbohydrates, Carboxylic Acids, Phynols and Polymers). The direction of the arrows indicates the gradient of the variables, and the length indicates the strength of their relationship with the ordination axes. The angle between arrows shows the correlation between variables. Points represent the sample sites (Red, 'Beneath'; Green, 'Control'), with their position relative to the arrows indicating the influence of the corresponding variables. The p values indicate model significance.

3.4. Conclusions

Community-level physiological profiles indicated that decomposing wildebeest carcasses create long-term functional changes to the soil microbiome beneath it.

- The overall microbial function was increased during 'active decay' and remained elevated for less than 12 months, by which time only remains of the carcass were left.
- Functional α-diversity and evenness increased and significantly differed from the Control soil microbiome (5m radius) for the entire experimental period (18 months).
- The microbiome beneath the carcasses remained functionally dissimilar (β-diversity) from the Control group for eighteen months, although the dissimilarity was reduced over time.
- Carbon-substrate utilisation succession was observed. Catabolism shifted from increased amino acid and amine utilisation (1 month) to carboxylic acid utilisation (6 and 12 months) to polymers, carbohydrate, and amine utilisation (18 months).
- Variations of soil nutrients (ammonium, nitrates and phosphorus) significantly explained the variation in carbon-substrate utilisation.

3.5. Study Limitations and Recommendations

Due to the high cost of Biolog™ EcoPlates™, limited samples could be taken. The first postmortem interval (one month) showed that amino acids and amine utilisation were significantly elevated. More frequent sampling during decomposition's 'bloated' and 'active decay' phases may have demarcated between amino acid and amine utilisation. Moreover, sampling ended at eighteen months when substrate utilisation differed significantly from Controls. Further sampling may have provided a clear indication of the duration of *hotspots* created by the carcasses.

The decrease in ultraviolet exposure and increase in humidity beneath the grids where the carcasses were placed, particularly during the 'dry' and 'remains' stages, as well as the intermittent urination and defecation by wildlife around the carcasses, represented confounding factors that could not be statistically controlled or addressed within the experimental design. The exact impact of these factors on the results remains unclear.

Although the carbon substrates in EcopPlate™ are not selected explicitly for the indication and monitoring of soil microbiome function associated with animal decomposition, it could significantly discriminate between the microbiomes. Microplates containing sole-carbon substrates specific to carcass decomposition, such as arginine, lysine, cadaverine, putrescine, and many others, may be beneficial for forensic microbiology.

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Chapter 4
Structural succession of mesic-grassland soil microbiomes beneath decomposing large herbivore carcasses

4.1. Introduction

The decomposition of organic matter is a critical ecosystem service performed by soil microbiota (Leff et al., 2015). Plant detritus, which is slow to decompose and relatively nutrient and energy-poor (Swift et al., 1979), is abundant in soil, contributing most of the organic matter in soil and is the focus point of most ecologically focused soil microbial studies. Carcass decomposition is rapid, requiring specific metabolic pathways from the soil microbiota that hydrolise animal proteins, lipids, and nucleic acids to volatile fatty acids, organic acids, organic nitrogen and phenols (Metcalf et al., 2013; Cobaugh et al., 2015). The nutritional contribution of carcasses to the ecosystem has historically been considered trivial (Putman, 1978; Owen-Smith, 1999; Parmenter and MacMahon, 2009), although it has been suggested that grassland-soil microbial diversity and structure are driven over decades by nutrient inputs other than grassland vegetation (Millard and Singh, 2010). Carcasses add energy-rich, low molecular weight labile proteins and lipids with a narrow carbon-nitrogen ratio (5:18:1) to soil (Metcalf et al., 2013; Cobaugh et al., 2015; Barton et al., 2013). The labile nutrients create ‘hot moments’ (short-term events that accelerate microbial processes) that result in ‘hotspots’ (a small volume of soil with faster process rates and intensive interaction), which 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 resulting ‘hotspots’, demonstrating their ecological relevance (Yang and Janssen, 2002; Carter et al., 2007; Benninger et al., 2008; Bradford et al., 2008; Strickland et al., 2009; Cotrufo et al., 2013; Macdonald et al. 2014). The 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 et al., 2003; Kuzyakov and Blagodatskaya, 2015; van Elsas et al., 2019). The competition for nutrients by these ecological groups can be observed through the

dynamic succession of their associated taxa. It may answer fundamental forensic questions, such as the postmortem interval and ecological questions, such as the ecological impact of carcass decomposition.

As described by Payne (1965), carcasses progress through six stages of decomposition ('fresh', 'bloated', 'active decay', 'advanced decay', 'dry' and 'remains'). The enteric microbiota initiates carcass decomposition, causing the carcass to bloat and eventually rupture with the extruded matter, initiating soil microbial succession. The rate at which carcasses progress through the decomposition stages and microbial succession occurs in the soil is influenced by the carcass microbiome, carcass mass (Spicka et al., 2011), the soil microbial community if placed on soil (Lauber et al., 2014), temperature (Meyer et al., 2013), precipitation (Archer, 2004; Singh et al., 2018), necrophagous invertebrates and scavengers (Crippen et al., 2016). Apart from human cadavers, which weigh relatively little, most decomposition studies use rodents and swine for their similarities to the human microbiome and size (Metcalf et al., 2013; Spicka et al., 2010; Lauber et al., 2014; Cobaugh et al., 2015). These carcasses are relatively small and do not reflect the majority of animals in a southern African natural Grassland. Southern African grasslands are dominated by a vast and diverse number of ruminant herbivores, many of which are particularly large (>200 kg), such as wildebeest (*Connochaetes .spp*), which also have naturally high mortality rates (Owen-Smith, 1999; Jones et al., 2015). Decomposition studies with comparable-sized carcasses are limited (Towne, 2000), especially those reporting on the necro and soil microbiomes. Moreover, most existing literature indicates relatively short sampling periods (< 1 year), as may be relevant to smaller carcasses and forensic purposes, with very few studies investigating succession after the 'remains' stage of decomposition (Singh et al., 2018). Thus, there is a paucity of empirical research on the structural succession of soil microbial communities over long periods using carcasses that exceed 50 kg (Weiss et al., 2016). Further research in structural succession using a variety of larger carcasses over more 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).

Besides estimating the time of death, human-associated biomarkers are a focal point of forensic microbiology. These biomarkers are rare in nature, exist in soil for a relatively short duration (Cobaugh et al., 2015) and have little ecological relevance due to their brief and low abundances.

From an ecological perspective, microbial biomarkers that explain the differences in soil microbial communities exposed to carcass decomposition are essential. Such biomarkers would require metagenomic analysis methods that support high dimensional class comparisons and determine the magnitude of the biologically relevant observed phenomenon (Segata et al., 2011), an aspect lacking in the current literature.

Statistically significant shifts in microbial community function have been observed in the soil beneath decomposing carcasses utilising culture-dependent methods (Chapter 3; Singh et al., 2018; Heo et al., 2021). Culture-dependent methods, however, only include the fraction of culturable microbiota present (Amann et al., 1995). One approach to characterising the metagenomic content of a community is to infer the community's functions by its taxonomic composition. Phylogenetic Investigation of Communities by Reconstruction of Unobserved State (PICRUSt) (Langille et al., 2013) software, in conjunction with the Kyoto Encyclopedia of Genes and Genomes (KEGG) orthologies (KOs) was successfully used by Metcalf et al. (2016) in predicting changes in bacterial enzymes in the abdominal cavity of decomposing mice. Their results predicted increases in nitrogen cycling, cadaverine and putrescine production, and amino acid degrading enzymes during relatively short-term (four-month) decomposition studies. More recently, *Tax4Fun* (Aßhauer et al., 2015) and *Vikodak* (*decoder* in Sanskrit) (Nagpal et al., 2016) have been utilised for the same purpose with comparable accuracy, although the approach cannot replace whole metagenome profiling. However, considering its accuracy, these techniques are practical when shotgun sequencing is prohibitively expensive (Aßhauer et al., 2015). Culture-dependent methods, in combination with culture-independent methods, may validate the link between microbial community functional traits and microbial community structure (Escalas et al., 2019).

This study aimed to characterise the structural succession of mesic-grassland soil microbiomes beneath *Connochaetes taurinus* (blue wildebeest) carcasses as they are large ruminant herbivores (180 ¬250 kg) and the most abundant antelope in eastern and southern Africa. They migrate in mass (>1 million) across grasslands annually and are subject to the most predation attempts, although their numbers remain stable (Stuart and Stuart, 2000). These characteristics make them the most appropriate subjects for carcass decomposition studies in natural southern African grassland settings. Sampling continued past carcass decomposition (eighteen months) when

minimal remains were observed to explore post-decomposition biomarkers. Microbial diversity and structural dissimilarity were compared to evaluate the transitory nature during and after carcass decomposition. Biologically relevant soil microbial biomarkers were identified, and their succession was monitored throughout the experimental period. Links between the observed functional succession from sole-carbon substrate utilisation (Chapter 3) and functional genes inferred from the microbial abundance related to carcass decomposition were evaluated.

4.2. Materials and Methods

4.2.1. Study area

The study occurred in a typical Mesic Highveld Grassland Bushveld ecotone at the Telperion Nature Reserve on the Mpumalanga and Gauteng provincial border in South Africa (25.7039° S, 28.9814° E). The region has a dry-winter subtropical highland climate (Mucina and Rutherford, 2006).

4.2.2. Experimental design and sampling

The experimental design is summarised in Figure 13. As described in Chapter 3, ten unspoiled and intact *Connochaetes taurinus* (wildebeest) carcasses (n = 4) were obtained from the reserve's culling process and secured to grids to prevent scavenging of large sections of the carcases. The carcasses were placed on level ground to prevent runoff and at least 900 meters apart so that the samples represented the diversity of grassland and soil conditions and to prevent spatial pseudoreplication (Schoenly et al., 2015). The carcasses' samples were not pooled to prevent sacrificial pseudoreplication (Schoenly et al., 2015). A sample consisted of three subsamples collected beneath the head, thorax and hindquarters, representing a carcass's 'Beneath' sample. Three subsamples were collected at an equal distance (5 meters) from each carcass to represent its 'Control' sample. A soil corer with a 2 cm diameter was used to collect the soil from 0¬10 cm aseptically. Sample collection occurred during the decay stages described by Payne (1965). The first samples were collected within one month postmortem. During that time, some carcasses were in 'active decay', having ruptured and maggot activity peaked, whilst others had reached 'advanced decay' as the maggots had migrated from the carcasses. Samples were also collected at six months postmortem ('dry' and 'remains' stages) and twelve and eighteen months postmortem when only pieces of dried-out hide, some bones, and hair remained. Each composite sample was placed in a Ziploc™ bag and stored at 4°C for 24 hours before processing the culture-dependent assay described in Chapter 3. The remaining soil from the samples was then frozen at -80°C for subsequent analysis.

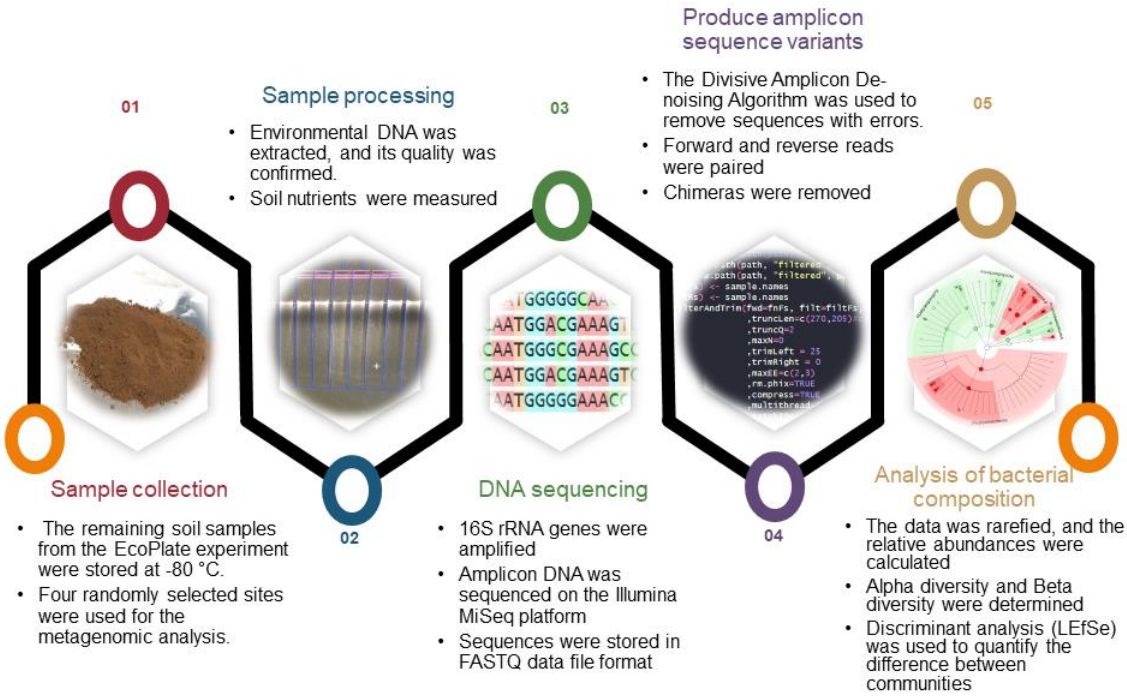


Figure 13: A workflow of the metagenomic experimental design consisting of sample collection, DNA extraction and soil nutrient analysis, marker gene sequencing, producing amplicon sequence variants and analysing the bacteria community structure.

4.2.3. Soil nutrient analysis

The Institute of Soil, Climate and Water of the Agricultural Research Council of South Africa analysed the soil samples from each site and time point. The potassium chloride extract method measured ammonium (NH_4) and nitrate (NO_3) ion contents. Phosphorus (P) content was measured using the Bray 1 method (Bray and Kurtz, 1945), and sulphate (SO4) was measured using a 1:10 soil water extraction method.

4.2.4. DNA extraction, sequencing

The environmental DNA from soil samples collected at four randomly selected sites, both beneath and at a five-meter radius of the carcasses, at intervals of one, six, twelve, and eighteen months postmortem (32 samples in total), was recovered and purified using the Dneasy™ PowerSoil™ DNA Isolation Kit (Qiagen, Valencia, CA, USA). The samples had been frozen at -80°C prior to this process. The concentration and purity of the DNA were quantified spectrophotometrically with a NanoDrop™ 2000 (Thermo Fisher Scientific, Inc., United States), and its quality and integrity were evaluated using 1% agarose gel electrophoresis (Appendix B).

Omega Bioservices (Norcross, GA, USA) used the KAPA HiFi HotStart ReadyMix PCR kit (Roche) with 341F (5'-CCTACGGGNGGCWGCAG-3') and 785R (5'-GACTACHVGGGTATCTAATCC-3') primers to amplify the V3¬V4 region of the 16S rRNA genes. The primers were selected as they allow the broadest range of bacterial taxa up to the genus level (Klindworth et al., 2013). An Illumina MiSeq v3 platform (Caporaso et al., 2012) sequenced the 32 samples, providing 300 base-pair (bp) forward and reverse sequence reads (adapters removed) at between 95000 to 157000 reads per sample as FASTQ data files.

The FASTQ files were processed with the *DADA2* package (version 1.26) (Callahan et al., 2016) in *R* version 4.2.1 (R Core Team, 2013). An inferred base call accuracy of 99.9% (referred to as Q30) (Ewing et al., 1998) was selected to ensure that accurate bp sequence reads were used. Therefore, the forward and reverse reads were trimmed 25 and 10 nucleotides from the 5'-end, respectively, and excluded from downstream analysis if they had less than 270 and 205 nucleotides, respectively, leaving 91000 to 101000 reads per sample. The forward and reverse read error rates were estimated, and the Divisive Amplicon De-noising Algorithm (DADA) removed sequence reads with errors. The de-noised forward and reverse reads of each sample were merged to form paired-end reads if the reads overlapped by >12 bp with no mismatches within overlapping bp. *De novo* chimaeras were detected and removed (6.7 %) based on sample consensus, after which 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). Twenty-four thousand five hundred and seventy unique ASVs were identified, and the data was imported to the *phyloseq* package version 1.41 (McMurdie and Holmes, 2013) in *R* for statistical analysis and visualisation.

4.2.5. Bioinformatics and statistical analyses.

A Control replicate of the eighteenth month was removed from the analysis due to inadequate sampling depth. Rarefaction curves indicated that the processed data adequately represented the diversity of ASVs of each sample (Appendix C). Differences in sampling depth between samples were normalised by random subsampling (rarefying) the data (Paulson et al., 2013; Weiss et al., 2016; Lin and Peddada, 2020), reducing the 16S rRNA gene inventory from 24570 to 24080 ASVs. The rarified data was used for all analyses. Observed ASV richness and Simpson's diversity index (Simpson, 1949) were selected to differentiate the Beneath and Control groups at the same time points and the same group at different time points (*estimate_richness* function in *phyloseq*). Differences in Observed richness and Simpson's diversity index values (α-diversity) were illustrated (*plot_richnes* function in *phyloseq*). Principal Coordinate Analysis (PCoA) (Gower, 1966) was computed (*ordinate* function in *phyloseq*) to illustrate the ordination results (c*ar* package version 3.1-0). The relative abundances of bacterial phyla between the Beneath and Control groups were compared by calculating the ASVs per phylum as a percentage of the total. Phyla that represented less than 2% of the total were aggregated. The linear discriminant analysis (LDA) of effect size (LEfSe) algorithm was applied to the data to indicate the taxa that were biologically most informative (biomarkers). Taxa with a relative abundance of more than three orders of magnitude (LDA log10 score > 3, $p < 0.05$) and most likely to explain the differences between the Beneath and Control groups were indicated. (Segata et al., 2011; Lin and Peddada, 2020). The analysis was performed in the *microbiomeMarker* package version 1.3.2 in *R* (Cao et al., 2022).

Inferred functional gene abundance was predicted with the *Tax4Fun* software (Aßhauer et al., 2015) through the *themetagenomics* package version 1.0.2 (Woloszynek et al., 2019) in *R*. *Tax4Fun* infers functional capabilities of microbial communities based on 16S rRNA gene sequences. The *t4F* function was used to estimate the relative abundance of specific enzymes relevant to carcass decomposition and Biolog™ Ecoplate™ sole-carbon sources metabolism (Chapter 3). The *t4f* function was applied to the normalised ASV output of the 16S rRNA analysis pipeline utilising the SILVA rDNA reference database (release 138.1). Dunnett's test (Dunnett, 1955) was used to compare the results of the Beneath group at different time points to

a composite control group for statistical differences (*DunnettTest* function from the *DescTools* package version 0.99.49).

For hypothesis testing of univariate data, the Mann-Whitney *U* test (*wilcox.test* in the *R* stats version 3.6.2) was used to compare the Beneath and Control group results of the same time point. The Kruskal-Wallis rank-sum test (*kruskal.test* in the *R stats*) was used to compare the same group at different time points (Hollander et al., 2013). The post-hoc Dunn's test (Dunn, 1964) was used for multiple comparisons (*DunnTest* function of *DescTools* package version 0.99.49), with the Benjamini and Hochberg (BH) *p*-value adjustment to Control for the false discovery rate (Benjamini and Hochberg, 1995). Permutational Multivariate Analysis of Variance (PERMANOVA) (Anderson, 2001) was used to test the null hypothesis that centroids and dispersion of the Beneath and Control groups are equivalent (β-diversity). The *adonis2* function from the *vegan* package version 2.6.2 (Dixon, 2003) was used to carry out the PERMANOVA. Pairwise PERMANOVA was performed with the BH *p*-value adjustment (*p.adjust* function in *R Stats*). Differences in beta-dispersion were evaluated by measuring multivariate homogeneity of group dispersions (Anderson et al., 2006) (*betadisper* and *permutest* functions in *vegan*). All permutation tests were done using 999 random permutations. A *p*-value of < 0.05 was considered statistically significant for all hypothesis tests.

4.3. Results and Discussion

4.3.1. Site description

The soil from the sampling sites had similar water-holding capacities (M = 29.8%, SD 4.41) and contained heterogeneous vegetation mainly consisting of *Fimbristylis hispidula*, *Cleome rubella*, *Eragrostis curvula*, *Phyllanthus parvulus* and *Cynodon dactylon* (Graham et al., 2020). During the sampling period (2019 – 2020), the area annually received a mean of 510 mm of rain during the wet seasons (November to March) and 40 mm during the dry seasons (May to September), with a mean ambient minimum and maximum temperatures of 5 and 23 °C during winter and 15 and 28°C during summer (SAWS, 2021) (Appendix A). Herbivores, small carnivores and scavengers frequented the area.

4.3.2. Soil nutrients

The five-meter Control group did not have statistically different (n = 4, p <0.05) measurements of ammonium, sulphate, nitrate, and phosphorus at the different time points (1, 6, 12, and 18 months), validating the radius of five meters as a boundary beyond which Control samples could be recovered as nutrients did not spread to that extent during the experiment (Figure 14). After one month, a 20-fold increase of ammonium (NH_4: χ^2= 27, df = 7, p <0.001) and an 8-fold increase in sulphate (SO_4: χ^2 = 18.3, df = 7 p <0.01) was measured below the carcasses. Ammonium and sulphate levels remained significantly elevated at six months but were reduced to levels similar to the Control group by twelve months. Nitrate levels were increased 33-fold at six months (NO_3: χ^2 = 24.6, df = 7, p <0.001) but were not significantly different at twelve months. Phosphorus increased after a month but was not statistically different from its Control group. At six months, phosphorous was increased 52-fold (P: χ^2 = 26.5, df = 7 p <0.001) and remained significantly elevated for the entire experimental period.

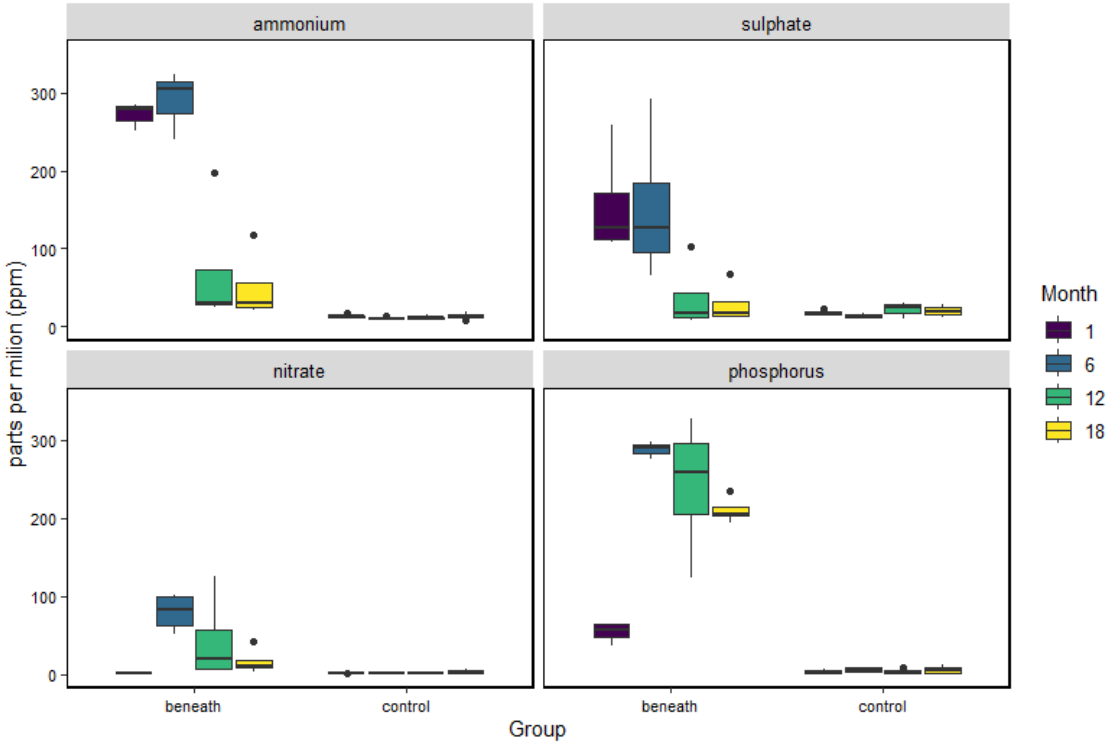


Figure 14: Box plots illustrating nutrients (ammonium, sulfate, nitrate, and phosphorus), as parts per million (ppm), measured from the soil beneath the carcasses and at a five-meter Control one, six, twelve, and eighteen months after carcass placement.

4.3.3. Microbiome structural changes

Richness and Diversity

Seasonal climate variations have a weak but significant effect on the diversity of soil bacteria (Zhao et al., 2022). Therefore, the Beneath and Control samples of the same time point were compared, eliminating seasonal variation as a confounding factor (Figure 15). A month after carcass placement, a four-fold reduction of richness and α-diversity were measured beneath the carcasses, which sigmoidally increased to Control levels during the experimental period. After twelve months, richness and eighteen months α-diversity (Simpsons index) were no longer significantly (Mann-Whitney: $U = 0$, $p < 0.05$) different from the Control soil. Decreased microbial diversity in the soil beneath rodent and swine carcasses has been observed by Metcalf et al. (2013) and Weiss et al. (2016) in short-term (< 2 months) studies and a long-term study (24 months) by Singh et al. (2018), using human cadavers. Conversely, Cobaugh et al. (2015) indicated that richness and α-diversity beneath human cadavers remained similar to Control soil during 'active decay' and only increased during the 'advanced decay' (3 – 6 months).

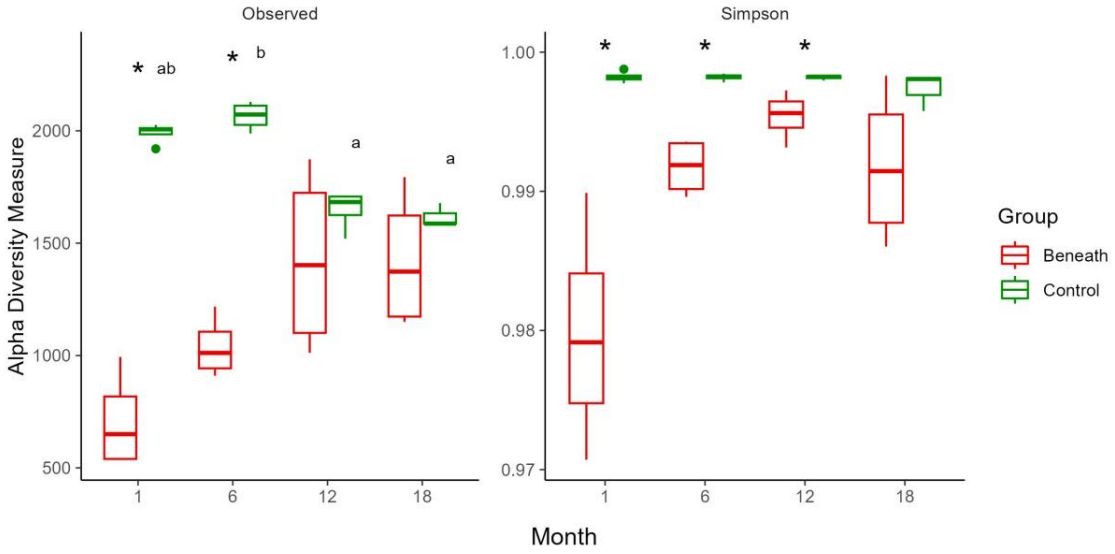


Figure 15: Box plots illustrating the Observed richness and Simpson's diversity index (α-diversity) values at one, six, twelve, and eighteen months. An asterisk (*) indicates a statistical difference between a 'Beneath' and 'Control' group of the same time point (Mann-Whitney *U* test: $n = 4$, $p < 0.05$). 'Control' group time points with the same letter are not significantly different (Dunn's test: $n = 4$, $p < 0.05$).

The species richness of the Control group was not equal at all the sampling time points (Kruscal-Wallis: $\chi^2 = 11.5$, $df = 3$ $p < 0.009$), with the twelfth and eighteenth month's richness being significantly (Dunn: BH-adjusted $p < 0.05$) reduced compared to the sixth month (Figure 15). Singh et al. (2018) found a significant decrease ($p < 0.05$) in richness at one meter, but not five meters, 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 of richness that we observed at five meters (Control soil) from larger carcasses (200250 Kg) was not a result of nutrients (ammonia, nitrate, sulphate and phosphorus) seeping from the carcass to a radius of five meters, indicated by the soil nutrient results (Figure 14). The reduced richness may result from seasonal climate variations (Zhao et al., 2022) or bioturbation of the surrounding Control soil (West and Whitman, 2022) by animals that frequent the carcasses. Species α-diversity, as determined by the Simpsons index, was not significantly different for Control soil during the eighteen months.

Beta Diversity and Dispersion

The Bray-Curtis-based PCoA plot (Figure 16) illustrates β-diversity as the variance of community dissimilarity and dispersion of the Beneath and Control soil bacterial communities of the four sampling time points. The Control group samples (Green) formed a single cluster (PERMANOVA: $F_{3,11} = 0.62$, $R^2 = 0.14$; $p = 0.993$) and were similarly dispersed ($F_{3,11} = 0.73$, $p = 0.572$), indicating compositional homogeneity and stability of their bacterial communities over eighteen months. The Beneath group formed distinct clusters at different time points (PERMANOVA: $F_{3,12} = 8.12$, $R^2 = 0.67$, $p = 0.001$). A pairwise comparison of Beneath clusters indicated that microbial communities at one month significantly differed from those at six months and that one and six months differed from bacterial communities at twelve and eighteen months. However, the Beneath communities at twelve and eighteen months were not significantly different ($F_{1,6} = 1.04$, $R^2 = 0.15$, BH-adjusted $p = 0.374$). In addition, the Beneath time points had different beta-dispersions ($F_{3,12} = 3.50$, $p = 0.039$). A pairwise comparison indicated that one and six-month samples had less dispersion than twelve and eighteen months (BH-*adjusted* $p < 0.05$), which overlap substantially (Figure 17). Thus, the Beneath group sample sites became more dissimilar as time progressed.

The ordination plots in Figure 17 illustrate the β-diversity and dispersion of the Beneath and Control groups of the same time points. Distinct clusters were formed on opposite sides of the ordination plots at all times except at eighteen months when an overlap occurs. PEMANOVA indicated significant differences between the Beneath and Control groups after one, six and twelve months. At eighteen months, the difference was no longer statistically significant (PERMANOVA: $F_{1,5} = 1.78$, $R^2 = 0.26$, $p = 0.143$). At six months, the data points for the Beneath group are more tightly clustered, indicating a more uniform community structure, whereas the Control group shows a wider spread of points, reflecting greater variability in community composition. The difference in this dispersion was statistically significant ($F_{1,6} = 19.44$, $p = 0.001$). At twelve months, the Beneath group was more dispersed than the C*ontrol* group, as some sites may be returning to a community structure resembling the Control group at a faster rate. Cobaugh et al. (2015) suggested a possible return to the original community structure of soil beneath human cadavers (5077 Kg), having monitored β-diversity and dispersion for seven months postmortem. Our results confirm that soil beneath large herbivore carcasses

(200 – 250 Kg) had returned to a community structure resembling the Control soil at eighteen months.

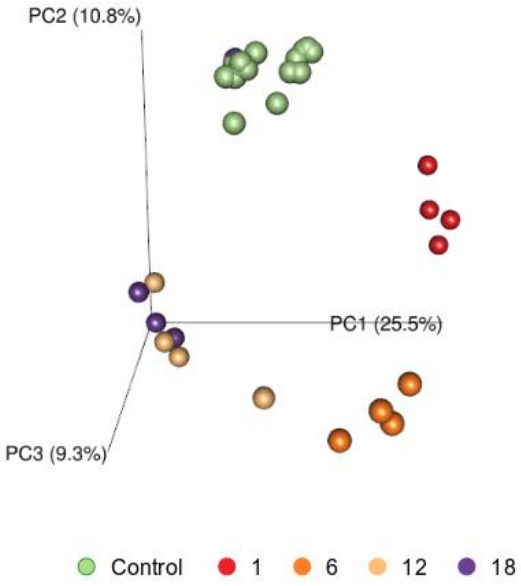


Figure 16: Bray-Curtis dissimilaritiy-based Principal Coordinate Analysis (PCoA) of the 'Beneath' and 'Control' groups at all time points (1, 6, 12 and 18 months).

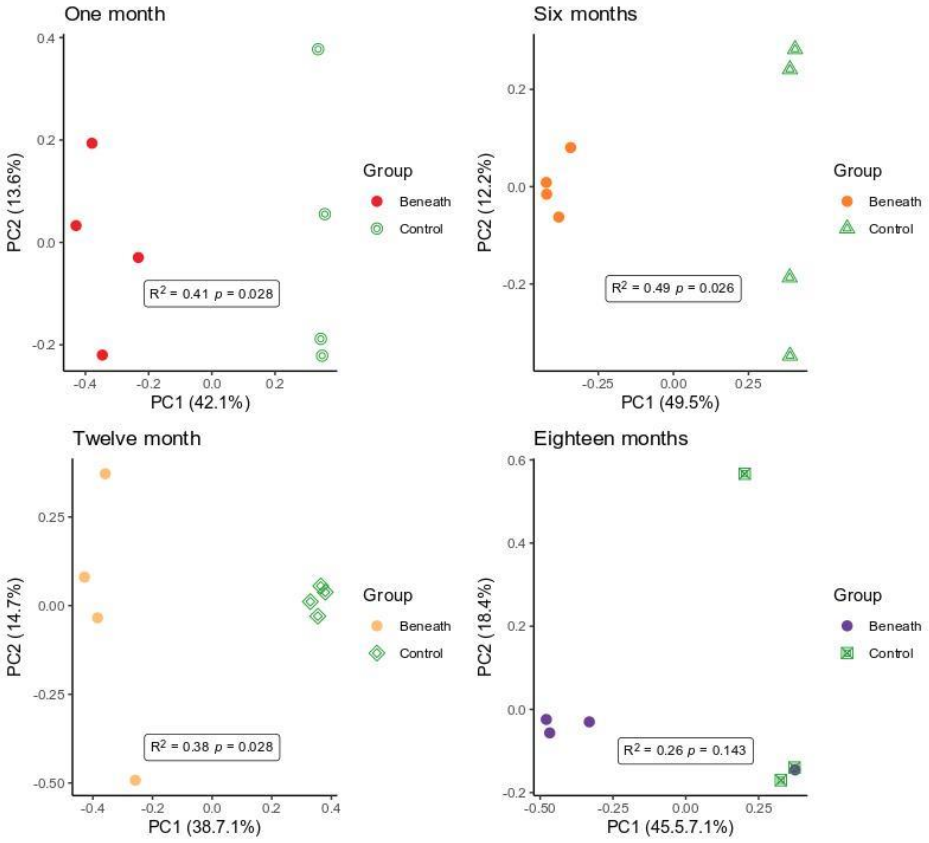


Figure 17: Bray-Curtis dissimilarity-based Principal Coordinate Analysis (PCoA) of the 'Beneath' and 'Control' groups of the same time point. PERMANOVA results (R^2 and *p* values) of each postmortem interval are indicated within the plot.

Phylum-level relative abundance

Comparing amplicon sequence variance counts of phyla, the Beneath and Control groups contained microbiota typical to the soil microbiome (Delgado-Baquerizo et al., 2016), with Acidobacteriota (Acidobacteria), Actinomycetota (Actinobacteria), Chloroflexota (Chloroflexi), Bacillota (Firmicutes), Planctomycetota (Planctomycetes), Pseudomonadota (Proteobacteria), and Verrucomicrobiota (Verrucomicrobia) representing 98% of the ASV (Figure 18). A month after the carcasses were placed, Firmicutes accounted for 55% of the ASVs beneath the carcasses, having been enriched 13-fold compared to Control soil at the same time point. Bacteroidetes was enriched 5-fold and Proteobacteria 2-fold compared to the Control group. Simultaneously, a reduction of Acidobacteria (12-fold), Chloroflexi, Planctomycetes and Verrucomicrobia (5-fold), Actinobacteria (3-fold) and other phyla representing less than 2% of the total ASVs were observed beneath the carcasses (Figure 18). After six months, Bacteroidetes accounted for 31% of the total ASVs of the Beneath group, being enriched 19-fold compared to the Control. Proteobacteria and Actinobacteria each represented ~20% of the total ASVs, almost double the Control values. Firmicutes was substantially reduced compared to the first month, although it was still enriched 2-fold compared to the Control. Chloroflexi, Planctomycetes and Verrucomicrobia were reduced 6-fold, and Acidobacteria was included in phyla, representing less than 2% of the total ASVs. At twelve months, Bacteroidetes (11-fold), Other phyla (4-fold), Firmicutes and Proteobacteria (2-fold) remained enriched, whilst Chloroflexi (10-fold) and Acidobacteria (3-fold) remained reduced. At eighteen months, Bacteroidetes was enriched 3-fold, and Chloroflexi and Verrucomicrobia were reduced 2-fold beneath the carcasses compared to the Control soil.

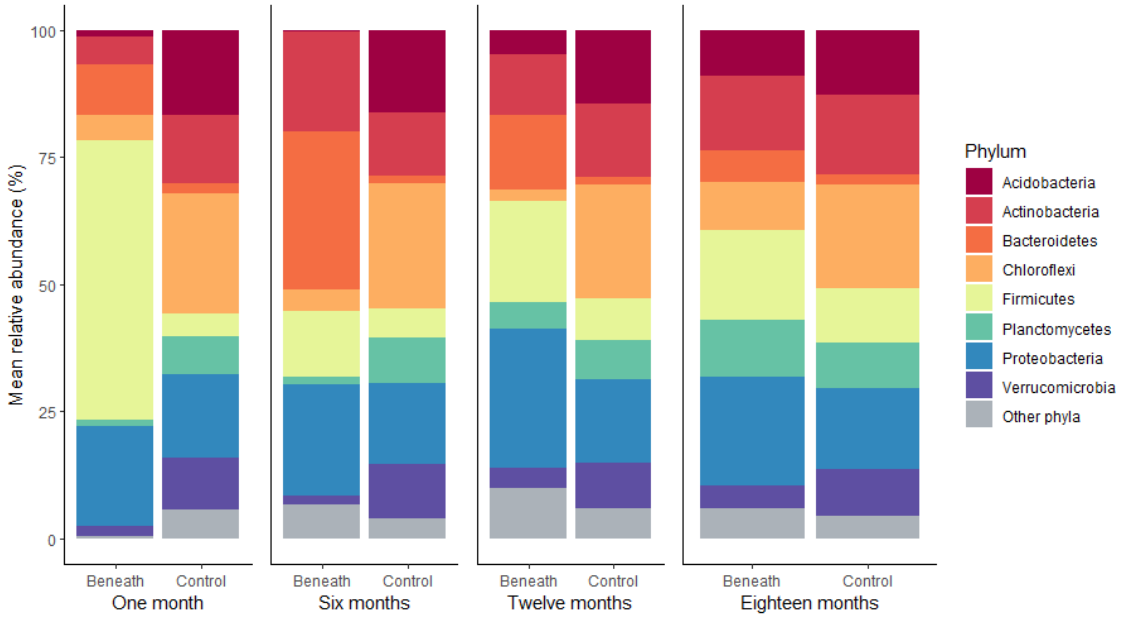


Figure 18: Stacked bars showing the phylum-level mean (n = 4) relative abundance of bacterial Amplicon Sequence Variants (ASVs) as a percentage of the total ASVs. Phyla representing less than two per cent of the total are indicated as Other phyla.

4.3.4. Biomarker succession

A month after placement, 238 taxa had statistically significant differences (Mann-Whitney *U:* $p < 0.05$) in relative abundance between the Beneath and Control groups. The number increased to 359 after six months, reduced to 271 after twelve months and 21 after eighteen months (Appendix D). The LEfSe algorithm (Lin and Peddada, 2020; Segata et al., 2011) determined the biologically meaningful taxa (biomarkers) most likely to explain the differences between the Beneath and Control groups of the same time point during and after carcass decomposition. The most prominent (LDA $\log_{10}$ score > 3, $p < 0.05$) biomarkers, resolved to family level after one, six, twelve and eighteen months postmortem, are indicated in cladograms, whereas the genus-level biomarkers are indicated in LEfSe plots (Figure 19 – 22).

After a month, Firmicutes and its classes Bacilli, Clostridia and Erysipelotrichia were the most prominent biomarkers highly enriched in the soil beneath the carcasses (Figure 19). Firmicutes, especially Clostridia, are particularly prevalent in the rumen as they hydrolyse cellulose (Barton

and Northup, 2011). Class Erysipelotrichia has been associated with lipid metabolism in the gut microbiome (Guo et al., 2021). Most Firmicutes also ferment amino acids, producing ammonia, hydrogen sulphide, fatty acids, amines and gases (van Elsas et al., 2019). In addition, Firmicutes increase inside a carcass during the 'bloated' phase of decomposition (Metcalf et al., 2013; Cobaugh et al., 2015). Once the carcass ruptures from the gas build-up, anaerobic Firmicutes are reduced because of their oxygen exposure. The nutrients and microbiota seeping into the soil change the pH and microbial structure (Forbes and Carter, 2015). *Planococcaceae* (genus *Savagea*), Tissierellaceae (*Tissierella*) and Erysipelotrichaceae (*Erysipelothix*) were identified as family-level biomarkers of the soil beneath the carcasses at one month (Figure 19); however, they were no longer statistically different from the Control group at six months. Our results (Figure 20¬22) showed that Clostridia and order Clostridiales remained enriched during the late advanced to post-decay stages for up to twelve months, with family Clostridiaceae identified as a biomarker at twelve months. Clostridia, an obligate anaerobe, can dominate the later stages (past six months) of decomposition (Kim et al., 2017). In the present study, the prolonged enrichment of Clostridia may have resulted from the extensive duration that the wildebeest hides were covering the soil, creating anaerobic conditions beneath it, and the depth (0¬10 cm) from which the soil samples were taken.

Despite the rumen intestinal tract containing relatively large amounts of Bacteroidetes (McCann et al., 2014) and the fact that Bacteroidetes increases during the 'bloated' phase (Metcalf et al., 2013; Cobaugh et al., 2015), the present study results (Figure 19) indicate that the mean relative abundance of Bacteroides beneath the carcasses was not statistically different (Mann-Whitney *U:* $p < 0.05$) from the Control soil due to a sizeable intersample variance of the Beneath group samples (Appendix E). A significant variance was also observed in the α-diversity results of the first month (Figure 15). Because changes to the soil microbiome are initiated after carcass rupture (Hyde et al., 2013; Pechal et al., 2013) and Bacteroidetes also contain numerous obligatory anaerobic genera rapidly reducing from oxygen exposure; the variance may stem from a variance in the time before rupture, which deviates largely even under controlled conditions (Lauber et al., 2014). Our results at the phylum level, using large herbivore carcasses, thus reflect observations during 'active decay' by Metcalf et al. (2013) and Cobaugh et al. (2015) using mice and human cadavers. However, at a family level, Flavobacteriaceae (*Myroides*) and Weeksellaceae (*Moheibacter*) were enriched beneath the carcasses after a month and remained

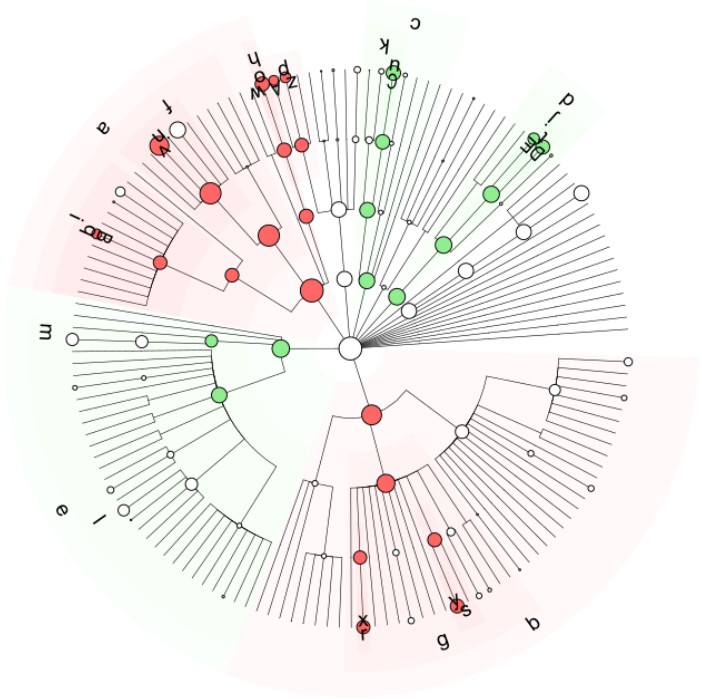
enriched for up to six months. At six months, Bacteroidetes was the predominant biomarker beneath the carcasses, including the family Sphingobacteriaceae (*Pedobacter*) (Figure 20). Cobaugh et al. (2015) also observed an increase in Bacteroidetes after six months when their study ended. The present study indicates that Bacteroidetes, especially the family Chitinophagaceae (Figure 21), remained a biomarker for up to twelve months.

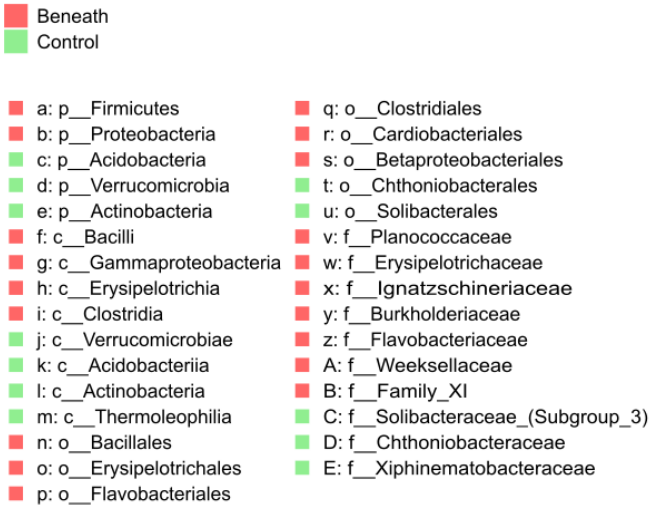
Proteobacteria and class Gammaproteobacteria were highly enriched after one month. They remained enriched beneath the carcasses for up to twelve months when it was the predominant biomarker (Figure 19¬21). Its increase in relative abundance has been observed in short-term (< 6 months) studies (Metcalf et al., 2013; Lauber et al., 2014; Cobaugh et al., 2015) and a long-term study by Singh et al. (2018) indicated a positive correlation between Gammaproteobacteria abundance and carcass mass. Beta and Gammaproteobacteria include many fast-growing r-strategists abundant under high nutrient conditions (van Elsas et al., 2019). Gammaproteobacteria is most prevalent on the skin of carcasses and the necrophagous dipteran larval microbiome (Pechal et al., 2013; Weatherbee et al., 2017). Family Ignatzschineriaceae, previously Xanthomonadaceae (*Ignatzschineria*) (Montecillo, 2023), and Betaproteobacteria Burkholderiaceae (*Paenalcaligenes*) were biomarkers of the first month. Burkholderiaceae was also a biomarker at six months, Rhodanobacteraceae was a biomarker at twelve, and the only biomarker from beneath the carcasses at eighteen months.

Actinobacteria and its classes, Actinomycetia and Thermoleophilia, were highly reduced beneath the carcasses after a month (Figure 19). However, Actinobacteria and Actinomycetia were enriched and identified as biomarkers of the Beneath group along with the family Micrococcaceae by the sixth month. In contrast, Thermoleophilia and the family Solirubrobacteriaceae remained reduced for up to twelve months. The reduction of Thermoleophilia correlated with a significant (LDA = 2.98, p = 0.013) reduction of phylum Deinococcus-Thermus, suggesting that the difference may be based on the reduced temperatures in the soil (Albuquerque and da Costa, 2014) beneath the carcasses and grids on which it was placed. Similarly, the reduction of Chloroflexi and family Ktedonobacteraceae may result from reduced temperatures and solar radiation.

The phylum Verrucomicrobia frequently dominates grassland soil (Bergmann et al., 2011). Verrucomicrobia and family Chthoniobacteraceae were highly reduced beneath the carcass at

every time point, whereas Xiphinematobacteraceae was reduced from the first to the twelfth month. Members of the Xiphinematobacteraceae family are endosymbionts of parasitic plant nematodes (Janssen, 2006), frequently observed in the study area (Girgan, 2019). The reduction of Xiphinematobacteracea may, to an extent, result from the soil and grass being covered. The decomposing carcasses added ammonium to the soil (Figure 14), which would increase the soil pH and reduce acidophilic Acidobacteria. Furthermore, Acidobacteria are oligotrophic K-strategists, which are slow-growing and prefer low nutrients (Metcalf et al., 2013; van Elsas et al., 2019). Our results indicated that Acidobacteria and family Solibacteraceae were highly reduced beneath the carcasses from the first to the twelfth month (Figures 19¬21), although the ammonium was substantially reduced by the twelfth month.





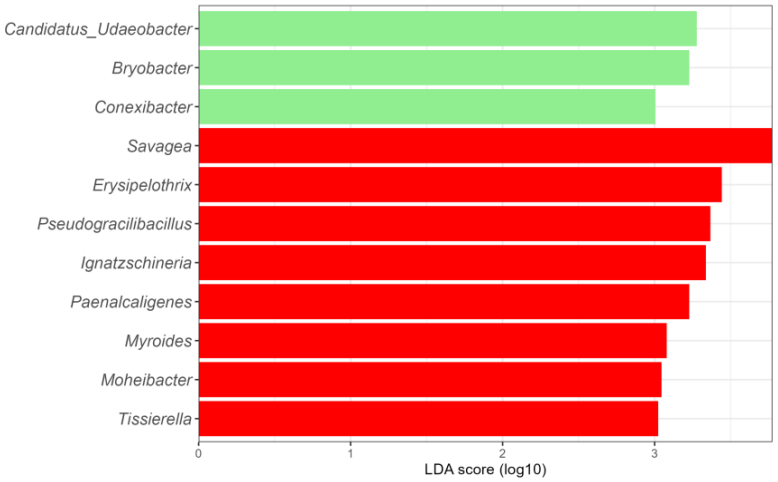


Figure 19: Least discriminant analysis (LDA) effect size (LEfSe) results of the soil microbiomes one month after carcass placement. The cladogram indicates biomarkers resolved to family level (LDA Score > 3; $p < 0.05$). Circle diameter indicates proportional taxonomic abundance. (White = $p > 0.05$). The LEfSe plots indicate the LDA $\log_{10}$ score of prominent genera.

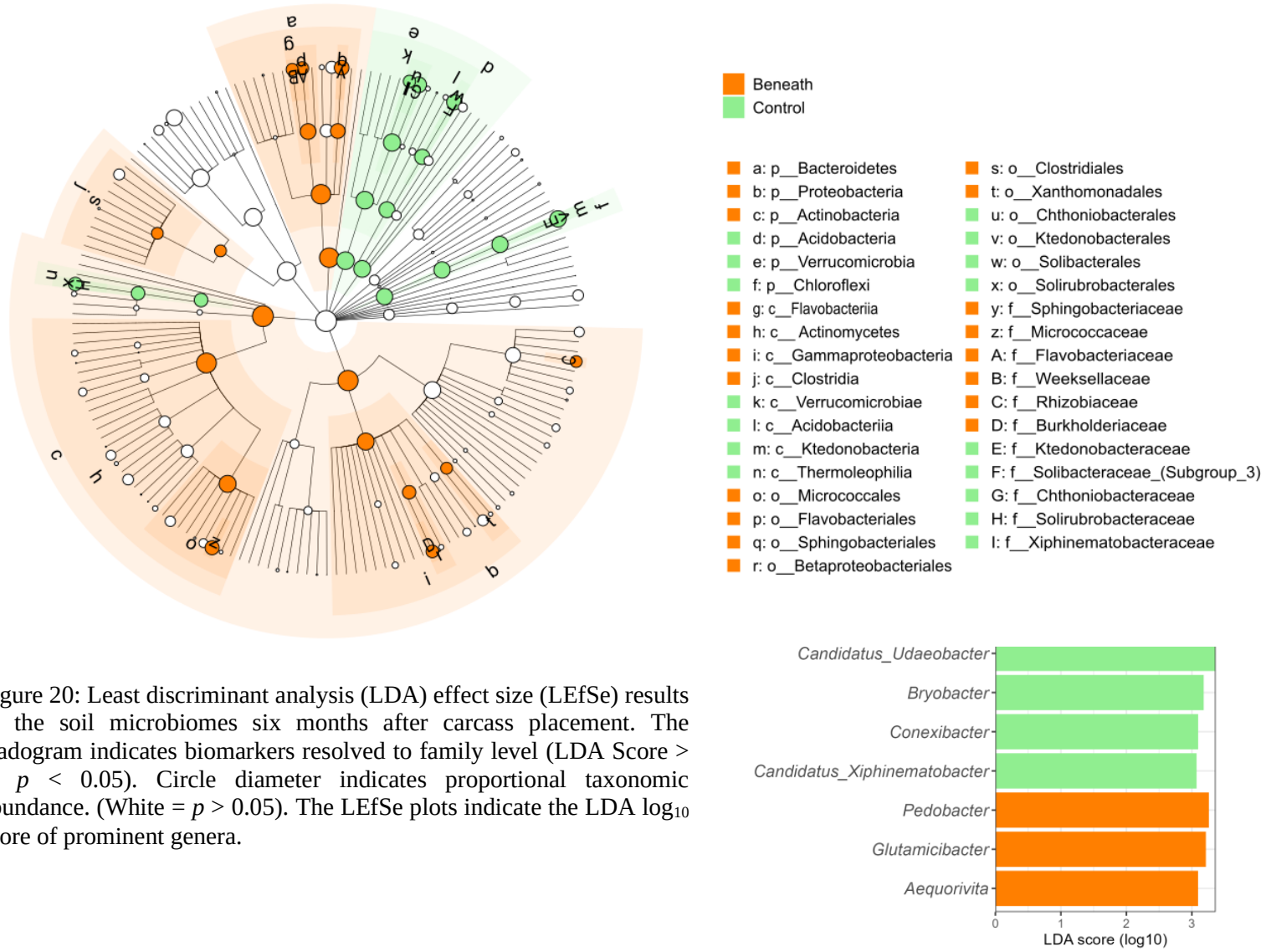


Figure 20: Least discriminant analysis (LDA) effect size (LEfSe) results of the soil microbiomes six months after carcass placement. The cladogram indicates biomarkers resolved to family level (LDA Score > 3; $p < 0.05$). Circle diameter indicates proportional taxonomic abundance. (White = $p > 0.05$). The LEfSe plots indicate the LDA $\log_{10}$ score of prominent genera.

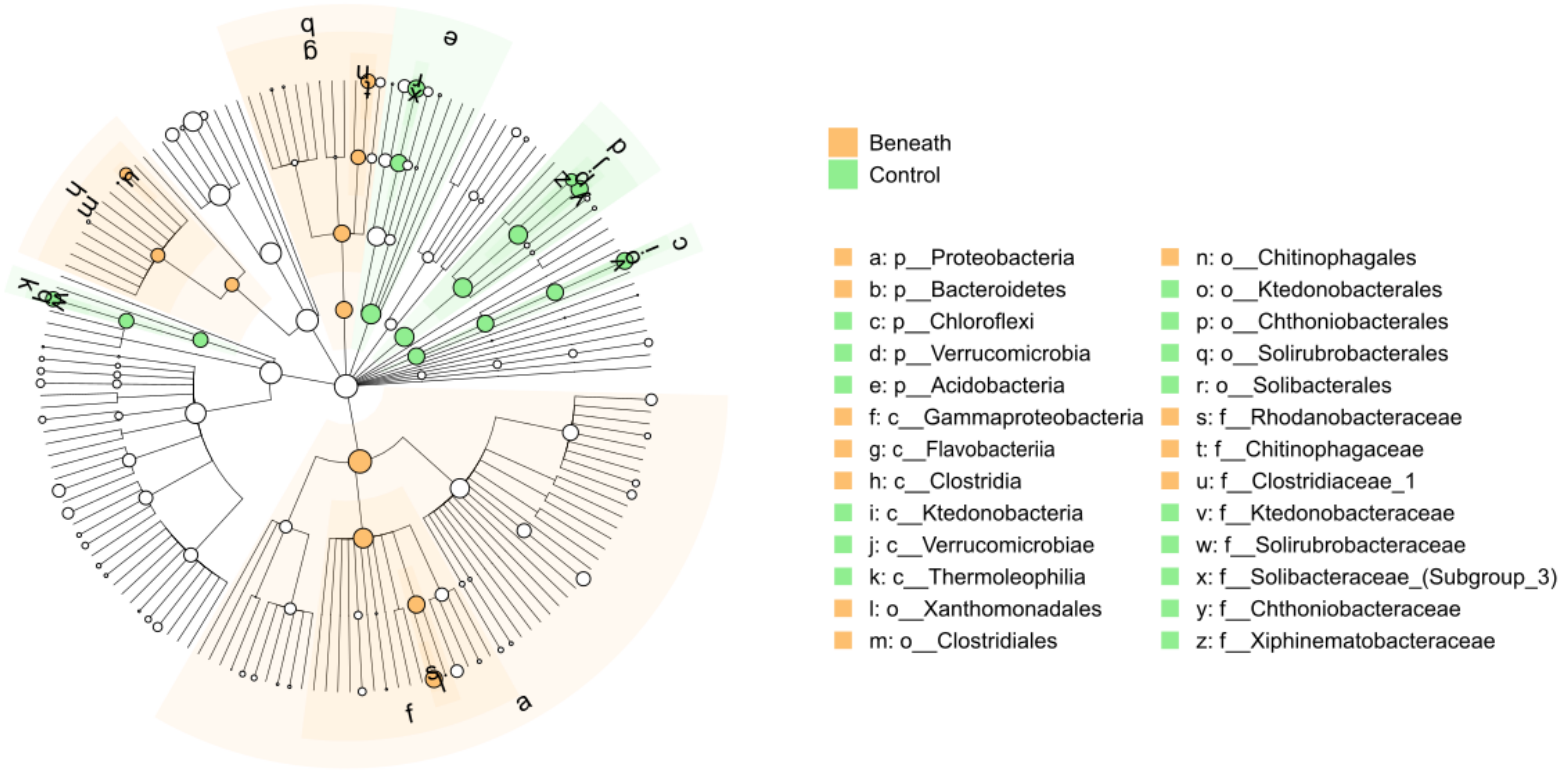
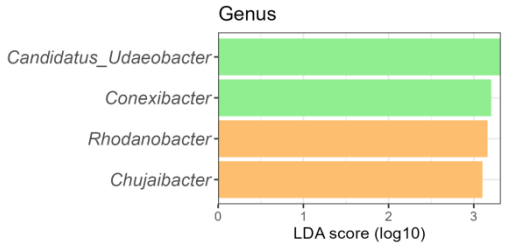


Figure 21: Least discriminant analysis (LDA) effect size (LEfSe) results of the soil microbiomes twelve months after carcass placement. The cladogram indicates biomarkers resolved to family level (LDA Score > 3; $p < 0.05$). Circle diameter indicates proportional taxonomic abundance. (White = $p > 0.05$). The LEfSe plots indicate the LDA $\log_{10}$ score of prominent genera.



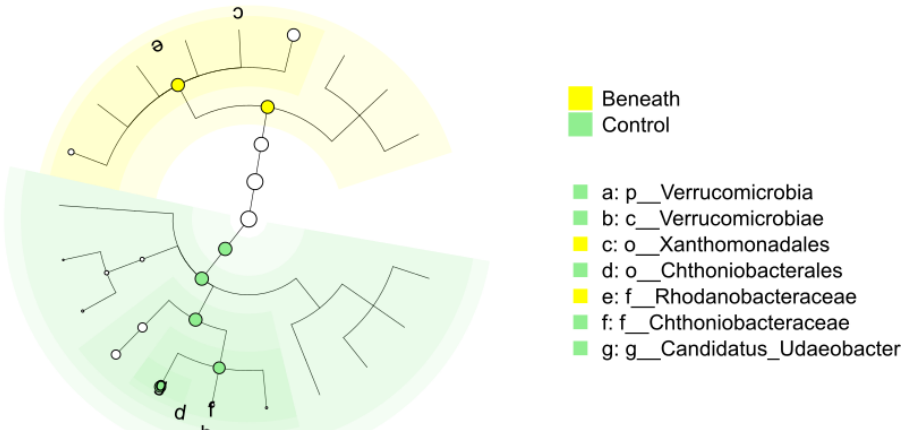


Figure 22: Least discriminant analysis (LDA) effect size (LEfSe) results of the soil microbiomes eighteen months after carcass placement. The cladogram indicates biomarkers resolved to family level (LDA Score > 3; $p < 0.05$). Circle diameter indicates proportional taxonomic abundance. (White = $p > 0.05$).

Family-level temporal succession

Distinct patterns were exhibited in the change in the relative abundance of the biologically relevant families over time (Figure 23). Families found to be highly enriched within the necrophagous dipteran larvae mass and gut microbiome (Weatherbee et al., 2017), Ignatzschineriaceae containing *Ignatzschineria* (Weiss et al., 2016; Montecillo, 2023), Tissierellaceae and Planococcaceae, reached an initial peak in abundance after one month and have very low abundance at six months and thereafter. Erysipelotrichaceae, which contains pathogenic genera to some ungulates (Mavrot et al., 2020) and has rarely been reported as enriched in necrobiome studies (Pascual et al., 2017), also exhibited an initial peak and an abrupt decrease. Burkholderiaceae (*Paenalcaligenes*), in contrast, was highly abundant in the first month and had a lengthy linear decrease over time. Burkholderiacea have been isolated from the gut of Stratiomyidae (Soldier Fly) larvae (Lee et al., 2013), which are present in the study area (Picker et al., 2012) and known to frequent carcasses during late advanced decay (Cobaugh et al., 2015). Therefore, the extended abundance of Burkholderiacea may result from continuous or delayed inoculation by its fly vector. In addition, Burkholderiacea can produce quorum-quenching compounds (Chan et al., 2011) that disrupt the quorum sensing of competitive bacteria in response to limited resources (Jordan et al., 2015), which may further prolong its abundance. Burkholderiaceae is a phosphate-solubilising bacteria (Stephen et al., 2015), and as phosphorus was increased during decomposition (Figure 14), its enrichment in the soil may

explain Burkholderiaceae longevity. The slow linear reduction of Burkholderiaceae observed in our long-term study has potential utility in postmortem microbial forensics (Figure 23).

The relative abundance of aerobic and lipolytic families Flavobacteriaceae, Weeksellaceae, Sphingobacteriaceae and Micrococcaceae (Steyn et al., 1998; Crippen et al., 2016), and Rhizobiaceae peaked during the 'dry' stage at six months (Figure 23) but were abruptly reduced by twelve months. Flavobacteriaceae and Weeksellaceae are also proteolytic (Crippen et al., 2016) and were highly enriched after one month. At twelve months, when only a few dry and scattered remains were left, Chitinophagaceae had reached peak relative abundance. A progressive linear increase of Chitinophagaceae was observed from the first month (Figure 23). Chitinophagaceae produce acid from sugars and hydrolyse chitin (Sangkhobol and Skerman, 1981), which is highest in the sheddings and cocoons of flies (Soetemans et al., 2020) and may account for the linear increase observed post-carcass decay. The relative abundance of Clostridiaceae, which hydrolyses proteins and carbohydrates, had a similar relative abundance from the first to the twelfth month and was reduced by eighteen months. Rhodanobacteracea, from the order Xanthomonadales, had a progressive linearly increase in relative abundance during the experimental period, reaching peak abundance after eighteen months. Rhodobacteraceae are deeply involved in sulphur and carbon biogeochemical cycling (Pujalte et al., 2014). Our results further substantiate observations (Pechal et al., 2014; Cobaugh et al., 2015) of its utility as a long-term estimator of postmortem intervals.

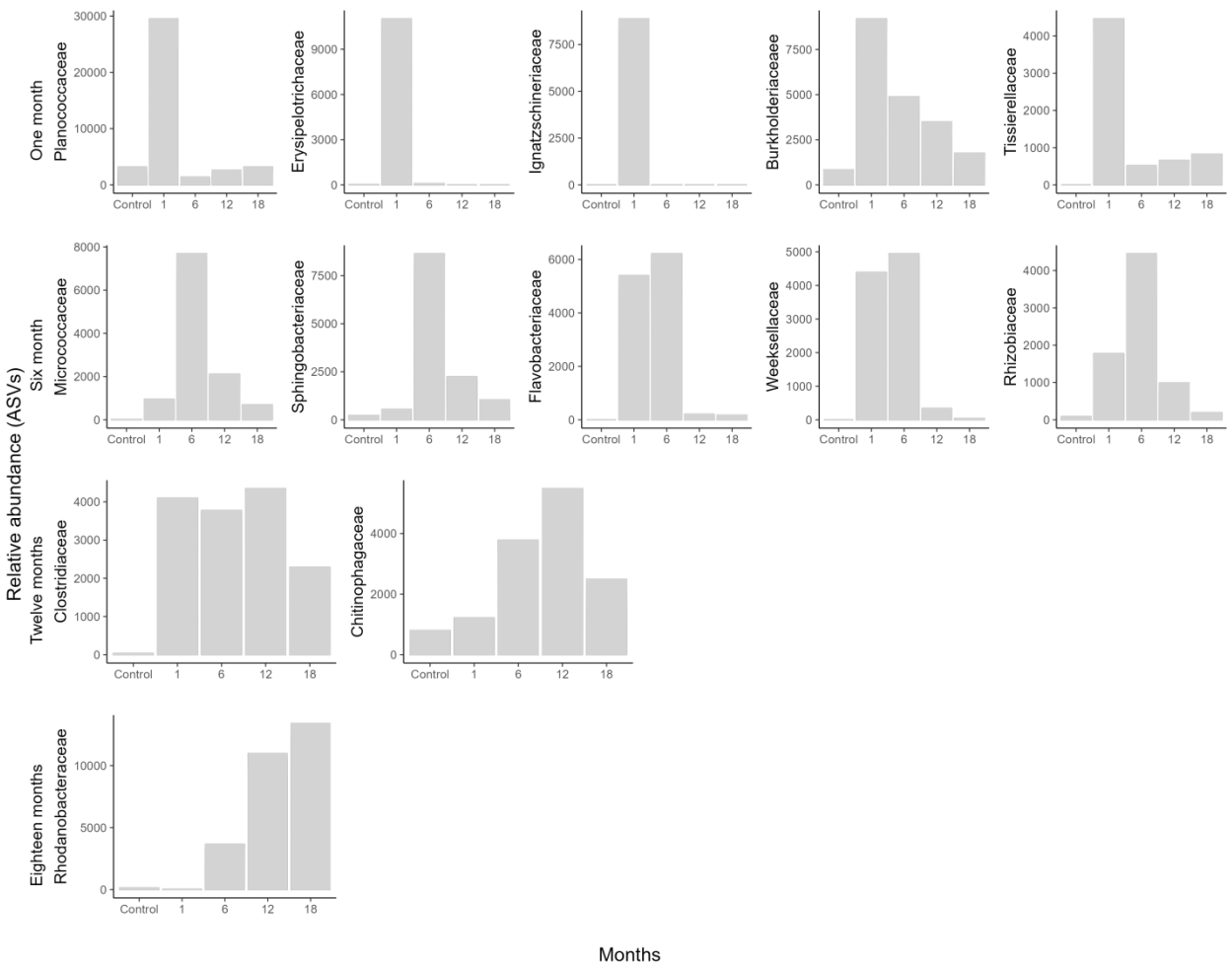


Figure 23: Relative abundances (ASV counts) of biologically relevant bacterial families (LDA log10 score > 3, p < 0.05) in the pooled control group and the 'Beneath' group at 1, 6, 12, and 18 months postmortem. Boxplots are organised based on the time point at which each family reached its peak abundance.

4.3.5. Inferred functional gene abundance

From the shifts in taxonomic abundances, *Tax4Fun* inferred that functional genes for the enzymes ammonia monooxygenase and nitrate reductase, which catalyses ammonia's oxidation and reduces nitrate to nitrite, was significantly (p < 0.001) increased in the microbial communities beneath the carcasses after six months, thus accelerating the rate at which nitrogen is cycled at that time (Figure 24). It was inferred that ammonia oxidation remained significantly elevated after twelve months (p = 0.019), whereas nitrite reduction was elevated up to eighteen months (p = 0.034). The predicted ammonia oxidation and nitrite reduction appear to follow the

significant increases in ammonium and nitrate observed in the soil samples chronologically (Figure 14).

A study by Heo et al. (2021), which analysed substrate utilisation of soil beneath swine carcasses with EcoPlates, indicated that the Glucose-1-phosphate was a consistent indicator of decomposition. Based on shifts in taxonomic abundances, the abundance of glucose-1-phosphatase, a catalyst of the carbohydrate Glucose-1-phosphate metabolism, increased significantly ($p < 0.001$) after a month and remained elevated after six months (Figure 24). The EcoPlate analysis described in Chapter 3 also indicated a significant increase in Glucose-1-phosphate utilisation beneath the carcasses in the first month ($t(18) = -3.542$, $p = 0.002$) that was not significant by six months ($t(18) = 0.776$, $p = 0.448$) and thereafter. Total carbohydrate utilisation was only significantly increased after eighteen months (Chapter 3 Figure 11). In this study, the utilisation of the carbohydrate D-xylose, also identified by Heo et al. (2021) as an indicator of carcass decomposition, was elevated by twelve months ($t(18)= -2.827$, $p = 0.011$) and remained significantly elevated after eighteen months ($t(18)= -2.185$, $p = 0.042$), isolating the elevation of D-xylose to the 'remains' and post-carcass decomposition stages. The taxonomic-based gene abundance analysis indicated that the enzyme D-xylose 1-dehydrogenase, a catalyst for the reduction of D-xylose to D-xylonolactone, increased by the sixth month and remained significantly elevated up to eighteen months (Figure 24).

The diamines, putrescine and cadaverine, are responsible for the odour of putrifying flesh (Crippen et al., 2016; Metcalf et al., 2016). Putricine can be produced by the decarboxylation of arginine to agmatine, which is then catalysed to putricine and urea by agmatinase, although other pathways exist (Metcalf et al., 2016). Based on inferred gene abundance, the enzymes in this pathway were abundant from the sixth to the twelfth month, and the enzyme spermidine synthase, which produces spermidine from putrescine, was elevated from the sixth to the eighteenth month (Figure 24). The utilisation of putricine as a sole-carbon substrate (Chapter 3), however, only significantly increased after eighteen months ($t(18)= -3.144$, $p = 0.005$). The production of cadaverine by the enzyme lysine decarboxylase was predicted to increase from the first month and remain elevated (Figure 24).

Although sampling time points that had elevated sole carbon-substrate utilisation were somewhat reflected by the inferred increase of related functional genes described above, analysis between

time-point paired samples of substrate utilisation and functional gene abundance only revealed slight positive correlations that were not statistically significant ($p > 0.05$). The accuracy of inferences made by software packages such as *Tax4Fun* depends on the accuracy and extent of the reference genome databases and the environments' complexity. Furthermore, the presence of functional genes, inferred from 16S rDNA, does not indicate that those functional genes are being expressed; therefore, a multi-omics approach would provide greater accuracy. (Xu et al., 2014). In addition, bacterial genomes contain accessory genes subject to horizontal gene transfer, even to distantly related species, and through which nitch-related functional traits can be transferred. Horizontal gene transfer is prevalent in soil, especially in the rhizo- and mycosperes (Nielsen and van Elsas, 2019).

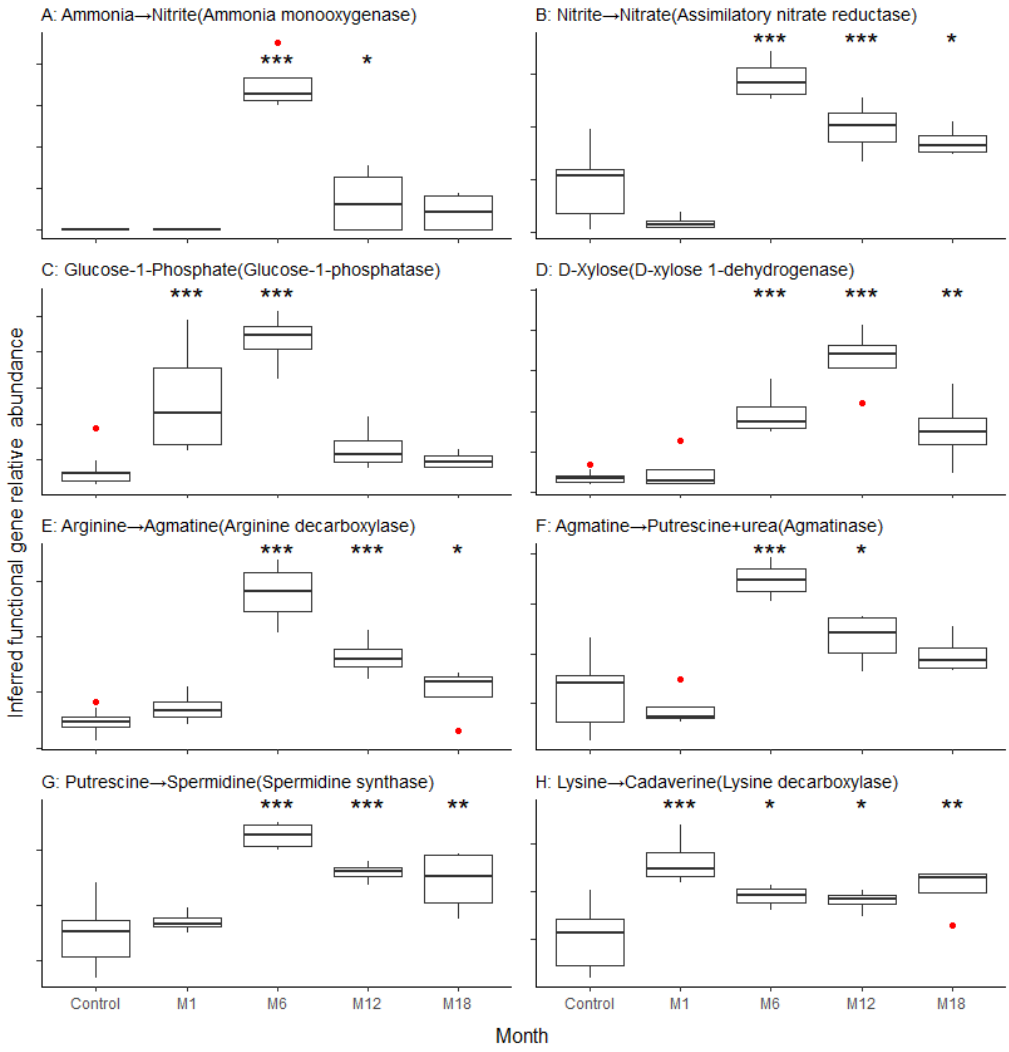


Figure 24: Inferred relative abundance of functional genes from the soil beneath the carcasses at different time points compared to a pooled control group. Significant differences from the control group (Dunnett's test) are indicated as follows: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$. Outliers are shown in red. (M1: one month, M6: six months, M12: twelve months, M18: eighteen months post-carcass placement).

4.4. Conclusions and recommendations

The results from this study contribute to our understanding of soil microbial community structure succession and postmortem microbial forensics. This study revealed that the mesic-grassland soil microbiome beneath large decomposing herbivore carcasses underwent successive structural changes that distinguished it from Control soil exceeding eighteen months. Species richness and diversity decreased during 'active decay', with richness recovering after twelve months and diversity by eighteen months. The microbiomes beneath the carcasses were significantly dissimilar at different time points for up to six months postmortem and significantly dissimilar from the Control soil for up to twelve months. Firmicutes families associated with necrophagous dipteral larvae dominated the soil microbiomes during 'active decay' but abruptly reduced afterwards. Bacteroidetes and then Proteobacteria dominated during advanced and post-carcass decay. Biologically relevant family-level biomarkers that differed by more than three orders of magnitude in abundance were identified. Biomarkers identified in literature from rodent, swine and human decomposition studies were confirmed, whereas others, such as Chitinophaceae, had notable enrichments beneath the wildebeest carcasses during the 'remains' and post-decay stage. These biomarkers may be related to the size of the carcasses, the extent of dipteran larva infestation, the extended sampling duration or the carcass species. There was a chronological similarity of the inferred functional gene abundance, the measured soil nutrients, and the utilisation of sole-carbon substrates. Increases in the relative abundance of putrescine and cadaverine-related enzymes were evident eighteen months postmortem.

It is recommended that soil sampling time points are synchronised with carcass rupture instead of carcass placement and that larger samples ($n > 4$) are collected to minimise within-sample variance, especially for the early sample comparisons. Studies beyond an eighteen-month time frame are recommended, as an increase in class Xanthomonadales, family Rhodanobacteracea, was observed even at the latest time point of this study. Because the same set of biomarkers was highly reduced beneath the carcasses throughout the experimental period, it is recommended that a control group should be included in carcass decomposition studies, not exposed to carcass decomposition but to confounding factors such as the microclimate created beneath the carcass. Improving microbial community functional characterisation requires a combined approach that includes a culture-dependent and multi-omics culture-independent approach.

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Chapter 5
Concluding remarks

5.1 Significance of the study

Soil microbiome changes beneath carcasses have been studied mainly through the decomposition of rodents, swine and humans. Samples are usually collected during the decomposition phases, diversity and structural dissimilarity are compared, and differentially abundance taxa are identified (Weiss et al., 2016). The carcass species selection is based on their similarity to humans because most studies are forensically focused. The decomposition of herbivores in excess of 200 kg has rarely been studied, although they represent most of the animal biomass in natural grasslands and pastures. In addition, most studies discontinue sampling once the decomposition has been completed. The long-term functional succession of the soil microbiomes during and after decomposition has received little attention. Given the rapid degradation of grasslands worldwide (Montanarella et al., 2015) and the significant contribution of energy-rich nutrients from carcasses to soil quality (Millard and Singh, 2010), it has become increasingly important to study the effects of large decomposing carcasses on soil microbial structure and function, particularly in grassland ecosystems.

This study characterised the functional and structural succession of soil microbiomes beneath large herbivore (*Connochaetes taurinus*) carcasses over long-term (one, six, twelve and eighteen-month) postmortem intervals (PMIs). Because most previous studies have involved omnivores with different intestinal microbiomes (Kobayashi et al., 2020), smaller mass, and a less robust skin structure than *Connochaetes*, this study adds to our knowledge of carcass decomposition. In addition, the natural mesic grassland where the study occurred has a history of intermittent exposure to similar decomposing species, thus naturally priming the soil microbiome for carcass decomposition, which may distinguish the results of this study from others. The Biolog™ Eoplate™ method, frequently utilised in agricultural and ecotoxicological studies but rarely utilised in carcass decomposition studies (Pechal et al., 2016; Heo et al., 2021), was proven to be an effective means of evaluating changes in carcass soil microbiomes. Significant differences in functional diversity and substrate guild utilisation could be detected even after eighteen months postmortem using ten sample replicates.

Structural succession was determined by 16S rRNA gene metagenomic analysis of the soil, as applied in other decomposition studies. Differential abundance analysis is considered the most accurate method for characterising metagenomic data when investigating microbiome associations. Numerous algorithms and software packages have been utilised for this purpose, with various advantages and disadvantages (Weiss et al., 2017; Lin and Pedadda, 2020). The Linear Discriminant Analysis Effect Size (LEfSe) algorithm was utilised in this study precisely as it quantifies the magnitude of the effect size of the differentially abundant microbial biomarkers. The algorithm identified and indicated the magnitude of biomarkers at various taxonomic levels depicted through cladograms.

5.2. Summary findings, contribution of the study and recommendations.

The overall function, observed from the mean substrate utilisation, was increased for six months postmortem as *hot moments* were created beneath the carcasses in which most microbial processes were accelerated. The duration of the *hot moments* coincided with the increased ammonium and sulphate in the soil. In testing the null hypotheses of no significant differences between the functional and structural diversities at different PMIs, it was revealed that functional diversity had increased in the soil beneath the carcasses for the entire experimental period (18 months). In contrast, structural diversity beneath the carcasses was reduced but recovered to control soil levels by eighteen months. Equally, the functional dissimilarity remained different from the control soil, whereas structural dissimilarity could not be discerned by eighteen months. The reviewed literature did not contain comparisons of functional or structural diversity at such an extended PMI, and the result contributes by indicating that functional changes brought about by large decomposing carcasses are enduring. The findings of this study highlight the long-term augmentation of phosphorus, a limiting nutrient, and the relatively short-term augmentation of nitrogen from large-carcass decomposition. The increased potential to degrade specific substrate groups was observed for an undetermined period, which may have broader ecological implications, such as the sustainability of grasslands. The study also highlights a disconnect between functional diversity and structural diversity. While functional diversity remained altered at eighteen months, with specific substrate guilds being utilized more than others, the structural diversity was no longer statistically dissimilar from the control soil. Therefore, further

metatranscriptomics research is recommended, as the disconnect between function and structure may originate from horizontal gene transfer (Nielsen and van Elsas, 2019).

In this study, the carbon source utilisation results of the 31 sole-carbon substrates were grouped into guilds and compared between soil from beneath carcasses and control soil with statistically significant results. However, it was noted that specific sole-carbon substrates (Glucose-1-phosphate, D-xylose and Putrescine) in isolation had significant differences between treatment groups, as also observed by Hoe et al. (2021). For further studies, it is recommended that a combination of sole-carbon substrates, selected explicitly for carcass decomposition, is utilised. Such a combination of sole-carbon substrates may be able to estimate PMIs accurately and has forensic utility.

Several biologically relevant biomarkers of various taxonomic levels were indicated, many of which correspond with related studies. The increase in Firmicutes at the onset of decomposition was no longer biologically relevant after six months, whereas increases in Proteobacteria and Bacteriodetes remained relevant biomarkers twelve months postmortem. At the one-month PMI, the most relevant microbial biomarkers in the soil were families and genera associated with necrophagous invertebrate vectors. Most of these were no longer relevant after six months when the carcasses were decomposed. Chitinophaceae, observed in short-term studies (Weiss et al., 2016) and related to the decomposition of insect remains and Rhodanobacteracea, were the most prominent family-level biomarkers at the twelve-month PMI. Rhodanobacteracea was the only relevant biomarker eighteen months postmortem. As Rhodanobacteracea was still increasing in abundance eighteen months postmortem, it is recommended that studies exceeding eighteen months be performed. It is further recommended that the soil microbial function and structure of grasslands in which herbivores are absent and present at different stocking rates be compared to establish if carcass decomposition has an effect at an ecological scale.

A balance between macro primary producers (vegetation) and grazers, predators, and scavengers (animals) is well-recognised in natural and sustainable grassland ecosystems. However, the long-term effects of anthropogenic activities, such as overstocking grazers, removing herbivores from the grassland before they expire, and the lack of predators and scavengers, are less understood. The impact of these activities on the soil microbiome and its sustainability is particularly underexplored. This study revealed that a large decomposing herbivore carcass affects the

immediate soil microbiome beyond the carcass's ephemeral decomposition, prompting a reevaluation of existing anthropogenic practices.

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Appendix A

Table 5: Minimum and maximum temperatures and rainfall measurements as recorded by the Bronkhorstspruit (0514408AX) and Witbank (0515320 8) weather stations of the South African Weather Services (SAWS) from 2016 to 2020. These weather stations are closest to the Telperion Nature Reserve.

Min

Weather station	Year	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
BRONKHORSTSPRUIT	2016		17.7	13.9	10.2	5.6	1.8	-0.5	1.2	9	11.8	14.7	16.7
BRONKHORSTSPRUIT	2017	16.1	16.3	12.4	10.3	4.4	0.8	0.8	3	8.3	10.5	12.8	14.9
BRONKHORSTSPRUIT	2018	14.1	15.4	13.7	7.8	2		-2	1.4	8	9.7	12.9	16.4
BRONKHORSTSPRUIT	2019	16.2	15.8	13.6	10.8	4.2	-0.5	-1.6	4.9	6.3	10.5	15.3	15.7
BRONKHORSTSPRUIT	2020	16.4	15.7	12.8	10	3.2	-1.2	-1.4	1	7.1	13.2	14.6	16.7
WITBANK	2016	15.3	15.7	14.2	10.9	7.5	5.3	3.4	5.8	8.8	12.1	13.5	15.3
WITBANK	2017	15	15	12.4	10.6	7	5	5.3	5.6	9.8	10.7	11.9	14.1
WITBANK	2018	13.9	15	13.4	12	7	4.5	4	7.6	9.6	10	12.2	15.4
WITBANK	2019	15.2	15.1	14.3	11.4	7.4	4.5	4.5	8.2	8.9	13	14.9	15
WITBANK	2020	15.2	14.9	12.4	10.2	6.2	3.2	3.1	5.4	9.8	13	14.7	16.1
AVG		15.3	15.7	13.3	10.4	5.5	2.6	1.6	4.4	8.6	11.5	13.8	15.6

Max

Weather station	Year	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
BRONKHORSTSPRUIT	2016		31.7	28.6	27.2	22.2	20.6	20.1	24.6	27.4	29.3	28	28.9
BRONKHORSTSPRUIT	2017	28.4	29.7	28.4	25.6	23	21.6	22.1	22.8	28.3	26.2	28	28
BRONKHORSTSPRUIT	2018	30.5	28	27.7	23.6	21.1		21.8	23.7	29.5	27.7	28.3	31.3
BRONKHORSTSPRUIT	2019	29.5	28.1	30.4	24.9	25.4	22.5	22.9	26.2	27.9	30.7	30.4	27.1
BRONKHORSTSPRUIT	2020	28.6	28.9	27.2	24.1	23.3	20	21.3	21.9	26.1	28.8	28.1	28.4
WITBANK	2016	27.8	29.5	26.8	25.6	20.9	19.4	18.9	23.3	22.6	27.6	26	27.5
WITBANK	2017	27.1	26.6	26.7	23.9	21.2	20.2	20.6	21.3	26.8	24.5	27	26.5
WITBANK	2018	27.9	26.3	26	23.9	21.4	20.1	18.6	23.6	27.2	26.2	26.8	29.7
WITBANK	2019	27.8	26.4	28	23.2	23.5	21.2	21.7	24.6	26.2	29.3	28.3	25.8
WITBANK	2020	26.7	26.7	25.6	22	21.7	18.4	19.9	21.8	26.1	26.6	26.5	27
AVG		28.3	28.2	27.5	24.4	22.4	20.4	20.8	23.4	26.8	27.7	27.7	28.0

Rainfall

Weather station	Year	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
BRONKHORSTSPRUIT	2016		27.2	176	30.6	56.6	11.2		0.4	8.6	73.2	199.6	62.4
BRONKHORSTSPRUIT	2017	205	4.6	11.2	96.6	30.6	0	0.6	0	35	83	89	87.8
BRONKHORSTSPRUIT	2018	21.6	65	196.4	41.6	22.6	0.2	5.4	5.2	9.8	65.6	55.4	101.8
BRONKHORSTSPRUIT	2019	140.6	153.8	8.8	49	1.8	0	0	0	2.2	3	69.2	267.2
BRONKHORSTSPRUIT	2020	98.6	82.2	76.2	128.4	0	5	0	0	8.8	0	59.6	86.8
WITBANK	2016	85.4	40.8	170.2	5.8	29	12	3	0.6	6.4	84	224.6	81.8
WITBANK	2017	181.2	127.8	35	113.6	56.8	0	12.4	3.8	27.2	83.8	109.2	153.2
WITBANK	2018	71.8	75.8	148.4	26	24.6	0.2	5.2	0	7.6	61.2	64	123
WITBANK	2019	119.4	98.2	46.6	65	1.4	0	0	0	6.8	22	111.6	156.2
WITBANK	2020	239.6	66.8	73.6	5.4	0	0.2	0	0	0	26.4	0.2	22.2
AVG		129.2	74.2	94.2	56.2	22.3	2.9	3.0	1.0	11.2	50.2	98.2	114.2
Yearly AVG		54.6	54.6	54.6	54.6	54.6	54.6	54.6	54.6	54.6	54.6	54.6	54.6

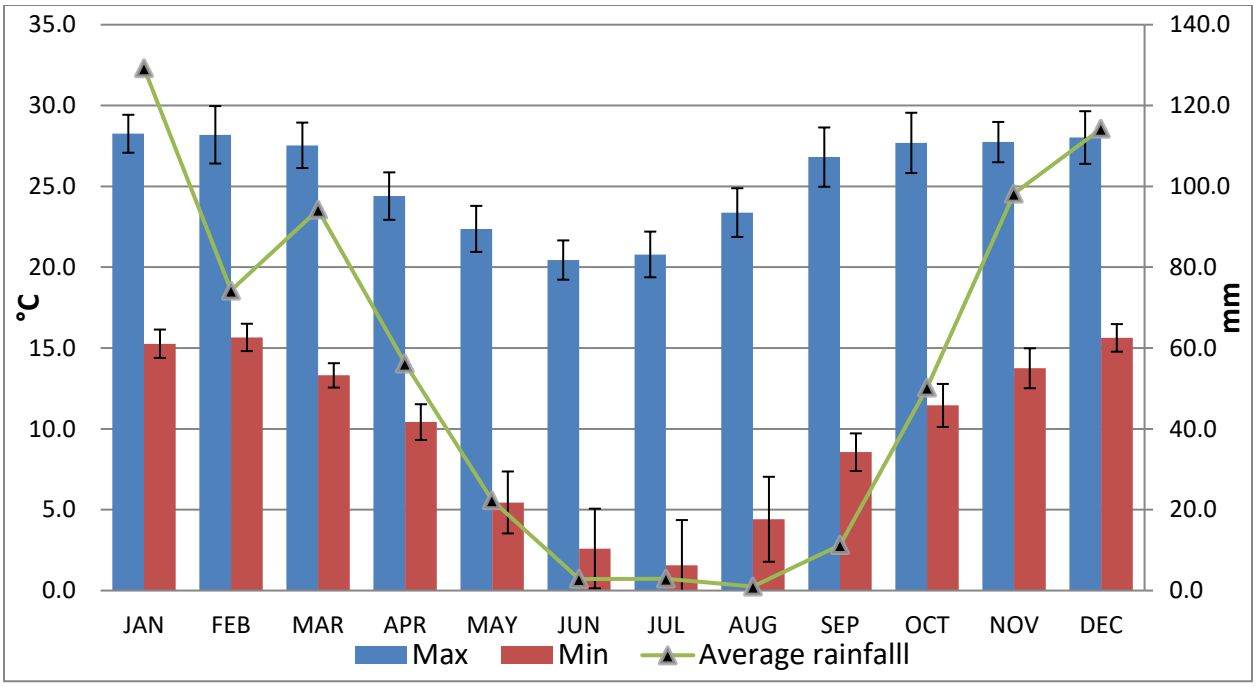

Figure 25: Average temperatures and rainfall recorded for the Telperion Nature Reserve.

Appendix B

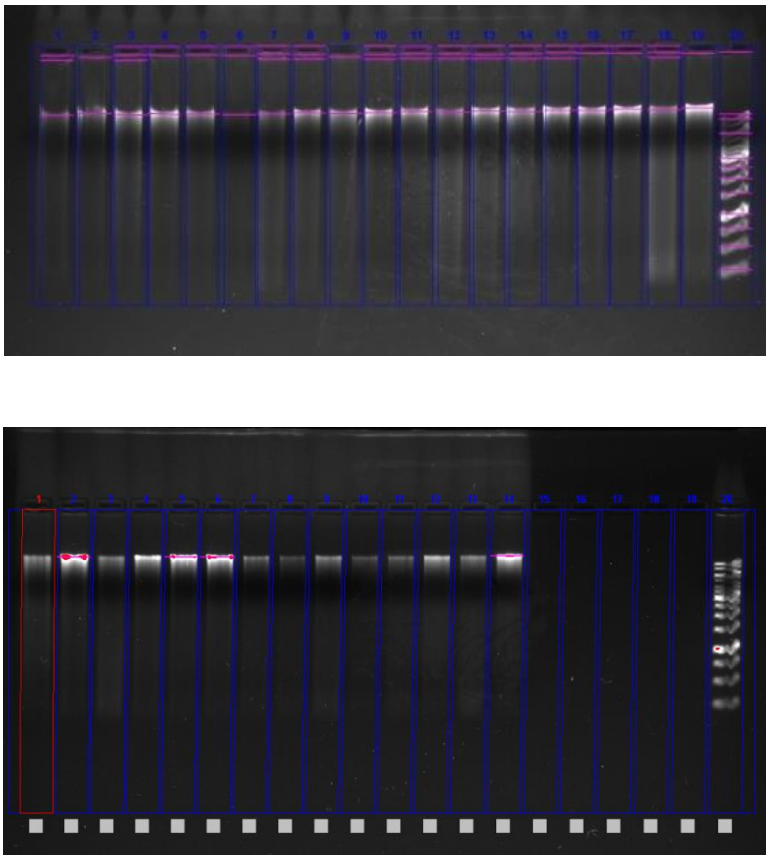

Figure 26: Quality and integrity evaluation of the environmental DNA of the 32 soil samples with 1% agarose gel electrophoresis.

Appendix C

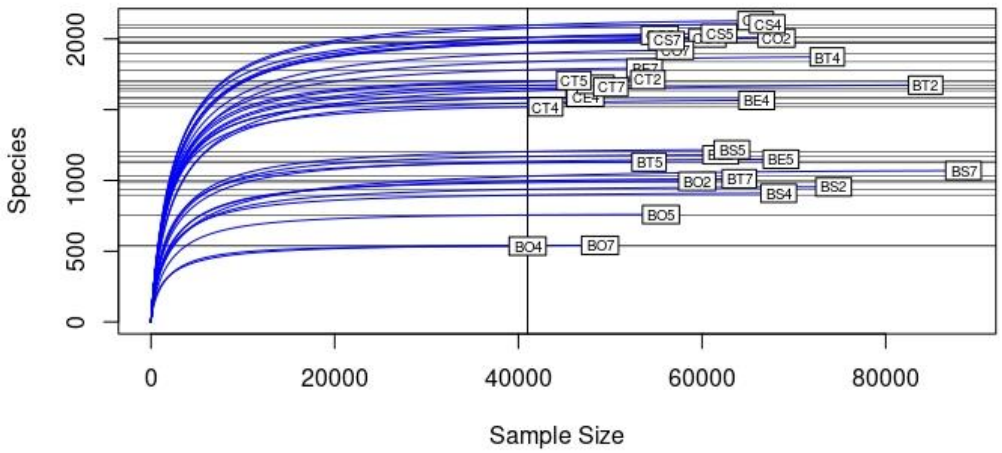

Figure 27: Rarefaction curves indicating that the processed data adequately represents the diversity of ASVs of each sample.

Appendix D

Table 6: Taxa with significantly different (Mann-Whitney *U:* $p < 0.05$) abundances between the 'Beneath' and 'Control' groups after one month of carcass decomposition. The data is sorted by descending linear discriminant analysis effect size (ef_lds).

No.	feature	enrich_group	ef_lda	pvalue
1	k__Bacteria|p__Firmicutes	Beneath	4.15	0.02
2	k__Bacteria|p__Firmicutes|c__Bacilli	Beneath	3.96	0.02
3	k__Bacteria|p__Firmicutes|c__Bacilli|o__Bacillales	Beneath	3.95	0.02
4	k__Bacteria	Beneath	3.93	0.02
5	k__Bacteria|p__Firmicutes|c__Bacilli|o__Bacillales|f__Planococcaceae	Beneath	3.88	0.02
6	k__Bacteria|p__Firmicutes|c__Bacilli|o__Bacillales|f__Planococcaceae|g__Savagea	Beneath	3.80	0.02
7	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria	Beneath	3.63	0.02
8	k__Bacteria|p__Proteobacteria	Beneath	3.53	0.02
9	k__Bacteria|p__Firmicutes|c__Erysipelotrichia|o__Erysipelotrichales|f__Erysipelotrichaceae|g__Erysipelothrix	Beneath	3.46	0.02
10	k__Bacteria|p__Firmicutes|c__Erysipelotrichia|o__Erysipelotrichales	Beneath	3.45	0.02
11	k__Bacteria|p__Firmicutes|c__Erysipelotrichia|o__Erysipelotrichales|f__Erysipelotrichaceae	Beneath	3.45	0.02
12	k__Bacteria|p__Firmicutes|c__Erysipelotrichia	Beneath	3.45	0.02
13	k__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales	Beneath	3.36	0.02
14	k__Bacteria|p__Firmicutes|c__Clostridia	Beneath	3.35	0.02
15	k__Bacteria|p__Firmicutes|c__Bacilli|o__Bacillales|f__Bacillaceae|g__Pseudogracilibacillus	Beneath	3.33	0.01
16	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Cardiobacteriales	Beneath	3.33	0.02
17	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Cardiobacteriales|f__Wohlfahrtiimonadaceae	Beneath	3.33	0.02
18	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Cardiobacteriales|f__Wohlfahrtiimonadaceae|g__Ignatzschineria	Beneath	3.30	0.02
19	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Flavobacteriales	Beneath	3.30	0.02
20	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Betaproteobacteriales|f__Burkholderiaceae	Beneath	3.26	0.02
21	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Betaproteobacteriales	Beneath	3.25	0.02
22	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Betaproteobacteriales|f__Burkholderiaceae|g__Paenalcaligenes	Beneath	3.15	0.01
23	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Flavobacteriales|f__Flavobacteriaceae	Beneath	3.05	0.01
24	k__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales|f__Family_XI	Beneath	3.03	0.01
25	k__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales|f__Family_XI|g__Tissierella	Beneath	3.01	0.01
26	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Flavobacteriales|f__Flavobacteriaceae|g__Myroides	Beneath	2.99	0.01
27	k__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales|f__Clostridiaceae_1	Beneath	2.99	0.02
28	k__Bacteria|p__Firmicutes|c__Bacilli|o__Bacillales|f__Planococcaceae|g__Sporosarcina	Beneath	2.96	0.02
29	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Flavobacteriales|f__Weeksellaceae	Beneath	2.92	0.02
30	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Flavobacteriales|f__Weeksellaceae|g__Moheibacter	Beneath	2.92	0.05
31	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Pseudomonadales	Beneath	2.83	0.02
32	k__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales|f__Clostridiaceae_1|g__Clostridium_sensu_stricto_7	Beneath	2.66	0.02
33	k__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales|f__Clostridiaceae_1|g__Hathewaya	Beneath	2.66	0.01
34	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Pseudomonadales|f__Moraxellaceae|g__Acinetobacter	Beneath	2.64	0.04
35	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Betaproteobacteriales|f__Burkholderiaceae|g__Pusillimonas	Beneath	2.51	0.01
36	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Micrococcales|f__Micrococcaceae	Beneath	2.46	0.02
37	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Pseudomonadales|f__Pseudomonadaceae|g__Oblitimonas	Beneath	2.39	0.01
38	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Pseudomonadales|f__Pseudomonadaceae	Beneath	2.38	0.02
39	k__Bacteria|p__Firmicutes|c__Bacilli|o__Lactobacillales	Beneath	2.37	0.02
40	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Chitinophagales|f__Chitinophagaceae|g__Taibaiella	Beneath	2.37	0.01
41	k__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales|f__Clostridiaceae_2	Beneath	2.28	0.05
42	k__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales|f__Clostridiaceae_2|g__Alkaliphilus	Beneath	2.27	0.05
43	k__Bacteria|p__Firmicutes|c__Bacilli|o__Lactobacillales|f__Enterococcaceae	Beneath	2.23	0.02
44	k__Bacteria|p__Firmicutes|c__Bacilli|o__Lactobacillales|f__Enterococcaceae|g__Vagococcus	Beneath	2.22	0.02
45	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Bacteroidales	Beneath	2.21	0.05
46	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Sphingobacteriales|f__Sphingobacteriaceae|g__Sphingobacterium	Beneath	2.07	0.01
47	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Enterobacteriales	Beneath	2.05	0.02
48	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Enterobacteriales|f__Enterobacteriaceae	Beneath	2.04	0.02
49	k__Bacteria|p__Firmicutes|c__Bacilli|o__Bacillales|f__Bacillaceae|g__Cerasibacillus	Beneath	2.02	0.01
50	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Cardiobacteriales|f__Wohlfahrtiimonadaceae|g__Wohlfahrtiimonas	Beneath	1.99	0.05
51	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Betaproteobacteriales|f__Burkholderiaceae|g__Comamonas	Beneath	1.97	0.05
52	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Enterobacteriales|f__Enterobacteriaceae|g__Providencia	Beneath	1.94	0.01
53	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Flavobacteriales|f__Flavobacteriaceae|g__Flavobacterium	Beneath	1.92	0.05
54	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Micrococcales|f__Microbacteriaceae|g__Leucobacter	Beneath	1.77	0.05
55	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Flavobacteriales|f__Flavobacteriaceae|g__Ulvibacter	Beneath	1.76	0.05
56	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Betaproteobacteriales|f__Burkholderiaceae|g__Alcaligenes	Beneath	1.75	0.05
57	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Micrococcales|f__Micrococcaceae|g__Enteractinococcus	Beneath	1.69	0.01
58	k__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales|f__Family_XI|g__Helcococcus	Beneath	1.69	0.01
59	k__Bacteria|p__Firmicutes|c__Bacilli|o__Lactobacillales|f__Carnobacteriaceae	Beneath	1.67	0.01
60	k__Bacteria|p__Firmicutes|c__Bacilli|o__Lactobacillales|f__Carnobacteriaceae|g__Atopostipes	Beneath	1.64	0.01
61	k__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales|f__Clostridiaceae_1|g__Clostridium_sensu_stricto_1	Beneath	1.59	0.04
62	k__Bacteria|p__Firmicutes|c__Bacilli|o__Bacillales|f__Staphylococcaceae	Beneath	1.52	0.01
63	k__Bacteria|p__Firmicutes|c__Bacilli|o__Bacillales|f__Staphylococcaceae|g__Nosocomiicoccus	Beneath	1.52	0.01
64	k__Bacteria|p__Firmicutes|c__Bacilli|o__Lactobacillales|f__Streptococcaceae	Beneath	1.42	0.05
65	k__Bacteria|p__Firmicutes|c__Bacilli|o__Lactobacillales|f__Streptococcaceae|g__Lactococcus	Beneath	1.38	0.05
66	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Caulobacterales|f__Caulobacteraceae|g__Brevundimonas	Beneath	1.35	0.05
67	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Corynebacteriales|f__Corynebacteriaceae	Beneath	1.33	0.05
68	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Corynebacteriales|f__Corynebacteriaceae|g__Corynebacterium_1	Beneath	1.33	0.05
69	k__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales|f__Clostridiaceae_1|g__Clostridium_sensu_stricto_15	Beneath	1.28	0.01
70	k__Bacteria|p__Firmicutes|c__Bacilli|o__Bacillales|f__Bacillaceae|g__Ornithinibacillus	Beneath	1.28	0.05
71	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Betaproteobacteriales|f__Burkholderiaceae|g__Bordetella	Beneath	1.19	0.05
72	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Flavobacteriales|f__Weeksellaceae|g__Weeksella	Beneath	1.08	0.01
73	k__Bacteria|p__Firmicutes|c__Bacilli|o__Bacillales|f__Bacillaceae|g__Amphibacillus	Beneath	1.08	0.05
74	k__Bacteria|p__Acidobacteria	Control	3.52	0.02
75	k__Bacteria|p__Verrucomicrobia|c__Verrucomicrobiae	Control	3.48	0.02
76	k__Bacteria|p__Verrucomicrobia	Control	3.47	0.02
77	k__Bacteria|p__Acidobacteria|c__Acidobacteriia	Control	3.47	0.02
78	k__Bacteria|p__Verrucomicrobia|c__Verrucomicrobiae|o__Chthoniobacterales	Control	3.43	0.02
79	k__Bacteria|p__Acidobacteria|c__Acidobacteriia|o__Solibacterales|f__Solibacteraceae_(Subgroup_3)	Control	3.40	0.02
80	k__Bacteria|p__Acidobacteria|c__Acidobacteriia|o__Solibacterales	Control	3.40	0.02
81	k__Bacteria|p__Actinobacteria	Control	3.39	0.02
82	k__Bacteria|p__Verrucomicrobia|c__Verrucomicrobiae|o__Chthoniobacterales|f__Chthoniobacteraceae	Control	3.28	0.02
83	k__Bacteria|p__Verrucomicrobia|c__Verrucomicrobiae|o__Chthoniobacterales|f__Chthoniobacteraceae|g__Candidatus_Udaeobacter	Control	3.26	0.02
84	k__Bacteria|p__Acidobacteria|c__Acidobacteriia|o__Solibacterales|f__Solibacteraceae_(Subgroup_3)|g__Bryobacter	Control	3.22	0.02
85	k__Bacteria|p__Actinobacteria|c__Actinobacteria	Control	3.16	0.04
86	k__Bacteria|p__Actinobacteria|c__Thermoleophilia	Control	3.03	0.02
87	k__Bacteria|p__Actinobacteria|c__Thermoleophilia|o__Solirubrobacterales|f__Solirubrobacteraceae	Control	3.01	0.02
88	k__Bacteria|p__Actinobacteria|c__Thermoleophilia|o__Solirubrobacterales	Control	3.01	0.02
89	k__Bacteria|p__Actinobacteria|c__Thermoleophilia|o__Solirubrobacterales|f__Solirubrobacteraceae|g__Conexibacter	Control	2.99	0.02
90	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Frankiales	Control	2.96	0.02
91	k__Bacteria|p__Verrucomicrobia|c__Verrucomicrobiae|o__Chthoniobacterales|f__Xiphinematobacteraceae	Control	2.94	0.04
92	k__Bacteria|p__Verrucomicrobia|c__Verrucomicrobiae|o__Chthoniobacterales|f__Xiphinematobacteraceae|g__Candidatus_Xiphinematobacter	Control	2.93	0.04
93	k__Bacteria|p__Acidobacteria|c__Acidobacteriia|o__Solibacterales|f__Solibacteraceae_(Subgroup_3)|g__Candidatus_Solibacter	Control	2.91	0.02
94	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Frankiales|f__Acidothermaceae|g__Acidothermus	Control	2.82	0.02
95	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Frankiales|f__Acidothermaceae	Control	2.82	0.02
96	k__Bacteria|p__Chloroflexi|c__Ktedonobacteria|o__Ktedonobacterales|f__Ktedonobacteraceae|g__1921-3	Control	2.75	0.02

No.	feature	enrich_group	ef_lda	pvalue
97	k__Bacteria|p__Chloroflexi|c__Ktedonobacteria|o__Ktedonobacterales|f__Ktedonobacteraceae|g__FCPS473	Control	2.70	0.04
98	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Rhizobiales|f__Xanthobacteraceae	Control	2.70	0.02
99	k__Bacteria|p__Acidobacteria|c__Acidobacteriia|o__Acidobacteriales	Control	2.68	0.02
100	k__Bacteria|p__Proteobacteria|c__Deltaproteobacteria	Control	2.61	0.02
101	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Rhizobiales|f__Xanthobacteraceae|g__Bradyrhizobium	Control	2.60	0.02
102	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Gammaproteobacteria_Incertae_Sedis|f__Gammaproteobacteria_Incertae_Sedis_Unknown_Family|g__Acidibacter	Control	2.59	0.02
103	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Gammaproteobacteria_Incertae_Sedis|f__Gammaproteobacteria_Incertae_Sedis_Unknown_Family	Control	2.59	0.02
104	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Gammaproteobacteria_Incertae_Sedis	Control	2.59	0.02
105	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Corynebacteriales|f__Mycobacteriaceae|g__Mycobacterium	Control	2.58	0.02
106	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Corynebacteriales|f__Mycobacteriaceae	Control	2.58	0.02
107	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Corynebacteriales	Control	2.57	0.02
108	k__Bacteria|p__Acidobacteria|c__Acidobacteriia|o__Acidobacteriales|f__Acidobacteriaceae_(Subgroup_1)	Control	2.54	0.02
109	k__Bacteria|p__Acidobacteria|c__Blastocatellia_(Subgroup_4)	Control	2.52	0.02
110	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Sphingomonadales	Control	2.51	0.02
111	k__Bacteria|p__Proteobacteria|c__Deltaproteobacteria|o__Myxococcales	Control	2.51	0.02
112	k__Bacteria|p__Acidobacteria|c__Blastocatellia_(Subgroup_4)|o__Pyrinomonadales|f__Pyrinomonadaceae	Control	2.50	0.04
113	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Sphingomonadales|f__Sphingomonadaceae	Control	2.50	0.02
114	k__Bacteria|p__Acidobacteria|c__Blastocatellia_(Subgroup_4)|o__Pyrinomonadales	Control	2.50	0.04
115	k__Bacteria|p__Acidobacteria|c__Blastocatellia_(Subgroup_4)|o__Pyrinomonadales|f__Pyrinomonadaceae|g__RB41	Control	2.50	0.04
116	k__Bacteria|p__Chloroflexi|c__Ktedonobacteria|o__Ktedonobacterales|f__Ktedonobacteraceae|g__1921-2	Control	2.49	0.02
117	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Sphingomonadales|f__Sphingomonadaceae|g__Sphingomonas	Control	2.39	0.02
118	k__Bacteria|p__Verrucomicrobia|c__Verrucomicrobiae|o__Pedosphaerales	Control	2.33	0.02
119	k__Bacteria|p__Verrucomicrobia|c__Verrucomicrobiae|o__Pedosphaerales|f__Pedosphaeraceae	Control	2.33	0.02
120	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Pseudonocardiales	Control	2.33	0.02
121	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Pseudonocardiales|f__Pseudonocardiaceae	Control	2.32	0.02
122	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Chitinophagales|f__Chitinophagaceae|g__Flavisolibacter	Control	2.25	0.02
123	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Frankiales|f__Frankiaceae	Control	2.22	0.04
124	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Frankiales|f__Frankiaceae|g__Jatrophihabitans	Control	2.22	0.04
125	k__Bacteria|p__Planctomycetes	Control	2.20	0.02
126	k__Bacteria|p__Planctomycetes|c__Planctomycetacia	Control	2.20	0.02
127	k__Bacteria|p__Verrucomicrobia|c__Verrucomicrobiae|o__Pedosphaerales|f__Pedosphaeraceae|g__ADurb.Bin063-1	Control	2.17	0.02
128	k__Bacteria|p__Proteobacteria|c__Deltaproteobacteria|o__Myxococcales|f__Haliangiaceae|g__Haliangium	Control	2.15	0.02
129	k__Bacteria|p__Proteobacteria|c__Deltaproteobacteria|o__Myxococcales|f__Haliangiaceae	Control	2.14	0.02
130	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Pseudonocardiales|f__Pseudonocardiaceae|g__Crossiella	Control	2.14	0.02
131	k__Bacteria|p__Acidobacteria|c__Acidobacteriia|o__Acidobacteriales|f__Koribacteraceae	Control	2.13	0.02
132	k__Bacteria|p__Acidobacteria|c__Acidobacteriia|o__Acidobacteriales|f__Koribacteraceae|g__Candidatus_Koribacter	Control	2.12	0.02
133	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Acetobacterales|f__Acetobacteraceae	Control	2.08	0.02
134	k__Bacteria|p__Proteobacteria|c__Deltaproteobacteria|o__Myxococcales|f__Archangiaceae	Control	2.07	0.03
135	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Acetobacterales	Control	2.07	0.02
136	k__Bacteria|p__Proteobacteria|c__Deltaproteobacteria|o__Myxococcales|f__Archangiaceae|g__Anaeromyxobacter	Control	2.05	0.02
137	k__Bacteria|p__Gemmatimonadetes|c__Gemmatimonadetes|o__Gemmatimonadales	Control	1.99	0.02
138	k__Bacteria|p__Gemmatimonadetes	Control	1.99	0.02
139	k__Bacteria|p__Gemmatimonadetes|c__Gemmatimonadetes|o__Gemmatimonadales|f__Gemmatimonadaceae	Control	1.99	0.02
140	k__Bacteria|p__Gemmatimonadetes|c__Gemmatimonadetes	Control	1.99	0.02
141	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Sphingobacteriales|f__Sphingobacteriaceae|g__Mucilaginibacter	Control	1.97	0.01
142	k__Bacteria|p__Acidobacteria|c__Acidobacteriia|o__Acidobacteriales|f__Acidobacteriaceae_(Subgroup_1)|g__Granulicella	Control	1.95	0.01
143	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Propionibacteriales	Control	1.94	0.02
144	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Rhizobiales|f__Beijerinckiaceae	Control	1.90	0.04
145	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Sphingomonadales|f__Sphingomonadaceae|g__Ellin6055	Control	1.89	0.01
146	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Frankiales|f__Geodermatophilaceae	Control	1.88	0.04
147	k__Bacteria|p__Gemmatimonadetes|c__Gemmatimonadetes|o__Gemmatimonadales|f__Gemmatimonadaceae|g__Gemmatimonas	Control	1.86	0.02
148	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Acetobacterales|f__Acetobacteraceae|g__Acidiphilium	Control	1.84	0.02
149	k__Bacteria|p__Acidobacteria|c__Acidobacteriia|o__Acidobacteriales|f__Acidobacteriaceae_(Subgroup_1)|g__Occallatibacter	Control	1.83	0.02
150	k__Bacteria|p__Proteobacteria|c__Deltaproteobacteria|o__Bdellovibrionales|f__Bdellovibrionaceae	Control	1.82	0.02
151	k__Bacteria|p__Proteobacteria|c__Deltaproteobacteria|o__Bdellovibrionales	Control	1.81	0.02
152	k__Bacteria|p__Proteobacteria|c__Deltaproteobacteria|o__Bdellovibrionales|f__Bdellovibrionaceae|g__Bdellovibrio	Control	1.81	0.02
153	k__Bacteria|p__Planctomycetes|c__Planctomycetacia|o__Gemmatales	Control	1.79	0.02
154	k__Bacteria|p__Planctomycetes|c__Planctomycetacia|o__Gemmatales|f__Gemmataceae	Control	1.78	0.02
155	k__Bacteria|p__Planctomycetes|c__Planctomycetacia|o__Gemmatales|f__Gemmataceae|g__Gemmata	Control	1.77	0.02
156	k__Bacteria|p__Verrucomicrobia|c__Verrucomicrobiae|o__Chthoniobacterales|f__Chthoniobacteraceae|g__Chthoniobacter	Control	1.76	0.02
157	k__Bacteria|p__Actinobacteria|c__Thermoleophilia|o__Gaiellales|f__Gaiellaceae|g__Gaiella	Control	1.76	0.02
158	k__Bacteria|p__Actinobacteria|c__Thermoleophilia|o__Gaiellales	Control	1.76	0.02
159	k__Bacteria|p__Acidobacteria|c__Acidobacteriia|o__Acidobacteriales|f__Acidobacteriaceae_(Subgroup_1)|g__Acidipila	Control	1.75	0.02
160	k__Bacteria|p__Actinobacteria|c__Thermoleophilia|o__Gaiellales|f__Gaiellaceae	Control	1.75	0.02
161	k__Bacteria|p__Proteobacteria|c__Deltaproteobacteria|o__Myxococcales|f__Polyangiaceae	Control	1.70	0.04
162	k__Bacteria|p__Proteobacteria|c__Deltaproteobacteria|o__Myxococcales|f__Polyangiaceae|g__Pajaroellobacter	Control	1.70	0.04
163	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Betaproteobacteriales|f__Nitrosomonadaceae	Control	1.69	0.02
164	k__Bacteria|p__Acidobacteria|c__Acidobacteriia|o__Acidobacteriales|f__Acidobacteriaceae_(Subgroup_1)|g__Bryocella	Control	1.67	0.05
165	k__Bacteria|p__Armatimonadetes	Control	1.67	0.02
166	k__Bacteria|p__Armatimonadetes|c__Chthonomonadetes	Control	1.65	0.02
167	k__Bacteria|p__Armatimonadetes|c__Chthonomonadetes|o__Chthonomonadales	Control	1.65	0.02
168	k__Bacteria|p__Armatimonadetes|c__Chthonomonadetes|o__Chthonomonadales|f__Chthonomonadaceae|g__Chthonomonas	Control	1.65	0.02
169	k__Bacteria|p__Armatimonadetes|c__Chthonomonadetes|o__Chthonomonadales|f__Chthonomonadaceae	Control	1.65	0.02
170	k__Bacteria|p__Verrucomicrobia|c__Verrucomicrobiae|o__Opitutales	Control	1.64	0.01
171	k__Bacteria|p__Verrucomicrobia|c__Verrucomicrobiae|o__Opitutales|f__Opitutaceae	Control	1.63	0.01
172	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Frankiales|f__Geodermatophilaceae|g__Blastococcus	Control	1.62	0.02
173	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Rhizobiales|f__Xanthobacteraceae|g__Pseudolabrys	Control	1.62	0.01
174	k__Bacteria|p__Planctomycetes|c__Planctomycetacia|o__Pirellulales|f__Pirellulaceae	Control	1.61	0.05
175	k__Bacteria|p__Planctomycetes|c__Planctomycetacia|o__Pirellulales	Control	1.61	0.05
176	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Propionibacteriales|f__Nocardioidaceae	Control	1.60	0.02
177	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Catenulisporales	Control	1.59	0.02
178	k__Bacteria|p__Verrucomicrobia|c__Verrucomicrobiae|o__Opitutales|f__Opitutaceae|g__Opitutus	Control	1.58	0.01
179	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Catenulisporales|f__Catenulisporaceae	Control	1.53	0.02
180	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Catenulisporales|f__Catenulisporaceae|g__Catenulispora	Control	1.53	0.02
181	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Reyranellales|f__Reyranellaceae|g__Reyranella	Control	1.53	0.04
182	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Rhizobiales|f__Beijerinckiaceae|g__Roseiarcus	Control	1.52	0.04
183	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Rhizobiales|f__Rhizobiales_Incertae_Sedis	Control	1.52	0.02
184	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Reyranellales|f__Reyranellaceae	Control	1.52	0.04
185	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Reyranellales	Control	1.52	0.04
186	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Betaproteobacteriales|f__Nitrosomonadaceae|g__Ellin6067	Control	1.51	0.01
187	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Xanthomonadales|f__Rhodanobacteraceae	Control	1.51	0.04
188	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Pseudonocardiales|f__Pseudonocardiaceae|g__Actinomycetospora	Control	1.50	0.04
189	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Propionibacteriales|f__Nocardioidaceae|g__Nocardioides	Control	1.50	0.02
190	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Chitinophagales|f__Chitinophagaceae|g__Parafilimonas	Control	1.48	0.05
191	k__Bacteria|p__Chlamydiae|c__Chlamydiae|o__Chlamydiales|f__Parachlamydiaceae	Control	1.48	0.02
192	k__Bacteria|p__Chlamydiae|c__Chlamydiae|o__Chlamydiales	Control	1.48	0.02
193	k__Bacteria|p__Chlamydiae|c__Chlamydiae	Control	1.47	0.02

No.	feature	enrich_group	ef_lda	pvalue
194	k__Bacteria|p__Chlamydiae|c__Chlamydiae|o__Chlamydiales|f__Parachlamydiaceae|g__Candidatus_Protochlamydia	Control	1.47	0.02
195	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Rhizobiales|f__Rhizobiales_Incertae_Sedis|g__Bauldia	Control	1.47	0.02
196	k__Bacteria|p__Chlamydiae	Control	1.47	0.02
197	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Betaproteobacteriales|f__Burkholderiaceae|g__Ramlibacter	Control	1.46	0.05
198	k__Bacteria|p__Acidobacteria|c__Thermoanaerobaculia|o__Thermoanaerobaculales|f__Thermoanaerobaculaceae	Control	1.46	0.01
199	k__Bacteria|p__Acidobacteria|c__Thermoanaerobaculia|o__Thermoanaerobaculales	Control	1.45	0.01
200	k__Bacteria|p__Acidobacteria|c__Thermoanaerobaculia|o__Thermoanaerobaculales|f__Thermoanaerobaculaceae|g__Subgroup_10	Control	1.45	0.01
201	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Micromonosporales	Control	1.45	0.01
202	k__Bacteria|p__Acidobacteria|c__Thermoanaerobaculia	Control	1.44	0.01
203	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Micromonosporales|f__Micromonosporaceae	Control	1.44	0.01
204	k__Bacteria|p__Gemmatimonadetes|c__Gemmatimonadetes|o__Gemmatimonadales|f__Gemmatimonadaceae|g__Gemmatirosa	Control	1.43	0.05
205	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Cytophagales|f__Cytophagaceae	Control	1.40	0.01
206	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Cytophagales	Control	1.40	0.01
207	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Rhizobiales|f__Xanthobacteraceae|g__Rhodoplanes	Control	1.40	0.05
208	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Rhizobiales|f__Xanthobacteraceae|g__GAS113	Control	1.40	0.02
209	k__Bacteria|p__Planctomycetes|c__Planctomycetacia|o__Pirellulales|f__Pirellulaceae|g__Pirellula	Control	1.34	0.05
210	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Cytophagales|f__Cytophagaceae|g__Sporocytophaga	Control	1.34	0.01
211	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Rhizobiales|f__Beijerinckiaceae|g__Methylobacterium	Control	1.32	0.04
212	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Xanthomonadales|f__Rhodanobacteraceae|g__Rhodanobacter	Control	1.32	0.01
213	k__Bacteria|p__Planctomycetes|c__Planctomycetacia|o__Pirellulales|f__Pirellulaceae|g__Pir4_lineage	Control	1.30	0.05
214	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Caulobacterales|f__Caulobacteraceae|g__Phenylobacterium	Control	1.27	0.03
215	k__Bacteria|p__Acidobacteria|c__Blastocatellia_(Subgroup_4)|o__Blastocatellales|f__Blastocatellaceae|g__JGI_0001001-H03	Control	1.27	0.02
216	k__Bacteria|p__Acidobacteria|c__Blastocatellia_(Subgroup_4)|o__Blastocatellales|f__Blastocatellaceae	Control	1.26	0.02
217	k__Bacteria|p__Acidobacteria|c__Blastocatellia_(Subgroup_4)|o__Blastocatellales	Control	1.26	0.02
218	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Streptosporangiales	Control	1.25	0.04
219	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Streptosporangiales|f__Thermomonosporaceae	Control	1.24	0.01
220	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Chitinophagales|f__Chitinophagaceae|g__Segetibacter	Control	1.24	0.01
221	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Xanthomonadales|f__Rhodanobacteraceae|g__Dyella	Control	1.22	0.05
222	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Micromonosporales|f__Micromonosporaceae|g__Actinoplanes	Control	1.22	0.01
223	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Chitinophagales|f__Chitinophagaceae|g__Niastella	Control	1.22	0.01
224	k__Bacteria|p__Proteobacteria|c__Deltaproteobacteria|o__Myxococcales|f__Phaselicystidaceae|g__Phaselicystis	Control	1.14	0.05
225	k__Bacteria|p__Proteobacteria|c__Deltaproteobacteria|o__Myxococcales|f__Phaselicystidaceae	Control	1.14	0.05
226	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Pseudonocardiales|f__Pseudonocardiaceae|g__Kutzneria	Control	1.14	0.02
227	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Rhizobiales|f__Hyphomicrobiaceae	Control	1.11	0.05
228	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Legionellales|f__Legionellaceae	Control	1.08	0.01
229	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Legionellales|f__Legionellaceae|g__Legionella	Control	1.07	0.01
230	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Legionellales	Control	1.06	0.01
231	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Sphingomonadales|f__Sphingomonadaceae|g__Altererythrobacter	Control	1.00	0.05
232	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Rhizobiales|f__Hyphomicrobiaceae|g__Hyphomicrobium	Control	0.97	0.05
233	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Diplorickettsiales	Control	0.95	0.01
234	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Diplorickettsiales|f__Diplorickettsiaceae|g__Aquicella	Control	0.94	0.01
235	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Diplorickettsiales|f__Diplorickettsiaceae	Control	0.94	0.01
236	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Streptosporangiales|f__Thermomonosporaceae|g__Actinomadura	Control	0.87	0.05
237	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Paracaedibacterales	Control	0.84	0.05
238	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Paracaedibacterales|f__Paracaedibacteraceae	Control	0.79	0.05

Table 7: Taxa with significantly different (Mann-Whitney *U:* $p < 0.05$) abundances between the 'Beneath' and 'Control' groups after six months. The data is sorted by descending linear discriminant analysis effect size (ef lds).

No.	feature	enrich_group	ef_lda	pvalue
1	k__Bacteria|p__Bacteroidetes	Beneath	3.76	0.02
2	k__Bacteria|p__Bacteroidetes|c__Bacteroidia	Beneath	3.76	0.02
3	k__Bacteria	Beneath	3.60	0.02
4	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Micrococcales	Beneath	3.56	0.02
5	k__Bacteria|p__Proteobacteria	Beneath	3.54	0.02
6	k__Bacteria|p__Actinobacteria|c__Actinobacteria	Beneath	3.52	0.02
7	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Flavobacteriales	Beneath	3.42	0.01
8	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria	Beneath	3.42	0.02
9	k__Bacteria|p__Actinobacteria	Beneath	3.35	0.04
10	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Sphingobacteriales|f__Sphingobacteriaceae	Beneath	3.30	0.02
11	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Sphingobacteriales	Beneath	3.30	0.02
12	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Micrococcales|f__Micrococcaceae	Beneath	3.28	0.02
13	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Sphingobacteriales|f__Sphingobacteriaceae|g__Pedobacter	Beneath	3.23	0.01
14	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Micrococcales|f__Micrococcaceae|g__Glutamicibacter	Beneath	3.21	0.01
15	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Flavobacteriales|f__Flavobacteriaceae	Beneath	3.16	0.01
16	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Flavobacteriales|f__Weeksellaceae	Beneath	3.08	0.01
17	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Xanthomonadales	Beneath	3.07	0.02
18	k__Bacteria|p__Firmicutes|c__Clostridia	Beneath	3.06	0.02
19	k__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales	Beneath	3.06	0.02
20	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Betaproteobacteriales	Beneath	3.04	0.02
21	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Flavobacteriales|f__Flavobacteriaceae|g__Aequorivita	Beneath	3.04	0.01
22	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Rhizobiales|f__Rhizobiaceae	Beneath	3.03	0.02
23	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Betaproteobacteriales|f__Burkholderiaceae	Beneath	3.00	0.02
24	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Rhizobiales|f__Rhizobiaceae|g__Aquamicrobium	Beneath	2.98	0.01
25	k__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales|f__Clostridiaceae_1	Beneath	2.98	0.02
26	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Xanthomonadales|f__Rhodanobacteraceae	Beneath	2.98	0.02
27	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Chitinophagales	Beneath	2.96	0.02
28	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria	Beneath	2.95	0.02
29	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Xanthomonadales|f__Rhodanobacteraceae|g__Rhodanobacter	Beneath	2.93	0.02
30	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Micrococcales|f__Brevibacteriaceae	Beneath	2.93	0.01
31	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Micrococcales|f__Brevibacteriaceae|g__Brevibacterium	Beneath	2.92	0.01
32	k__Bacteria|p__Deinococcus-Thermus|c__Deinococci|o__Deinococcales|f__Trueperaceae|g__Truepera	Beneath	2.92	0.01
33	k__Bacteria|p__Deinococcus-Thermus	Beneath	2.92	0.01
34	k__Bacteria|p__Deinococcus-Thermus|c__Deinococci|o__Deinococcales|f__Trueperaceae	Beneath	2.91	0.01
35	k__Bacteria|p__Deinococcus-Thermus|c__Deinococci|o__Deinococcales	Beneath	2.91	0.01
36	k__Bacteria|p__Deinococcus-Thermus|c__Deinococci	Beneath	2.91	0.01
37	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Rhizobiales	Beneath	2.90	0.02
38	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Flavobacteriales|f__Weeksellaceae|g__Moheibacter	Beneath	2.89	0.01
39	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Chitinophagales|f__Chitinophagaceae	Beneath	2.84	0.02
40	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Chitinophagales|f__Chitinophagaceae|g__Taibaiella	Beneath	2.80	0.01
41	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Pseudomonadales	Beneath	2.78	0.01
42	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Betaproteobacteriales|f__Burkholderiaceae|g__Castellaniella	Beneath	2.78	0.01
43	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Micrococcales|f__Microbacteriaceae	Beneath	2.74	0.02
44	k__Bacteria|p__Firmicutes|c__Bacilli|o__Bacillales|f__Bacillaceae|g__Pseudogracilibacillus	Beneath	2.74	0.01
45	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Pseudomonadales|f__Moraxellaceae	Beneath	2.71	0.01
46	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Propionibacteriales|f__Nocardioidaceae	Beneath	2.70	0.02
47	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Propionibacteriales	Beneath	2.68	0.02
48	k__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales|f__Clostridiaceae_1|g__Clostridium_sensu_stricto_7	Beneath	2.67	0.01
49	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Propionibacteriales|f__Nocardioidaceae|g__Aeromicrobium	Beneath	2.67	0.01
50	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Flavobacteriales|f__Weeksellaceae|g__Chryseobacterium	Beneath	2.64	0.01
51	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Rhizobiales|f__Devosiaceae	Beneath	2.58	0.02
52	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Rhizobiales|f__Devosiaceae|g__Devosia	Beneath	2.57	0.02
53	k__Bacteria|p__Verrucomicrobia|c__Verrucomicrobiae|o__Chthoniobacterales|f__Chthoniobacteraceae|g__LD29	Beneath	2.56	0.02
54	k__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales|f__Clostridiaceae_1|g__Hathewaya	Beneath	2.55	0.01
55	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Micrococcales|f__Microbacteriaceae|g__Microbacterium	Beneath	2.48	0.02
56	k__Bacteria|p__Actinobacteria|c__Acidimicrobiia|o__Microtrichales	Beneath	2.48	0.01
57	k__Bacteria|p__Actinobacteria|c__Acidimicrobiia	Beneath	2.47	0.01
58	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Betaproteobacteriales|f__Burkholderiaceae|g__Pusillimonas	Beneath	2.46	0.01
59	k__Bacteria|p__Actinobacteria|c__Acidimicrobiia|o__Microtrichales|f__Iamiaceae|g__Iamia	Beneath	2.46	0.01
60	k__Bacteria|p__Actinobacteria|c__Acidimicrobiia|o__Microtrichales|f__Iamiaceae	Beneath	2.45	0.01
61	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Micrococcales|f__Micrococcaceae|g__Enteractinococcus	Beneath	2.45	0.01
62	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Frankiales|f__Nakamurellaceae	Beneath	2.41	0.01
63	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Frankiales|f__Nakamurellaceae|g__Nakamurella	Beneath	2.40	0.01
64	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Rhodobacterales|f__Rhodobacteraceae	Beneath	2.40	0.01
65	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Rhodobacterales	Beneath	2.39	0.01
66	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Pseudomonadales|f__Moraxellaceae|g__Alkanindiges	Beneath	2.39	0.01
67	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Micrococcales|f__Microbacteriaceae|g__Leucobacter	Beneath	2.39	0.01
68	k__Bacteria|p__Firmicutes|c__Bacilli|o__Bacillales|f__Bacillaceae|g__Cerasibacillus	Beneath	2.36	0.01
69	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Xanthomonadales|f__Xanthomonadaceae	Beneath	2.34	0.01
70	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Corynebacteriales|f__Nocardiaceae	Beneath	2.32	0.02
71	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Corynebacteriales|f__Nocardiaceae|g__Gordonia	Beneath	2.31	0.01
72	k__Bacteria|p__Planctomycetes|c__Planctomycetacia|o__Isosphaerales|f__Isosphaeraceae|g__Isosphaera	Beneath	2.31	0.01
73	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Pseudomonadales|f__Moraxellaceae|g__Acinetobacter	Beneath	2.29	0.01
74	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Rhodobacterales|f__Rhodobacteraceae|g__Paracoccus	Beneath	2.28	0.01
75	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Chitinophagales|f__Chitinophagaceae|g__Arachidicoccus	Beneath	2.28	0.01
76	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Micrococcales|f__Ruaniaceae	Beneath	2.27	0.01
77	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Micrococcales|f__Ruaniaceae|g__Haloactinobacterium	Beneath	2.27	0.01
78	k__Bacteria|p__Planctomycetes|c__Planctomycetacia|o__Planctomycetales|f__Rubinisphaeraceae	Beneath	2.26	0.01
79	k__Bacteria|p__Planctomycetes|c__Planctomycetacia|o__Planctomycetales|f__Rubinisphaeraceae|g__Planctomicrobium	Beneath	2.26	0.01
80	k__Bacteria|p__Planctomycetes|c__Planctomycetacia|o__Planctomycetales	Beneath	2.26	0.02
81	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Sphingobacteriales|f__Sphingobacteriaceae|g__Sphingobacterium	Beneath	2.26	0.01
82	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Cytophagales	Beneath	2.20	0.02
83	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Betaproteobacteriales|f__Nitrosomonadaceae|g__Nitrosomonas	Beneath	2.18	0.01
84	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Micrococcales|f__Intrasporangiaceae	Beneath	2.16	0.01

No.	feature	enrich_group	ef_lda	pvalue
85	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Caulobacterales|f__Caulobacteraceae|g__Brevundimonas	Beneath	2.15	0.01
86	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Caulobacterales	Beneath	2.15	0.02
87	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Caulobacterales|f__Caulobacteraceae	Beneath	2.15	0.02
88	k__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales|f__Family_XI	Beneath	2.14	0.01
89	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Micrococcales|f__Intrasporangiaceae|g__Ornithinimicrobium	Beneath	2.13	0.01
90	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Flavobacteriales|f__Flavobacteriaceae|g__Flavobacterium	Beneath	2.11	0.01
91	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Sphingobacteriales|f__Sphingobacteriaceae|g__Parapedobacter	Beneath	2.10	0.01
92	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Cytophagales|f__Cytophagaceae	Beneath	2.09	0.02
93	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Cytophagales|f__Cytophagaceae|g__Cytophaga	Beneath	2.08	0.02
94	k__Bacteria|p__Firmicutes|c__Bacilli|o__Lactobacillales	Beneath	2.07	0.02
95	k__Bacteria|p__Firmicutes|c__Bacilli|o__Lactobacillales|f__Carnobacteriaceae	Beneath	2.07	0.01
96	k__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales|f__Family_XI|g__Tissierella	Beneath	2.06	0.01
97	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Corynebacteriales|f__Dietziaceae	Beneath	1.97	0.01
98	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Corynebacteriales|f__Dietziaceae|g__Dietzia	Beneath	1.97	0.01
99	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Pseudomonadales|f__Pseudomonadaceae	Beneath	1.97	0.01
100	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Pseudomonadales|f__Pseudomonadaceae|g__Pseudomonas	Beneath	1.96	0.05
101	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Xanthomonadales|f__Xanthomonadaceae|g__Stenotrophomonas	Beneath	1.96	0.01
102	k__Bacteria|p__Firmicutes|c__Bacilli|o__Lactobacillales|f__Carnobacteriaceae|g__Atopostipes	Beneath	1.95	0.01
103	k__Bacteria|p__Firmicutes|c__Bacilli|o__Bacillales|f__Staphylococcaceae	Beneath	1.95	0.05
104	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Chitinophagales|f__Chitinophagaceae|g__Niabella	Beneath	1.92	0.01
105	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Flavobacteriales|f__Flavobacteriaceae|g__Myroides	Beneath	1.92	0.01
106	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Chitinophagales|f__Chitinophagaceae|g__Ferruginibacter	Beneath	1.88	0.05
107	k__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales|f__Clostridiaceae_1|g__Clostridium_sensu_stricto_1	Beneath	1.87	0.02
108	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Pseudomonadales|f__Moraxellaceae|g__Psychrobacter	Beneath	1.87	0.01
109	k__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales|f__Clostridiaceae_1|g__Clostridium_sensu_stricto_15	Beneath	1.85	0.02
110	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Xanthomonadales|f__Xanthomonadaceae|g__Luteimonas	Beneath	1.84	0.05
111	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Flavobacteriales|f__Flavobacteriaceae|g__Ulvibacter	Beneath	1.82	0.05
112	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Betaproteobacteriales|f__Burkholderiaceae|g__Ottowia	Beneath	1.80	0.01
113	k__Bacteria|p__Firmicutes|c__Bacilli|o__Bacillales|f__Staphylococcaceae|g__Staphylococcus	Beneath	1.80	0.05
114	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Micrococcales|f__Dermabacteraceae|g__Brachybacterium	Beneath	1.80	0.01
115	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Micrococcales|f__Dermabacteraceae	Beneath	1.79	0.01
116	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Betaproteobacteriales|f__Burkholderiaceae|g__Paenalcaligenes	Beneath	1.79	0.05
117	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Rhizobiales|f__Rhizobiaceae|g__Aminobacter	Beneath	1.76	0.01
118	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Sphingobacteriales|f__Sphingobacteriaceae|g__Pseudopedobacter	Beneath	1.75	0.01
119	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Rhodobacterales|f__Rhodobacteraceae|g__Pontibaca	Beneath	1.75	0.01
120	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Sphingomonadales|f__Sphingomonadaceae|g__Altererythrobacter	Beneath	1.73	0.01
121	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Xanthomonadales|f__Rhodanobacteraceae|g__Dokdonella	Beneath	1.69	0.02
122	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Salinisphaerales|f__Solimonadaceae|g__Alkanibacter	Beneath	1.68	0.01
123	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Salinisphaerales|f__Solimonadaceae	Beneath	1.68	0.03
124	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Salinisphaerales	Beneath	1.67	0.03
125	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Sphingomonadales|f__Sphingomonadaceae|g__Sandaracinobacter	Beneath	1.67	0.01
126	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Frankiales|f__Geodermatophilaceae|g__Antricoccus	Beneath	1.66	0.01
127	k__Bacteria|p__Verrucomicrobia|c__Verrucomicrobiae|o__Verrucomicrobiales	Beneath	1.62	0.04
128	k__Bacteria|p__Verrucomicrobia|c__Verrucomicrobiae|o__Verrucomicrobiales|f__Rubritaleaceae	Beneath	1.61	0.01
129	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Micrococcales|f__Sanguibacteraceae|g__Sanguibacter	Beneath	1.61	0.01
130	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Micrococcales|f__Sanguibacteraceae	Beneath	1.60	0.01
131	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Cytophagales|f__Cyclobacteriaceae	Beneath	1.59	0.01
132	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Cytophagales|f__Cyclobacteriaceae|g__Mariniradius	Beneath	1.59	0.01
133	k__Bacteria|p__Verrucomicrobia|c__Verrucomicrobiae|o__Verrucomicrobiales|f__Rubritaleaceae|g__Luteolibacter	Beneath	1.59	0.01
134	k__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales|f__Clostridiaceae_2|g__Alkaliphilus	Beneath	1.56	0.01
135	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Flavobacteriales|f__Flavobacteriaceae|g__Gelidibacter	Beneath	1.56	0.05
136	k__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales|f__Clostridiaceae_2	Beneath	1.56	0.01
137	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Rhizobiales|f__Rhizobiaceae|g__Falsochrobactrum	Beneath	1.52	0.01
138	k__Bacteria|p__Proteobacteria|c__Deltaproteobacteria|o__Bdellovibrionales|f__Bacteriovoracaceae|g__Peredibacter	Beneath	1.50	0.02
139	k__Bacteria|p__Proteobacteria|c__Deltaproteobacteria|o__Bdellovibrionales|f__Bacteriovoracaceae	Beneath	1.49	0.02
140	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Sphingomonadales|f__Sphingomonadaceae|g__Sphingopyxis	Beneath	1.46	0.05
141	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Betaproteobacteriales|f__Burkholderiaceae|g__Candidimonas	Beneath	1.45	0.01
142	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Rhizobiales|f__Xanthobacteraceae|g__Afipia	Beneath	1.41	0.01
143	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Flavobacteriales|f__Crocinitomicaceae	Beneath	1.41	0.05
144	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Flavobacteriales|f__Crocinitomicaceae|g__Fluviicola	Beneath	1.41	0.05
145	k__Bacteria|p__Firmicutes|c__Bacilli|o__Bacillales|f__Staphylococcaceae|g__Jeotgalicoccus	Beneath	1.41	0.05
146	k__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales|f__Family_XI|g__Anaerosalibacter	Beneath	1.41	0.01
147	k__Bacteria|p__Firmicutes|c__Bacilli|o__Lactobacillales|f__Carnobacteriaceae|g__Carnobacterium	Beneath	1.39	0.05
148	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Flavobacteriales|f__Flavobacteriaceae|g__Arenibacter	Beneath	1.37	0.05
149	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Corynebacteriales|f__Corynebacteriaceae|g__Corynebacterium_1	Beneath	1.35	0.05
150	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Corynebacteriales|f__Corynebacteriaceae	Beneath	1.35	0.05
151	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Betaproteobacteriales|f__Burkholderiaceae|g__Parapusillimonas	Beneath	1.34	0.02
152	k__Bacteria|p__Chloroflexi|c__Chloroflexia|o__Thermomicrobiales|f__Thermomicrobiaceae|g__Sphaerobacter	Beneath	1.32	0.05
153	k__Bacteria|p__Firmicutes|c__Erysipelotrichia|o__Erysipelotrichales|f__Erysipelotrichaceae	Beneath	1.32	0.05
154	k__Bacteria|p__Firmicutes|c__Erysipelotrichia	Beneath	1.32	0.05
155	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Actinomycetales|f__Actinomycetaceae	Beneath	1.31	0.01
156	k__Bacteria|p__Firmicutes|c__Erysipelotrichia|o__Erysipelotrichales|f__Erysipelotrichaceae|g__Erysipelothrix	Beneath	1.31	0.05
157	k__Bacteria|p__Firmicutes|c__Erysipelotrichia|o__Erysipelotrichales	Beneath	1.31	0.05
158	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Actinomycetales	Beneath	1.30	0.01
159	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Actinomycetales|f__Actinomycetaceae|g__Flaviflexus	Beneath	1.30	0.01
160	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Micrococcales|f__Cellulomonadaceae|g__Actinotalea	Beneath	1.25	0.05
161	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Acetobacterales|f__Acetobacteraceae|g__Roseococcus	Beneath	1.23	0.05
162	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Betaproteobacteriales|f__Burkholderiaceae|g__Verticia	Beneath	1.20	0.01
163	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Oceanospirillales	Beneath	1.20	0.01
164	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Oceanospirillales|f__Pseudohongiellaceae	Beneath	1.19	0.05
165	k__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales|f__Caldicoprobacteraceae	Beneath	1.18	0.05
166	k__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales|f__Caldicoprobacteraceae|g__Caldicoprobacter	Beneath	1.18	0.05

No.	feature	enrich_group	ef_lda	pvalue
167	k__Bacteria|p__Verrucomicrobia|c__Verrucomicrobiae|o__Opitutales|f__Puniceicoccaceae	Beneath	1.08	0.05
168	k__Bacteria|p__Verrucomicrobia|c__Verrucomicrobiae|o__Opitutales|f__Puniceicoccaceae|g__Cerasicoccus	Beneath	1.08	0.05
169	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Micrococcales|f__Microbacteriaceae|g__Huakuichenia	Beneath	1.06	0.01
170	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Rhizobiales|f__Hyphomicrobiaceae|g__Filomicrobium	Beneath	1.02	0.05
171	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Rhizobiales|f__Rhizobiaceae|g__Pseudochrobactrum	Beneath	0.99	0.01
172	k__Bacteria|p__Tenericutes	Beneath	0.99	0.05
173	k__Bacteria|p__Tenericutes|c__Mollicutes	Beneath	0.98	0.05
174	k__Bacteria|p__Firmicutes|c__Bacilli|o__Bacillales|f__Planococcaceae|g__Rummeliibacillus	Beneath	0.98	0.02
175	k__Bacteria|p__Tenericutes|c__Mollicutes|o__Acholeplasmatales|f__Acholeplasmataceae|g__Acholeplasma	Beneath	0.97	0.05
176	k__Bacteria|p__Tenericutes|c__Mollicutes|o__Acholeplasmatales|f__Acholeplasmataceae	Beneath	0.96	0.05
177	k__Bacteria|p__Tenericutes|c__Mollicutes|o__Acholeplasmatales	Beneath	0.96	0.05
178	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Parvibaculales	Beneath	0.95	0.05
179	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Parvibaculales|f__Parvibaculaceae	Beneath	0.94	0.05
180	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Rhizobiales|f__Beijerinckiaceae|g__Bosea	Beneath	0.94	0.05
181	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Rhizobiales|f__Beijerinckiaceae|g__Camelimonas	Beneath	0.92	0.01
182	k__Bacteria|p__Firmicutes|c__Bacilli|o__Bacillales|f__Bacillaceae|g__Ornithinibacillus	Beneath	0.90	0.05
183	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Alteromonadales	Beneath	0.88	0.05
184	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Alteromonadales|f__Idiomarinaceae	Beneath	0.86	0.05
185	k__Bacteria|p__Firmicutes|c__Bacilli|o__Bacillales|f__Bacillaceae|g__Amphibacillus	Beneath	0.84	0.05
186	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Cytophagales|f__Spirosomaceae	Beneath	0.78	0.05
187	k__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales|f__Peptostreptococcaceae|g__Paeniclostridium	Beneath	0.74	0.05
188	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Cytophagales|f__Spirosomaceae|g__Larkinella	Beneath	0.71	0.05
189	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Parvibaculales|f__Parvibaculaceae|g__Rhodoligotrophos	Beneath	0.64	0.05
190	k__Bacteria|p__Actinobacteria|c__Acidimicrobiia|o__Microtrichales|f__Ilumatobacteraceae|g__CL500-29_marine_group	Beneath	0.64	0.05
191	k__Bacteria|p__Actinobacteria|c__Acidimicrobiia|o__Microtrichales|f__Ilumatobacteraceae	Beneath	0.63	0.05
192	k__Bacteria|p__Firmicutes|c__Clostridia|o__Thermoanaerobacterales|f__Family_III	Beneath	0.61	0.01
193	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Corynebacteriales|f__Nocardiaceae|g__Rhodococcus	Beneath	0.59	0.05
194	k__Bacteria|p__Firmicutes|c__Clostridia|o__Thermoanaerobacterales|f__Family_III|g__Tepidanaerobacter	Beneath	0.59	0.01
195	k__Bacteria|p__Firmicutes|c__Clostridia|o__Thermoanaerobacterales	Beneath	0.58	0.01
196	k__Bacteria|p__Acidobacteria	Control	3.55	0.02
197	k__Bacteria|p__Verrucomicrobia	Control	3.52	0.02
198	k__Bacteria|p__Verrucomicrobia|c__Verrucomicrobiae	Control	3.51	0.02
199	k__Bacteria|p__Verrucomicrobia|c__Verrucomicrobiae|o__Chthoniobacterales	Control	3.49	0.02
200	k__Bacteria|p__Acidobacteria|c__Acidobacteriia	Control	3.45	0.02
201	k__Bacteria|p__Chloroflexi|c__Ktedonobacteria|o__Ktedonobacterales	Control	3.41	0.02
202	k__Bacteria|p__Chloroflexi	Control	3.41	0.02
203	k__Bacteria|p__Chloroflexi|c__Ktedonobacteria|o__Ktedonobacterales|f__Ktedonobacteraceae	Control	3.41	0.02
204	k__Bacteria|p__Chloroflexi|c__Ktedonobacteria	Control	3.40	0.02
205	k__Bacteria|p__Acidobacteria|c__Acidobacteriia|o__Solibacterales	Control	3.40	0.02
206	k__Bacteria|p__Acidobacteria|c__Acidobacteriia|o__Solibacterales|f__Solibacteraceae_(Subgroup_3)	Control	3.40	0.02
207	k__Bacteria|p__Verrucomicrobia|c__Verrucomicrobiae|o__Chthoniobacterales|f__Chthoniobacteraceae|g__Candidatus_Udaeobacter	Control	3.34	0.02
208	k__Bacteria|p__Verrucomicrobia|c__Verrucomicrobiae|o__Chthoniobacterales|f__Chthoniobacteraceae	Control	3.28	0.02
209	k__Bacteria|p__Acidobacteria|c__Acidobacteriia|o__Solibacterales|f__Solibacteraceae_(Subgroup_3)|g__Bryobacter	Control	3.17	0.02
210	k__Bacteria|p__Actinobacteria|c__Thermoleophilia	Control	3.10	0.02
211	k__Bacteria|p__Actinobacteria|c__Thermoleophilia|o__Solirubrobacterales|f__Solirubrobacteraceae|g__Conexibacter	Control	3.09	0.02
212	k__Bacteria|p__Actinobacteria|c__Thermoleophilia|o__Solirubrobacterales	Control	3.09	0.02
213	k__Bacteria|p__Actinobacteria|c__Thermoleophilia|o__Solirubrobacterales|f__Solirubrobacteraceae	Control	3.09	0.02
214	k__Bacteria|p__Verrucomicrobia|c__Verrucomicrobiae|o__Chthoniobacterales|f__Xiphinematobacteraceae|g__Candidatus_Xiphinematobacter	Control	3.07	0.02
215	k__Bacteria|p__Verrucomicrobia|c__Verrucomicrobiae|o__Chthoniobacterales|f__Xiphinematobacteraceae	Control	3.07	0.02
216	k__Bacteria|p__Acidobacteria|c__Acidobacteriia|o__Solibacterales|f__Solibacteraceae_(Subgroup_3)|g__Candidatus_Solibacter	Control	3.00	0.02
217	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Frankiales|f__Acidothermaceae	Control	2.88	0.02
218	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Frankiales|f__Acidothermaceae|g__Acidothermus	Control	2.88	0.02
219	k__Bacteria|p__Chloroflexi|c__Ktedonobacteria|o__Ktedonobacterales|f__Ktedonobacteraceae|g__1921-2	Control	2.85	0.02
220	k__Bacteria|p__Acidobacteria|c__Blastocatellia_(Subgroup_4)	Control	2.82	0.01
221	k__Bacteria|p__Acidobacteria|c__Blastocatellia_(Subgroup_4)|o__Pyrinomonadales|f__Pyrinomonadaceae	Control	2.78	0.01
222	k__Bacteria|p__Acidobacteria|c__Blastocatellia_(Subgroup_4)|o__Pyrinomonadales	Control	2.78	0.01
223	k__Bacteria|p__Acidobacteria|c__Blastocatellia_(Subgroup_4)|o__Pyrinomonadales|f__Pyrinomonadaceae|g__RB41	Control	2.78	0.01
224	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Frankiales	Control	2.77	0.02
225	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Rhizobiales|f__Xanthobacteraceae	Control	2.74	0.02
226	k__Bacteria|p__Chloroflexi|c__Ktedonobacteria|o__Ktedonobacterales|f__Ktedonobacteraceae|g__1921-3	Control	2.71	0.02
227	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Rhizobiales|f__Xanthobacteraceae|g__Bradyrhizobium	Control	2.69	0.02
228	k__Bacteria|p__Chloroflexi|c__Ktedonobacteria|o__Ktedonobacterales|f__Ktedonobacteraceae|g__FCPS473	Control	2.64	0.02
229	k__Bacteria|p__Acidobacteria|c__Acidobacteriia|o__Acidobacteriales	Control	2.52	0.02
230	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Gammaproteobacteria_Incertae_Sedis	Control	2.49	0.02
231	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Gammaproteobacteria_Incertae_Sedis|f__Gammaproteobacteria_Incertae_Sedis_Unknown_Family	Control	2.48	0.02
232	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Gammaproteobacteria_Incertae_Sedis|f__Gammaproteobacteria_Incertae_Sedis_Unknown_Family|g__Acidibacter	Control	2.48	0.02
233	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Pseudonocardiales|f__Pseudonocardiaceae	Control	2.44	0.02
234	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Pseudonocardiales	Control	2.44	0.02
235	k__Bacteria|p__Verrucomicrobia|c__Verrucomicrobiae|o__Pedosphaerales|f__Pedosphaeraceae	Control	2.37	0.02
236	k__Bacteria|p__Verrucomicrobia|c__Verrucomicrobiae|o__Pedosphaerales	Control	2.37	0.02
237	k__Bacteria|p__Acidobacteria|c__Acidobacteriia|o__Acidobacteriales|f__Acidobacteriaceae_(Subgroup_1)	Control	2.35	0.02
238	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Pseudonocardiales|f__Pseudonocardiaceae|g__Crossiella	Control	2.33	0.02
239	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Chitinophagales|f__Chitinophagaceae|g__Flavisolibacter	Control	2.31	0.02
240	k__Bacteria|p__Verrucomicrobia|c__Verrucomicrobiae|o__Pedosphaerales|f__Pedosphaeraceae|g__ADurb.Bin063-1	Control	2.24	0.02
241	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Sphingomonadales|f__Sphingomonadaceae|g__Sphingomonas	Control	2.23	0.02
242	k__Bacteria|p__Armatimonadetes	Control	2.14	0.01
243	k__Bacteria|p__Armatimonadetes|c__Chthonomonadetes|o__Chthonomonadales|f__Chthonomonadaceae	Control	2.14	0.01
244	k__Bacteria|p__Armatimonadetes|c__Chthonomonadetes	Control	2.13	0.01
245	k__Bacteria|p__Armatimonadetes|c__Chthonomonadetes|o__Chthonomonadales|f__Chthonomonadaceae|g__Chthonomonas	Control	2.13	0.01
246	k__Bacteria|p__Armatimonadetes|c__Chthonomonadetes|o__Chthonomonadales	Control	2.13	0.01
247	k__Bacteria|p__Verrucomicrobia|c__Verrucomicrobiae|o__Chthoniobacterales|f__Chthoniobacteraceae|g__Chthoniobacter	Control	2.10	0.02
248	k__Bacteria|p__Planctomycetes|c__Planctomycetacia|o__Gemmatales|f__Gemmataceae	Control	2.09	0.02
249	k__Bacteria|p__Planctomycetes|c__Planctomycetacia|o__Gemmatales	Control	2.09	0.02

No.	feature	enrich_group	ef_lda	pvalue
250	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Acetobacterales|f__Acetobacteraceae	Control	2.09	0.02
251	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Acetobacterales	Control	2.08	0.02
252	k__Bacteria|p__Planctomycetes|c__Planctomycetacia|o__Gemmatales|f__Gemmataceae|g__Gemmata	Control	2.06	0.02
253	k__Bacteria|p__Acidobacteria|c__Acidobacteriia|o__Acidobacteriales|f__Koribacteraceae	Control	2.03	0.02
254	k__Bacteria|p__Acidobacteria|c__Acidobacteriia|o__Acidobacteriales|f__Koribacteraceae|g__Candidatus_Koribacter	Control	2.03	0.02
255	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Betaproteobacteriales|f__Burkholderiaceae|g__Burkholderia-Caballeronia-Paraburkholderia	Control	2.01	0.02
256	k__Bacteria|p__Planctomycetes|c__Planctomycetacia|o__Isosphaerales|f__Isosphaeraceae|g__Singulisphaera	Control	2.01	0.02
257	k__Bacteria|p__Chloroflexi|c__Ktedonobacteria|o__Ktedonobacterales|f__Ktedonobacteraceae|g__G12-WMSP1	Control	1.99	0.04
258	k__Bacteria|p__Proteobacteria|c__Deltaproteobacteria|o__Myxococcales|f__Haliangiaceae|g__Haliangium	Control	1.99	0.02
259	k__Bacteria|p__Proteobacteria|c__Deltaproteobacteria|o__Myxococcales|f__Haliangiaceae	Control	1.99	0.02
260	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Rhizobiales|f__Beijerinckiaceae	Control	1.95	0.02
261	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Frankiales|f__Frankiaceae|g__Jatrophihabitans	Control	1.93	0.04
262	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Frankiales|f__Frankiaceae	Control	1.92	0.04
263	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Streptomycetales	Control	1.89	0.02
264	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Streptomycetales|f__Streptomycetaceae	Control	1.89	0.02
265	k__Bacteria|p__Planctomycetes|c__Planctomycetacia|o__Isosphaerales|f__Isosphaeraceae|g__Aquisphaera	Control	1.88	0.01
266	k__Bacteria|p__Chloroflexi|c__Ktedonobacteria|o__Ktedonobacterales|f__Ktedonobacteraceae|g__1959-1	Control	1.86	0.02
267	k__Bacteria|p__Gemmatimonadetes|c__Gemmatimonadetes|o__Gemmatimonadales|f__Gemmatimonadaceae	Control	1.85	0.02
268	k__Bacteria|p__Gemmatimonadetes|c__Gemmatimonadetes|o__Gemmatimonadales	Control	1.85	0.02
269	k__Bacteria|p__Gemmatimonadetes|c__Gemmatimonadetes	Control	1.85	0.02
270	k__Bacteria|p__Gemmatimonadetes	Control	1.85	0.02
271	k__Bacteria|p__Firmicutes|c__Bacilli|o__Bacillales|f__Alicyclobacillaceae	Control	1.83	0.04
272	k__Bacteria|p__Proteobacteria|c__Deltaproteobacteria|o__Myxococcales|f__Archangiaceae	Control	1.82	0.02
273	k__Bacteria|p__Gemmatimonadetes|c__Gemmatimonadetes|o__Gemmatimonadales|f__Gemmatimonadaceae|g__Gemmatimonas	Control	1.78	0.04
274	k__Bacteria|p__Verrucomicrobia|c__Verrucomicrobiae|o__Pedosphaerales|f__Pedosphaeraceae|g__Pedosphaera	Control	1.78	0.01
275	k__Bacteria|p__Acidobacteria|c__Acidobacteriia|o__Acidobacteriales|f__Acidobacteriaceae_(Subgroup_1)|g__Granulicella	Control	1.77	0.02
276	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Reyranellales	Control	1.77	0.02
277	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Reyranellales|f__Reyranellaceae	Control	1.76	0.02
278	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Reyranellales|f__Reyranellaceae|g__Reyranella	Control	1.76	0.02
279	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Rhizobiales|f__Xanthobacteraceae|g__Rhodoplanes	Control	1.76	0.02
280	k__Bacteria|p__Acidobacteria|c__Acidobacteriia|o__Acidobacteriales|f__Acidobacteriaceae_(Subgroup_1)|g__Edaphobacter	Control	1.75	0.02
281	k__Bacteria|p__Acidobacteria|c__Acidobacteriia|o__Acidobacteriales|f__Acidobacteriaceae_(Subgroup_1)|g__Occallatibacter	Control	1.74	0.01
282	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Sphingomonadales|f__Sphingomonadaceae|g__Ellin6055	Control	1.71	0.02
283	k__Bacteria|p__Proteobacteria|c__Deltaproteobacteria|o__Myxococcales|f__Archangiaceae|g__Anaeromyxobacter	Control	1.70	0.03
284	k__Bacteria|p__Acidobacteria|c__Blastocatellia_(Subgroup_4)|o__Blastocatellales|f__Blastocatellaceae	Control	1.69	0.01
285	k__Bacteria|p__Acidobacteria|c__Blastocatellia_(Subgroup_4)|o__Blastocatellales	Control	1.69	0.01
286	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Kineosporiales|f__Kineosporiaceae|g__Quadrisphaera	Control	1.68	0.02
287	k__Bacteria|p__Acidobacteria|c__Acidobacteriia|o__Acidobacteriales|f__Acidobacteriaceae_(Subgroup_1)|g__Acidipila	Control	1.67	0.01
288	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Betaproteobacteriales|f__Nitrosomonadaceae|g__Ellin6067	Control	1.67	0.01
289	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Acetobacterales|f__Acetobacteraceae|g__Acidisphaera	Control	1.67	0.02
290	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Acetobacterales|f__Acetobacteraceae|g__Acidiphilium	Control	1.67	0.01
291	k__Bacteria|p__Chlamydiae|c__Chlamydiae|o__Chlamydiales	Control	1.66	0.02
292	k__Bacteria|p__Chlamydiae|c__Chlamydiae	Control	1.66	0.02
293	k__Bacteria|p__Chloroflexi|c__Ktedonobacteria|o__Ktedonobacterales|f__Ktedonobacteraceae|g__Ktedonobacter	Control	1.66	0.04
294	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Kineosporiales	Control	1.66	0.02
295	k__Bacteria|p__Chlamydiae	Control	1.66	0.02
296	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Kineosporiales|f__Kineosporiaceae	Control	1.65	0.02
297	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Sphingobacteriales|f__Sphingobacteriaceae|g__Mucilaginibacter	Control	1.63	0.02
298	k__Bacteria|p__Chlamydiae|c__Chlamydiae|o__Chlamydiales|f__Parachlamydiaceae|g__Candidatus_Protochlamydia	Control	1.63	0.01
299	k__Bacteria|p__Chlamydiae|c__Chlamydiae|o__Chlamydiales|f__Parachlamydiaceae	Control	1.61	0.02
300	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Catenulisporales	Control	1.61	0.02
301	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Streptomycetales|f__Streptomycetaceae|g__Streptomyces	Control	1.59	0.04
302	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Rhizobiales|f__Beijerinckiaceae|g__Roseiarcus	Control	1.56	0.01
303	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Catenulisporales|f__Catenulisporaceae|g__Catenulispora	Control	1.56	0.02
304	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Rhizobiales|f__Rhizobiales_Incertae_Sedis	Control	1.56	0.02
305	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Streptomycetales|f__Streptomycetaceae|g__Kitasatospora	Control	1.55	0.01
306	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Catenulisporales|f__Catenulisporaceae	Control	1.55	0.02
307	k__Bacteria|p__Proteobacteria|c__Deltaproteobacteria|o__Myxococcales|f__Polyangiaceae	Control	1.54	0.03
308	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Rhizobiales|f__Rhizobiales_Incertae_Sedis|g__Bauldia	Control	1.53	0.02
309	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Rhizobiales|f__Xanthobacteraceae|g__GAS113	Control	1.51	0.01
310	k__Bacteria|p__Acidobacteria|c__Thermoanaerobaculia|o__Thermoanaerobaculales|f__Thermoanaerobaculaceae	Control	1.51	0.01
311	k__Bacteria|p__Proteobacteria|c__Deltaproteobacteria|o__Myxococcales|f__Polyangiaceae|g__Pajaroellobacter	Control	1.51	0.03
312	k__Bacteria|p__Acidobacteria|c__Thermoanaerobaculia|o__Thermoanaerobaculales	Control	1.50	0.01
313	k__Bacteria|p__Acidobacteria|c__Thermoanaerobaculia|o__Thermoanaerobaculales|f__Thermoanaerobaculaceae|g__Subgroup_10	Control	1.50	0.01
314	k__Bacteria|p__Acidobacteria|c__Thermoanaerobaculia	Control	1.50	0.01
315	k__Bacteria|p__Acidobacteria|c__Blastocatellia_(Subgroup_4)|o__Blastocatellales|f__Blastocatellaceae|g__JGI_0001001-H03	Control	1.49	0.05
316	k__Bacteria|p__Actinobacteria|c__Rubrobacteria|o__Rubrobacterales	Control	1.47	0.02
317	k__Bacteria|p__Actinobacteria|c__Rubrobacteria|o__Rubrobacterales|f__Rubrobacteriaceae|g__Rubrobacter	Control	1.47	0.02
318	k__Bacteria|p__Actinobacteria|c__Rubrobacteria	Control	1.47	0.02
319	k__Bacteria|p__Actinobacteria|c__Rubrobacteria|o__Rubrobacterales|f__Rubrobacteriaceae	Control	1.47	0.02
320	k__Bacteria|p__Firmicutes|c__Bacilli|o__Bacillales|f__Paenibacillaceae|g__Cohnella	Control	1.46	0.02
321	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Propionibacteriales|f__Propionibacteriaceae|g__Microlunatus	Control	1.43	0.05
322	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Micromonosporales	Control	1.41	0.05
323	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Micromonosporales|f__Micromonosporaceae	Control	1.41	0.05
324	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Betaproteobacteriales|f__Nitrosomonadaceae|g__MND1	Control	1.37	0.01
325	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Acetobacterales|f__Acetobacteraceae|g__Acidicaldus	Control	1.35	0.05
326	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Chitinophagales|f__Chitinophagaceae|g__Segetibacter	Control	1.34	0.01
327	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Frankiales|f__Geodermatophilaceae|g__Geodermatophilus	Control	1.32	0.02
328	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Pseudonocardiales|f__Pseudonocardiaceae|g__Actinomycetospora	Control	1.32	0.04
329	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Frankiales|f__Geodermatophilaceae|g__Blastococcus	Control	1.27	0.02
330	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Betaproteobacteriales|f__Burkholderiaceae|g__Ramlibacter	Control	1.26	0.05
331	k__Bacteria|p__Proteobacteria|c__Deltaproteobacteria|o__Myxococcales|f__Archangiaceae|g__Archangium	Control	1.25	0.04
332	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Micrococcales|f__Cellulomonadaceae|g__Cellulomonas	Control	1.24	0.05
333	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Chitinophagales|f__Chitinophagaceae|g__Parafilimonas	Control	1.22	0.01
334	k__Bacteria|p__Planctomycetes|c__Planctomycetacia|o__Pirellulales|f__Pirellulaceae|g__Pir4_lineage	Control	1.21	0.05
335	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Chitinophagales|f__Chitinophagaceae|g__Niastella	Control	1.20	0.01
336	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Rhizobiales|f__Beijerinckiaceae|g__Methylobacterium	Control	1.20	0.01
337	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Pseudonocardiales|f__Pseudonocardiaceae|g__Kutzneria	Control	1.14	0.01
338	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Rhizobiales|f__Beijerinckiaceae|g__1174-901-12	Control	1.10	0.05
339	k__Bacteria|p__Gemmatimonadetes|c__Gemmatimonadetes|o__Gemmatimonadales|f__Gemmatimonadaceae|g__Gemmatirosa	Control	1.10	0.01
340	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Micromonosporales|f__Micromonosporaceae|g__Planosporangium	Control	1.10	0.05
341	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Diplorickettsiales	Control	1.07	0.04
342	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Diplorickettsiales|f__Diplorickettsiaceae	Control	1.07	0.04
343	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Diplorickettsiales|f__Diplorickettsiaceae|g__Aquicella	Control	1.07	0.04
344	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Legionellales	Control	1.04	0.02
345	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Legionellales|f__Legionellaceae	Control	1.04	0.02
346	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Legionellales|f__Legionellaceae|g__Legionella	Control	1.04	0.02
347	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Pseudonocardiales|f__Pseudonocardiaceae|g__Kibdelosporangium	Control	1.01	0.05
348	k__Bacteria|p__Planctomycetes|c__Planctomycetacia|o__Gemmatales|f__Gemmataceae|g__Fimbriiglobus	Control	0.87	0.05
349	k__Bacteria|p__Fibrobacteres|c__Fibrobacteria	Control	0.84	0.05
350	k__Bacteria|p__Fibrobacteres|c__Fibrobacteria|o__Fibrobacterales	Control	0.84	0.05
351	k__Bacteria|p__Fibrobacteres|c__Fibrobacteria|o__Fibrobacterales|f__Fibrobacteraceae	Control	0.84	0.05
352	k__Bacteria|p__Fibrobacteres	Control	0.84	0.05
353	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Rhizobiales|f__Beijerinckiaceae|g__Psychroglaciecola	Control	0.84	0.01
354	k__Bacteria|p__Fibrobacteres|c__Fibrobacteria|o__Fibrobacterales|f__Fibrobacteraceae|g__possible_genus_04	Control	0.84	0.05
355	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Coxiellales|f__Coxiellaceae	Control	0.81	0.05
356	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Coxiellales	Control	0.81	0.05
357	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Coxiellales|f__Coxiellaceae|g__Coxiella	Control	0.80	0.05
358	k__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales|f__Peptococcaceae	Control	0.72	0.05
359	k__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales|f__Peptococcaceae|g__Desulfosporosinus	Control	0.72	0.05

Table 8: Taxa with significantly different (Mann-Whitney U: $p < 0.05$) abundances between the 'Beneath' and 'Control' groups after twelve months. The data is sorted by descending linear discriminant analysis effect size (ef_lds).

No.	feature	enrich_group	ef_lda	pvalue
1	k__Bacteria|p__Proteobacteria	Beneath	3.65	0.02
2	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria	Beneath	3.53	0.02
3	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Xanthomonadales	Beneath	3.43	0.02
4	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Xanthomonadales|f__Rhodanobacteraceae	Beneath	3.42	0.02
5	k__Bacteria|p__Bacteroidetes|c__Bacteroidia	Beneath	3.31	0.02
6	k__Bacteria|p__Bacteroidetes	Beneath	3.31	0.02
7	k__Bacteria|p__Firmicutes|c__Clostridia	Beneath	3.15	0.02
8	k__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales	Beneath	3.14	0.02
9	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Xanthomonadales|f__Rhodanobacteraceae|g__Rhodanobacter	Beneath	3.09	0.02
10	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Xanthomonadales|f__Rhodanobacteraceae|g__Chujaibacter	Beneath	3.09	0.01
11	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Chitinophagales|f__Chitinophagaceae	Beneath	3.06	0.02
12	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Chitinophagales	Beneath	3.06	0.02
13	k__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales|f__Clostridiaceae_1	Beneath	3.03	0.02
14	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Micrococcales	Beneath	2.97	0.02
15	k__Bacteria|p__Firmicutes|c__Bacilli|o__Bacillales|f__Bacillaceae|g__Pseudogracilibacillus	Beneath	2.83	0.01
16	k__Bacteria|p__Acidobacteria|c__Acidobacteriia|o__Acidobacteriales|f__Acidobacteriaceae_(Subgroup_1)	Beneath	2.82	0.02
17	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Betaproteobacteriales|f__Burkholderiaceae	Beneath	2.82	0.02
18	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Betaproteobacteriales	Beneath	2.80	0.02
19	k__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales|f__Clostridiaceae_1|g__Clostridium_sensu_stricto_7	Beneath	2.73	0.01
20	k__Bacteria|p__Acidobacteria|c__Acidobacteriia|o__Acidobacteriales	Beneath	2.73	0.02
21	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Sphingobacteriales|f__Sphingobacteriaceae	Beneath	2.73	0.02
22	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Sphingobacteriales	Beneath	2.72	0.02
23	k__Bacteria|p__Firmicutes|c__Bacilli|o__Bacillales|f__Planococcaceae|g__Sporosarcina	Beneath	2.71	0.02
24	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Micrococcales|f__Micrococcaceae	Beneath	2.71	0.02
25	k__Bacteria|p__Acidobacteria|c__Acidobacteriia|o__Acidobacteriales|f__Acidobacteriaceae_(Subgroup_1)|g__Acidipila	Beneath	2.64	0.02
26	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Micrococcales|f__Micrococcaceae|g__Glutamicibacter	Beneath	2.63	0.01
27	k__Bacteria|p__Planctomycetes	Beneath	2.58	0.02
28	k__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales|f__Clostridiaceae_1|g__Hathewaya	Beneath	2.57	0.01
29	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Chitinophagales|f__Chitinophagaceae|g__Segetibacter	Beneath	2.55	0.04
30	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Rhizobiales|f__Devosiaceae	Beneath	2.52	0.02
31	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Rhizobiales|f__Devosiaceae|g__Devosia	Beneath	2.51	0.02
32	k__Bacteria|p__Planctomycetes|c__Planctomycetacia	Beneath	2.49	0.04
33	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Betaproteobacteriales|f__Burkholderiaceae|g__Castellaniella	Beneath	2.43	0.01
34	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Micrococcales|f__Microbacteriaceae	Beneath	2.41	0.02
35	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Cytophagales	Beneath	2.40	0.02
36	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Streptomycetales|f__Streptomycetaceae|g__Streptomyces	Beneath	2.38	0.04
37	k__Bacteria|p__Firmicutes|c__Bacilli|o__Bacillales|f__Bacillaceae|g__Cerasibacillus	Beneath	2.37	0.01
38	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Propionibacteriales|f__Nocardioidaceae	Beneath	2.35	0.04
39	k__Bacteria|p__Verrucomicrobia|c__Verrucomicrobiae|o__Chthoniobacterales|f__Chthoniobacteraceae|g__LD29	Beneath	2.29	0.03
40	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Rhizobiales|f__Rhizobiaceae	Beneath	2.29	0.04
41	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Sphingobacteriales|f__Sphingobacteriaceae|g__Pedobacter	Beneath	2.29	0.01
42	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Propionibacteriales	Beneath	2.28	0.04
43	k__Bacteria|p__Planctomycetes|c__Planctomycetacia|o__Isosphaerales|f__Isosphaeraceae|g__Isosphaera	Beneath	2.28	0.01
44	k__Bacteria|p__Acidobacteria|c__Acidobacteriia|o__Acidobacteriales|f__Acidobacteriaceae_(Subgroup_1)|g__Granulicella	Beneath	2.27	0.02
45	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Propionibacteriales|f__Nocardioidaceae|g__Nocardioides	Beneath	2.24	0.02
46	k__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales|f__Family_XI	Beneath	2.21	0.01
47	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Micrococcales|f__Brevibacteriaceae|g__Brevibacterium	Beneath	2.21	0.05
48	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Micrococcales|f__Brevibacteriaceae	Beneath	2.20	0.05
49	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Caulobacterales|f__Caulobacteraceae	Beneath	2.14	0.02
50	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Caulobacterales	Beneath	2.14	0.02
51	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Cytophagales|f__Hymenobacteraceae	Beneath	2.14	0.01
52	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Micrococcales|f__Microbacteriaceae|g__Microbacterium	Beneath	2.13	0.01
53	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Flavobacteriales	Beneath	2.11	0.01
54	k__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales|f__Clostridiaceae_2	Beneath	2.11	0.01
55	k__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales|f__Clostridiaceae_2|g__Alkaliphilus	Beneath	2.10	0.01
56	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Micrococcales|f__Intrasporangiaceae	Beneath	2.09	0.02
57	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Chitinophagales|f__Chitinophagaceae|g__Ferruginibacter	Beneath	2.09	0.02
58	k__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales|f__Family_XI|g__Tissierella	Beneath	2.06	0.01
59	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Cytophagales|f__Cytophagaceae	Beneath	2.05	0.02
60	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Salinisphaerales	Beneath	2.04	0.02
61	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Salinisphaerales|f__Solimonadaceae	Beneath	2.03	0.02
62	k__Bacteria|p__Proteobacteria|c__Deltaproteobacteria|o__Myxococcales|f__Phaselicystidaceae	Beneath	2.03	0.02
63	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Xanthomonadales|f__Xanthomonadaceae	Beneath	2.02	0.01
64	k__Bacteria|p__Proteobacteria|c__Deltaproteobacteria|o__Myxococcales|f__Phaselicystidaceae|g__Phaselicystis	Beneath	2.02	0.02
65	k__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales|f__Clostridiaceae_1|g__Clostridium_sensu_stricto_1	Beneath	2.01	0.01
66	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Salinisphaerales|f__Solimonadaceae|g__Alkanibacter	Beneath	1.95	0.05
67	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Chitinophagales|f__Chitinophagaceae|g__Taibaiella	Beneath	1.90	0.01
68	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Flavobacteriales|f__Weeksellaceae	Beneath	1.89	0.05
69	k__Bacteria|p__Planctomycetes|c__Phycisphaerae|o__Tepidisphaerales	Beneath	1.89	0.01
70	k__Bacteria|p__Planctomycetes|c__Phycisphaerae|o__Tepidisphaerales|f__Tepidisphaeraceae	Beneath	1.88	0.01
71	k__Bacteria|p__Planctomycetes|c__Phycisphaerae|o__Tepidisphaerales|f__Tepidisphaeraceae|g__Tepidisphaera	Beneath	1.88	0.01
72	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Flavobacteriales|f__Weeksellaceae|g__Chryseobacterium	Beneath	1.87	0.05
73	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Xanthomonadales|f__Rhodanobacteraceae|g__Dokdonella	Beneath	1.86	0.04
74	k__Bacteria|p__Deinococcus-Thermus|c__Deinococci	Beneath	1.85	0.01
75	k__Bacteria|p__Deinococcus-Thermus	Beneath	1.85	0.01
76	k__Bacteria|p__Deinococcus-Thermus|c__Deinococci|o__Deinococcales	Beneath	1.85	0.01
77	k__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales|f__Clostridiaceae_1|g__Clostridium_sensu_stricto_15	Beneath	1.85	0.01
78	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Frankiales|f__Nakamurellaceae	Beneath	1.84	0.01
79	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Frankiales|f__Nakamurellaceae|g__Nakamurella	Beneath	1.84	0.01
80	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Corynebacteriales|f__Nocardiaceae	Beneath	1.84	0.02
81	k__Bacteria|p__Planctomycetes|c__Phycisphaerae	Beneath	1.83	0.04
82	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Cytophagales|f__Hymenobacteraceae|g__Hymenobacter	Beneath	1.80	0.01
83	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Rhizobiales|f__Xanthobacteraceae|g__Rhodopseudomonas	Beneath	1.76	0.01
84	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Sphingobacteriales|f__Sphingobacteriaceae|g__Sphingobacterium	Beneath	1.75	0.05
85	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Micrococcales|f__Intrasporangiaceae|g__Ornithinimicrobium	Beneath	1.75	0.01
86	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Steroidobacterales	Beneath	1.74	0.01
87	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Steroidobacterales|f__Steroidobacteraceae|g__Steroidobacter	Beneath	1.74	0.01
88	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Steroidobacterales|f__Steroidobacteraceae	Beneath	1.73	0.01

No.	feature	enrich_group	ef_lda	pvalue
89	k__Bacteria|p__Proteobacteria|c__Deltaproteobacteria|o__Myxococcales|f__Myxococcaceae	Beneath	1.73	0.01
90	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Rhizobiales|f__Xanthobacteraceae|g__Afipia	Beneath	1.72	0.05
91	k__Bacteria|p__Acidobacteria|c__Acidobacteriia|o__Acidobacteriales|f__Acidobacteriaceae_(Subgroup_1)|g__Terriglobus	Beneath	1.72	0.02
92	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Flavobacteriales|f__Flavobacteriaceae	Beneath	1.71	0.01
93	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Betaproteobacteriales|f__Nitrosomonadaceae|g__Nitrosospira	Beneath	1.71	0.02
94	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Caulobacterales|f__Caulobacteraceae|g__Brevundimonas	Beneath	1.71	0.01
95	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Pseudomonadales	Beneath	1.71	0.02
96	k__Bacteria|p__Acidobacteria|c__Blastocatellia_(Subgroup_4)|o__Blastocatellales|f__Blastocatellaceae|g__Blastocatella	Beneath	1.70	0.01
97	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Micrococcales|f__Microbacteriaceae|g__Humibacter	Beneath	1.69	0.01
98	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Betaproteobacteriales|f__Burkholderiaceae|g__Eoetvoesia	Beneath	1.67	0.05
99	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Micrococcales|f__Microbacteriaceae|g__Leifsonia	Beneath	1.66	0.01
100	k__Bacteria|p__Actinobacteria|c__Acidimicrobiia|o__Microtrichales	Beneath	1.66	0.04
101	k__Bacteria|p__Actinobacteria|c__Acidimicrobiia	Beneath	1.66	0.04
102	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Cytophagales|f__Cytophagaceae|g__Cytophaga	Beneath	1.65	0.05
103	k__Bacteria|p__Proteobacteria|c__Deltaproteobacteria|o__Myxococcales|f__Myxococcaceae|g__Corallococcus	Beneath	1.65	0.01
104	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Xanthomonadales|f__Xanthomonadaceae|g__Lysobacter	Beneath	1.64	0.01
105	k__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales|f__Family_XI|g__Anaerosalibacter	Beneath	1.63	0.01
106	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Pseudomonadales|f__Moraxellaceae	Beneath	1.62	0.05
107	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Cytophagales|f__Hymenobacteraceae|g__Adhaeribacter	Beneath	1.62	0.05
108	k__Bacteria|p__Deinococcus-Thermus|c__Deinococci|o__Deinococcales|f__Deinococcaceae|g__Deinococcus	Beneath	1.60	0.01
109	k__Bacteria|p__Deinococcus-Thermus|c__Deinococci|o__Deinococcales|f__Deinococcaceae	Beneath	1.60	0.01
110	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Micrococcales|f__Microbacteriaceae|g__Amnibacterium	Beneath	1.58	0.05
111	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Micrococcales|f__Dermacoccaceae|g__Flexivirga	Beneath	1.58	0.01
112	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Micrococcales|f__Dermacoccaceae	Beneath	1.58	0.01
113	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Corynebacteriales|f__Nocardiaceae|g__Gordonia	Beneath	1.57	0.01
114	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Sphingomonadales|f__Sphingomonadaceae|g__Porphyrobacter	Beneath	1.57	0.01
115	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Rhizobiales|f__Rhizobiaceae|g__Shinella	Beneath	1.57	0.05
116	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Rhizobiales|f__Rhizobiaceae|g__Aminobacter	Beneath	1.54	0.05
117	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Micrococcales|f__Intrasporangiaceae|g__Oryzihumus	Beneath	1.53	0.05
118	k__Bacteria|p__Deinococcus-Thermus|c__Deinococci|o__Deinococcales|f__Trueperaceae	Beneath	1.52	0.05
119	k__Bacteria|p__Deinococcus-Thermus|c__Deinococci|o__Deinococcales|f__Trueperaceae|g__Truepera	Beneath	1.51	0.05
120	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Corynebacteriales|f__Nocardiaceae|g__Rhodococcus	Beneath	1.51	0.04
121	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Xanthomonadales|f__Xanthomonadaceae|g__Luteimonas	Beneath	1.51	0.05
122	k__Bacteria|p__Planctomycetes|c__Planctomycetacia|o__Isosphaerales|f__Isosphaeraceae|g__Paludisphaera	Beneath	1.46	0.05
123	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Flavobacteriales|f__Flavobacteriaceae|g__Flavobacterium	Beneath	1.45	0.05
124	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Propionibacteriales|f__Nocardioidaceae|g__Aeromicrobium	Beneath	1.44	0.01
125	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Acetobacterales|f__Acetobacteraceae|g__Roseomonas	Beneath	1.44	0.04
126	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Chitinophagales|f__Chitinophagaceae|g__Flavitalea	Beneath	1.43	0.05
127	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Micrococcales|f__Micrococcaceae|g__Arthrobacter	Beneath	1.42	0.01
128	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Micrococcales|f__Sanguibacteraceae|g__Sanguibacter	Beneath	1.42	0.05
129	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Micrococcales|f__Sanguibacteraceae	Beneath	1.42	0.05
130	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Rhizobiales|f__Hyphomicrobiaceae|g__Hyphomicrobium	Beneath	1.41	0.02
131	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Xanthomonadales|f__Rhodanobacteraceae|g__Frateuria	Beneath	1.41	0.05
132	k__Bacteria|p__Firmicutes|c__Bacilli|o__Bacillales|f__Bacillaceae|g__Virgibacillus	Beneath	1.39	0.05
133	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Salinisphaerales|f__Solimonadaceae|g__Nevskia	Beneath	1.39	0.05
134	k__Bacteria|p__Actinobacteria|c__Acidimicrobiia|o__Microtrichales|f__Microtrichaceae|g__IMCC26207	Beneath	1.39	0.05
135	k__Bacteria|p__Actinobacteria|c__Acidimicrobiia|o__Microtrichales|f__Microtrichaceae	Beneath	1.39	0.05
136	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Corynebacteriales|f__Dietziaceae	Beneath	1.39	0.05
137	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Cellvibrionales|f__Cellvibrionaceae|g__Cellvibrio	Beneath	1.38	0.05
138	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Cellvibrionales	Beneath	1.38	0.05
139	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Corynebacteriales|f__Dietziaceae|g__Dietzia	Beneath	1.38	0.05
140	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Frankiales|f__Geodermatophilaceae|g__Antricoccus	Beneath	1.37	0.05
141	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Cellvibrionales|f__Cellvibrionaceae	Beneath	1.37	0.05
142	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Rhizobiales|f__Beijerinckiaceae|g__Bosea	Beneath	1.36	0.01
143	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Betaproteobacteriales|f__Burkholderiaceae|g__Aquabacterium	Beneath	1.34	0.01
144	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Rhodobacterales|f__Rhodobacteraceae	Beneath	1.29	0.05
145	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Sphingomonadales|f__Sphingomonadaceae|g__Altererythrobacter	Beneath	1.29	0.02
146	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Rhodobacterales	Beneath	1.29	0.05
147	k__Bacteria|p__Proteobacteria|c__Deltaproteobacteria|o__Oligoflexales|f__Oligoflexaceae	Beneath	1.25	0.01
148	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Pseudomonadales|f__Moraxellaceae|g__Acinetobacter	Beneath	1.25	0.05
149	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Xanthomonadales|f__Xanthomonadaceae|g__Stenotrophomonas	Beneath	1.25	0.05
150	k__Bacteria|p__Proteobacteria|c__Deltaproteobacteria|o__Oligoflexales	Beneath	1.25	0.01
151	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Betaproteobacteriales|f__Burkholderiaceae|g__Bordetella	Beneath	1.22	0.05
152	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Micrococcales|f__Ruaniaceae|g__Haloactinobacterium	Beneath	1.22	0.05
153	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Micrococcales|f__Ruaniaceae	Beneath	1.22	0.05
154	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Rhizobiales|f__Rhizobiales_Incertae_Sedis|g__Alsobacter	Beneath	1.18	0.05
155	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Rhizobiales|f__Beijerinckiaceae|g__Methylovirgula	Beneath	1.17	0.05
156	k__Bacteria|p__Firmicutes|c__Bacilli|o__Bacillales|f__Bacillaceae|g__Ornithinibacillus	Beneath	1.15	0.05
157	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Rhodobacterales|f__Rhodobacteraceae|g__Paracoccus	Beneath	1.14	0.05
158	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Frankiales|f__Cryptosporangiaceae	Beneath	1.13	0.05
159	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Frankiales|f__Cryptosporangiaceae|g__Fodinicola	Beneath	1.12	0.05
160	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Micrococcales|f__Promicromonosporaceae	Beneath	1.08	0.05
161	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Micrococcales|f__Promicromonosporaceae|g__Cellulosimicrobium	Beneath	1.07	0.05
162	k__Bacteria|p__Spirochaetes|c__Spirochaetia|o__Spirochaetales|f__Spirochaetaceae	Beneath	1.07	0.05
163	k__Bacteria|p__Spirochaetes|c__Spirochaetia|o__Spirochaetales	Beneath	1.07	0.05
164	k__Bacteria|p__Spirochaetes|c__Spirochaetia	Beneath	1.07	0.05
165	k__Bacteria|p__Spirochaetes	Beneath	1.06	0.05
166	k__Bacteria|p__Proteobacteria|c__Deltaproteobacteria|o__Oligoflexales|f__Oligoflexaceae|g__Oligoflexus	Beneath	1.05	0.01
167	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Pseudomonadales|f__Pseudomonadaceae	Beneath	1.04	0.02
168	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Pseudomonadales|f__Pseudomonadaceae|g__Pseudomonas	Beneath	1.03	0.02
169	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Sphingomonadales|f__Sphingomonadaceae|g__Sphingobium	Beneath	1.02	0.01
170	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Paracaedibacterales|f__Paracaedibacteraceae	Beneath	1.00	0.05
171	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Paracaedibacterales	Beneath	1.00	0.05
172	k__Bacteria|p__Armatimonadetes|c__Fimbriimonadia|o__Fimbriimonadales	Beneath	0.93	0.05
173	k__Bacteria|p__Armatimonadetes|c__Fimbriimonadia|o__Fimbriimonadales|f__Fimbriimonadaceae	Beneath	0.93	0.05
174	k__Bacteria|p__Armatimonadetes|c__Fimbriimonadia|o__Fimbriimonadales|f__Fimbriimonadaceae|g__Fimbriimonas	Beneath	0.93	0.05
175	k__Bacteria|p__Armatimonadetes|c__Fimbriimonadia	Beneath	0.93	0.05
176	k__Bacteria|p__Firmicutes|c__Bacilli|o__Bacillales|f__Planococcaceae|g__Bhargavaea	Beneath	0.91	0.05
177	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Rhizobiales|f__Beijerinckiaceae|g__Camelimonas	Beneath	0.90	0.05

No.	feature	enrich_group	ef_lda	pvalue
178	k__Bacteria|p__Proteobacteria|c__Deltaproteobacteria|o__Oligoflexales|f__Oligoflexaceae|g__Silvanigrella	Beneath	0.90	0.05
179	k__Bacteria|p__Planctomycetes|c__Planctomycetacia|o__Planctomycetales|f__Rubinisphaeraceae	Beneath	0.89	0.01
180	k__Bacteria|p__Chloroflexi|c__Chloroflexia|o__Thermomicrobiales|f__Thermomicrobiaceae|g__Sphaerobacter	Beneath	0.89	0.05
181	k__Bacteria|p__Planctomycetes|c__Planctomycetacia|o__Planctomycetales	Beneath	0.87	0.04
182	k__Bacteria|p__Planctomycetes|c__Planctomycetacia|o__Planctomycetales|f__Rubinisphaeraceae|g__Planctomicrobium	Beneath	0.84	0.05
183	k__Bacteria|p__Proteobacteria|c__Deltaproteobacteria|o__Bdellovibrionales|f__Bacteriovoracaceae	Beneath	0.82	0.05
184	k__Bacteria|p__Proteobacteria|c__Deltaproteobacteria|o__Bdellovibrionales|f__Bacteriovoracaceae|g__Peredibacter	Beneath	0.81	0.05
185	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Paracaedibacterales|f__Paracaedibacteraceae|g__Candidatus_Captivus	Beneath	0.75	0.05
186	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Cytophagales|f__Spirosomaceae	Beneath	0.68	0.05
187	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Cytophagales|f__Spirosomaceae|g__Dyadobacter	Beneath	0.66	0.05
188	k__Bacteria|p__Chloroflexi|c__Ktedonobacteria|o__Ktedonobacterales|f__Ktedonobacteraceae	Control	3.42	0.02
189	k__Bacteria|p__Chloroflexi|c__Ktedonobacteria	Control	3.42	0.02
190	k__Bacteria|p__Chloroflexi|c__Ktedonobacteria|o__Ktedonobacterales	Control	3.41	0.02
191	k__Bacteria|p__Chloroflexi	Control	3.41	0.02
192	k__Bacteria|p__Verrucomicrobia|c__Verrucomicrobiae|o__Chthoniobacterales	Control	3.36	0.02
193	k__Bacteria|p__Verrucomicrobia|c__Verrucomicrobiae	Control	3.33	0.02
194	k__Bacteria|p__Verrucomicrobia	Control	3.33	0.02
195	k__Bacteria|p__Verrucomicrobia|c__Verrucomicrobiae|o__Chthoniobacterales|f__Chthoniobacteraceae|g__Candidatus_Udaeobacter	Control	3.29	0.02
196	k__Bacteria|p__Actinobacteria|c__Thermoleophilia	Control	3.21	0.02
197	k__Bacteria|p__Actinobacteria|c__Thermoleophilia|o__Solirubrobacterales	Control	3.20	0.02
198	k__Bacteria|p__Actinobacteria|c__Thermoleophilia|o__Solirubrobacterales|f__Solirubrobacteraceae	Control	3.20	0.02
199	k__Bacteria|p__Actinobacteria|c__Thermoleophilia|o__Solirubrobacterales|f__Solirubrobacteraceae|g__Conexibacter	Control	3.19	0.02
200	k__Bacteria|p__Acidobacteria|c__Acidobacteriia|o__Solibacterales	Control	3.16	0.02
201	k__Bacteria|p__Acidobacteria|c__Acidobacteriia|o__Solibacterales|f__Solibacteraceae_(Subgroup_3)	Control	3.16	0.02
202	k__Bacteria|p__Acidobacteria	Control	3.14	0.02
203	k__Bacteria|p__Verrucomicrobia|c__Verrucomicrobiae|o__Chthoniobacterales|f__Chthoniobacteraceae	Control	3.12	0.02
204	k__Bacteria|p__Verrucomicrobia|c__Verrucomicrobiae|o__Chthoniobacterales|f__Xiphinematobacteraceae|g__Candidatus_Xiphinematobacter	Control	2.99	0.02
205	k__Bacteria|p__Verrucomicrobia|c__Verrucomicrobiae|o__Chthoniobacterales|f__Xiphinematobacteraceae	Control	2.99	0.02
206	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Frankiales|f__Acidothermaceae|g__Acidothermus	Control	2.90	0.02
207	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Frankiales|f__Acidothermaceae	Control	2.90	0.02
208	k__Bacteria|p__Chloroflexi|c__Ktedonobacteria|o__Ktedonobacterales|f__Ktedonobacteraceae|g__HSB_OF53-F07	Control	2.86	0.01
209	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Frankiales	Control	2.85	0.02
210	k__Bacteria|p__Acidobacteria|c__Acidobacteriia|o__Solibacterales|f__Solibacteraceae_(Subgroup_3)|g__Candidatus_Solibacter	Control	2.80	0.02
211	k__Bacteria|p__Acidobacteria|c__Blastocatellia_(Subgroup_4)|o__Pyrinomonadales|f__Pyrinomonadaceae|g__RB41	Control	2.75	0.01
212	k__Bacteria|p__Acidobacteria|c__Blastocatellia_(Subgroup_4)|o__Pyrinomonadales|f__Pyrinomonadaceae	Control	2.75	0.01
213	k__Bacteria|p__Acidobacteria|c__Blastocatellia_(Subgroup_4)|o__Pyrinomonadales	Control	2.75	0.01
214	k__Bacteria|p__Chloroflexi|c__Ktedonobacteria|o__Ktedonobacterales|f__Ktedonobacteraceae|g__1921-3	Control	2.74	0.02
215	k__Bacteria|p__Chloroflexi|c__Ktedonobacteria|o__Ktedonobacterales|f__Ktedonobacteraceae|g__FCPS473	Control	2.68	0.02
216	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Rhizobiales|f__Xanthobacteraceae|g__Bradyrhizobium	Control	2.66	0.02
217	k__Bacteria|p__Acidobacteria|c__Blastocatellia_(Subgroup_4)	Control	2.64	0.04
218	k__Bacteria|p__Firmicutes|c__Bacilli|o__Bacillales|f__Planococcaceae|g__Lysinibacillus	Control	2.62	0.02
219	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Rhizobiales|f__Xanthobacteraceae	Control	2.62	0.02
220	k__Bacteria|p__Chloroflexi|c__Ktedonobacteria|o__Ktedonobacterales|f__Ktedonobacteraceae|g__1921-2	Control	2.59	0.02
221	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Pseudonocardiales	Control	2.45	0.02
222	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Pseudonocardiales|f__Pseudonocardiaceae	Control	2.45	0.02
223	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Gammaproteobacteria_Incertae_Sedis|f__Gammaproteobacteria_Incertae_Sedis_Unknown_Family|g__Acidibacter	Control	2.44	0.02
224	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Gammaproteobacteria_Incertae_Sedis	Control	2.43	0.02
225	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Gammaproteobacteria_Incertae_Sedis|f__Gammaproteobacteria_Incertae_Sedis_Unknown_Family	Control	2.42	0.02
226	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Micrococcales|f__Bogoriellaceae	Control	2.29	0.04
227	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Micrococcales|f__Bogoriellaceae|g__Georgenia	Control	2.29	0.04
228	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Pseudonocardiales|f__Pseudonocardiaceae|g__Crossiella	Control	2.28	0.02
229	k__Bacteria|p__Chloroflexi|c__Ktedonobacteria|o__Ktedonobacterales|f__Ktedonobacteraceae|g__JG30a-KF-32	Control	2.23	0.02
230	k__Bacteria|p__Chloroflexi|c__Ktedonobacteria|o__Ktedonobacterales|f__Ktedonobacteraceae|g__G12-WMSP1	Control	2.18	0.02
231	k__Bacteria|p__Proteobacteria|c__Deltaproteobacteria|o__Myxococcales|f__Archangiaceae	Control	2.15	0.04
232	k__Bacteria|p__Acidobacteria|c__Acidobacteriia|o__Acidobacteriales|f__Koribacteraceae	Control	2.08	0.02
233	k__Bacteria|p__Acidobacteria|c__Acidobacteriia|o__Acidobacteriales|f__Koribacteraceae|g__Candidatus_Koribacter	Control	2.08	0.02
234	k__Bacteria|p__Proteobacteria|c__Deltaproteobacteria|o__Myxococcales|f__Archangiaceae|g__Anaeromyxobacter	Control	2.04	0.04
235	k__Bacteria|p__Firmicutes|c__Bacilli|o__Bacillales|f__Paenibacillaceae	Control	1.99	0.02
236	k__Bacteria|p__Chloroflexi|c__Ktedonobacteria|o__Ktedonobacterales|f__Ktedonobacteraceae|g__Ktedonobacter	Control	1.92	0.05
237	k__Bacteria|p__Firmicutes|c__Bacilli|o__Bacillales|f__Planococcaceae|g__Chungangia	Control	1.89	0.02
238	k__Bacteria|p__Chloroflexi|c__Ktedonobacteria|o__Ktedonobacterales|f__Ktedonobacteraceae|g__1959-1	Control	1.74	0.01
239	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Acetobacterales|f__Acetobacteraceae|g__Acidisphaera	Control	1.71	0.01
240	k__Bacteria|p__Actinobacteria|c__Thermoleophilia|o__Gaiellales	Control	1.71	0.01
241	k__Bacteria|p__Actinobacteria|c__Thermoleophilia|o__Gaiellales|f__Gaiellaceae	Control	1.71	0.01
242	k__Bacteria|p__Actinobacteria|c__Thermoleophilia|o__Gaiellales|f__Gaiellaceae|g__Gaiella	Control	1.71	0.01
243	k__Bacteria|p__Firmicutes|c__Bacilli|o__Bacillales|f__Paenibacillaceae|g__Cohnella	Control	1.68	0.02
244	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Betaproteobacteriales|f__Nitrosomonadaceae|g__MND1	Control	1.55	0.01
245	k__Bacteria|p__Actinobacteria|c__Rubrobacteria|o__Rubrobacterales|f__Rubrobacteriaceae|g__Rubrobacter	Control	1.49	0.01
246	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Rhizobiales|f__Rhizobiales_Incertae_Sedis|g__Baldia	Control	1.49	0.02
247	k__Bacteria|p__Actinobacteria|c__Rubrobacteria|o__Rubrobacterales	Control	1.49	0.01
248	k__Bacteria|p__Actinobacteria|c__Rubrobacteria|o__Rubrobacterales|f__Rubrobacteriaceae	Control	1.49	0.01
249	k__Bacteria|p__Actinobacteria|c__Rubrobacteria	Control	1.49	0.01
250	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Acetobacterales|f__Acetobacteraceae|g__Rhodopila	Control	1.47	0.05
251	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Pseudonocardiales|f__Pseudonocardiaceae|g__Pseudonocardia	Control	1.47	0.02
252	k__Bacteria|p__Planctomycetes|c__Planctomycetacia|o__Pirellulales	Control	1.47	0.02
253	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Chitinophagales|f__Chitinophagaceae|g__Parafilimonas	Control	1.46	0.04
254	k__Bacteria|p__Planctomycetes|c__Planctomycetacia|o__Pirellulales|f__Pirellulaceae	Control	1.46	0.02
255	k__Bacteria|p__Firmicutes|c__Bacilli|o__Bacillales|f__Paenibacillaceae|g__Paenibacillus	Control	1.41	0.03
256	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Streptomycetales|f__Streptomycetaceae|g__Kitasatospora	Control	1.34	0.05
257	k__Bacteria|p__Acidobacteria|c__Subgroup_6	Control	1.32	0.05
258	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Catenulisporales|f__Catenulisporaceae|g__Catenulispora	Control	1.32	0.02
259	k__Bacteria|p__Acidobacteria|c__Subgroup_6|o__Unknown_Order	Control	1.32	0.05
260	k__Bacteria|p__Acidobacteria|c__Subgroup_6|o__Unknown_Order|f__Unknown_Order_Unknown_Family|g__Vicinamibacter	Control	1.31	0.05
261	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Catenulisporales|f__Catenulisporaceae	Control	1.31	0.02
262	k__Bacteria|p__Acidobacteria|c__Subgroup_6|o__Unknown_Order|f__Unknown_Order_Unknown_Family	Control	1.31	0.05
263	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Rhizobiales|f__Xanthobacteraceae|g__GAS113	Control	1.31	0.05
264	k__Bacteria|p__Planctomycetes|c__Planctomycetacia|o__Pirellulales|f__Pirellulaceae|g__Pirellula	Control	1.30	0.02
265	k__Bacteria|p__Nitrospirae|c__Nitrospira|o__Nitrospirales|f__Nitrospiraceae	Control	1.18	0.05
266	k__Bacteria|p__Nitrospirae|c__Nitrospira|o__Nitrospirales	Control	1.17	0.05
267	k__Bacteria|p__Nitrospirae|c__Nitrospira	Control	1.17	0.05
268	k__Bacteria|p__Nitrospirae	Control	1.17	0.05
269	k__Bacteria|p__Nitrospirae|c__Nitrospira|o__Nitrospirales|f__Nitrospiraceae|g__Nitrospira	Control	1.17	0.05
270	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Pseudonocardiales|f__Pseudonocardiaceae|g__Kutzneria	Control	1.15	0.05
271	k__Bacteria|p__Acidobacteria|c__Acidobacteriia|o__Solibacterales|f__Solibacteraceae_(Subgroup_3)|g__TSBb06	Control	1.01	0.05

Table 9: Taxa with significantly different (Mann-Whitney U: $p < 0.05$) abundances between the 'Beneath' and 'Control' groups after eighteen months. The data is sorted by descending linear discriminant analysis effect size (ef_lds).

No.	feature	enrich_group	ef_lda	pvalue
1	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Xanthomonadales|f__Rhodanobacteraceae	Beneath	3.48	0.03
2	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Xanthomonadales	Beneath	3.48	0.03
3	k__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Xanthomonadales|f__Rhodanobacteraceae|g__Rhodanobacter	Beneath	2.54	0.03
4	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Sphingobacteriales|f__Sphingobacteriaceae	Beneath	2.29	0.03
5	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Sphingobacteriales	Beneath	2.27	0.03
6	k__Bacteria|p__Verrucomicrobia|c__Verrucomicrobiae|o__Chthoniobacterales|f__Chthoniobacteraceae|g__LD29	Beneath	2.05	0.05
7	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Micrococcales|f__Intrasporangiaceae	Beneath	1.80	0.03
8	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Rhizobiales|f__Beijerinckiaceae|g__Psychroglaciecola	Beneath	1.58	0.03
9	k__Bacteria|p__Actinobacteria|c__Acidimicrobiia	Beneath	1.31	0.03
10	k__Bacteria|p__Actinobacteria|c__Acidimicrobiia|o__Microtrichales	Beneath	1.31	0.03
11	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Paracaedibacterales	Beneath	1.19	0.03
12	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Paracaedibacterales|f__Paracaedibacteraceae	Beneath	1.18	0.03
13	k__Bacteria|p__Verrucomicrobia|c__Verrucomicrobiae|o__Chthoniobacterales	Control	3.32	0.03
14	k__Bacteria|p__Verrucomicrobia	Control	3.32	0.03
15	k__Bacteria|p__Verrucomicrobia|c__Verrucomicrobiae	Control	3.32	0.03
16	k__Bacteria|p__Verrucomicrobia|c__Verrucomicrobiae|o__Chthoniobacterales|f__Chthoniobacteraceae|g__Candidatus_Udaeobacter	Control	3.26	0.03
17	k__Bacteria|p__Verrucomicrobia|c__Verrucomicrobiae|o__Chthoniobacterales|f__Chthoniobacteraceae	Control	3.19	0.03
18	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Rhizobiales|f__Xanthobacteraceae|g__Bradyrhizobium	Control	2.36	0.03
19	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Kineosporiales|f__Kineosporiaceae|g__Quadrisphaera	Control	1.96	0.03
20	k__Bacteria|p__Verrucomicrobia|c__Verrucomicrobiae|o__Pedosphaerales|f__Pedosphaeraceae|g__ADurb.Bin063-1	Control	1.92	0.03
21	k__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Acetobacterales|f__Acetobacteraceae|g__Rhodopila	Control	1.66	0.03

Appendix E

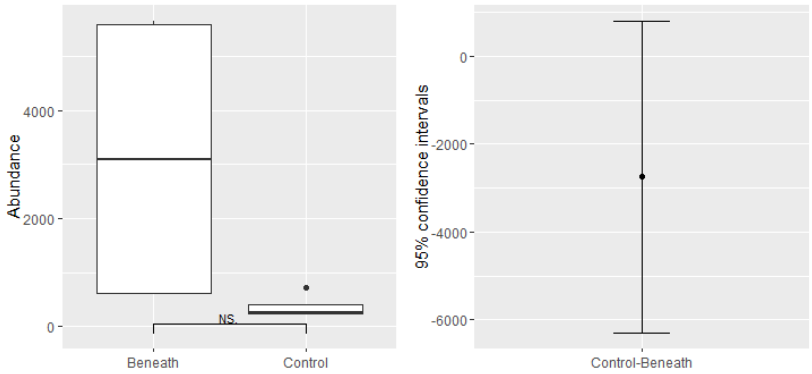

Figure 28: Bacteroidetes increases after one month of decomposition beneath the carcasses were not statistically different (Mann-Whitney *U*: $p < 0.05$) from the 'Control' soil due to the sizeable intersample variance of the 'Beneath' group samples.