**Links between bacteria derived from penguin guts and deposited guano and the surrounding soil microbiota**

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**Abstract:**

Penguins are an important indicator of marine ecosystem health and a major contributor of nutrients to terrestrial ecosystems in Antarctica. Their stomach microbiota is influenced by both the prey consumed and their foraging environment in the sea. As penguins feed at sea and breed on land, they might be expected to transfer microbes (e.g. prey-associated and marine bacteria) as well as nutrients from their stomachs while regurgitating food or in their guano to the surrounding terrestrial environment. However, most research attention to date has focused separately on the penguin gut microbiota (via cloacal/guano samples) and the terrestrial soil microbiota, and any relationship between them has yet to be established. Here we analysed the bacterial communities in stomach regurgitates and cloacal swabs from the same individual birds, freshly deposited guano and rookery soils of two *Pygoscelis* penguins that breed sympatrically on Signy Island (South Orkney Islands, maritime Antarctic) using a high-throughput DNA sequencing method. Our data do not support the hypothesis that bacteria transferred from penguin guts and/or deposited guano make a significant contribution to the communities of the surrounding terrestrial microbial ecosystem. In both penguin species, composition of bacterial communities differed between the four sample types, with Jaccard similarities ranging between 10 and 36%. Assemblages of the dominant and co-occurring bacterial communities in rookery soils were either significantly negatively correlated or not correlated with the three other sample types. Sample-specific communities were also identified in this study, contributing around 63% of the identified diversity overall.

**Keywords:** Antarctic • Bacterial input • Microbial diversity • Terrestrial environment

**Introduction:**

Penguins are an important indicator species of marine ecosystem health in Antarctica, being amongst the top marine consumers (Brooke 2004), with populations strongly influenced by environmental factors (Boersma et al. 2009; Forcada and Trathan 2009; Boersma and Rebstock 2014). In our recent study (Yew et al. 2017), prey-associated and marine bacteria were confirmed to be present in the microbial communities of penguin stomach regurgitates, suggesting an influence of their diet and foraging environment on the gut microbial community.

Penguins are also a key contributor of nutrients to terrestrial ecosystems in Antarctica (Heine and Speir 1989). Guano deposited by penguins fertilises the typically nutrient-poor Antarctic soils by providing the organic materials, such as nitrogen, organic carbon and phosphorus, required to start the development of ornithogenic soils in and around their rookeries (Heine and Speir 1989; Hofstee et al. 2006; Kim et al. 2012; Ball et al. 2015). These nutrient-rich ornithogenic soils are often linked with the succession of a variety of bacteria (Aislabie et al. 2009; Chong et al. 2009; Kim et al. 2012; Ma et al. 2013; Wang et al. 2015) and other microflora (Vidal et al. 2003; Bokhorst et al. 2007; Zwolicki et al. 2015) and microfauna (Raymond et al. 2013; Bokhorst and Convey 2016) in Antarctica. In studies of other bird species, the nest environment has been reported to have significant effects on the success of egg incubation and the gut microbiota of nestlings (Lucas and Heeb 2005; Brandl et al. 2014). The ornithogenic soil environment potentially in turn may also impact the development of the gut microbial community in penguin chicks.

Previously, avian faecal indicator bacteria, such as *Escherichia coli*, *Enterococcus* spp. and *Enterobacter* spp., have been identified in bird-impacted soil (Trawińska et al. 2016), sand (Whitman and Nevers 2003; Staley et al. 2016) and aquatic (Jiang et al. 2007) environments. As penguins feed in the sea and breed on land, the growth of certain bacteria (e.g. prey-associated and marine bacteria) in the stomach may have an influence on penguin gut microbiomes, and further subsequently be input to the soil microbiota either through defecation or during regurgitation of food to chicks. However, although the establishment of gut microbes in the faeces and surrounding soils has been reported previously in mammals (Faedo et al. 2002) and in termites (Otani et al. 2016), no similar reports appear to exist for bird species including penguins, with studies being limited to the interactions between bird gut microbiota and the nest environment (Hird et al. 2014; Goodenough et al. 2016).

In penguins, researchers have used studies of deposited guano or cloacal swabs to make inferences about gut microbiota (Zdanowski et al. 2004; Banks et al. 2009; Dewar et al. 2013, 2014; Barbosa et al. 2016), and also on the correlations between soil physio-chemical properties and microbiota in rookery soils, in order to study potential microbial roles in ornithogenic soil nutrient cycling (Chong et al. 2009; Kim et al. 2012; Ma et al. 2013). But research is yet to address the converse process of transfer of penguin internal gut microbes into their deposited faecal material and hence potentially into the surrounding terrestrial microbial community. Understanding the role of any link between penguin gut microbes and those of the surrounding terrestrial ecosystem is important as there could be multiple impacts, including on the soil nutrient cycling-associated microbes, the penguin chicks, and the surrounding free-living microfloral and microfaunal communities.

The objectives of this study were 1) to compare the bacterial community compositions between stomach regurgitates, cloacal swabs, freshly deposited guano and rookery soils of two *Pygoscelis* penguin species, 2) to correlate assemblage patterns of the dominant and the co-occurring bacterial communities between the four sample types, and 3) to characterise whether and if so which gut/ guano bacterial community members are input to the surrounding soils. As penguins clearly input biogenic material to the surrounding terrestrial environment through regurgitation and defecation, we hypothesised that correlations between the bacterial communities across the four sample types would be present.

**Materials and methods:**

**Sample collection and DNA extraction**

This study was conducted at the Gourlay Peninsula (60°43.586’ S, 45°35.063’ W) breeding colony of Adélie (*Pygoscelis adeliae*) and Chinstrap (*Pygoscelis antarctica*) Penguins on Signy Island, South Orkney Islands (Fig. 1) during the 2013/14 breeding season. A total of six stomach regurgitates, six cloacal swabs, six guano and six rookery soil samples were collected from the two *Pygoscelis* penguins (*n* = 3 for each penguin species) into sterile tubes using tools that were cleaned with 70% ethanol. Penguin stomach regurgitates and cloacal swabs were sampled at the shore close to the breeding bird colonies (i.e. the main route of penguin travel between the sea and the nearby colonies) (Lynnes et al. 2004). Regurgitated ingesta samples of penguins returned from the sea were obtained by stomach flushing (Wilson 1984) following CEMP Standard Methods (CCAMLR 2003), as part of the standard sampling protocol of the long-term monitoring programme of the Convention for the Conservation of Antarctic Marine Living Resources (CCAMLR) Ecosystem Monitoring Programme (CEMP) on Signy Island. On the spot, cloacal swabs were sampled using sterile swabs from the birds whose stomachs had been flushed for the stomach regurgitate sampling, whilst freshly deposited guano of the breeding birds were collected within the nearby colonies. On the same day or not later than a week depending on weather and logistic constraints, rookery soils (2-5 cm depth from the soil surface) were collected within the same colonies of the breeding birds. As soon as possible after returning to the laboratory at the British Antarctic Survey’s Signy Island research station (1-3 h after collection), total DNA was extracted from the stomach regurgitates using the DNeasy® Blood & Tissue Kit (QIAGEN, Hilden, Germany) (Yew et al. 2017), and from the cloacal swabs, guano and rookery soil samples using the Power Soil® DNA Isolation Kit (MoBio Laboratories, Carlsbad, California, USA), following the manufacturers’ instructions.

**16S V4 gene fragment amplification, sequencing and quality filtering**

We employed a high-throughput sequencing approach (Illumina MiSeq) to access the bacterial community composition in the samples studied based on the variable region 4 (V4) of the bacterial 16S rDNA. The use of this high-throughput sequencing approach enabled us to produce a high resolution profile of bacterial community composition (Caporaso et al. 2011; Suenaga 2012) for each sample type, and hence allowed a robust comparative analysis across the samples studied. The V4 region of the 16S rRNA gene, which was widely used to study bacterial and archaeal communities in the Earth Microbiome Project, was amplified using the adapted PCR primers (F515 and R806) and the polymerase chain reaction (PCR) as described by Caporaso et al. (2011). DNA quality was checked using a NanoDrop 2000c (Thermo Scientific, Waltham, Massachusetts, USA) and quantified using a Qubit® 2.0 Fluorometer (Invitrogen, Carlsbad, California, USA). DNA libraries were prepared and performed in the MiSeq system for paired-end runs following the manufacturer’s instructions (Illumina Inc., San Diego, California, USA). The generated sequences were trimmed and demultiplexed using MiSeq Reporter v2.5 (Illumina Inc., San Diego, California, USA). Forward and reverse reads were merged using the make.contigs command in Mothur with default parameters (Kozich et al. 2013). The generated fasta files were uploaded to an open source metagenomics RAST (MG-RAST) server (Meyer et al. 2008) for quality filtering processes and downstream analyses.

**Classification of bacterial community composition in individual samples**

Bacterial species-level classification based on the 16S rDNA short regions is imperfect, due to insufficient separation of closely related species (Janda and Abbott 2007; Tindall et al. 2010), even though a commonly accepted 97% sequence similarity threshold has been used elsewhere (Faith et al. 2013; Nakayama et al. 2013). However, the aim of this study was simply to generate composition profiles of bacterial communities that were present in the samples studied to enable comparison between different sample types, rather than provide a detailed taxonomic assessment of surviving or growing bacteria in each sample. In this study, bacterial community compositions of penguin stomach regurgitates, cloacal swabs, guano and rookery soils were classified at an operational taxonomic unit (OTU) level, with a minimum sequence identity threshold of 99%. OTUs were identified using the BLAST-like alignment tool (BLAT) search to the ribosomal database project (RDP) classifier implemented in the MG-RAST server, with maximum e-value set to 10−5 and minimum alignment length set to 50 nucleotides (Meyer et al. 2008). In order to limit the impact of sequencing errors, OTUs represented by less than three reads (singletons and doubletons) were removed following Andersson et al. (2008) and Lazarevic et al. (2009), and were not considered further.

**Comparative analyses of bacterial communities across the four sample types**

To visualise the overall bacterial community relationships across all 24 individual samples studied, principal coordinate analysis (PCoA) was performed based on the Bray-Curtis distance on normalised OTU annotation data (Meyer et al. 2008).

To test whether there were any significant differences in the mean values of OTU richness among the four types of samples (stomach regurgitates, cloacal swabs, guano and rookery soils) in Adélie and Chinstrap Penguins, the total OTUs annotated (with 3 or more reads) for all samples were checked for normal distribution, prior to one-way analysis of variance (ANOVA) with *post hoc* comparisons using Tukey’s honestly significant difference (HSD) test (IBM SPSS Windows version 19.0, Armonk, New York, USA).

As Illumina MiSeq is a semi-quantitative method (Hirsch et al. 2010), the bacterial community composition in Adélie and Chinstrap Penguin samples was analysed based on presence/absence data of the annotated OTUs. The Jaccard index was used to calculate the percentage of bacterial composition similarity between any two sample types across the four types of samples obtained. A Venn diagram was generated using a web tool (<http://bioinformatics.psb.ugent.be/webtools/Venn/>) to show the proportions of unique and shared OTUs between penguin stomach regurgitates, cloacal swabs, guano and rookery soils.

To examine assemblage patterns of the dominant and the co-occurring bacterial communities between the four sample types, Spearman rank multiple correlation analysis was conducted on the relative abundance of the frequently encountered OTUs (≥0.1%) and the shared OTUs. Any two sample types were considered positively correlated when a statistically significant (*p* < 0.05) Spearman coefficient (*rs*) > 0.19 was obtained (Fowler et al. 1998).

**Nucleotide sequence accession numbers**

Sequences were deposited in MG-RAST (Meyer et al. 2008) with accession numbers listed in Table 1.

**Results:**

**OTU classification and sample coverage**A total of 1,381 and 1,261 bacterial species-level OTUs were annotated from Adélie and Chinstrap Penguins, respectively, with individual samples ranging from 31 to 337 OTUs in Adélie Penguins, and from 31 to 243 in Chinstrap Penguins (Table 1). Besides unclassified bacteria, the annotated OTUs from Adélie Penguins were closely matched to a total of 260 bacterial genera belonging to 17 phyla. In Chinstrap Penguins, a total of 247 bacterial genera were assigned, also belonging to 17 phyla. Following the rare-biosphere threshold proposed by Lynch and Neufeld (2015), approximately 24.2% (6-70 OTUs per sample) of these OTUs in Adélie Penguins, and 32.7% (8-72 OTUs per sample) in Chinstrap Penguins accounted for ≥0.1% of relative abundance, and were considered as the dominant community members. OTUs accounting for <0.1% were considered representative of rare community members. The complete lists of annotated OTUs in individual samples of Adélie and Chinstrap Penguins, along with the abundance of each OTU, are provided in Online Resources 1 and 2.

Good’s coverage (Good 1953) showed that the sampling completeness averaged 99.8% (ranging from 97.8 to 100.0%) (Table 1), confirming that the coverage for all samples studied was sufficient to permit comparative bacterial community analysis.

**Overall bacterial community relationships**

Across all 24 samples obtained from the two penguin species, PCoA (Fig. 2) revealed closer bacterial community relationships between stomach regurgitates and cloacal swabs, and between cloacal swabs and guano. However, lower bacterial community relationships were apparent between rookery soils and the three other sample types, particularly the stomach regurgitates.

**Comparative analyses between the four sample types in Adélie Penguins**

In Adélie Penguins, significant differences were observed in the OTU richness between the four sample types studied (one-way ANOVA, *F*(3,8) = 5.320, *p* = 0.026). *Post hoc* comparisons with Tukey’s HSD (Table 2) showed that the mean values of OTU richness of rookery soils (*X* ± SE = 230 ± 66 OTUs, *n* = 3) was significantly higher (*p* < 0.05) than that of stomach regurgitates (*X* ± SE = 42 ± 6 OTUs, *n* = 3) or cloacal swabs (*X* ± SE = 51 ± 8 OTUs, *n* = 3), but was not significantly higher than guano (*X* ± SE = 138 ± 38 OTUs, *n* = 3). There were no significant differences in the mean values of OTU richness between stomach regurgitates, cloacal swabs and guano.

Excluding unclassified bacteria, paired comparisons of the bacterial community compositions using the Jaccard index (Fig. 3) showed significant differences between the four sample types in Adélie Penguins, with the highest similarity being present between guano and rookery soils (36%) and the lowest similarity between stomach regurgitates and rookery soils (10%). A total of 76, 105, 274 and 384 distinct OTUs were identified in the stomach regurgitates, cloacal swabs, guano and rookery soils, respectively (Fig. 3). Of the 532 distinct OTUs identified, 4.3% were the shared OTUs between the four sample types. Approximately 13% of these shared OTUs were frequently encountered in all four sample types, belonging to the phyla Actinobacteria, Bacteroidetes, Firmicutes, Fusobacteria, Proteobacteria and Tenericutes. The unique OTUs (62% in total) present in stomach regurgitates, cloacal swabs, guano or rookery soils accounted for 3.9%, 4.1%, 16.4% and 37.0%, respectively. About 9.5% of the unique OTUs in stomach regurgitates, 4.5% in cloacal swabs, 6.9% in guano, and 10.2% in rookery soils, belonged to the frequently encountered classification. The unique and the shared OTUs are listed in Online Resource 3.

Spearman rank multiple correlation analysis (Table 3) identified a significant positive correlation in assemblage patterns of the frequently encountered OTUs between Adélie Penguin stomach regurgitates and cloacal swabs (*rs* = 0.297, *n* = 122, *p* = 0.001), but a significant negative correlation between stomach regurgitates and rookery soils (*rs* = -0.275, *n* = 122, *p* = 0.002). No significant correlations were observed between the frequently encountered OTU assemblages of stomach regurgitates and cloacal swabs with guano or rookery soils. In addition, clear differences were apparent at bacterial phylum level (Fig. 4), where a shift in assemblage patterns appeared in the dominant phyla. Community members whose representation increased between stomach regurgitates and rookery soils included Actinobacteria (<0.1 to 1.6%), Bacteroidetes (0.6 to 35.0%), Proteobacteria (5.6 to 11.1%) and unclassified bacteria (0.8 to 28.2%). Firmicutes (65.6 to 22.9%) and Tenericutes (27.0 to 0.1%), in contrast, showed a decrease between stomach regurgitates and rookery soils.

In the assemblage patterns of shared OTUs (Table 3), a significant positive correlation was found between Adélie Penguin stomach regurgitates and cloacal swabs (*rs* = 0.445, *n* = 23, *p* = 0.033). Stomach regurgitates and cloacal swabs showed no significant correlations with guano or rookery soils. Amongst the co-occurring community members (Fig. 5), those which increased in occurrence between stomach regurgitates and rookery soils were closely related to *Psychrobacter* (1.1 to 6.1%) and *Tissierella* (<0.1 to 11.0%). OTUs assigned to *Clostridium* (61.7 to 2.5%) and *Mycoplasma* (30.8 to <0.1%) decreased in frequency between stomach regurgitates and rookery soils. OTUs assigned to *Helicobacter* were relatively abundant in cloacal swabs and guano but not in stomach regurgitates or rookery soils.

**Comparative analyses between the four sample types in Chinstrap Penguins**

In Chinstrap Penguins, again there were significant differences in the OTU richness between the four sample types studied (one-way ANOVA, *F*(3,8) = 7.317, *p* = 0.011). *Post hoc* comparisons with Tukey’s HSD (Table 2) indicated that the mean value of OTU richness of rookery soils (*X* ± SE = 202 ±23 OTUs, *n* = 3) was significantly higher (*p* < 0.05) than those of stomach regurgitates (*X* ± SE = 56 ±3 OTUs, *n* = 3), cloacal swabs (*X* ± SE = 80 ± 41 OTUs, *n* = 3) and guano (*X* ± SE = 83 ± 11 OTUs, *n* = 3). There were no significant differences in the mean values of OTU richness between stomach regurgitates, cloacal swabs and guano.

Excluding unclassified bacteria, Chinstrap Penguins harboured significantly different community compositions in the four sample types. The Jaccard index (Fig. 3) showed the highest similarity was present between cloacal swabs and guano (28%) and the lowest similarity was between stomach regurgitates and rookery soils (13%). A total of 106, 184, 154 and 325 distinct OTUs were identified in the stomach regurgitates, cloacal swabs, guano and rookery soils, respectively (Fig. 3). Approximately 5.3% of the 490 distinct OTUs identified were shared between the four sample types, and 7.7% of these shared OTUs were frequently encountered in all four sample types. Again, these included community members of Actinobacteria, Bacteroidetes, Firmicutes, Fusobacteria, Proteobacteria and Tenericutes. The unique OTUs (64% in total) present in stomach regurgitates, cloacal swabs, guano or rookery soils accounted for 5.7%, 13.5%, 6.5% and 38.0%, respectively. About 25% of the unique OTUs in stomach regurgitates, 12.1% in cloacal swabs, 15.6% in guano, and 5.9% in rookery soils were frequently encountered. The unique and the shared OTUs are listed inOnline resource 4.

Spearman rank multiple correlation analysis (Table 3) identified significant negative correlations in assemblage patterns of the frequently encountered OTUs between Chinstrap Penguin rookery soils and stomach regurgitates (*rs* = -0.434, *n* = 119, *p* < 0.0001) or cloacal swabs (*rs* = -0.399, *n* = 119, *p* < 0.0001). No significant correlations were detected in the frequently encountered OTU assemblages between stomach regurgitates, cloacal swabs and guano. Community members whose representation increased between stomach regurgitates and rookery soils included the phyla Actinobacteria (<0.1 to 1.7%), Bacteroidetes (0.7 to 56.3%) and unclassified bacteria (10.5 to 17.6%) (Fig. 4). Proteobacteria (20.2 to 14.9%) and Tenericutes (58.5 to <0.1%) showed a decrease between stomach regurgitates and rookery soils.

In the assemblage patterns of shared OTUs (Table 3), Chinstrap Penguin cloacal swabs showed a significant positive correlation with stomach regurgitates (*rs* = 0.497, *n* = 26, *p* = 0.010), and a significant negative correlation with rookery soils (*rs* = -0.399, *n* = 26, *p* = 0.043). No significant correlations were detected between stomach regurgitates and guano or rookery soils. Amongst the co-occurring community members (Fig. 5), OTUs closely related to *Flavobacterium* (<0.1 to 9.1%) and *Tissierella* (<0.1 to 5.6%) showed an increase between Chinstrap Penguin stomach regurgitates and rookery soils. OTUs assigned to *Clostridium* (7.2 to 0.9%) and *Mycoplasma* (67.9 to <0.1%) decreased in occurrence between stomach regurgitates and rookery soils. OTUs assigned to *Fusobacterium* were relatively abundant in cloacal swabs and guano but not in stomach regurgitates or rookery soils.

**Discussion:**

Both *Pygoscelis* penguins examined here revealed similar results, despite potential inter- and intra-specific variations in the microbiota of the samples studied between the two penguin species (Yew et al. 2017). Our data showed that the bacterial communities in penguin stomach regurgitates, cloacal swabs, freshly deposited guano and rookery soils were significantly different in terms of the OTU richness, community composition, and assemblage patterns of the dominant and the co-occurring community members. In addition, while Actinobacteria, Bacteroidetes, Firmicutes, Fusobacteria, Proteobacteria and Tenericutes were frequently found in all four sample types, the representation of these phyla shifted across the different sample types. Although interactions between avian gut microbiota and the nest environments have previously been reported (Lucas and Heeb 2005; Goodenough et al. 2016; Otani et al. 2016), despite the detection of avian faecal-indicator bacteria (e.g. *Escherichia coli*, *Enterococcus* and *Enterobacter*) in bird-impacted areas (Whitman and Nevers 2003; Jiang et al. 2007; Staley et al. 2016; Trawińska et al. 2016), there appear to be no published comparative analyses between the microbiota of guts, faeces and the habitat soils of birds (including penguins). Previously, in studies of a very different taxonomic group, detailed comparative investigations between the microbiota of gut, deposited materials and surrounding soils have been reported in termites (Fall et al. 2007; Makonde et al. 2015; Manjula et al. 2016). While different methods were used to generate the data, these studies reported similar findings to those obtained here, with significant differences being observed in the bacterial communities obtained from termite guts, mounds and the surrounding soils. These studies also reported a frequency shift in co-occurring bacterial phyla between termite guts and the surrounding soils. Our main finding is consistent with these comparative investigations on termites, although in detail the bacterial community compositions (particularly in terms of genera) identified in penguin samples were different from those of termites.

Rookery soils of both *Pygoscelis* penguins harboured the richest bacterial communities, followed by guano, cloacal swabs and stomach regurgitates. This is also in accordance with the termite study reported by Makonde et al. (2015), where termite-associated soils had higher bacterial diversity than termite guts. Rookery soil bacterial phyla composition in this study was similar to previously described rookery soils on Signy Island (Chong et al. 2009) and other locations in Antarctica (Kim et al. 2012; Bottos et al. 2014; Rampelotto et al. 2014), with the predominant community members being Actinobacteria, Aquificae, Bacteroidetes, Firmicutes, Fusobacteria, Gemmatimonadetes, Planctomycetes, Proteobacteria, Spirochaetes, Tenericutes and Verrucomicrobia. Although 78% of the assigned bacterial phyla in total that were present in stomach regurgitates, cloacal swabs and/or guano were also present in rookery soils, the bacterial OTU composition of rookery soils was clearly different from stomach regurgitates, cloacal swabs and guano (Jaccard similarities ranged between 10 and 36%). The dominant and the co-occurring community assemblages of rookery soils were either significantly negatively correlated or not correlated with the three other sample types. Together, these data suggest that there is very low establishment probability of microbes from penguin internal gut and/or deposited guano into the surrounding terrestrial microbial ecosystem, where these microbes might possibly be constrained by the differences in physicochemical properties and nutrient availabilities between the two distinct environments.

Amongst the co-occurring bacterial community members between rookery soils and of stomach regurgitates, cloacal swabs, and/or deposited guano, about 12% belonged to previously identified groups of Antarctic marine origins (MacCormack and Fraile 1990; Zdanowski and Donachie 1993; Bowman et al. 1997; Staley and Gosink 1999; Purdy et al. 2003; Dickinson et al. 2016). These included OTUs closely related to the genera *Aeromonas*, *Arthrobacter*, *Brachybacterium*, *Corynebacterium*, *Cytophaga*, *Desulfobacterium*, *Desulfobulbus*, *Leeuwenhoekiella*, *Marinobacter*, *Oceanimonas*, *Octadecabacter*, *Planococcus*, *Polaribacter*, *Psychrobacter*, *Psychroflexus*, *Sphingobacterium*, *Sphingomonas* and *Xanthomonas*. OTUs annotated to Antarctic fish and krill-associated bacteria (Kelly et al. 1978; MacCormack and Fraile 1990; Ward et al. 2009) accounted for 5% of diversity overall, included the genera *Acinetobacter*, *Bacillus*, *Flavobacterium*, *Micrococcus*, *Mycoplasma*, *Nocardioides*, *Pseudomonas* and *Staphylococcus*. Approximately 9% of the OTUs were assigned to bird/penguin host-associated bacteria (Soucek and Mushin 1970; Barbosa and Palacios 2009; Dewar et al. 2013; Trawińska et al. 2016), belonging to the genera *Campylobacter*, *Clostridium*, *Edwardsiella*, *Enterococcus*, *Erysipelothrix*, *Eubacterium*, *Helicobacter*, *Lactobacillus*, *Plesiomonas*, *Sporosarcina*, *Streptobacillus*, *Streptococcus* and *Veillonella*. These findings indicate that, besides penguin host-associated bacteria, the establishment of transferred prey-associated and marine bacteria from penguins to the surrounding soils during regurgitation and defecation does occur, even though the establishment probability is low. As a majority of these bacterial groups have been reported to degrade and/or produce organic (Bowman et al. 1997; Wong et al. 2000; Mancuso Nichols et al. 2005; Andreína Pacheco et al. 2008, Yau et al. 2013) and/or inorganic (Kelly et al. 1978; Zdanowski and Donachie 1993; Bowman et al. 1997; Purdy et al. 2003; Yau et al. 2013) matter, thus they may potentially play a role in nutrient cycles and transfer processes (Schime and Schaeffer 2012; Cavicchioli 2015) between Antarctic marine and terrestrial environments.

Guano freshly deposited by both *Pygoscelis* penguins had predominant community members including Actinobacteria, Bacteroidetes, Firmicutes, Fusobacteria, Proteobacteria and Tenericutes. This is similar to the bacterial phyla composition in previously reported penguin faecal samples (Banks et al. 2009; Dewar et al. 2013, 2014). Besides rookery soils, freshly deposited penguin guano also showed significant differences in bacterial community composition from those of stomach regurgitates (Jaccard similarity = 14 and 26% in Adélie and Chinstrap Penguins, respectively) and cloacal swabs (22 and 28%, respectively). No significant correlations in the dominant and co-occurring community assemblages were observed between deposited guano and stomach regurgitates or cloacal swabs. In a recent study, chicken faecal and caecal microbiota were reported to be qualitatively similar but quantitatively different (Stanley et al. 2015). Although, previously, faecal samples have been widely used as an indicator for the microbial study of the gastrointestinal tract of mammals, our data suggest that, in penguins in particular, deposited guano does not provide a suitable proxy for the study of the stomach microbiota. Similar findings have previously been reported in studies of other bird species (Wilkinson et al. 2016) and of humans (Durbán et al. 2011), where it was suggested that faecal microbiota are not closely representative of gut microbiota.

While data generated using MiSeq do not provide a robust quantitative assessment of community composition at specific level (Hirsch et al. 2010), clear shifts in the assemblages of co-occurring bacteria were observed across the sample types. For instance, in both Adélie and Chinstrap Penguins, the co-occurring OTUs that were assigned to *Clostridium* and *Mycoplasma* decreased in proportional representation from stomach regurgitates to cloacal swabs/guano and rookery soils. These bacteria are commonly found in homeothermic animal hosts, such as birds and mammals (Craven et al. 2001; Elfaki et al. 2002; Aldape et al. 2006). Penguin stomachs are warm (38°C) (Thouzeau et al. 2003) while, at Signy Island, daily soil temperatures measured across a number of locations ranged between -8 and 20°C throughout the year (Davey et al. 1992). Previously, Zdanowski et al. (2005) proposed that temperature is the most important physical factor influencing bacterial transformations during penguin guano decomposition. Factors such as the changes of temperature between the sampled environments might underlie the decrease in co-occurring bacteria across the three other sample types, and limit the establishment of stomach-specific bacteria in the surrounding soils.

In our study, sample-specific bacterial communities accounted for 62 and 64% of the overall identified community members in Adélie and Chinstrap Penguins, respectively. Previously, in a comparative analysis of termite guts and the associated surrounding soils, Manjula et al. (2016) suggested that such differences in microbial community composition and structure may be due to the roles of different microbes in each environment. The potential functions of distinct bacterial groups identified in penguin guts (Soucek and Mushin 1970; Barbosa and Palacios 2009; Dewar et al. 2013), deposited guano (Banks et al. 2009; Dewar et al. 2014) and rookery soils (Chong et al. 2009; Kim et al. 2012; Bottos et al. 2014; Rampelotto et al. 2014) have been inferred previously. In the current study, the main functions of these sample-specific bacteria remain unknown, as the sequencing approach used does not provide reliable species-level information. Further research is required to isolate these sample-specific bacteria for functional gene studies in order to understand their roles in the environment.

**Conclusions:**

Our study revealed clear differences in the bacterial communities of stomach regurgitates, cloacal swabs, freshly deposited guano and rookery soils of two *Pygoscelis* penguins that breed sympatrically on Signy Island. More than half the OTUs identified were sample-specific, suggesting that input/survival of microbes from penguin internal gut and/or deposited materials into the surrounding terrestrial microbial ecosystem might not provide a significant *in situ* contribution to terrestrial soil microbial community composition.

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**References:**

Aislabie J, Jordan S, Ayton J, Klassen JL, Barker GM, Turner S (2009) Bacterial diversity associated with ornithogenic soil of the Ross Sea region Antarctica. Can J Microbiol 55: 21-36

Aldape MJ, Bryant AE, Stevens DL (2006) *Clostridium sordellii* infection: Epidemiology clinical findings and current perspectives on diagnosis and treatment. Clin Infect Dis 43: 1436-1446

Andersson AF, Lindberg M, Jakobsson H, Bäckhed F, Nyrén P, Engstrand L (2008) Comparative analysis of human gut microbiota by barcoded pyrosequencing. PLoS ONE 3: e2836. doi: 10.1371/journal.pone.0002836

Andreína Pacheco M, Lentino M, Mata C, Barreto S, Araque M (2008) Microflora in the crop of adult dusky-billed parrotlets (*Forpus Modestus*). J Ornithol 149: 621-628

Ball BA, Tellez CR, Virginia RA (2015) Penguin activity influences soil biogeochemistry and soil respiration in rookeries on Ross Island Antarctica. Polar Biol 38: 1357-1368

Banks JC, Craig S, Cary I, Hogg D (2009) The phylogeography of Adélie Penguin faecal flora. Environ Microbiol 11: 577-588

Barbosa A, Palacios MJ (2009) Health of Antarctic birds: A review of their parasites pathogens and diseases. Polar Biol 32: 1095-1115

Barbosa A, Balagué V, Valera F, Martínez A, Benzal J, Motas M, Diaz JI, Mira A, Pedrós-Alió C (2016) Age-Related differences in the gastrointestinal microbiota of Chinstrap Penguins (*Pygoscelis antarctica*). PLoS ONE 11: e0153215. doi: 10.1371/journal.pone.0153215

Boersma PD, Rebstock GA, Frere E, Moore SE (2009) Following the fish: Penguins and productivity in the South Atlantic. Ecol Monogr 79: 59-76

Boersma PD, Rebstock GA (2014) Climate change increases reproductive failure in Magellanic Penguins. PLoS ONE 9: e85602. doi: 10.1371/journal.pone.0085602

Bokhorst S, Huiskes A, Convey P, Aerts R (2007) External nutrient inputs into terrestrial ecosystems of the Falkland Islands and the Maritime Antarctic. Polar Biol 30: 1315-1321

Bokhorst S, Convey P (2016) Impact of marine vertebrates on Antarctic terrestrial microarthropods. Antarct Sci 28: 175-186

Bottos EM, Scarrow JW, Archer SDJ, McDonald IR, Cary SC (2014) Bacterial community structures of Antarctic soils. In: Cowan DA (ed) Antarctic Terrestrial Microbiology, Springer, Berlin, pp 9-33

Bowman JP, Nichols DS, McMeekin TA (1997) *Psychrobacter glacincola* sp. nov a halotolerant psychrophilic bacterium isolated from Antarctic sea ice. Syst Appl Microbiol 20: 209-215

Brandl HB, van Dongen WFD, Darolová A, Krištofík J, Majtan J, Hoi H (2014) Composition of bacterial assemblages in different components of reed warbler nests and a possible role of egg incubation in pathogen regulation. PLoS ONE 12: e114861. doi: 10.1371/journal.pone.0114861

Brooke MD (2004) The food consumption of the world’s seabirds. P Roy Soc B 271: 246-248

Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Lozupone CA, Turnbaugh PA, Fierer N, Knight R (2011) Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. P Natl Acad Sci 108: 1-7

Cavicchioli R (2015) Microbial ecology of Antarctic aquatic systems. Nat Rev Microbiol 13: 691-706

CCAMLR (2003) CEMP Standard Methods. CCAMLR, Hobart

Chong CW, Dunn MJ, Convey P, Tan GYA, Wong RCS, Tan IKP (2009) Environment influences on bacterial diversity of soils on Signy Island maritime Antarctic. Polar Biol 32: 1571-1582

Craven SE, Stern NJ, Bailey JS, Cox NA (2001) Incidence of *Clostridium perfringens* in broiler chickens and their environment during production and processing. Avian Dis 45: 887-896

Davey MC, Pickup J, Block W (1992) Temperature variation and its biological significance in fellfield habitats on a maritime Antarctic island. Antarct Sci 4: 383-388

Dewar ML, Arnould JPY, Dann P, Trathan P, Groscolas R, Smith S (2013) Interspecific variations in the gastrointestinal microbiota in penguins. Microbiol Open 2: 195-204

Dewar ML, Arnould JP, Krause L, Trathan P, Dann P, Smith SC (2014) Influence of fasting during moult on the faecal microbiota of penguins. PLoSONE 9: e99996. doi: 10.1371/journal.pone.0099996

Dickinson I, Goodall-Copestake W, Thorne MAS, Schlitt T, Ávila-Jiménez ML, Pearce DA (2016) Extremophiles in an Antarctic marine ecosystem. Microorganisms 4: 8. doi: 10.3390/microorganisms4010008

Durbán A, Abellán JJ, Jiménez-Hernández N, Ponce M, Ponce J, Sala T, D’Auria G, Latorre A, Moya A (2011) Assessing gut microbial diversity from feces and rectal mucosa. Microbial Ecol 61: 123-133

Elfaki MG, Abas B, Mahmoud OM, Kleven SH (2002) Isolation and characterization of *Mycoplasma arginini* from camels (*Camelus dromedaries*) pneumonia. Comp Immunol Microb 25: 49-57

Faedo M, Larsen M, Dimander SO, Yeates GW, Höglund J, Waller PJ (2002) Growth of the fungus *Duddingtonia flagrans* in soil surrounding feces deposited by cattle or sheep fed the fungus to control nematode parasites. Biol Control 23: 64-70

Faith JJ, Guruge JL, Charbonneau M, Subramanian S, Seedorf H, Goodman AL, Clemente JC, Knight R, Heath AC, Leibel RL, Rosenbaum M, Gordon JI (2013) The long-term stability of the human gut microbiota. Science 341: 1237439

Fall S, Hamelin J, Ndiaye F, Assigbetse K, Aragno M, Chotte JL, Brauman A (2007) Differences between bacterial communities in the gut of a soil-feeding termite (*Cubitermes niokolo ensis*) and its mounds. Appl Environ Microbiol 73: 5199-5208

Forcada J, Trathan PN (2009) Penguin responses to climate change in the Southern Ocean. Glob Change Biol 15: 1618-1630

Fowler J, Cohen L, Jarvis P (1998) Practical statistics for field biology. John Wiley and Sons, Chichester

Good IJ (1953) The population frequencies of species and the estimation of population parameters. Biometrika 40: 237-264

Goodenough AE, Stallwood B, Dandy S, Nicholson TE, Stubbs H, Coker DG (2016) Like mother like nest: similarity in microbial communities of adult female Pied Flycatchers and their nests. J Ornithol. doi: 101007/s10336-016-1371-1

Heine JC, Speir TW (1989) Ornithogenic soils of the cape bird Adélie Penguin rookeries Antarctica. Polar Biol 10: 89-99

Hird SM, Carstens BC, Cardiff SW, Dittmann DL, Brumfield RT (2014) Sampling locality is more detectable than taxonomy or ecology in the gut microbiota of the brood-parasitic Brown-headed Cowbird (*Molothrus ater*). PeerJ 2: e321. doi: 10.7717/peerj.321

Hirsch PR, Mauchline TH, Clark IM (2010) Culture-independent molecular techniques for soil microbial ecology. Soil Biol Biochem 42: 878-887

Hofstee EH, Balks MR, Petchey F, Campbell DI (2006) Soils of Seabee Hook Cape Hallett northern Victoria Land Antarctica. Antarct Sci 18: 473-486

Janda JM, Abbott SL (2007) 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: Pluses perils and pitfalls. J Clin Microbiol 45: 2761-2764

Jiang SC, Chu W, Olson BH, He JW, Choi S, Zhang J, Le JY, Gedalanga PB (2007) Microbial source tracking in a small southern California urban watershed indicates wild animals and growth as the source of fecal bacteria. Appl Microbiol Biot 76: 927-934

Kelly MD, Lukaschewsky S, Anderson CG (1978) Bacterial flora of Antarctic krill (*Euphasia superba*) and some of their enzymatic properties. J Food Sci 43: 1196-1197

Kim OS, Chae N, Lim HS, Cho A, Kim JH, Hong SG, Oh J (2012) Bacterial diversity in ornithogenic soils compared to mineral soils on King George Island Antarctica. J Microbiol 50: 1081-1085

Kozich JJ, Westcott SL, Baxter NT, Highlander SK, Schloss PD (2013) Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. Appl Environ Microbiol 79: 5112-5120

Lazarevic V, Whiteson K, Huse S, Hernandez D, Farinelli L, Østerås M, Schrenzel J, François P (2009) Metagenomic study of the oral microbiota by Illumina high-throughput sequencing. J Microbiol Meth 79: 266-271

Lucas FS, Heeb P (2005) Environmental factors shape cloacal bacterial assemblages in great tit *Parus major* and blue tit *P caeruleus* nestlings. J Avian Biol 36: 510-516

Lynch MD, Neufeld JD (2015) Ecology and exploration of the rare biosphere. Nat Rev Microbiol 13: 217-229

Lynnes AS, Reid EK, Croxall JP (2004) Diet and reproductive success of Adélie and Chinstrap Penguins: linking response of predators to prey population dynamics. Polar Biol 27:544–554

Ma D, Zhu R, Ding W, Shen C, Chu H, Lin X (2013) Ex-situ enzyme activity and bacterial community diversity through soil depth profiles in penguin and seal colonies on Vestfold Hills East Antarctica. Polar Biol 36: 1347-1361

MacCormack WP, Fraile ER (1990) Bacterial flora of newly caught Antarctic fish *Notothenia neglecta*. Polar Biol 10: 413-417

Makonde HM, Mwirichia R, Osiemo Z, Boga HI, Klenk HN (2015) 454 Pyrosequencing based assessment of bacterial diversity and community structure in termite guts mounds and surrounding soils. Springer Plus 4: 471. doi: 10.1186/s40064-015-1262-6

Mancuso Nichols CA, Guezennec J, Bowman JP (2005) Bacterial exopolysaccharides from extreme marine environments with special consideration of the Southern Ocean sea ice and deep-sea hydrothermal vents: A review. Mar Biotechnol 7: 253-271

Manjula A, Pushpanathan M, Sathyavathi S, Gunasekaran P, Rajendhran J (2016) Comparative analysis of microbial diversity in termite gut and termite nest using Ion sequencing. Curr Microbiol 72: 267-275

Meyer F, Paarmann D, D'Souza M, Olson R, Glass EM, Kubal M, Paczian T, Rodriguez A, Stevens R, Wilke A, Wilkening J, Edwards RA (2008) The MG-RAST server: A public resource for the automatic phylogenetic and functional analysis of metagenomes. BMC Bioinformatics 9: 386. doi: 10.1186/1471-2105-9-386

Nakayama J, Jiang J, Watanabe K, Chen K, Ninxin H, Matsuda K, Kurakawa T, Tsuji H, Sonomoto K, Lee YK (2013) Up to species-level community analysis of human gut microbiota by 16S rRNA amplicon pyrosequencing. Bioscience of Microbiota Food and Health 32: 69-76

Otani S, Hansen LH, Sørensen SJ, Poulsen M (2016) Bacterial communities in termite fungus combs are comprised of consistent gut deposits and contributions from the environment Microbial Ecol 71: 207-220

Purdy KJ, Nedwell DB, Embley TM (2003) Analysis of the sulfate-reducing bacterial and methanogenic archaeal populations in contrasting Antarctic sediments. Appl Environ Microbiol 69: 3181-3191

Rampelotto PH, Barboza ADM, Pereira AB, Triplett EW, Schaefer CEGR, Camargo FAO, Roesch LFW (2014) Distribution and interaction patterns of bacterial communities in an ornithogenic soil of Seymour Island Antarctica. Microbial Ecol 69: 684-694

Raymond MR, Wharton DA, Marshall CJ (2013) Factors determining nematode distributions at Cape Hallett and Gondwana station Antarctica. Antarct Sci 25: 347-357

Schime JP, Schaeffer SM (2012) Microbial control over carbon cycling in soil. Front Microbiol 3: 348

Soucek Z, Mushin R (1970) Gastrointestinal bacteria of certain Antarctic birds and mammals. Appl Microbiol 20: 561-566

Staley JT, Gosink JJ (1999) Poles apart: Biodiversity and biogeography of sea ice bacteria. Annu Rev Microbiol 53: 189-215

Staley ZR, Robinson C, Edge TA (2016) Comparison of the occurrence and survival of fecal indicator bacteria in recreational sand between urban beach playground and sandbox settings in Toronto Ontario. Sci Total Environ 541: 520-527

Stanley D, Geier MS, Chen H, Hughes RJ, Moore RJ (2015) Comparison of fecal and cecal microbiotas reveals qualitative similarities but quantitative differences. BMC Microbiology 15: 51. doi: 10.1186/s12866-015-0388-6

Suenaga H (2012) Targeted metagenomics: a high-resolution metagenomics approach for specific gene clusters in complex microbial communities. Environ Microbiol 14: 13-22

Thouzeau C, Froget G, Monteil H, LeMaho Y, Harf-Monteil C (2003) Evidence of stress in bacteria associated with long-term preservation of food in the stomach of incubating King Penguins (*Aptenodytes patagonicus*). Polar Biol 26: 115-123

Tindall BJ, Rosselló-Móra R, Busse HJ, Ludwig W, Kämpfer P (2010) Notes on the characterization of prokaryote strains for taxonomic purposes. Int J Syst Evol Micr 60: 249-266

Trawińska B, Chmielowiec-Korzeniowska A, Nowakowicz-Dębek B, Tymczyna L, Bombik T, Pyrz M, Tymczyna-Sobotka M (2016) Evaluation of microbial contamination of feces and soil on a laying-hen farm depending on sampling site and season. Revista Brasileira de Zootecnia 45: 190-194

Vidal E, Jouventin P, Frenot Y (2003) Contribution of alien and indigenous species to plant-community assemblages near penguin rookeries at Crozet archipelago. Polar Biol 26: 432-437

Wang NF, Zhang T, Zhang F, Wang ET, He JF, Ding H, Zhang BT, Liu J, Ran XB, Zang JY (2015) Diversity and structure of soil bacterial communities in the Fildes Region (maritime Antarctica) as revealed by 454 pyrosequencing. Front Microbiol 6: 1188. doi: 10.3389/fmicb.2015.01188

Ward NL, Steven B, Penn K, Methé BA, Detrich WH (2009) Characterization of the intestinal microbiota of two Antarctic notothenioid fish species. Extremophiles 13: 679-685

Whitman RL, Nevers MB (2003) Foreshore sand as a source of *Escherichia coli* in nearshore water of a Lake Michigan Beach. Appl Environ Microbiol 69: 5555-5562

Wilkinson N, Hughes RJ, Aspden WJ, Chapman J, Moore RJ, Stanley D (2016) The gastrointestinal tract microbiota of the Japanese quail, *Coturnix japonica*. Appl Microbiol Biotechnol 100: 4201-4209

Wilson RP (1984) An improved stomach pump for penguins and other seabirds. J Field Ornithol 55: 109-112

Wong TY, Preston LA, Schiller NL (2000) Alginate lyase: Review of major sources and enzyme characteristics structure-function analysis biological roles and applications. Annu Rev Microbiol 54: 289-340

Yau S, Lauro FM, Williams TJ, DeMaere MZ, Brown MV, Rich J, Gibson JAE, Cavicchioli R (2013) Metagenomic insights into strategies of carbon conservation and unusual sulphur biogeochemistry in a hypersaline Antarctic lake. Int Soc Microbial Ecol 7: 1944-1961

Yew WC, Pearce DA, Dunn MJ, Samah AA, Convey P (2017) Bacterial community composition in Adélie (*Pygoscelis adeliae*) and Chinstrap (*Pygoscelis antarctica*) Penguin stomach contents from Signy Island, South Orkney Islands. Polar Biology. doi: 10.1007/s00300-017-2162-8

Zdanowski MK, Donachie SP (1993) Bacteria in the sea-ice zone between Elephant Island and the South Orkneys during the Polish sea-ice zone expedition (December 1988 to January 1989). Polar Biol 13: 245-254

Zdanowski MK, Weglenski P, Golik P, Sasin JM, Borsuk P, Zmuda MJ, Stankovic A (2004) Bacterial diversity in Adélie Penguin *Pygoscelis adeliae* guano: Molecular and morpho-physiological approaches. FEMS Microbiol Ecol 50: 163-173

Zdanowski MK, Zmuda MJ, Zwolska I (2005) Bacterial role in the decomposition of marine-derived material (penguin guano) in the terrestrial maritime Antarctic. Soil Biol Biochem 37: 581-595

Zwolicki A, Barcikowski M, Barcikowski A, Cymerski M, Stempniewicz L, Convey P (2015) Seabird colony effects on soil properties and vegetation zonation patterns on King George Island Maritime Antarctic. Polar Biol 38: 1645-1655

**Figure captions**

**Fig. 1** The locations of **a** South Orkney Islands in the maritime Antarctic, **b** Signy Island within the South Orkney Island archipelago, and **c** Gourlay Peninsula on Signy Island. Map provided by Laura Gerrish, Mapping and Geographic Information Centre, British Antarctic Survey.

**Fig. 2** Principal coordinates analysis (PCoA) of individual samples studied using Bray-Curtis distance on normalised OTU annotation data.

**Fig. 3** Venn diagram showed the proportion of unique and shared OTUs between stomach regurgitates (S), cloacal swabs (CS), guano (G) and rookery soils (RS) of Adélie (A) and Chinstrap (C) Penguins. The numbers (in brackets) outside Venn diagram indicate total OTUs found in each type of sample. The numbers inside Venn diagram indicate unique and shared OTUs between the four sample types. Bacterial community composition similarity was calculated using the Jaccard index.

**Fig. 4** Assemblage patterns of the frequently encountered (≥0.1%) bacterial phyla in Adélie (A) and Chinstrap (C) Penguin stomach regurgitates (S), cloacal swabs (CS), guano (G) and rookery soils (RS).

**Fig. 5** Assemblage patterns of the co-occurring bacterial genera community members in Adélie (A) and Chinstrap (C) Penguin stomach regurgitates (S), cloacal swabs (CS), guano (G) and rookery soils (RS). Only bacterial genera with relative abundance ≥1% in any sample typs are shown.