Arthropods, nematodes, fungi, and bacteria associated with penguin carrion in Barton Peninsula, King George Island, Antarctica

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Abstract

Carrion decomposition contributes to the soil microbial community structure. This research aimed to identify the soil arthropod, nematode, bacterial, and fungal communities associated with penguin carrion on King George Island, Antarctica. Soil samples were collected around and beneath fresh (freshly killed penguins by the predators) and dried (decomposed more than a year) penguin carrion. Soil bacterial and fungal communities associated with the penguin carrion were analyzed using the 16S rRNA Illumina MiSeq sequencing. Arthropod identification was using Sanger sequencing and nematodes were determined using morphological identification. This study demonstrated that there are no significant differences in arthropod and nematode ($p = 0.415$), bacteria ($p = 0.386$), and fungi ($p = 0.635$) communities between decomposition stages, soil location, and species of penguin carrion. This is the first study to identify soil arthropods, nematodes, bacterial, and fungal communities associated with penguin carrion, offering important insights into the initial documentation of the necrobiome communities in the polar region.

1.0 Introduction

Carrion decomposition is a fundamental ecological process that includes the breakdown of dead animals and the recycling of their embodied nutrients through other organisms and their environment. Previous studies have demonstrated that microbial decomposers (i.e. bacteria, archaea, fungi, protists) play a critical role in carrion decomposition (Carter and Tibbett 2008; Lauber et al. 2014; Picard et al. 2015a; Metcalf et al. 2016). The diversity of the carrion microbial community is impressive, with thousands of taxa associated with such ephemeral resources (Pechal et al. 2013, 2014; Picard et al. 2015a). Soil microbial communities play an important role during carrion decomposition in terrestrial ecosystems by maintaining the soil quality through their involvement in organic matter biochemical dynamics, nutrient cycling, aiding in the pathogenic spread, and decomposition (Martı´nez et al. 2008; Parmenter and Macmahon 2009; Howard et al. 2010; Yang et al. 2017; Harrison et al. 2020).

Necrophagous arthropods further accelerate the decomposition rate of carrion in terrestrial environments (Payne 1965; Pechal et al. 2013, 2014). Their attraction, colonization, development, and migration to carrion can affect nutrient transformation and release, and thus, local biodiversity (Hocking and Reimchen 2006; Lisi and Schindler 2011; Tomberlin et al. 2011; Hawlena et al. 2012). Meanwhile, nematodes comprise up to 80% of all multicellular organisms found in the soil environment (Bongers and Ferris 1999). During the active stage of decomposition, the carrion is dominated by the bacterial-feeding nematodes specifically Rhabditidae and Diplogasteridae (Id et al. 2020). The soil nematode population is likely responding to the proliferation of bacteria communities associated with carrion (Benninger et al. 2008; Carter and Tibbett 2008).

The decomposition of carrion by these organisms is partially regulated by various abiotic and biotic factors such as vegetation and inter-specific competition among scavengers (Philip S. Barton and Joseph K. Bump 2019), temperature (Ward et al. 1998; Carter and Tibbett 2006; Barton et al. 2013), moisture, and

humidity (Schimel et al. 1999), tissue type (Dickson et al. 2011), surrounding vegetation (Ibekwe et al. 2002; Kuske et al. 2002) and soil pH (Haslam and Tibbett 2009), which influence the microbial community assembly and function during carrion decomposition.

Temperature plays an integral role in the rate of tissue decomposition (Mann et al. 1990; Bass III 1996; Gill-King 1996; Megyesi et al. 2005; Vass 2011; Zhou and Byard 2011) as it impacts the activity of invertebrates (Campobasso et al. 2001; Archer and Elgar 2003; Joy et al. 2006; Sharanowski et al. 2008; Voss et al. 2009), vertebrates scavengers (O'Brien et al. 2010; Dabbs and Martin 2013; Young et al. 2014), and bacteria and fungi (Carter et al. 2008; Hopkins 2008; Kasper et al. 2012; Lauber et al. 2014). Although extensive research has been carried out on the effects of abiotic factors on microbial diversity during carrion decomposition, no single study focuses on the decomposer diversity of Antarctica despite its relatively simple ecosystem (Convey 2010).

The diversity of microorganisms is crucial to the ecosystem's functioning since it is required to support ecological processes such as organic matter decomposition, nutrient cycling, and soil aggregation within the ecosystem (Kennedy 1999). Therefore, this study aims to identify and understand the association of necrobiome (i.e., arthropods, nematodes, bacterial, and fungal communities) with penguin carrions in Antarctica. We hypothesize that different penguin species, soil locations, and the stage of decomposition will be associated with different types of necrobiome communities.

2.0 Methods

2.1 Sample collections

Soil samples were collected from December 2019 to January 2020 at Narębski Point, Antarctic Specially Protected Area (ASPA) 171), Barton Peninsula, King George Island, Antarctica (62° 13' S, 58 ° 47' W, 10 m a.s.l.) (Fig. 1 (a)). The sample collection was based on haphazard sampling, which was dependent on the available carrion samples during the sampling time. Approximately 30 g of soil from beneath and 5 m away (around) from penguin carrion were collected and placed in zip-lock bags. Both soil samples (beneath and around) collected share similar environments and fall within the purview of ASPA 171. This study was limited to the observation of just two distinct penguin species which were *Pygoscelis antarcticus* (Forster, 1781) (Chinstrap) (Fig. 1(b)) and *Pygoscelis papua* (Forster, 1781) (Gentoo) (Fig. 1(c)) that exhibited only two stages of decomposition: - fresh (n = 2) and dry (n = 17) (Fig. 1(d) and (e)). Note that only fresh carrion of Gentoo penguins was available during field sampling, whereas the rest were dried carrion. The fresh stage of penguin decomposition was characterized by freshly killed penguins (primarily by predators such as skuas or seals), where soft tissues and internal organs were remained intact. In contrast, the dry stage was defined as the skeletonization stage of the penguin carrion, where only bones and feathers were left visible *in-situ*. Samples were kept in the − 20°C freezer at King Sejong Station. Samples were then brought back to Malaysia in an ice cooler box with an import permission and stored permanently in a -20°C freezer at the Institute of Medical Molecular Biotechnology (IMMB), Faculty of Medicine, Universiti Teknologi MARA.

2.2 Arthropod and nematode isolation and identification.

Approximately 15mL of 70% ethanol was poured into a petri dish containing 15 grams of soil sample and observed under a stereo microscope (Olympus SZX7, Japan). The arthropods and nematodes were then isolated using an applicator stick and preserved in 95% ethanol and nematode preservation solution (70% ethanol and glycerine) (Bauer 2022) before identification. Arthropods were stored in 95% ethanol until further processing (molecular identification); meanwhile, the nematode basic identification was conducted using the 'Interactive diagnostic key to nematodes' (UNL Nematology Lab 1977). Experts in respective taxonomy groups were consulted for the identity of the nematodes and arthropods collected (see acknowledgments).

2.4 DNA extraction

Arthropod DNA was isolated using the DNeasy® Blood & Tissue kit (QIAGEN, Germany), while soil bacteria and fungi DNA was extracted using the DNeasy® PowerSoil® kit (QIAGEN, Germany). Following DNA extraction, the genomic DNA (gDNA) was purified, and run-on gel electrophoresis was performed using 1% TAE agarose gel at 100kV for 60 minutes. The DNA concentration was subsequently measured using a SpectraMax QuickDrop UV-Vis spectrophotometer (Molecular Devices, USA).

2.5 DNA amplification and generation

The amplification of the arthropod mitochondrial COI was performed in a final volume of 25µL containing < 250 ng genomic DNA, 12.5 µL GoTaq® Hot Start Master Mix (Promega, USA), and 10 µM of each forward and reverse primer. Meanwhile, soil bacteria and fungi gDNA was amplified using REDiant 2X PCR Master Mix (1st Base, Singapore). Two parts of library construction were conducted. The selected regions (16S V3-V4 and ITS2) were amplified using locus-specific sequence primers with overhang adapters. Then, the dual indices were attached to the amplicon PCR using Illumina Nextera XT Index Kit v2 (Illumina, USA). The quality of the libraries was measured using Agilent Bioanalyzer 2100 System (Agilent, USA) by Agilent DNA 1000 Kit and fluorometric quantification by Helixyte GreenTM Quantifying Reagent (AAT Bioquest, USA).

2.6 Next-Generation sequencing

The libraries were normalized and pooled according to the protocol recommended by Illumina and then sequenced using the MiSeq platform using 300 PE.

2.7 Data analysis

Paired-end reads are first removed from sequence adaptors, and then low-quality reads using BBDuk of the BBTools package. Then, the forward and reverse reads are merged using USEARCH v11.0.667. All sequences that are shorter than 150 bp or longer than 600 bp (sequenced on the MiSeq platform) were removed from downstream processing. Reads are then aligned with 16S rRNA (SILVA Release 132) and UNITE ITS database and inspected for chimeric errors using VSEARCH v2.6.2. Reads are clustered de novo into OTUs at 97% similarity using UPARSE v11.0.667; rare OTUs with less than two reads were

deleted from downstream processing. Taxonomic assignment of OTU is achieved using QIIME V1.9.1 against the Silva 16S rRNA database.

2.8 Statistical analysis

Statistical analysis was conducted using a vegan package in R (v.4.1.3). The α-diversity of each community was measured by calculating the Shannon–Wiener (H'), Simpson (D-1), and inverse Simpson (1/λ) indexes. Bray-Curtis dissimilarity was used for the arthropods and nematode's β-diversity. Bacteria and fungi principal coordinate analysis (PCoA) was identified using Jaccard and Jensen Shannon Divergence. The evenness distribution of species was measured in mean relative abundance. Statistical results with $p < 0.05$ were considered a significant difference.

3.0 Results

3.1 Arthropods and nematodes

Arthropods isolated from the soil samples were identified as mites; *Alaskozetes antarcticus*, *Halozetes belgicae*, and collembola; *Cryptopygus antarcticus* (Fig. 2 (a), (b), and (c)). Meanwhile, nematodes were identified morphologically according to order level, and were identified as Mononchida (Fig. 3 (a)), Rhabditida (Fig. 3 (b)), and Dorylaimida (Fig. 3 (c)). The mean relative abundance is distributed unevenly, with the most abundant taxa being Mononchida nematodes (n = 635). Two penguin species (i.e., Chinstrap (*Pygoscelis antarcticus* (Forster, 1781)), and Gentoo (*Pygoscelis papua* (Forster, 1781))), soil sample location (around (five meters away) and beneath penguin carrion), and stage of decomposition (fresh and dry) were grouped accordingly into Gentoo dry around (GDA), Gentoo dry beneath (GDB), Gentoo fresh around (GFA), Gentoo fresh beneath (GFB), Chinstrap dry around (CDA), and Chinstrap dry beneath (CDB). All carcass's weight and sex were unidentified. The fresh stage groups of decomposition (GFA (arthropods (n = 5), nematodes (n = 24)) and GFB (arthropods (n = 0), nematodes (n = 15))) had the lowest abundance of arthropods and nematodes in comparison to the dry stage groups (GDA (arthropods (n = 88), nematodes (n = 134)), GDB (arthropods (n = 231), nematodes (n = 266)), CDA (arthropods (n = 400), nematodes (n = 426)), and CDB (arthropods (n = 454), nematodes (n = 440))) (Fig. 4 (a)).

The alpha diversity indexes (Shannon–Wiener (H')) of arthropod and nematode communities in GFB (H' = 0.000) showed the lowest diversity compared to CDA (H' = 1.173), CDB (H' = 1.139), GDA (H' = 0.787), GDB (H' = 0.598), and GFA (H' = 0.937) samples (Fig. 4 (b)). The intercommunity comparison assessed by Non-Metric Multidimensional Scaling (NMDS) (Fig. 4 (c)) showed that all groups were overlapping in the composition of arthropods and nematodes. Overall, the arthropod and nematode communities did not show significant differences between the groups ($p = 0.415$).

3.2 Bacteria and fungi

Proteobacteria had the highest mean relative abundance at the bacterial phylum level in all groups (CDB = 0.451, CDA 0.600, GDB = 0.284, GDA = 0.490, GFB = 0.463 and GFA = 0.376) compared to Cyanobacteria (CDB = 0.095, CDA 0.015, GDB = 0.259, GDA = 0.075, GFB = 0.106 and GFA = 0.049). On the species level,

Clostridium esterthicum was the most abundant bacteria found in all groups, followed by *Carnobacterium inhibens, Simplicispira psychrophile*, uncultured cyanobacterium, and sphingobacteriales (Fig. 5 (a) and (c)). Figure 5 (b) shows the two main fungal phyla associated with the penguin carrion, namely Ascomycota and Basidiomycota. As shown in Fig. 5 (d), *Mrakia frigida* was the most abundant fungi species in all groups (CDB = 0.582, CDA 0.643; GDB = 0.650, GDA = 0.681, GFB = 0.550 and GFA = 0.555).

Analysis of Variance (ANOVA) showed no significant differences in alpha diversity among groups for the bacterial ($p = 0.386$) and fungal ($p = 0.635$) communities. Principal Coordinate Analysis (PCoA) was carried out using the Jensen-Shannon Divergence distance metric and showed that samples did not cluster by the group (Fig. 5 (e) and (f)). To compare microbial composition among the six groups, permutational multivariate analysis of variance (PERMANOVA) with Jensen Shannon Divergence distance metric was used. There was no significant difference between the group distances for bacterial ($R2 = 0.2$, $p = 0.6$) or fungal communities ($R2 = 0.18$, $p = 0.785$).

4.0 Discussion

This study has been unable to demonstrate the hypothesis posed, in which, different penguin species, soil location, and the stage of decomposition were associated with different types of necrobiome communities. The current study found no significant differences in arthropod, nematode, bacterial, and fungal communities between penguin species, soil location, and the stage of decomposition. It is well known that environmental conditions contribute to organism abundance and diversity. Antarctica is constantly subjected to harsh climatic conditions such as limited organic nutrients, low humidity, frequent freeze-thaw and wet-dry cycles, low temperatures, fluctuating UV radiation, and desiccating solid winds (Wynn-Williams 1990; Cowan and Tow 2004). Moreover, earlier studies recorded that few arthropod species in Antarctic soils were dependent on vegetation in the environment (Davis R C 1981; Usher and Booth 1986; Sinclair 2001; Convey 2010). When plant production is increased, food availability is enhanced and promotes greater populations of herbivores (Siemann 1998; Haddad et al. 2009; La Pierre and Smith 2016), supporting larger predator and parasitoid populations (Langellotto and Denno 2004; Hairston et al. 2009; Fretwell 2012). This is likely a reason for the lower arthropod abundance as the vegetation biomass in Antarctica is low.

Meanwhile, in this study, only three arthropod species were identified, with *Cryptopygus antarcticus* being the most dominant. They are known to feed on algae, decaying organic matter, and fungi, making them specialist feeders (Tillbrook 1968; Broady 1979). It is important to note that within the soil food web, arthropods and nematodes represent the most abundant (85%) invertebrates (Culliney 2013). They display important regulations on soil food webs as they directly control the microbial biomass (Lussenhop 1992). For example, bacterial-feeding nematodes, primarily Rhabditidae, respond positively to nutrient enrichment, such as water and organic matter, compared to other bacterivores such as the Cephalobidae (Bongers and Ferris 1999; Georgieva et al. 2002; Ferris and Matute 2003; Blanc et al. 2006; Steel et al. 2010).

The result of this study indicates that the identified microbes from our samples are mostly psychrophilic. Due to the polar environment, the psychrophilic microbes undergo a condition in which the cellular membranes become more rigid as temperature decreases, thus limiting their ability to take up nutrients and release by-products of cellular metabolism (Yarzábal 2016). These findings further support the idea of Deming (2003), who reported that cold-adapted microorganisms achieve physiological and ecological successes in cold environments due to unique features in their proteins and membranes and their genetic responses to thermal shifts (Deming 2003). Psychrophiles have evolved necessary cellular adaptations to survive the freezing temperatures, including mechanisms to maintain membrane fluidity (Russell 1997; Jagannadham et al. 2000), synthesis of cold-acclimation proteins (Hébraud and Potier 1999), freeze tolerance strategies (Slaper Harry, Guss J. Velders 1996), and cold-active enzymes. The result also accords with previous research suggesting that microbial abundance and activity in epinecrotic communities are patchy and dependent on environmental factors such as temperature and habitat(Pechal et al. 2013). Similarly, Yergeau et al., (2007) found that the community structure, bacterial diversity, abundance, and functional gene density are affected by environmental conditions (Yergeau et al. 2007).

This study indicates a non-significant difference in soil bacteria in the sample beneath and around the carrion. The results of this study match those observed in earlier studies that described major bacterial phyla found consistently across the Antarctic continent. Current research finds that Proteobacteria were the most abundant bacteria isolated from the samples. The abundance of Proteobacteria identified during carrion decomposition was consistent with other relevant studies (Dickson et al. 2011; Benbow et al. 2015; Jing He et al. 2019). The current study is also in line with Metcalf et al. (2013), who reported that Proteobacteria and Firmicutes dominate small vertebrate (mouse) carrion (Metcalf et al. 2013). This finding agrees with Picard et al., (2015b), who noted that the Proteobacteria dominated the abdominal cavity community in later phases of decomposition and was the predominant taxon on skin communities throughout the decomposition process (Picard et al. 2015b).

Ascomycota fungi are decomposers that break down animal tissues into major organic compounds and are particularly active during the active decay of carrion (Metcalf et al. 2016). The stochastic spatial and temporal nature of carrion microbial communities contributes to the variation within and across carrion (Ramette and Tiedje 2007). Biotic and abiotic factors in the soil environment are altered due to the release of organic compounds in the soil at different decomposition stages (Vass et al. 2004). Fungal species present in the environment and fungi originating from the body's microflora are subjected to growth inhibition and potentially to inter-specific competition that can result in sequential changes to the composition of the fungal community (Parkinson et al. 2009; Singh et al. 2018). This study found that Ascomycota was the most abundant fungal taxa associated with penguin carrion, consistent with previous studies that observed an increase in the abundance of Ascomycota during the first stages of decomposition (Fu et al. 2019).

5.0 Conclusion

This study aimed to identify soil arthropods, nematodes, and bacterial and fungal communities associated with penguin carrion. The results show that the necrobiome communities did not have any significant differences between penguin species, stages of decomposition, and soil locations. Given the limitations imposed by our restricted time in the field, we recognize that the distribution of samples between fresh (n = 2) and dry (n = 17) carrion was unbalanced. We acknowledge that this unbalanced representation, particularly the lack of data on dry carrion movement, may be viewed as a limitation of this research. However, this initial report will pave the way for further progress in this area of investigation, offering opportunities for future advancements and a more comprehensive understanding of the necrobiome community structure in Antarctica. These results make a noteworthy contribution, for the first time, to the initial documentation of necrobiome communities in response to the penguin decomposition process under the Antarctica climate.

Declarations

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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Authors contributions

The authors contributed to the different parts of this manuscript as follows:

N.A.Z. methodology, investigation, formal analysis, visualization, and writing - original draft.

V.L.L. methodology, and writing – review, and editing.

S.S.G. statistical analysis, writing – review, and editing.

S.S.I. writing – review and editing.

M.H.M. writing – review, and editing.

J.H. writing – review and editing.

W.Y.L. writing – review and editing.

J.K.T. writing – review and editing.

C.C.H. conceptualization, methodology, and writing – review, and editing.

Competing interest

The authors declare no competing interest.

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Figures

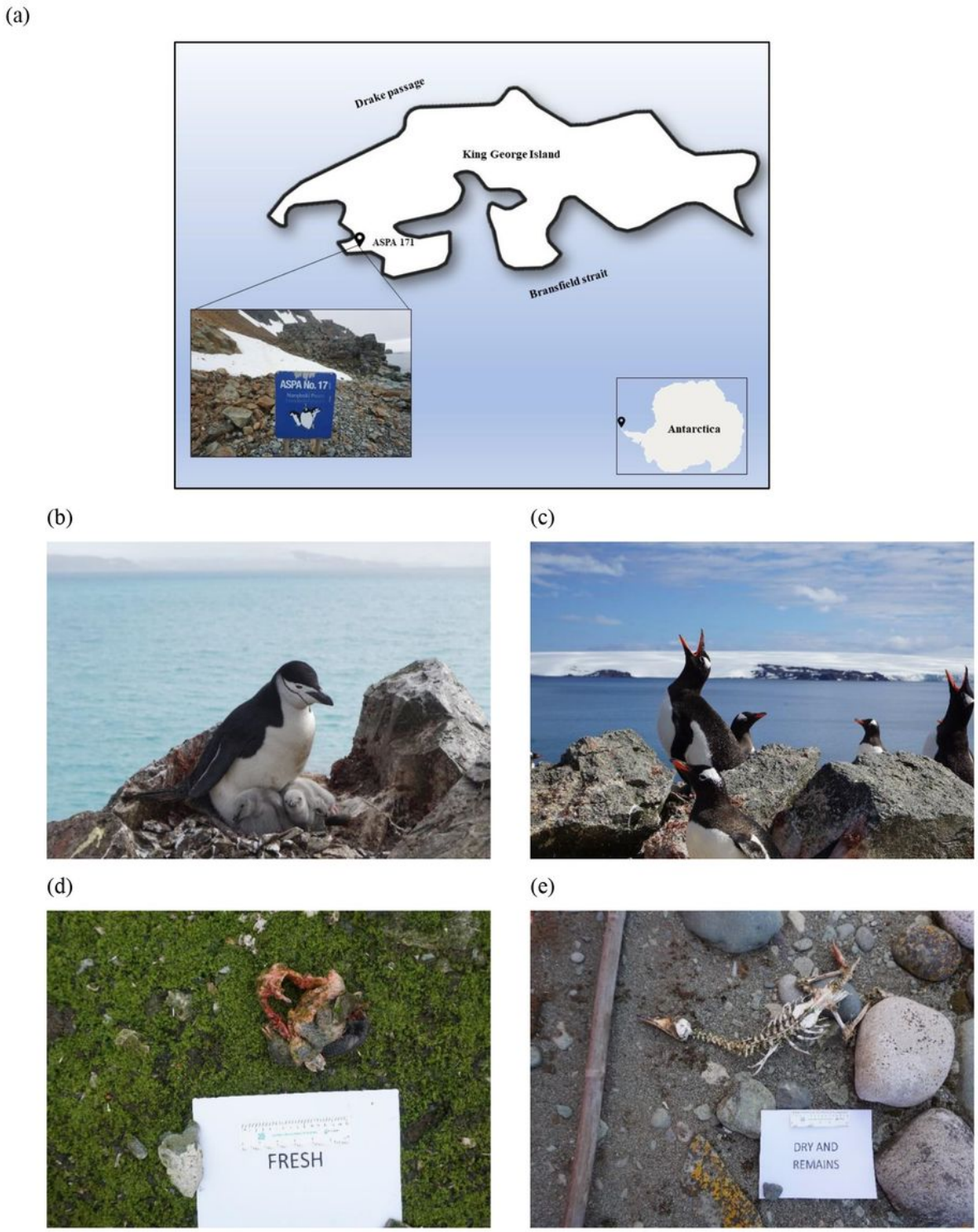

Figure 1

(a) Sample collection was conducted in Antarctic Specially Protected Area (ASPA) 171 on King George Island, Antarctica. The map was generated using QGIS 3.24.3. The Chinstrap (*Pygoscelis antarcticus* (Forster, 1781)) penguin (b) is characterized by black plumage on the top of the head with a white face, while the Gentoo (*Pygoscelis papua* (Forster, 1781) penguin (c) has a bright orange-red bill and white patches above the eyes. The fresh stage (d) of penguin carrion is characterized by freshly killed penguins,

where many soft tissues and internal organs remain intact. In contrast, the dry decay stage (e) is the skeletonization stage of the penguin carrion, where only bones and feathers are left visible.

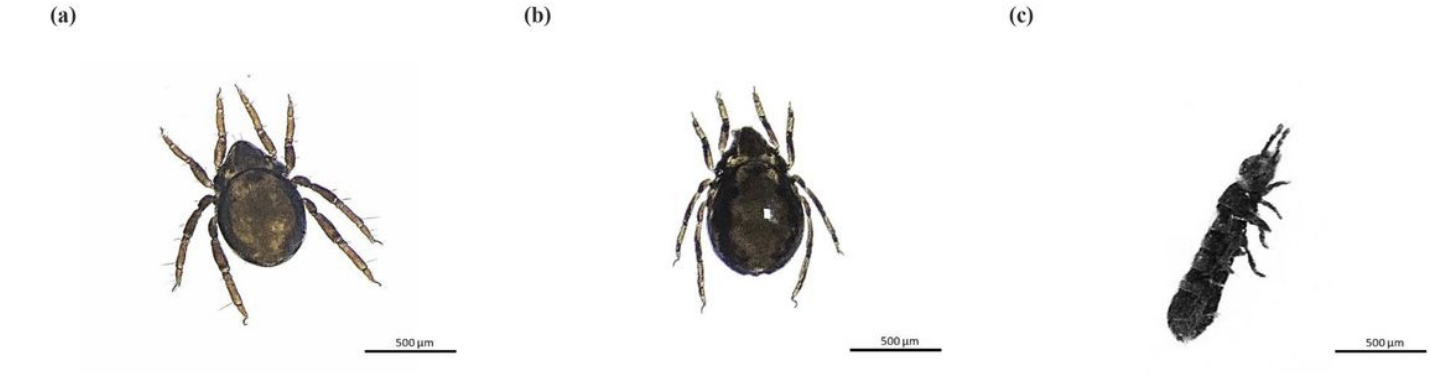

Figure 2

Arthropods isolated from the soil beneath penguin carrion in Antarctica including **(a)** *Alaskozetes antarcticus* mite **(b)** *Halozetes belgicae* mite and, (**c**) *Cryptopygus antarcticus,* an Antarctic collembolan which was found abundantly at the study site.

Figure 3

Nematodes isolated from the soil beneath penguin carrion in Antarctica including **(a)** a Mononchida nematode, a group of predatory nematodes that feed on other soil nematodes, (b) Rhabditida, mainly fungus or bacteria feeders, and **(c)** a free-living feeding juvenile Dorylaimida nematode.

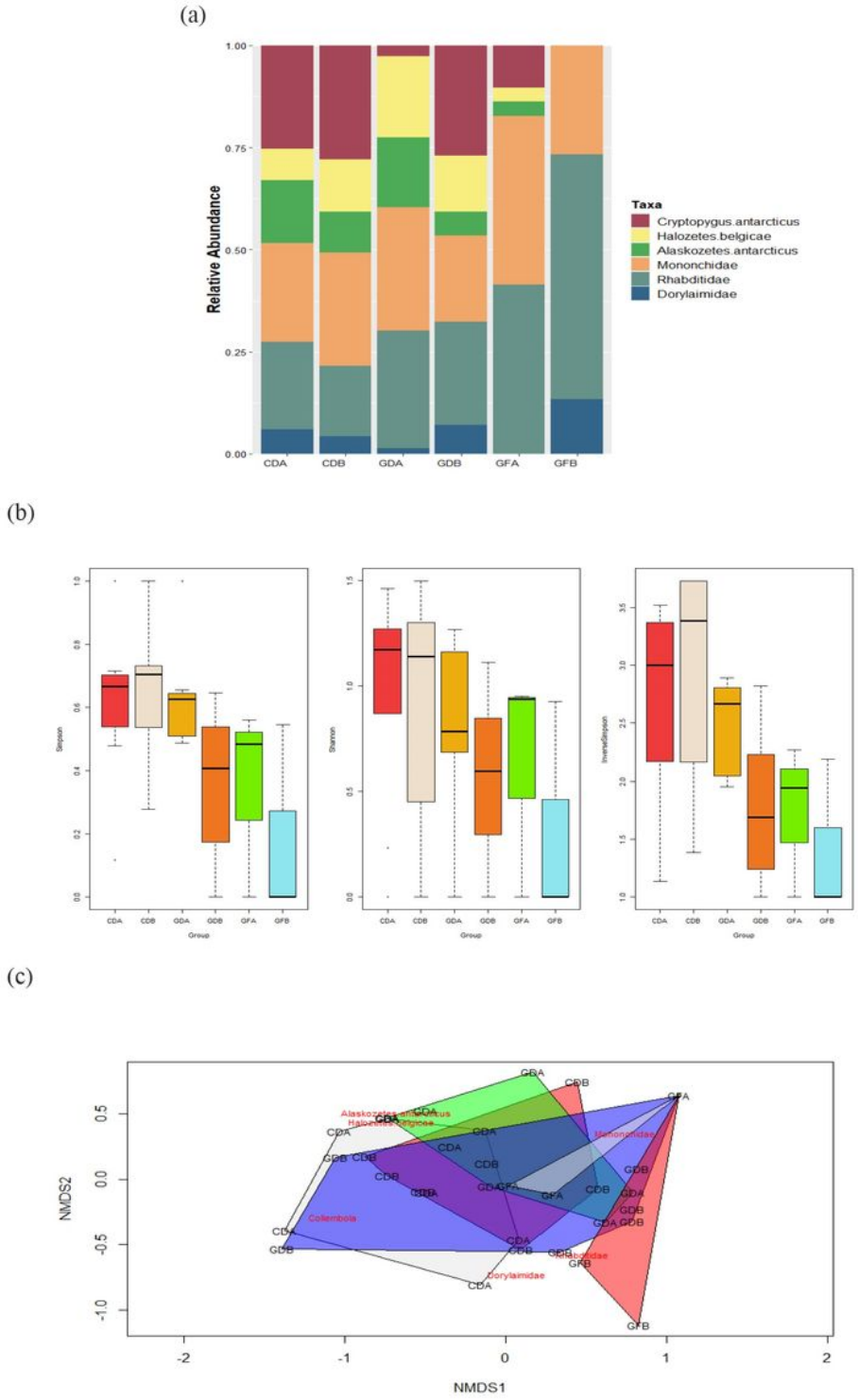

Figure 4

(a) Histograms showing arthropod and nematode mean relative abundance from the soil samples collected from penguin carrion. **(b)** Box plots are clustered according to Shannon–Wiener, Simpson, and inverse Simpson diversity indexes, and **(c)** comparison of arthropods and nematodes composition among groups in non-metric multidimensional scaling (NMDS), stress value: 0.14. Sample abbreviations:

Gentoo dry around (GDA), Gentoo dry beneath (GDB), Gentoo fresh around (GFA), Gentoo fresh beneath (GFB), Chinstrap dry around (CDA), and Chinstrap dry beneath (CDB).

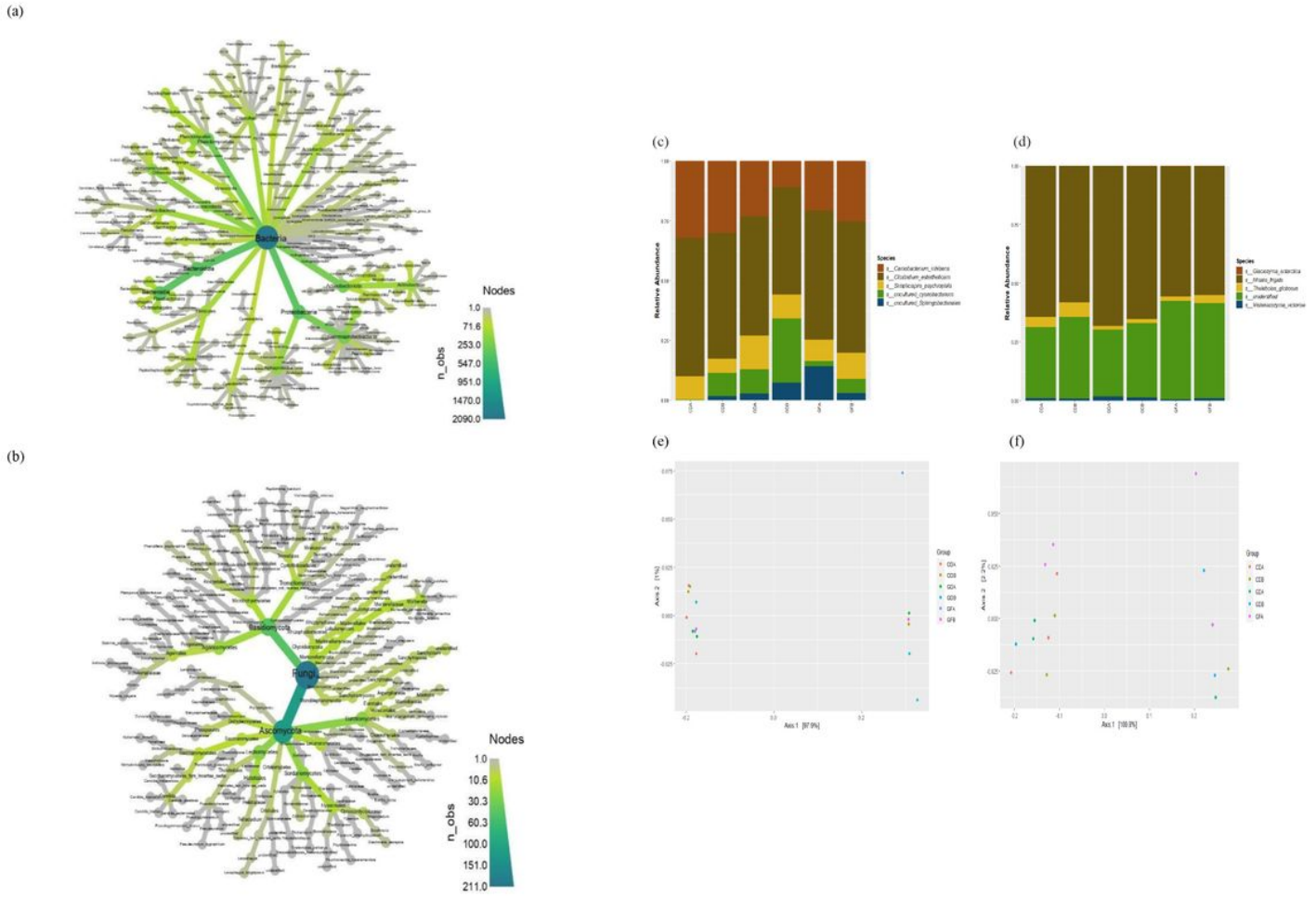

Figure 5

Heat treesshowing overall bacteria **(a)** and fungi **(b)** taxonomic hierarchy from phyla level from the soil collected from penguin carrion in Antarctica. The size and color of nodes and edges represent taxa abundance across all investigated samples. Histograms show the top five most abundant bacteria **(c)**and fungi **(d)** species within groups. The X-axis represents the sample group meanwhile Y-axis the relative abundance. *Clostridium estherticum* and *Mrakia fragida*are the group's most dominant bacteria and fungi, respectively. The ordination plot of the PCoA analysis was conducted on the distances of bacterial **(e)** and fungi **(f)**community composition that samples do not cluster by the group. Each group is represented by a different color: green = Gentoo dry around (GDA), teal = Gentoo dry beneath (GDB), blue = Gentoo fresh around (GFA), pink = Gentoo fresh beneath (GFB), orange = Chinstrap dry around (CDA), and brown = Chinstrap dry beneath (CDB).