Soil Microbiome Diversity on Sable Island, Nova Scotia

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Abstract

The soil microbiome is essential for ecosystem functioning and influences both above- and below-ground communities. Sable Island is a sand bar with a large feral horse population, which has been shown to affect marram grasslands more than heathlands. Feral horses can have negative effects on soil microbiomes by reducing microbial diversity and abundance, but there has been no previous research on soil microbes on Sable Island. Determining the microbial composition and the driving factors of diversity is crucial for effective ecosystem management. Our goal was to determine differences in microbial α -diversity and β -diversity across vegetation types, soil pH, organic matter, and phosphorous. This study also serves as an overall survey of bacterial and fungal diversity. We expected microbial α -diversity to be lower in marram grasslands than in heathlands, and the microbial β-diversity to differ between vegetation types. Using eDNA from soil samples, we identified bacteria with 16S and fungi with ITS2 DNA barcodes and performed soil analyses from 80 samples from 16 sites on the island. We identified a total of 812 bacterial and 336 fungal ASVs. Our results indicate that bacterial α -diversity was significantly (p < 0.05) affected by the vegetation type, soil pH, and organic matter content, but not phosphorous concentration. In contrast, fungi were robust to variation in the environmental factors and fungal a-diversity was not significantly affected by any variables. Microbial α-diversity was not lower in marram grasslands than in heathlands, potentially due to horse activities damaging the vegetation types similarly and an increase in nutrient deposition in the marram. Microbial β-diversity of presence/absence was not significantly affected by any variable. Identifying drivers of soil microbiome diversity is important for effective ecosystem management in vulnerable ecosystems such as Sable Island.

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1. Introduction

1.1 Microbes in the environment

Microbes in the soil consist of bacteria, fungi, archaea, viruses, and protozoa; together these microbes form the soil microbiome (Fierer, 2017). Soil contains a high abundance of diverse taxa even though only approximately 0.000001% of the soil surface area is inhabited by microbes (Young & Crawford, 2004). Of the microbes that are present, only 0.1-5% are active at a given moment, and 10-60% can be activated within hours of stimuli (Blagodatskaya & Kuzyakov, 2013). This means that most microbes are dormant until conditions permit, at which point they become engaged in processes such as decomposition. The microbiome composition varies widely depending on many factors, including the vegetation type and soil characteristics such as aggregate size, texture, nutrient content, and pH; however, no particular factor is consistently an overarching determinant of the composition (Fierer, 2017).

The soil microbiome is fundamental to ecosystem functioning; it is responsible for nutrient cycling, decomposition, and carbon sequestration (Mishra et al., 2023). These three processes often work simultaneously when breaking down organic matter. Nitrogen is cycled by ammonification followed by nitrification; this is when organic nitrogen is converted to ammonia by one set of microorganisms, and then another group of microorganisms convert the ammonia into nitrites and nitrates (Mishra et al., 2023). Microorganisms convert phosphorous from an organic to an inorganic form which is soluble and more bioavailable to plants (Mishra et al., 2023). Microbes feed on the organic carbon in decomposing organic material to use it for their biomass. Additionally, the microbiome affects atmospheric carbon dioxide (CO₂) concentrations despite their low cumulative biomass (Liang et al., 2017). Carbon sequestration occurs when autotrophic microbes take CO₂ from the atmosphere to build biomass or release non-gaseous carbon forms

into the rhizosphere (Jansson & Hofmockel, 2020). Dead microbial biomass, containing carbon, adheres to soil substrates and acts as a carbon sink (Liang et al., 2017). Some microbe species can also transform CO_2 into carbonate minerals that remain in the soil for extensive periods (Jiang et al., 2022). However, when the microbial pool is affected by increased temperature or nitrogen, the microbial contribution to the carbon sink is decreased which can result in a carbon source-sink imbalance (Liang & Balser, 2012).

Shifts in the microbial taxa can impact how the microbiome operates and what ecological function is served (Fierer, 2017). Diversity of the microbiome can depend on abiotic factors such as pH, soil nutrient concentrations, precipitation, and temperature; physical disturbance such as compaction and erosion; and anthropogenic factors such as pollutants, invasive species, and climate change (Mishra et al., 2023). As the soil microbiome is important for the functioning of ecosystems, impacts on the microbiome can lead to reduced functionality with downstream consequences.

1.1.1 Bacteria and Fungi

Bacterial and fungal taxonomic diversity is affected by abiotic and biotic factors. As bacteria and fungi both live in similar environments, they are competing for the same resources (Bahram et al., 2018). Bacterial taxa differ among climatic regions suggesting niche specialization, which may make them more susceptible to climate change (Bahram et al., 2018). Bahram et al. (2018) suggested that fungi are more tolerant to nutrient and water limitations than bacteria, as they possess structures like hyphae which improve their ability to access water and can use more complex carbon sources. Both taxonomic groups have evolved mechanisms to increase their competitive advantage: fungi will express antimicrobial compounds, and in response, bacteria

express antibiotic-resistance genes (ARGs) (Bahram et al., 2018). Bacteria expressing ARGs are found more often when in competition with fungi than with other bacteria (Bahram et al., 2018).

The relative abundance of bacteria or fungi in the soil fluctuates based on environmental factors. This is often due to pH, nutrients, soil moisture, soil temperature, disturbance, and the presence of organic matter; however, the outcome of bacterial or fungal dominance can be inconsistent with the general expectations (Strickland & Rousk, 2010). This is likely due to both groups having similar functions in ecosystem processes, as they can be symbiotic with plants, break down organic matter, or cause disease (Clark, 2012; Money, 2016). Also, the high diversity within the microbiome can allow shifts in dominance depending on environmental conditions (i.e., saprophytic fungi decrease with nitrogen limitation, but mycorrhizal fungi are unaffected) (Strickland & Rousk, 2010). Bacterial or fungal dominance can not be directly related to functionality within the soil; the specific functional groups of bacteria or fungi must be compared to assess relevant dominances and functional shifts (Strickland & Rousk, 2010). However, determining the functionality of specific taxa remains a difficult task (Fierer, 2017).

Some taxa of bacteria and fungi form mutualistic associations that benefit both their plant host and the microbe itself. These associations help the plant and promote growth by providing nutrients that are difficult to access, making plants more stress tolerant and providing resistance to disease by pathogenic microbes (Amoo et al., 2023; Trillas & Segarra, 2009). Protection of the plant by a beneficial microorganism is done by parasitizing the pathogenic microorganism, antibiosis (i.e., production of antibiotics), or competition (Amoo et al., 2023).

The association some bacteria form with plants results in a nitrogen-carbon exchange. Two major groups of bacteria are nitrogen-fixing: rhizobia and *Frankia*; these bacteria form associations with plants and receive carbon while providing nitrogen to the plant (Franche et al.,

2009). Both groups of bacteria enter the plant through root hairs intracellularly or intercellularly, and once the infection is established the plant forms a nodule at that site (Vessey et al., 2004). Rhizobia forms a symbiosis with leguminous plants or non-leguminous *Parasponia* sp. where plants form root nodules for the bacteria to live in (Franche et al., 2009); the rhizobia give the plant ammonium and receive carbon in return (Prell & Poole, 2006). *Frankia* bacteria form a symbiosis with actinorhizal plants, leading to a similar exchange of nitrogen and carbon (Vessey et al., 2004).

Several types of fungi, such as endophytic and ectophytic, form beneficial associations with plants, which live in and outside the plant's body, respectively. Examples of endophytic fungi include arbuscular mycorrhizae and ericoid mycorrhizae. Arbuscular and ericoid mycorrhizae form associations via penetrating the plant cells by growing branched or coiled structures, respectively (Trillas & Segarra, 2009). Fungi that form an association without penetrating the host are known as ectomycorrhizal, or ectophytic, fungi which form structures around the root of the plant (Trillas & Segarra, 2009). Like rhizobia and *Frankia*, the associated fungi receive carbon from the host plant, while providing nutrients such as phosphorous, nitrogen, potassium, and other metals to the plant (Marschner & Dell, 1994). The colonization of the plant with the fungi elicits a defence response by the plant to prevent invasion into the endodermis and vessels by pathogenic microbes, and thereby help protect the plant from pathogenic fungi (Trillas & Segarra, 2009).

1.2 Grazer effects on the environment

Terrestrial grazers are animals that feed on plants like grasses; often, these animals form herds such as cattle and horses. Grazers affect biotic and abiotic factors in their environment through activities such as grazing, trampling, and producing excrement (Eldridge et al., 2020). Biotic effects of grazing include the herbivory of plant species which reduces plant relative abundances, facilitates exotic species, and alters the plant community composition (e.g., grasslands to shrublands) with increasing grazing intensity (Mikola et al., 2009; Eldridge et al., 2020; McSherry & Ritchie, 2013). Grazers also have biotic effects on the presence of soil macrofauna (e.g., nematodes, ants), and can induce the growth of mycorrhizal hyphae (Mikola et al., 2009; Eldridge et al., 2020; McSherry & Ritchie, 2013). Abiotic effects of grazing consist of increased soil pH, increased soil density (as trampling removes soil pores), increased soil moisture, increased erosion, and reduced litter cover and depth (Mikola et al., 2009; Eldridge et al., 2020). Grazers also cause nutrient patchiness due to herbivory; they remove plant biomass and thus nutrients, and later return the nutrients in patches through excrement (Mikola et al., 2009).

Depending on vegetation and soil type, the effects of grazers can differ. Intense grazing on sandy soils reduces vegetation, which can make the soil more vulnerable to erosion and potentially lead to net carbon release from the soil (McSherry & Ritchie, 2013). Whether plants use C₃ or C₄ photosynthesis affects whether the carbon is sequestered or released under grazing pressures. The C₃ and C₄ photosynthesis pathways differ in the enzymes (RuBP carboxylase *vs.* PEP carboxylase, respectively) and mechanisms used to create the sugar, allowing for different adaptations to their environments, such as cold tolerance (Pearcy & Ehleringer, 1984). Under heavy grazing intensity, C₄ grasses have a positive interaction by increasing soil carbon sequestration, this is in contrast to C₃ grasses which have a negative interaction by decreasing the soil carbon (McSherry & Ritchie, 2013). This may partly be due to mycorrhizal associations with C₄ grasses which enhance soil stability by fungal glomalin production (McSherry & Ritchie, 2013).

Detrimental environmental effects on the vegetation and soil are caused by overstocking grazing animals which leads to the over-utilization of land and food resources (Rickert, 1996). Horses spend more time foraging and travelling than cattle resulting in increased damage to vegetation and soil through their varied grazing and trampling at high stocking densities (Nolte et

al., 2017). Feral horses significantly negatively affect environmental quality by causing reductions in plant biomass, increases in erosion, reductions in animal richness, and reductions in microbial abundances which leads to ecosystem functioning disruptions (Eldridge et al., 2020).

1.3 Sable Island

Sable Island is a sand bar southeast of Nova Scotia, Canada. The crescent-shaped island is approximately 40 km long and has a maximum width of 1.5 km, located at 43.9333 °N, -60.0000 °W in the North Atlantic Ocean (Eamer et al., 2022; Catling et al., 2009). As the island is a sand bar, there is accumulation and erosion of sediment, both of which affect the vegetation communities; currently, the island has more retreat occurring than sedimentation (Eamer et al., 2022). There is a possibility of eastward migration of the island due to how the sediment is accumulating, though the retreat trends are stronger which indicates the island is shrinking, rather than migrating (Eamer et al., 2022). Sable Island experiences strong winds averaging 25.7 km/h which help shape the dunescape as well as the vegetation communities (Byrne & McCann, 1995).

From 1553 to 1633, animals such as cattle and pigs were transported by Portuguese settlers to live on the island, but the animals were then removed and slaughtered by Acadians and New Englanders (Christie, 1980). Reverend Andrew Le Mercier brought cattle, sheep, hogs, and horses to the island in 1737, but abandoned them by 1753, and just before 1760, Thomas Hancock also brought horses, hogs, sheep, and goats to the island (Christie, 1980). Though many animals were taken to the island over the years, they were often stolen or sold, with only horses establishing long-term survival (Christie, 1980). Either the introduction of horses by Le Mercier or Hancock led to an established population of horses which persists today (Christie, 1980).

1.3.1 Sable Island Biodiversity

Sable Island is an important habitat on which a number of animal species, including birds and seals, depend. Bird species include the Least Sandpiper (*Calidris minutilla*), Spotted Sandpiper (*Actitis macularia*), Ipswich Sparrow (*Passerculus sandwichensis princeps*), and endangered Roseate Tern (*Sterna dougallii*) (Miller, 1983; Ross, 1980; Gochfeld, 1983). Herring Gulls (*Larus argentatus*) also frequent this island, although they are not native (Miller, 1983). This island is an important breeding ground for grey seals (*Halichoerus grypus*) and harbour seals (*Phoca vitulina*) (Freedman et al. 2011). One of the most prominent species is the introduced feral horse (*Equus caballus*). There are also at least 573 documented invertebrates, of which 480 are native (Chow, 2020). Therefore, Sable Island is home to a diverse array of animal species, some of which are endemic and highly dependent on the island.

Vegetation on Sable Island covers 40% of the surface area with the unvegetated portion consisting of bare fine-medium textured sand (Byrne & McCann, 1995). The island was previously thought to have been mostly covered by vegetation, but that has decreased since the establishment of horses in the 18th century (Byrne & McCann, 1995). There are 158 native species and 82 introduced species of flora (Catling et al., 1984; Catling et al., 2009) forming communities of marram and shrub heathland which dominate the island, alongside sandwort and pond-edge vegetation (Catling et al., 1984; Eamer et al., 2022). Marram grass grows in wind-blown sandy substrate, shrub heathland is in protected inland sites, sandwort is found closer to the ocean where it is prone to inundation and sea spray during storms, and pond-edge vegetation is found surrounding brackish or freshwater ponds (Catling et al., 1984). The distribution of these communities on Sable Island is related to distance from shore and the slope of the dune which is associated with sand erosion or accumulation (Tissier et al., 2013).

In dunescapes in Nova Scotia, marram grass (e.g., *Calamagrostis breviligulata*) is responsible for the stabilization of the sand which leads to the formation of sand ridges (Davis, 1996). Heathland vegetation (e.g., *Juniperus horizontalis*) can then form communities in the stabilized substrate after there is an increase in organic matter (Davis, 1996). Vegetation types that are sensitive to sand accumulation, such as heathlands, are inland and away from potential sand burial sites, and plants with salt adaptations are near brackish water or by the shore (Tissier et al., 2013). Although marram grasslands are the most tolerant to sand accumulation, they are the most susceptible to horse damage (Freedman et al., 2011).

The horse population was introduced to this island by humans but has been feral for generations and is genetically distinct from other breeds, though they are not considered a distinct breed (Freedman et al., 2011; Plante et al., 2007). As of 2019, the population consisted of approximately 590 horses (Johnsen, 2022). Given the island's small size and vulnerability to erosion, having such a significant number of horses grazing and trampling the soil can cause substantial damage. These horses have been extensively studied in terms of their history, foraging habits, gut microbiome, population genetics, and negative effects on the vegetation communities, especially the marram grasslands (Christie, 1980; Johnsen, 2022; Stothart et al., 2020; Lucas et al., 2009; Plante et al., 2007; Freedman et al., 2011). However, the effects of the horses on the soil microbiome on Sable Island remain unclear due to a lack of research on the soil microbiome.

1.4 Objectives and Hypotheses

We used eDNA metabarcoding to assess the differences in the microbiomes of Sable Island. α -diversity and β -diversity were compared to assess differences in local species richness and the turnover in microbial communities across sites, respectively. Our objectives were to 1) conduct an overall survey of bacteria and fungi, and 2) assess the effects of vegetation type (marram vs. heath), pH, organic matter, and phosphorous (P_2O_5) on microbial α -diversity and β -diversity.

Plant species richness positively affects microbial biomass, activity, and composition in other grasslands (Strecker et al., 2015; Chung et al., 2007). Therefore, we hypothesized that the α -diversity of the microbes would be lower in the marram grasslands than in the shrub heathlands due to the lower plant species richness, and the negative effects on marram by horses (Freedman et al., 2011). Similarly, as specific vegetation is expected to support different microbial taxa, and horse activity impacts the vegetation types differently, we hypothesized there would be more similarities in the microbial communities within the vegetation types than between them.

2. Materials and Methods

2.1 Site Design

In the spring of 2021, 16 sites were selected across Sable Island based on evidence of horse activity (e.g., grazing, trails, rubbing, or feces) (Figure 1). The plant communities and the geomorphology vary across sites, but each site was paired with a corresponding, similar site to create a total of eight pairs. The sites ranged in size from 0.25 ha to 2.36 ha, and five, 5 m x 5 m plots were evenly distributed within each site. The vegetation in these plots was categorized as marram grassland, heathland, intermediate, or unvegetated (marram n = 35, heath n = 31, intermediate n = 12, unvegetated n = 2). Plots dominated with marram grass (*Calamagrostis breviligulata*) or beach pea (*Lathyrus japonicus*) were categorized as marram, while plots with dominant species such as bayberry (*Morella pensylvanica*) or crowberry (*Empetrum nigrum*) were categorized as heath. Plots dominated by yarrow (*Achillea millefolium*) or Kentucky bluegrass (*Poa pratensis*) were categorized as intermediate, and unvegetated plots were categorized as

unvegetated. The plots were placed at least 5 m from the edge of the site to account for edge effects. In total, 80 plots representing five replicate plots for each of the 16 sites across Sable Island were used in this study.



Figure 1. Locations of the 16 sites established on Sable Island, Nova Scotia in the spring of 2021. The size of each site ranged from 0.25 ha to 2.36 ha.

2.2 Soil Sampling and Analyses

2.2.1 eDNA

Vegetation was removed with pruning shears from a circular patch, 26 cm in diameter, near the centre of each plot (Figure 2). A bulb planter (12.7 cm width x 12.7 cm depth) was then used to take a soil sample for eDNA analysis. The bulb planter was cleaned between samples with a dilute, unscented soap solution and a rag to prevent cross-contamination. The samples were stored individually in resealable plastic bags, kept in a cooler while in the field, and then stored at -20 °C for the duration of collection between mid-July and the end of August 2021. Samples were shipped to Dalhousie University and Saint Mary's University, NS where they were stored at -80 °C.

2.2.2 Soil Characteristics

Immediately outside the centre of the western edge of the plot, vegetation was removed from a 30 cm x 30 cm, 900 cm² area (Figure 2). A soil sample was taken with the same bulb planter (12.7 cm width x 12.7 cm depth) for pH, phosphorous concentration (kg/ha) and organic matter (%) analysis. The bulb planter was cleaned between samples with a dilute, unscented soap solution to prevent cross-contamination. The samples were stored individually in resealable plastic bags and kept in a cooler while in the field, and then stored at -20 °C for the duration of collection. Samples were shipped to the Department of Agriculture, Truro, NS for analysis. All soil samples were collected between mid-July and the end of August 2021.



Figure 2. Plot placement set up for Sable Island soil sampling. The sample for soil eDNA came from the 12.7 cm diameter area inside the plot where vegetation was removed. The sample for soil characteristics (pH, phosphorous, and organic matter) came from the 30 cm x 30 cm area outside the west side of the plot where vegetation was removed. The measurements reported are accurate, but the figure is not to scale.

After the soil characteristics were analyzed, the samples were grouped into appropriately sized categories for analysis. For pH, the categories were 4.00-4.99 (n = 17), 5.00-5.99 (n = 33), and >6.00 (n = 28). For organic matter content (%) the categories were <1 (n = 48), 1-2 (n = 16), and >2 (n = 14). Finally, the phosphorous concentration (kg/ha) categories were 10-20 (n = 8), 21-30 (n = 53), and >30 (n = 17). One plot was not analyzed for any soil characteristics as no soil sample was taken. It was categorized as N/A (n = 1).

2.3 eDNA Extraction, Metabarcoding, and Bioinformatics Processing

The soil samples for eDNA were stored at -80 °C and were thawed and homogenized before DNA extraction. Environmental DNA was extracted from approximately 0.25 g of soil with a DNeasy® PowerSoil® Pro Kit (QIAGEN) following their recommended protocols and using a QIAGEN TissueLyser II at 25 Hz for 10 minutes for cell lysis as recommended. After the DNA was isolated and eluted, the samples were stored at -80 °C until sequencing.

The extracted eDNA was sent to the Integrated Microbiome Resource lab at Dalhousie University (Halifax, NS) for sequencing and bioinformatics processing (Comeau, 2022; Comeau et al., 2017). Briefly, the targeted DNA was PCR-amplified with the Phusion Plus polymerase and separate primers designed to amplify bacteria and fungi (Comeau, 2022). For bacteria, the 16S V4-V5 region was amplified (forward primer: 515FB = GTGYCAGCMGCCGCGGTAA, reverse primer: 926R = CCGYCAATTYMTTTRAGTTT (Quince et al., 2011)). For fungi, the ITS2 region was amplified (forward primer: ITS86(F) = GTGAATCATCGAATCTTTGAA (Turenne et al., 1999), reverse primer: ITS4(R) = TCCTCCGCTTATTGATATGC (White et al., 1990)). The PCR began with an initial denaturation at 98 °C for 30 seconds, followed by 25 cycles of 98 °C for 10 s, 55 °C for 30 s, 72 °C for 30 s, a final extension at 72 °C for 4 min 30 s and then held at 4 °C (Comeau, 2022). PCR products were verified by the Hamilton Nimbus Select robot using Coastal

Genomics Analytical Gels and normalized with the Charm Biotech Just-a-Plate 96-well Normalization Kit (Comeau, 2022). PCR products were quantified fluorometrically with the Invitrogen Qubit dsDNA HS assay before sequencing on an Illumina MiSeq (v3 2 x 300 bp pairedend reads) (Comeau et al., 2017).

After sequencing was completed, the sequence reads were processed according to Comeau et al. (2017). Briefly, the paired-end reads were merged using PEAR (v0.9.10) and any low-quality sequences (quality score <30 in 90% of bases) were identified by FastQC (v0.11.5) and removed by FASTX-Toolkit (v0.0.14). Any potentially chimeric reads were removed with VSEARCH (v1.11.1). Then, open-reference ASV (amplicon sequence variant) picking was done with QIIME wrapper scripts (v1.91) by comparing the sequence reads obtained to reference sequences to identify an ASV for each amplicon when possible. An ASV for bacteria (16S) includes reads that have 97% similar sequence identity. ASVs that were less than 0.1% of the total number of sequences were removed. Finally, QIIME2 (Bolyen et al., 2019) was used to compare the diversity of taxa found across samples.

2.4 Statistical Analyses

 α -diversity of bacteria and fungi was determined using Shannon entropy which was performed using QIIME2 (Bolyen et al., 2019). Shannon entropy accounts for the evenness and abundance of taxa within a community by measuring the uncertainty and creating equivalencies which can be compared (Jost, 2006). Two communities which have the same entropy may differ in terms of evenness or abundance of taxa (Jost, 2006). The Shannon entropies were compared with a Kruskal-Wallis test to determine significance using QIIME2 (Bolyen et al., 2019). The microbial community composition (β -diversity) of bacteria and fungi was determined by calculating Jaccard and Bray-Curtis distance matrixes and performing a Principal Coordinate Analysis (PCoA) using QIIME2 (Bolyen et al., 2019). Jaccard is based on presence/absence data and thus weights rare taxa equal to abundant taxa; Bray-Curtis is abundance-based and thus is less sensitive to changes in rare species (Jaccard, 1912; Bray & Curtis, 1957). The Jaccard index is the most robust to errors across indices, and Bray-Curtis is the most robust to errors for abundancebased indices (Schroeder & Jenkins, 2018). The variables' effects on the microbial communities were assessed with a permutational multivariate analysis of variance (PERMANOVA) using the Adonis2 function from the vegan package in R (Oksanen et al., 2022).

All data visualizations and the PERMANOVAs were performed in R v.4.3.2 (R Core Team, 2023) and R Studio v.2023.12.1+402 (Posit team, 2023). Packages not already stated are qiime2R (Bisanz, 2018) and phyloseq (McMurdie & Holmes, 2013) for reading qza files from QIIME2, tidyverse (Wickham et al., 2019) for data manipulation and visualizations, and RColorBrewer for colour-bind-friendly palettes (Neuwirth, 2022).

3. Results

3.1 Overview

Across all 80 samples from the 16 sites on Sable Island, there were a total of 3,057,553, and 2,282,503 raw DNA sequence reads for bacteria and fungi respectively. From those reads, we identified 37 bacterial and 12 fungal phyla with a total of 812 and 336 bacterial and fungal ASVs, respectively (Appendix Tables C1 & C2). Of the 80 samples, one unvegetated sample failed to amplify for bacteria and fungi, and one marram sample failed to be sequenced for fungi (bacteria n = 79, fungi n = 78). The bacterial communities had higher α -diversity than the fungal

communities. α -diversity of bacteria differed significantly (p < 0.05) across vegetation types and between most environmental factors, whereas α -diversity of fungi did not differ significantly. β -diversity in each vegetation type and across environmental factors were not significantly different for bacteria or fungi with the Jaccard distance. However, the β -diversity of bacterial taxa was significantly different across vegetation types with the Bray-Curtis distance.

3.2 Relative Abundance

Across vegetation types, the relative abundance of bacterial and fungal phyla differed, however, the dominant phyla were similar. The two most abundant bacterial phyla were the same across all three vegetation types (Table 1). Firmicutes was the most abundant bacterial phylum in all vegetation types and was more relatively abundant in heath than marram and intermediate (Table 1, Figure 3). Similarly, Basidiomycota was the most abundant fungal phylum across all vegetation types (Table 1). However, Basidiomycota were more relatively abundant in the intermediate sites than marram and heath (Table 1, Figure 3). The same bacterial and fungal phyla were in the top six most relatively abundant phyla across the vegetation types but differed in their abundance rank depending on the vegetation type (Table 1). There were fewer similarities in the phyla relative abundance rank in fungi between vegetation types compared to bacteria (Table 1).



Figure 3. The relative abundances of taxa at the phylum classification in each vegetation type on Sable Island, NS. The most abundant 10 phyla are displayed, in order of decreasing relative abundance, with the remaining less abundant phyla grouped into the 'Other' category. (a) Bacterial taxa; (b) Fungal taxa.

Table 1. Ranking of the most relatively abundant bacteria and fungi phyla across the vegetation types on Sable Island, NS in 2021.

Abundance rank	Н	Heath		Intermediate		Marram	
Tulik	Bacteria	Fungi	Bacteria	Fungi	Bacteria	Fungi	
1	Firmicutes	Basidiomycota	Firmicutes	Basidiomycota	Firmicutes	Basidiomycota	
2	Proteobacteria	Mortierellomycota	Proteobacteria	Chytridiomycota	Proteobacteria	Mortierellomycota	
3	Acidobacteriota	Mucoromycota	Planctomycetota	Ascomycota	Planctomycetota	Chytridiomycota	
4	Planctomycetota	Glomeromycota	Acidobacteriota	Mortierellomycota	Acidobacteriota	Ascomycota	
5	Actinobacteriota	Ascomycota	Actinobacteriota	Glomeromycota	Actinobacteriota	Glomeromycota	
6	Bacteroidota	Chytridiomycota	Bacteroidota	Mucoromycota	Bacteroidota	Mucoromycota	
7	Verrucomicrobiota	Calcarisporiellomycota	Other	Olpidiomycota	Other	Monoblepharomycota	
8	Other	Monoblepharomycota	Verrucomicrobiota	Monoblepharomycota	Verrucomicrobiota	Rozellomycota	
9	Myxococcota	Other	Chloroflexi	Rozellomycota	Chloroflexi	Olpidiomycota	
10	Chloroflexi	Olpidiomycota	Gemmatimonadota	Other	Myxococcota	Other	
11	Gemmatimonadota	Rozellomycota	Myxococcota	Calcarisporiellomycota	Gemmatimonadota	Calcarisporiellomycota	

3.3 α-Diversity

The intermediate vegetation type had the highest bacterial α -diversity, followed by the unvegetated, and then the marram (Figure 4a, Appendix Table A1). The heath bacterial diversity was significantly lower than the marram and the intermediate (p = 0.043, p = 0.006) (Figure 4a, Appendix Table A2). In fungi, the α -diversity was not significantly different across the vegetation types (p = 0.61) (Figure 4b, Appendix Table A1).

Bacterial α -diversity was similar in soil samples with pH 4.00-4.99 and >6, with 4.00-4.99 being slightly higher; the lowest diversity was in pH 5.00-5.99 (Figure 4c, Appendix Table A1). There was a significant difference from pH 4.00-4.99 to both pH 5.00-5.99 and >6 (p = 0.008, p = 0.004) (Appendix Table A2). In fungi, the α -diversity was not significantly affected by pH (p = 0.80) (Figure 4d, Appendix Table A1).

The bacterial α -diversity in soil samples with an organic matter content <1% and 1-2% was similar, with 1-2% being slightly higher (Figure 4e, Appendix Table A1). The bacterial diversity in organic matter >2% was significantly lower than <1% and 1-2% (p = 0.005, p = 0.011) (Figure 4e, Appendix Table A2). In fungi, the α -diversity was not significantly affected by organic matter content (p = 0.73) (Figure 4f, Appendix Table A1).

There were no significant differences in the α -diversity for bacteria or fungi across phosphorous concentrations (p = 0.44, p = 0.17) (Figure 4g, h, Appendix Table A1).



Figure 4. The mean Shannon entropy (α -diversity) of soil microbial communities in vegetation types and across environmental factors on Sable Island, NS. (**a**, **c**, **e**, **g**) Bacterial taxa; (**b**, **d**, **f**, **h**) Fungal taxa; (**a**, **b**) Vegetation types; (**c**, **d**) pH; (**e**, **f**) Organic matter content (%); (**g**, **h**) Phosphorous (P₂O₅) (kg/ha). N/A represents plots where soil analyses were not completed. Error bars represent standard error.

3.4 β-Diversity

β-diversity was compared using two different dissimilarity methods with a PCoA. When using the Jaccard distance on bacterial communities, the PC1 axis explained 14.28% of the variation, and PC2 explained 6.92%, compared to using the Bray-Curtis distance where the PC1 axis explained 17.43%, and PC2 explained 8.74%. When using the Jaccard distance on fungal communities, the PC1 axis explained 10.1% and PC2 explained 6.73%, whereas the Bray-Curtis distance PC1 axis explained 7.91% and, PC2 explained 6.84%. The microbial composition of vegetation types was not significantly different for either bacteria or fungi with the Jaccard distance (p = 0.076, p = 0.85) (Figure 5a, b, Appendix Table A3). However, with the Bray-Curtis distance, bacterial communities across vegetation types were significantly different, but not for fungi (p =0.038, p = 0.53) (Figure 5c, d, Appendix Table A3). Both Jaccard and Bray-Curtis distances explained a low amount of variation (<5%) for bacteria and fungi (Appendix Table A3). The variance was not explained by the environmental factors (pH, organic matter, phosphorous) for Jaccard or Bray-Curtis distances for bacteria or fungi with all R² below 0.03, and all p-values >0.05 (Appendix Table A3, Appendix Figure B1, B2, B3).



Figure 5. The beta diversity of soil microbes in vegetation types on Sable Island, NS with a principal coordinate analysis (PCoA) with a Jaccard (a, b), and a Bray-Curtis distance matrix (c, d). (a, c) Bacterial taxa; (b, d) Fungal taxa. N/A represents plots where soil analyses were not completed.

4. Discussion

Bacterial diversity differed based on several factors whereas fungal diversity did not change across any factor. We found significant effects of vegetation types, soil pH, and organic matter content, but not phosphorous concentration, on bacterial α -diversity. We found no significant effects of any factors on fungal α -diversity. Additionally, for β -diversity, the bacterial and fungal taxa in each vegetation type, soil pH, organic matter content, and phosphorous category were not significantly different, and each factor explained less than 5% of the variation observed. Although the bacterial taxa abundances differed significantly across vegetation types, the vegetation still explains a low amount (3.7%) of the variation. The most relatively abundant phyla of bacteria and fungi were the same in each vegetation type further reflecting the lack of significant differences.

4.1 Effects of Vegetation Type on Microbial Diversity

4.1.1 Relative Abundance

Firmicutes had the highest bacterial relative abundance in all vegetation types, likely due to their close association with plant roots in the endorhizosphere and rhizosphere soil (Hashmi et al., 2020). The most abundant family of Firmicutes found was *Bacillaceae* in both marram grasslands and heathlands. This family is tolerant to a variety of conditions including drought, salinity and other extremes allowing them to survive in the different soils in the different vegetation types (Mandic-Mulec et al., 2016). Proteobacteria also had a high relative abundance in marram grasslands and intermediate vegetation. In another study, proteobacteria were also common in the soil with grasses (Qiao et al., 2022). Some classes of proteobacteria are copiotrophic and thus found in nutrient-rich soils, and copiotrophs are more abundant in herbs than shrubs (Ma et al., 2023). However, marram grass grows in a sandy substrate rather than soil which is typically nutrient-limited by nitrogen and phosphorous, which would not be preferable for copiotrophs. However, fecal excrement by horses along with nutrient transfer mediated by grey seal movements would enrich the soils facilitating copiotrophic bacteria such as proteobacteria to flourish (Read, 1989; McLoughlin et al., 2016).

Basidiomycota had the highest relative abundance in all vegetation types by far. This may be due to their ability to form associations with plants and their saprotrophic ability allowing them to create symbioses with plants, and then continue to thrive after the plant has died (Watkinson, 2016). Arbuscular mycorrhizal fungi are from the phylum Glomeromycota and are associated with grasses often and trees, ectomycorrhizal fungi are from Basidiomycota and Ascomycota and are associated with trees, and ericoid mycorrhiza are from Ascomycota and are associated with heath plants (Watkinson, 2016). Based on those symbiotic relationships, we expected significant differences in the fungal phyla and their relative abundances between vegetation types; however, we found no significant differences. Though similar to our results, another study found a higher abundance of Ascomycota in grasslands and mixed vegetation sites (Qiao et al., 2022). Interestingly, there were no significant differences in fungal taxa turnover across vegetation types suggesting symbioses with plants may not be eliciting as much of an effect on fungi as previously expected.

4.1.2 α -Diversity

The vegetation type had significant effects on the bacterial α -diversity but not in the way we hypothesized. Instead, the intermediate vegetation had the highest α -diversity among the four vegetation types. One study also found that shrub-grass mixed communities had higher bacterial α -diversity than grass communities (Qiao et al., 2022). This is potentially due to having a variety of plant functional groups in this community (i.e., both grasses and shrubs), allowing for a higher variety of different plant-microbe associations. In contrast, the marram grass vegetation type mainly contained grasses, while the heath vegetation type mainly contained shrubs.

Aside from the symbioses occurring between the plant and microbes, the bacterial diversity results could be explained by the effects of horse activities. We initially hypothesized that marram

grasslands would have a decreased diversity due to horse activities such as frequent grazing, but our results show a lower diversity in heath than marram. The horses on Sable Island prefer to consume marram grass as it is a higher quality food, but when population density is high, heathlands are increasingly selected (Van Beest et al., 2014). This results in the horses foraging and thus trampling the soil and causing mechanical damage to the microbes in both marram and heathlands which was not accounted for previously. In unvegetated plots, there may be less trampling and damage which could contribute to the higher diversity in those plots.

4.1.3 β -Diversity

We hypothesized the microbial communities would differ between the vegetation types, which was partially supported. Bacteria in vegetation types were significantly different in abundance, but not in community composition. This indicates some vegetation types were more favourable for certain taxa allowing their dominance over other taxa. Other studies have also found significant differences in the microbial communities in different vegetation types (e.g., Ma et al., 2023), though we did not see the same differences in fungi. Many of the bacterial and fungal taxa in our samples inhabit a wide range of environments which is why we saw them in all vegetation types (Hashmi et al., 2020; Tedersoo et al., 2014; He et al., 2022). Our soil samples contained bulk and rhizospheric soil which means we were unable to detect microbial community differences at a finer scale. This could partially explain the overall microbial community homogeneity observed.

4.2 Effects of Soil Characteristics on Microbial Diversity

4.2.1 α -Diversity

Bacterial α -diversity was affected by the pH and organic matter content but not phosphorus. There were no significant effects of these factors on fungal α -diversity. There was a significantly lower bacterial α -diversity in soil with a pH of 5-5.99 than in the lower and higher pH category, which is different from another study in which the bacterial α -diversity increased with pH from 3 to 9 (Griffiths et al., 2011). In other studies, fungal diversity also increased with increased pH from 3 to 6.5 (Liu et al., 2018). We also observed a lower bacterial α -diversity in organic matter >2%, which differs from other studies where more organic matter leads to an increase in microbial diversity (Sul et al., 2013). We found bacterial and fungal α -diversity were not affected by phosphorous, even though the taxa in our study were similar to a study which found an effect of phosphorus (Kuramae et al., 2011). Research has also shown that fungi tend to be more tolerant of environmental stressors than bacteria (Coleine et al., 2022). This could explain why there is a lack of significant effects on the fungal α -diversity.

4.2.2 β -Diversity

There were no significant differences in the turnover of bacteria or fungal communities across the various soil characteristics. Many of the bacterial and fungal taxa are ubiquitous in soil environments which is why we observed them across the full ranges of soil pH, organic matter content and phosphorous concentration (Hashmi et al., 2020; Ward et al., 2009; Tedersoo et al., 2014; He et al., 2022).

4.3 Ecosystem Management

Our research found bacteria to be more variable than fungi across the abiotic and biotic environment on Sable Island. Soil microbes influence the health of plants and may encourage growth through symbioses thus improving soil quality and reducing erosion (Clark, 2012; Money, 2016; Normaniza et al., 2018). As the vegetation on Sable Island is affected by the horses (Freedman et al., 2011), understanding how the microbes are affected and implementing protection for them is essential for ecosystem functionality. The microbial communities are important for ecosystem management and can aid in the protection of vegetation, and resistance of the soil to erosion (Watkinson, 2016; Normaniza et al., 2018). The positive effects of microbes on vegetation and soil can also help vulnerable organisms such as the Roseate Terns, and the grey seals by protecting their nesting habitat from degradation.

4.4 Limitations and Future Research

This study was able to identify a wide variety of bacterial and fungal diversity by using eDNA. This methodology can identify taxa which are low in abundance or are unculturable giving us a deeper insight into the community (Torsvik & Øvreås, 2002). However, it does detect taxa which are inactive and in their dormant state, which means there may be more differentiation in active microbial communities between vegetation types and soil characteristics than we were able to detect (Locey et al., 2020). Accounting for microbial dormancy is a common issue when dealing with microorganisms which calls for different methods of analyzing microbial communities (Locey, 2010). Also, we sampled with a soil core and homogenized the soil samples, which makes identifying fine-scale relationships difficult as microbial communities can differ between soil aggregates and between bulk soil and the rhizosphere (Fierer, 2017; Philippot et al., 2013). It is important to keep in mind the complexity of the microbiome and that there are likely many interactions that were not identified.

Additionally, there may be more species that were not identified due to either sampling sites not encompassing enough areas to identify all taxa on Sable Island, or due to limitations of primers. Some taxa may not have been amplified consistently, or at all by the primers leading to a misrepresentation or a lack of representation overall. Even though some taxa may be left out or misrepresented, using DNA is still able to identify more than other methods such as culturing. This study has resulted in a baseline survey of bacterial and fungal diversity across Sable Island, particularly on the microbiome diversity and composition between vegetation types. The next step for the microbiome on Sable Island is to investigate the direct and indirect effects the horses have on the microbiome and soil characteristics. As this study has found significant differences in the bacterial communities already, further determining the impacts of the horses on the microbiome will aid in ecosystem management to support the island's biodiversity.

5. Conclusion

The soil microbiome is responsible for driving many ecosystem functions, affecting aboveground organisms, and it is also affected by abiotic and biotic factors (Mishra et al., 2023; Van Der Heijden et al., 2008; Fierer, 2017). The microbiome taxa can adapt to changes in the abiotic and biotic environment by shifting to exploit new resources and by forming symbioses or other interactions (Li et al., 2022). Our results indicate bacterial α -diversity to be more variable than fungi due to environmental differences including vegetation types and soil characteristics. We found very few differences in the β -diversity of bacteria, and no differences in fungal diversity suggesting robust microbial communities or an indistinguishable variety of active and dormant taxa. Many of the taxa identified on Sable Island are common throughout the soil and occupy a wide range of environmental conditions. Some of our results are consistent with other studies, particularly regarding plant-microbe associations, while other results regarding soil characteristics found no significance or inconsistent patterns compared to other studies. Sable Island has allowed a unique study of a soil microbiome which is not affected by anthropogenic activity directly but rather by feral horses. Further research can identify the direct impacts of the horses on the microbiome. Identifying the key players of the microbiome along with what is driving the diversity

and composition is important for ecosystem management, especially in vulnerable ecosystems such as Sable Island (Fierer, 2017).

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Appendix

Appendix A

Table A1. Shannon entropy mean values for the vegetation types and environmental factors for each microbe. The *H* and *p* values are reported for each variable for bacteria and fungi. Significant *p* values are in bold (p<0.05).

Microbe	Variable	Shannon entropy	Standard error	Н	p value
	Vegetation type				
Bacteria	Heath	7.536	0.432		
	Intermediate	8.803	0.075	0.140	0.027
	Marram	7.949	0.403	9.140	0.027
	Unvegetated	8.357			
Fungi	Heath	3.926	0.205		
	Intermediate	3.488	0.367	1.026	0.607
	Marram	3.576	0.216	1.830	0.007
	Unvegetated	3.965			
	рН				
Bacteria	4.00-4.99	8.374	0.281		
	5.00-5.99	6.652	0.725	12.240	0.007
	>6	8.130	0.378		
Fungi	4.00-4.99	4.019	0.226		
C	5.00-5.99	3.648	0.236	1.027	0.795
	>6	3.594	0.233		
	Organic matter content (%)				
Bacteria	<1	8.147	0.297		
	1-2	8.280	0.382	11.300	0.010
	>2	6.652	0.808		
Fungi	<1	3.607	0.183		
	1-2	3.888	0.355	1.314	0.726
	>2	3.855	0.210		
	Phosphorous (P ₂ O ₅) (kg/ha)				
Bacteria	10-20	6.850	1.238		
	21-30	8.058	0.276	2.690	0.442
	>30	7.927	0.521		
Fungi	10-20	4.616	0.269		
	21-30	3.612	0.167	4.992	0.172
	>30	3.644	0.310		

Table A2. Pair-wise Kruskal Wallis (post hoc) test results to determine significant differences within variables. The *H*, *p* values, and *q* values (adjusted *p* value for false discovery rate) are reported for interactions within bacterial variables which were significant. Significant *p* values are in bold (p < 0.05).

Var	Н	p value	q value	
Group 1 Group 2				
Vegetation type				
Unvegetated	Heath	0.355	0.551	0.551
	Intermediate	2.570	0.109	0.218
	Marram	0.669	0.413	0.496
Heath	Intermediate	7.628	0.006	0.034
	Marram	4.100	0.043	0.129
Intermediate	Marram	1.101	0.294	0.441
рН				
4.00-4.99	5.00-5.99	7.030	0.008	0.024
	>6.00	8.427	0.004	0.022
5.00-5.99	>6.00	0.681	0.409	0.409
Organic matter content (%)				
<1	>2	7.901	0.005	0.030
	1-2	0.312	0.577	0.577
>2	1-2	6.430	0.011	0.034

Table A3. Permutational multivariate analysis of variance results of *F*, variance explained (R^2), and *p* value for each variable, each microbe and with Jaccard or Bray-Curtis distances. Significant *p* values are in bold (p < 0.05).

Dissimilarity Measure	Microbe	Variable	F	R^2	p value
Jaccard	Bacteria	Vegetation type	1.260	0.033	0.076
		pН	1.062	0.028	0.284
		Organic matter content	1.070	0.028	0.275
		Phosphorous	0.888	0.023	0.739
	Fungi	Vegetation type	0.880	0.023	0.851
		pН	0.866	0.023	0.890
		Organic matter content	1.066	0.028	0.252
		Phosphorous	1.085	0.029	0.208
Bray-Curtis	Bacteria	Vegetation type	1.454	0.037	0.038
		pН	1.036	0.027	0.366
		Organic matter content	0.986	0.026	0.459
		Phosphorous	0.657	0.017	0.988
	Fungi	Vegetation type	0.977	0.026	0.531
		pН	0.833	0.022	0.925
		Organic matter content	1.035	0.027	0.346
		Phosphorous	1.097	0.029	0.195

Appendix **B**



Figure B1. The beta diversity of soil microbes in different soil pH on Sable Island, NS with a principal coordinate analysis (PCoA) with a Jaccard (**a**, **b**), and a Bray-Curtis distance matrix (**c**, **d**) for (**a**, **c**) Bacterial taxa and (**b**, **d**) Fungal taxa. N/A represents plots where soil analyses were not completed.



Figure B2. The beta diversity of soil microbes in different soil organic matter contents on Sable Island, NS with a principal coordinate analysis (PCoA) with a Jaccard (**a**, **b**), and a Bray-Curtis distance matrix (**c**, **d**) for (**a**, **c**) Bacterial taxa and (**b**, **d**) Fungal taxa. N/A represents plots where soil analyses were not completed.



Figure B3. The beta diversity of soil microbes in different soil phosphorous (P_2O_5) concentrations on Sable Island, NS with a principle coordinate analysis (PCoA) with a Jaccard (**a**, **b**), and a Bray-Curtis distance matrix (**c**, **d**) for (**a**, **c**) Bacterial taxa and (**b**, **d**) Fungal taxa. N/A represents plots where soil analyses were not completed.

Appendix C

C.1. Bacteria and Archaea

Table	C1. All archaeal	and bacterial A	SVs identifi	ied on Sabl	e Island,	NS in 2021	in alphabe	tical
order.								

Kingdom	Phylum	Family	Genus	Species
Archaea Bacteria	Crenarchaeota	Bathyarchaeia Group_1.1c Nitrosotaleaceae Nitrososphaeraceae Abditibacteriaceae	Bathyarchaeia Group_1.1c Candidatus_Nitrosotalea Candidatus_Nitrocosmicus Abditibacterium	uncultured_methanogenic uncultured_crenarchaeote uncultured_archaeon uncultured_archaeon utsteinense
				bacterium
				bacterium_LY17
				metagenome
				uncultured_actinobacterium
Bacteria	Acidobacteriota	Acidobacteriaceae_(Subgroup_1)	Acidicapsa	uncultured_bacterium acidisoli
				borealis
				Acidicapsa_sp.
			Acidipila	Acidobacteria_bacterium
				metagenome
				uncultured_bacterium
			Bryocella	elongata
				uncultured_Acidobacteria
				uncultured_bacterium
			Edaphobacter	Edaphobacter_sp.
				uncultured_Acidobacterium
			Granulicella	bacterium_enrichment
				paludicola
				Granulicella_sp.
			Occallatibacter	bacterium_enrichment
				uncultured_bacterium
				uncultured_forest
			uncultured	Acidobacteria_bacterium
				uncultured_Acidobacterium
		Acidobacteriae	Paludibaculum	uncultured_forest metagenome
				uncultured_Acidobacteria
		AKIW659 Blastocatellaceae	AKIW659 Aridibacter	uncultured_bacterium uncultured_bacterium uncultured_bacterium
		Bryobacteraceae	Blastocatella Bryobacter	uncultured_organism Acidobacteria_bacterium
				Acidobacteriaceae_bacterium
				metagenome
				uncultured_eubacterium
				uncultured_Holophaga
				uncultured_Solibacteraceae

Kingdom	Phylum	Family	Genus	Species
		DS-100	DS-100	uncultured_bacterium
		Holophagaceae Koribacteraceae	Holophagaceae Candidatus_Koribacter	uncultured_bacterium uncultured_Acidobacteria
				uncultured_bacterium
		Solibacteraceae	Candidatus_Solibacter	uncultured_eubacterium bacterium_Ellin7504
				uncultured_Acidobacteriaceae
				uncultured_bacterium
				uncultured_forest
		Subgroup_12 Subgroup_13	Subgroup_12 Subgroup_13	uncultured_Holophaga uncultured_bacterium uncultured_bacterium
		Subgroup_17 Subgroup_2	Subgroup_17 Subgroup_2	uncultured_Holophaga metagenome hacterium_enrichment
		Subgroup_2	Subgroup_2	matagenoma
				uncultured_Acidobacteria
				uncultured_bacterium
				uncultured_eubacterium
				uncultured_forest
		Subgroup_5 Subgroup_7	Subgroup_5 Subgroup_7	uncultured_Holophaga uncultured_bacterium uncultured_Desulfovirga
		Thermoanaerobaculaceae	Subgroup_10	uncultured_proteobacterium Acidobacteria_bacterium
				Holophaga_sp.
				uncultured_soil
		uncultured	Thermoanaerobaculum uncultured	uncultured_bacterium metagenome
				metagenome
				uncultured_Acidobacteriaceae
				uncultured_Acidobacteriales
				uncultured_Acidobacteriales
				uncultured_bacterium
				uncultured_Candidatus
				uncultured_forest
Bacteria	Actinobacteriota	Vicinamibacteraceae 67-14	Vicinamibacteraceae 67-14	uncultured_Holophaga Acidobacteria_bacterium bacterium_enrichment
				metagenome
				Solirubrobacterales_bacterium
				uncultured_bacterium
		Acidothermaceae	Acidothermus	uncultured_Conexibacteraceae Actinomycetales_bacterium
				metagenome
				uncultured_Actinoallomurus
				uncultured_actinobacterium
				uncultured_bacterium
				uncultured_soil
				uncultured Thermomonosporaceae

Table C1	(Continued)
Table C1.	(Commueu)

Kingdom	Phylum	Family	Genus	Species
		Actinospicaceae	Actinospica	Actinospica_sp.
				Persicaria_minor
				uncultured_actinobacterium
		Catenulisporaceae	Catenulispora	uncultured_bacterium Catenulispora_acidiphila
		Demequinaceae Dermabacteraceae Dermacoccaceae	Demequina Brachybacterium Flexivirga	Catenulispora_sp. globuliformis tyrofermentans alba
		Frankiaceae	Jatrophihabitans	bacterium_Ellin6090
				Jatrophihabitans_sp.
				metagenome
				uncultured_actinobacterium
		Gaiellaceae Iamiaceae	Gaiella Iamia	uncultured_bacterium metagenome uncultured_Acidimicrobiales
		Ilumatobacteraceae	CL500-29_marine_group	Aquihabitans_daechungensis Hevea_brasiliensis
				metagenome
			Ilumatobacter	nonamiensis
			uncultured	uncultured_Actinomycetales
		IMCC26256	IMCC26256	uncultured_bacterium actinobacterium_BGR
				bacterium_enrichment
				uncultured_Acidimicrobiia
				uncultured_Acidothermaceae
				uncultured_actinobacterium
		Kineosporiaceae	Kineosporia	uncultured_Ferrimicrobium Kineosporia_aurantiaca
				uncultured_Kineosporiaceae
		MB-A2-108 Microbacteriaceae	Quadrisphaera MB-A2-108 Agrococcus	granulorum metagenome jejuensis
		Micromonosporaceae	Amnibacterium Actinocatenispora	uncultured_bacterium Actinocatenispora_rupis
			Dactylosporangium	uncultured_bacterium
		Microtrichaceae Microtrichales Nakamurellaceae	Luedemannella uncultured Microtrichales Nakamurella	metagenome uncultured_Acidimicrobiia metagenome uncultured_Nakamurellaceae
		Nocardiaceae Nocardioidaceae	Rhodococcus Marmoricola Nocardioides	uncultured_organism aerolatus uncultured_bacterium Kribbella_sn
			110000 0000000	dilutus
				ununus kalotolorar -
				nalotolerans
		Propionibacteriaceae	Aestuariimicrobium	uncultured_organism actinobacterium_MH3-4
			Friedmanniella	uncultured_bacterium
		Pseudonocardiaceae	uncultured Amycolatopsis	uncultured_endolithic balhimycina

Kingdom	Phylum	Family	Genus	Species
				saalfeldensis
			Pseudonocardia	spinosispora
		Solirubrobacteraceae	Conexibacter	uncultured_bacterium woesei
				Rubrobacterineae_bacterium
				uncultured_bacterium
				uncultured_Conexibacteraceae
			JCM_18997	Conexibacter_sp.
			uncultured	Phytoplasma_sp.
		Sporichthyaceae uncultured	uncultured uncultured	Solirubrobacterales_bacterium uncultured_bacterium bacterium_Ellin6515
				bacterium_Ellin6517
				Gaiella_sp.
				metagenome
				uncultured_Conexibacter
				uncultured_forest
Bacteria	Armatimonadota	Armatimonadales	Armatimonadales	uncultured_Pseudonocardiaceae uncultured_Armatimonadetes
			uncultured_bacterium	
				uncultured_Chloroflexi
				uncultured_eubacterium
		Chthonomonadaceae	Chthonomonas	uncultured_soil uncultured_Armatimonadetes
				uncultured_bacterium
		Chthonomonadales Fimbriimonadaceae	Chthonomonadales Fimbriimonadaceae	unidentified_marine uncultured_bacterium metagenome
		uncultured	uncultured	uncultured_bacterium metagenome
				uncultured_actinobacterium
Bacteria	Bacteroidota	37-13 AKYH767	37-13 AKYH767	uncultured_bacterium uncultured_Chitinophagaceae metagenome
		Chitinophagaceae	Arachidicoccus	uncultured_Bacteroidetes Arachidicoccus_sp.
			Aurantisolimonas	uncultured_bacterium
				uncultured_soil
			Chitinophaga	metagenome
			Dinghuibacter	silviterrae
			Edaphobaculum	uncultured_bacterium
				uncultured_Bacteroidetes
				uncultured_Chitinophaga
				uncultured_Chitinophagaceae
			Filimonas	Filimonas_sp.
			Heliimonas	uncultured_bacterium
			Niabella	hibiscisoli
			Niastella	koreensis
				metagenome

Kingdom	Phylum	Family	Genus	Species
			Parafilimonas	metagenome
				uncultured_bacterium
				uncultured_Bacteroidetes
			Pseudoflavitalea	metagenome
				uncultured_bacterium
			Puia	metagenome
			Rurimicrobium	uncultured_bacterium
			Segetibacter	aerophilus
				uncultured_bacterium
				uncultured_Chitinophagaceae
			Taibaiella	smilacinae
			Terrimonas	metagenome
			uncultured	Panacibacter_ginsenosidivorans
				Panacibacter_sp.
				uncultured_Chitinophaga
				uncultured_Ferruginibacter
		Crocinitomicaceae	UTBCD1 Fluviicola	uncultured_bacterium Fluviicola_sp.
		CWT_CU03-E12	CWT_CU03-E12	uncultured_bacterium bacterium_enrichment
				uncultured_bacterium
		Cytophagaceae	Cytophaga	uncultured_Bacteroidetes metagenome
				uncultured_bacterium
			Cytophagaceae	uncultured_bacterium
		env.OPS_17 FFCH9454 Flavobacteriaceae	Sporocytophaga env.OPS_17 FFCH9454 Flavobacterium	myxococcoides metagenome uncultured_bacterium hauense
				ceti
			Sediminicola	uncultured_Flavobacteriaceae
		Hymenobacteraceae	Vitellibacter Adhaeribacter	uncultured_Aequorivita uncultured_bacterium
			Hymenobacter	algoricola
				metagenome
				psychrophilus
				Siccationidurans_ginsengisoli
		Ignavibacteriaceae Kapabacteriales	Ignavibacterium Kapabacteriales	uncultured_bacterium uncultured_Chlorobi uncultured_bacterium
		KD3-93	KD3-93	uncultured_soil metagenome
		Microscillaceae	Chryseolinea	metagenome
				uncultured_bacterium
			Ohtaekwangia	metagenome
			OLB12	uncultured_bacterium
			uncultured	Algoriphagus_sp.
				Cytophagales_bacterium
				Musa_ABB

Kingdom	Phylum	Family	Genus	Species
				uncultured_Flammeovirgaceae uncultured_Flexibacter
		NS9_marine_group Paludibacteraceae Rhodothermaceae	NS9_marine_group Paludibacter Rubrivirga	uncultured_Flexibacteraceae metagenome uncultured_bacterium uncultured_bacterium
		S15-21 Saprospiraceae SB-5 SJA-28	uncultured S15-21 uncultured SB-5 SJA-28	uncultured_bacterium uncultured_bacterium wastewater_metagenome uncultured_soil uncultured_bacterium
		Sphingsbasteriasaa	Musilasinihastor	uncultured_Ignavibacterium
		Sphingobacteriaceae	Olivibacter	soli
			Padobactar	arcticus
			Teuobucier	heparinus
		Spirosomaceae	Dyadobacter	Sphingobacteriia_bacterium fermentans
				Dyadobacter_sp.
			Persicitalea	uncultured_bacterium
			Spirosoma	fluminis
				horti
				jeollabukense
				linguale
				luteum
				spitsbergense
				uncultured_bacterium
				unidentified
		uncultured	uncultured uncultured	uncultured_Bacteroidetes uncultured_bacterium
				uncultured_Bacteroidetes
Bacteria	Bdellovibrionota	Weeksellaceae Bdellovibrionaceae	Chryseobacterium OM27_clade	uncultured_Cyclobacteriaceae piscium uncultured_bacterium
		0319-0620	0319-0G20	marine_metagenome
		Bdellovibrionaceae	Bdellovibrio	uncuturea_gamma uncultured_Syntrophobacteraceae uncultured_bacterium
				unidentified
		Silvanigrellaceae	Silvanigrella	metagenome
		Oligoflexales	Oligoflexus	Thymallus_thymallus
Bacteria	Chloroflexi	A4b	A4b	uncultured_bacterium metagenome
				 uncultured_bacterium
				uncultured_soil
		AD3	AD3	wastewater_metagenome uncultured_Chloroflexi
		AKIW781	AKIW781	uncultured_soil uncultured_bacterium
		Anaerolineaceae	Leptolinea	uncultured_Kouleothrix uncultured_soil

Kingdom	Phylum	Family	Genus	Species
-		Anaerolineae	Anaerolineae	uncultured_Bellilinea
		C0119	<i>C0119</i>	uncultured_bacterium
		Fl. 1554	El., 1554	uncultured_Chloroflexi
		Elev-1554 JG30-KF-AS9	Elev-1554 JG30-KF-AS9	unculturea_bacterium Chloroflexi bacterium
				uncultured bacterium
		JG30-KF-CM45	JG30-KF-CM45	metagenome
				uncultured_bacterium
				uncultured_soil
				uncultured_Sphaerobacter
				uncultured_Thermomicrobium
		JG30-KF-CM66	JG30-KF-CM66	uncultured_Caldilinea
		Ktedonobacteraceae	1959-1	uncultured_bacterium
			Ktedonobacter	uncultured_Chloroflexi
			Thermosporothrix	uncultured_bacterium
			uncultured	uncultured_bacterium
				uncultured_soil
		Roseiflexaceae	uncultured	uncultured_bacterium
		5085 TK10	5085 TK10	metagenome metagenome
Bacteria	Cloacimonadota	PBS-18	PBS-18	uncultured_bacterium
Bacteria	Cyanobacteria	Gastranaerophilales	Gastranaerophilales	uncultured_bacterium
		Leptolyngbyaceae Nostocaceae	Leptolyngbya_PCC-6306 Anabaena_PCC-7122	Plectolyngbya_hodgsonu Anabaena_sp
		Obscuribacteraceae	Candidatus_Obscuribacter	uncultured_bacterium
			Obscuribacteraceae	metagenome
				uncultured_bacterium
		Sericytochromatia	Sericytochromatia	metagenome
		1 7		uncultured_bacterium
		Vampirovibrionaceae	Vampirovibrio	metagenome
				unculturea_bacterium
Bacteria	Deinococcota	Deinococcaceae	Vampirovibrionaceae Deinococcus	uncultured_bacterium bacterium_Ellin510
				aerolatus
				aerophilus
				alpinitundrae
				irradiatisoli
				persicinus
				radiomollis
				radioresistens
				ruber
				swuensis
Bacteria	Dependentiae	Babeliaceae Babeliales	Babeliaceae Babelialor	uncultured_bacterium
		UBA12409	UBA12409	uncultured bacterium
		Vermiphilaceae	Vermiphilaceae	uncultured_bacterium
				uncultured_Candidatus
Bacteria	Desulfobacterota	Desulfobulbaceae Desulforgering	uncultured Desulfativhahdium	uncultured_bacterium
		Syntrophorhabdaceae	Syntrophorhabdus	uncultured_bacterium uncultured_bacterium
				uncultured_delta
		uncultured	uncultured	uncultured_Dongia
Bacteria	Elusimicrobiota	Endomicrobiaceae	Endomicrobium	uncultured_bacterium

Table C1.	(Continued)
14010 011	Continueur

Kingdom	Phylum	Family	Genus	Species
		Lineage_IIa	Lineage_IIa	uncultured_actinobacterium
				uncultured_bacterium
Bacteria Bacteria	FCPU426 Fibrobacterota	Lineage_IIb Lineage_IV FCPU426 B122 Fibrobacteraceae	Lineage_IIb Lineage_IV FCPU426 B122 uncultured	uncultured_soil uncultured_bacterium uncultured_Termite uncultured_bacterium uncultured_bacterium bacterium_enrichment
				metagenome
			possible_genus_04	Juncus_effusus
				metagenome
Bacteria	Firmicutes	uncultured Alicyclobacillaceae	uncultured Tumebacillus	uncultured_bacterium uncultured_bacterium avium
		Bacillaceae	Bacillus	Tumebacillus_sp. murimartini
				psychrosaccharolyticus
		Caloramatoraceae Clostridia_vadinBB60_group Clostridiaceae	Fonticella Clostridia_vadinBB60_group Clostridium_sensu_stricto_12	thermoamylovorans uncultured_bacterium uncultured_bacterium Clostridium_akagii
			Clostridium_sensu_stricto_9	uncultured_Clostridium
		Erysipelotrichaceae	uncultured Turicibacter	uncultured_bacterium uncultured_bacterium
		Ethanoligenenaceae Hungateiclostridiaceae Lachnospiraceae Paenibacillaceae	ZOR0006 Incertae_Sedis uncultured Tyzzerella Paenibacillus	uncultured_organism uncultured_bacterium uncultured_Ruminococcaceae uncultured_Clostridium alvei
				daejeonensis
				kobensis
				lautus
				oryzae
				pectinilyticus
				populi
				turicensis
				uliginis
				xylanilyticus
		Planococcaceae Sporomusaceae	Domibacillus Dendrosporobacter	uncultured_actinobacterium metagenome unidentified_eubacterium
Bacteria Bacteria	GAL15 Gemmatimonadota	Thermoactinomycetaceae type_III uncultured GAL15 Gemmatimonadaceae	Pelosinus Pasteuria type_III uncultured GAL15 Gemmatimonas	uncultured_bacterium uncultured_Firmicutes uncultured_Mollicutes low_GC uncultured_Firmicutes bacterium_Ellin5290
				Gemmatimonadetes_bacterium
				uncultured_Gemmatimonadaceae
				uncultured_soil
			Roseisolibacter	Gemmatirosa_kalamazoonesis agri

Kingdom	Phylum	Family	Genus	Species
				uncultured_bacterium
				uncultured_forest
			uncultured	Gemmatimonadetes_bacterium
				metagenome
				uncultured_Alphaproteobacteria
Bacteria Bacteria	Methylomirabilota Myxococcota	Longimicrobiaceae Methylomirabilaceae Amb-16S-1034 BIrii41 Haliangiaceae	Longimicrobiaceae Sh765B-TzT-35 Amb-16S-1034 BIrii41 Haliangium	uncultured_proteobacterium uncultured_bacterium uncultured_bacterium uncultured_Polyangiaceae metagenome myxobacterium_AT3-03
				uncultured_Myxococcales
				uncultured_Nannocystineae
		mle1-27 Myxococcaceae	mle1-27 KD3-10	uncultured_proteobacterium uncultured_Polyangiaceae metagenome
				uncultured_bacterium
			uncultured	metagenome
		Nannocystaceae Phaselicystidaceae Polyangiaceae	Nannocystis Phaselicystis Aetherobacter	uncultured_delta unidentified uncultured_bacterium uncultured_bacterium
			Minicystis	uncultured_bacterium
			Pajaroellobacter	Sorangiineae_bacterium
				uncultured_bacterium
				uncultured_Polyangiaceae
		Sandaracinaceae	uncultured	uncultured_proteobacterium delta_proteobacterium
				metagenome
Bacteria	Nitrospirota	Nitrospiraceae	Nitrospira	uncultured_soil moscoviensis
				uncultured_Green
Bacteria Bacteria	Patescibacteria Planctomycetota	uncultured Parcubacteria Saccharimonadales BD7-11 CPla-3_termite_group	uncultured Parcubacteria Saccharimonadales BD7-11 CPla-3_termite_group	uncultured_Nitrospirales uncultured_bacterium uncultured_bacterium uncultured_bacterium uncultured_bacterium metagenome
				uncultured_bacterium
		Gemmataceae	Fimbriiglobus	uncultured_planctomycete Gemmata_sp.
				metagenome
				uncultured_bacterium
				uncultured_Gemmata
			Gemmata	bacterium_enrichment
				Gemmata_sp.
				metagenome
				uncultured_bacterium
				uncultured_Planctomycetaceae
				uncultured Planctomycetales

Kingdom	Phylum	Family	Genus	Species
				uncultured_planctomycete
				uncultured_soil
			uncultured	bacterium_enrichment
				metagenome
				Planctomycetaceae_bacterium
				planctomycete_WY69
				uncultured_bacterium
				uncultured_planctomycete
				uncultured_Planctomycetia
				uncultured_sludge
				uncultured_thermal
				uncultured_Zavarzinella
			Zavarzinella	uncultured_bacterium
				Zavarzinella_formosa
		Gimesiaceae	uncultured	uncultured_bacterium
		Isosphaeraceae	Aquisphaera	uncultured_bacterium
				uncultured_planctomycete
			Candidatus_Nostocoida	Candidatus_Nostocoida
				uncultured_bacterium
			Paludisphaera	metagenome
				Paludisphaera_borealis
				uncultured_bacterium
			Singulisphaera	Isosphaera_sp.
				acidiphila
				rosea
				uncultured_Planctomycetales
				uncultured_planctomycete
			Tundrisphaera	metagenome
				lichenicola
				uncultured_bacterium
				uncultured_eubacterium
				uncultured_Planctomycetaceae
				uncultured_planctomycete
			uncultured	Isosphaera_sp.
				metagenome
				uncultured_bacterium
				uncultured_Planctomycetaceae
				uncultured_Planctomycetales
		ОМ190	OM190	uncultured_Singulisphaera uncultured_bacterium
		Dhunianha	7.0	uncultured_soil
		Pnycispnaeraceae	I-ð	unculturea_bacterium
			Phycisphaera	uncultured_bacterium
			SM1AU2	metagenome
		Pirellulaceae	uncutturea Blastopirellula	metagenome marina
			Pir4_lineage	metagenome

Kingdom	Phylum	Family	Genus	Species
				uncultured_bacterium
				uncultured_Planctomycetaceae
				uncultured_Planctomycetales
				uncultured_soil
			Pirellula	agricultural_soil
				metagenome
				Pirellula_sp.
				Planctomycetes_bacterium
				uncultured_bacterium
				uncultured_Pasteuria
				uncultured_Planctomycetaceae
				uncultured_Planctomycetales
			Rhodopirellula	planctomycete str.
			1	uncultured Planctomycetaceae
			uncultured	 bacterium_enrichment
				metagenome
				uncultured bacterium
				uncultured Firmicutes
				uncultured Planctomycetaceae
				uncultured Planctomycetales
		Debision	Dimensionalism	uncultured_sludge
		Kubinisphäeraceae	Planciomicrobium	unculturea_bacterium
			SH-PL14	metagenome
				uncultured_bacterium
				uncultured_Planctomycetaceae
		Schlesneriaceae	Planctopirus	uncultured_planctomycete metagenome
				uncultured bacterium
			Schlesneria	metagenome
				uncultured bacterium
				uncultured planctomycete
				uncultured Schlesneria
		Tepidisphaeraceae	Tepidisphaeraceae	uncultured_bacterium
		uncultured	uncultured	uncultured_planctomycete agricultural_soil
				metagenome
				uncultured_bacterium
				uncultured_bacterium
				uncultured_Planctomyces
				uncultured_soil
		vadinHA49	vadinHA49	uncultured_bacterium
		WD2101_soil_group	WD2101_soil_group	Planctomycetales_bacterium
				uncultured_bacterium
				uncultured_eubacterium
				uncultured_Planctomycetaceae
				uncultured_Planctomycetales
				uncultured planctomycete

Kingdom	Phylum	Family	Genus	Species
Bacteria	Proteobacteria	A0839	A0839	uncultured_soil metagenome
				uncultured_Phyllobacteriaceae
		A21b	A21b	uncultured_proteobacterium uncultured_bacterium
		Acetobacteraceae	Acetobacteraceae	uncultured_soil Acidisphaera_sp.
			Acidicaldus	uncultured_forest
			Acidiphilium	metagenome
				uncultured_bacterium
				uncultured_eubacterium
				uncultured_soil
			Acidisoma	tundrae
				uncultured_bacterium
			Acidisphaera	metagenome
				uncultured_soil
			Acidocella	uncultured_bacterium
			Belnapia	uncultured_bacterium
			Rhodopila	uncultured_bacterium
			Rhodovastum	Acetobacteraceae_bacterium
				metagenome
				uncultured_bacterium
			Roseomonas	arctica
				lacus
				ludipueritiae
				uncultured_organism
			uncultured	metagenome
		Amb-16S-1323 Beijerinckiaceae	Amb-16S-1323 1174-901-12	uncultured_soil uncultured_bacterium Methyloferula_sp.
				Rhizobiales_bacterium
				uncultured_Alphaproteobacteria
				uncultured_bacterium
				uncultured_Rhizobiales
				uncultured_soil
			alphaI_cluster	Alsobacter_sp.
			Beijerinckiaceae	Chelatococcus_asaccharovorans
			Camelimonas	uncultured_bacterium
			Methylobacterium-Methylorubrum	Methylobacterium_adhaesivum
				Methylobacterium_aerolatum
				Methylobacterium_cerastii
				Methylobacterium_komagatae
				uncultured_organism
			Methylocapsa	uncultured_bacterium
			Methylocella	Methylocella_palustris
			Methylocystis	uncultured_bacterium
			Methylorosula	Beijerinckia_sp.

Kingdom	Phylum	Family	Genus	Species
				uncultured_bacterium
			Methylovirgula	uncultured_bacterium
			Psychroglaciecola	Psychroglaciecola_arctica
				uncultured_bacterium
			Rhodoblastus	uncultured_bacterium
			Roseiarcus	$uncultured_Alphaproteobacteria$
				uncultured_bacterium
			uncultured	Methylobacterium_sp.
				uncultured_bacterium
		Burkholderiaceae	Limnobacter	uncultured_forest metagenome
		Caulobacteraceae	Pandoraea Asticcacaulis	Burkholderia_sp. uncultured_bacterium
			Brevundimonas	abyssalis
			Caulobacter	uncultured_soil
			Phenylobacterium	Afipia_genosp.
			uncultured	uncultured_Alphaproteobacteria
				uncultured_bacterium
		CCD24 Cellvibrionaceae Comamonadaceae	CCD24 Cellvibrio Aquabacterium	uncultured_Caulobacteraceae metagenome uncultured_bacterium citratiphilum
		Coxiellaceae	Variovorax Coxiella	soli uncultured_bacterium
		Devosiaceae Diplorickettsiaceae	Devosia Aquicella	uncultured_Coxiellaceae neptuniae metagenome
				uncultured_bacterium
			uncultured	metagenome
				Rickettsiella_sp.
				uncultured_bacterium
		Dongiaceae	Dongia	uncultured_Coxiellaceae metagenome
		Elsteraceae	Aliidongia	uncultured_Rhodospirillaceae dinghuensis
		Geminicoccaceae	uncultured Candidatus_Alysiosphaera	uncultured_bacterium metagenome
			Candidatus_Alysiosphaera	uncultured_bacterium
		Granulosicoccaceae Holosporaceae Hyphomicrobiaceae	Geminicoccus Granulosicoccus uncultured Hyphomicrobium	uncultured_bacterium marinus uncultured_bacterium methylovorum
		Hyphomonadaceae	Hirschia	Hyphomicrobium_sp. metagenome
		Inquilinaceae	SWB02 Inquilinus	metagenome uncultured_Alphaproteobacteria
		JG36-TzT-191	JG36-TzT-191	uncultured_eubacterium uncultured_bacterium
		KF-JG30-C25	KF-JG30-C25	uncultured_proteobacterium uncultured_gamma

Kingdom	Phylum	Family	Genus	Species
		Labraceae	Labrys	uncultured_soil methylaminiphilus
				uncultured_Phyllobacteriaceae
		Legionellaceae Magnetospirillaceae Methyloligellaceae Methylomonadaceae Methylophilaceae	Legionella uncultured uncultured Crenothrix uncultured	Xanthobacteraceae_bacterium uncultured_bacterium uncultured_bacterium metagenome uncultured_bacterium metagenome
		Methylopilaceae Micropepsaceae	Methylotenera Albibacter Micropepsaceae	uncultured_bacterium Methylopila_sp. uncultured_Hyphomicrobiaceae
				uncultured_proteobacterium
			Micropepsis	uncultured_bacterium
			Rhizomicrobium	uncultured_bacterium
			uncultured	bacterium_enrichment
				metagenome
				uncultured_Acidobacterium
				uncultured_bacterium
				uncultured_Hyphomicrobiaceae
		Moraxellaceae	Psychrobacter	uncultured_proteobacterium alimentarius
		Nitrosomonadaceae	uncultured Ellin6067	metagenome alpha_proteobacterium
			GOUTA6	uncultured_bacterium
			IS-44	metagenome
			mle1-7	uncultured_Rhodocyclaceae
			MND1	metagenome
		Oxalobacteraceae Paracaedibacteraceae Pleomorphomonadaceae Pseudomonadaceae R7C24	uncultured Massilia Candidatus_Paracaedibacter uncultured Pseudomonas R7C24	metagenome albidiflava uncultured_bacterium uncultured_Rhizobiales marincola metagenome
		Revranellaceae	Revranella	uncultured_bacterium metagenome
		Rhizobiaceae	uncultured Mesorhizobium	uncultured_Alphaproteobacteria thiogangeticum
				Phyllobacteriaceae_bacterium
				Rhodospirillales_bacterium
		Rhizobiales_Incertae_Sedis	Tianweitania Alsobacter	uncultured_bacterium metallidurans
			Bauldia	metagenome
				uncultured_bacterium
				uncultured_Methylocystaceae
				uncultured_proteobacterium
			Nordella	uncultured_bacterium
			uncultured	metagenome
		Rhodanobacteraceae	Dokdonella	uncultured_Alphaproteobacteria uncultured_Dokdonella
			Dyella	uncultured_Dyella

Kingdom	Phylum	Family	Genus	Species
			Luteibacter	Luteibacter_sp.
			Mizugakiibacter	uncultured_Xanthomonadaceae
			Rhodanobacter	uncultured_Dokdonella
				uncultured_Rudaea
				uncultured_Xanthomonadaceae
			Rudaea	uncultured_gamma
		Rhodobacteraceae	Amaricoccus	tamworthensis
		Rhodocyclaceae Rhodomicrobiaceae Rhodospirillaceae SC-1-84	Uliginosibacterium Rhodomicrobium uncultured SC-I-84	uncultured_bacterium metagenome uncultured_Hyphomicrobiaceae uncultured_bacterium metagenome
				uncultured Nitrosomonadaceae
		Solimonadaceae	Alkanibacter	uncultured_soil gamma_proteobacterium
				metagenome
			Nevskia	Persicaria_minor
				uncultured_bacterium
			Polycyclovorans	uncultured_bacterium
				uncultured_Xanthomonadaceae
		Sphingomonadaceae	uncultured Altererythrobacter	uncultured_bacterium bacterium
				Porphyrobacter_mercurialis
				uncultured_Altererythrobacter
			Hephaestia	uncultured_bacterium
			Novosphingobium	rosa
				uncultured_soil
			Parablastomonas	uncultured_bacterium
			Plot4-2H12	uncultured_Sphingomonadaceae
			Polymorphobacter	uncultured_Sphingomonas
			Qipengyuania	uncultured_bacterium
			Rhizorhapis	Sphingomonas_gotjawalisoli
			Sphingobium	Sphingomonas_azotofornans
				sufflavum
			Sphingomonas	Afipia_sp.
				crusticola
				ginsengisoli
				oligoaromativorans
				panacis
				phyllosphaerae
				polyaromaticivorans
				uncultured_eubacterium
			Sphingopyxis	baekryungensis
				taejonensis
		Steroidobacteraceae	Steroidobacter	bacterium_MI-37
				bacterium_MI-8
			uncultured	Hypsibius_dujardini
				metagenome

Kingdom	Phylum	Family	Genus	Species
		Sutterellaceae	AAP99	metagenome
		uncultured	uncultured uncultured	uncultured_bacterium metagenome
				metagenome
				Rhodospirillaceae_bacterium
				uncultured_Alphaproteobacteria
				uncultured_bacterium
				uncultured_Bradyrhizobiaceae
				uncultured_forest
				uncultured_Hyphomicrobiaceae
				uncultured_proteobacterium
				uncultured_Rhizobiales
				uncultured_Rhodospirillaceae
		Unknown_Family	Acidibacter	uncultured_soil metagenome
				Steroidobacter_sp.
				uncultured_bacterium
				uncultured_gamma
				uncultured_proteobacterium
				uncultured_Steroidobacter
				$uncultured_Xanthomonadales$
			Candidatus_Berkiella	uncultured_Thiotrichales
				uncultured_bacterium
			$Candidatus_Ovatus bacter$	uncultured_bacterium
		WD260 Xanthobacteraceae	Unknown_Family WD260 Afipia	uncultured_soil uncultured_bacterium uncultured_Rhodopseudomonas
			Rhodoplanes	uncultured_Hyphomicrobiaceae
			uncultured	uncultured_Bradyrhizobiaceae
				uncultured_forest
		Xanthomonadaceae	Xanthobacteraceae Pseudoxanthomonas	Rhizobiales_bacterium Pseudomonas_sp.
			Stenotrophomonas	rhizophila
Bacteria	RCP2-54	Yersiniaceae RCP2-54	uncultured Serratia RCP2-54	uncultured_bacterium symbiotica uncultured_bacterium
				uncultured_Syntrophobacterales
Bacteria	Spirochaetota	Spirochaetaceae	Spirochaeta	Spirochaeta_sp.
			Spirochaeta_2	metagenome
Bacteria	Sumerlaeota	V2072-189E03 Sumerlaeaceae	uncultured V2072-189E03 Sumerlaea	uncultured_soil uncultured_Spirochaetales uncultured_Acidobacteria
				uncultured_bacterium
Bacteria	Verrucomicrobiota	Chthoniobacteraceae	Candidatus_Udaeobacter	uncultured_Desulfocaldus uncultured_Verrucomicrobia
			Chthoniobacter	bacterium_Ellin506
				bacterium_Ellin507
				uncultured_bacterium
				uncultured_soil

Kingdom	Phylum	Family	Genus	Species
				Verrucomicrobia_bacterium
			Chthoniobacteraceae	uncultured_bacterium
		Methylacidiphilaceae Omnitrophaceae Omnitrophales Opitutaceae Pedosphaeraceae	LD29 uncultured Candidatus_Omnitrophus Omnitrophales uncultured ADurb.Bin063-1	uncultured_bacterium uncultured_bacterium uncultured_planctomycete uncultured_planctomycete uncultured_bacterium uncultured_bacterium
			Ellin516	metagenome
				uncultured_bacterium
			Pedosphaera	uncultured_bacterium
			Pedosphaeraceae	bacterium_enrichment
			uncultured	bacterium_enrichment
		Puniceicoccaceae S-BQ2-57_soil_group Verrucomicrobiaceae	Cerasicoccus S-BQ2-57_soil_group Roseimicrobium	uncultured_bacterium uncultured_organism uncultured_Verrucomicrobia metagenome
		Xiphinematobacteraceae	uncultured Candidatus_Xiphinematobacter	uncultured_soil uncultured_Verrucomicrobia
Bacteria	WPS-2	WPS-2	WPS-2	uncultured_Xiphinematobacteriaceae hydrothermal_vent
				metagenome
				uncultured_bacterium
				uncultured_Firmicutes
Bacteria	WS2	WS2	WS2	uncultured_forest uncultured_soil

C.2 Fungi

Table C2. All fungal ASVs identified on Sable Island, NS in 2021 in alphabetical order.

Phylum	Family	Genus	Species
Ascomycota	Celotheliaceae	Celotheliaceae_gen_Incertae_sedis	Celotheliaceae_sp
	Clavicipitaceae	Clavicipitaceae_gen_Incertae_sedis	Clavicipitaceae_sp
		Metapochonia	suchlasporia
	Cyphellophoraceae	Cyphellophoraceae_gen_Incertae_sedis	Cyphellophoraceae_sp
	Herpotrichiellaceae	Exophiala	moniliae
			opportunistica
			quercina
	Hypocreaceae	Hypocreaceae_gen_Incertae_sedis	Hypocreaceae_sp
	Hypocreales_fam_Incertae_sedis	Hypocreales_gen_Incertae_sedis	Hypocreales_sp
	Ophiocordycipitaceae	Drechmeria	campanulata
		Hirsutella	rostrata
		Perennicordyceps	paracuboidea
	Orbiliales_fam_Incertae_sedis	Orbiliales_gen_Incertae_sedis	Orbiliales_sp
	Pezizales_fam_Incertae_sedis	Pezizales_gen_Incertae_sedis	Pezizales_sp

Phylum	Family	Genus	Species
		Coprotus	Coprotus_sp
	Pezizomycotina_fam_Incertae_sedis	Cytosporella	Cytosporella_sp
	Saccharomycetales_fam_Incertae_sedis	Candida	Candida_fluviatilis
	Taphrinaceae	Taphrina	inositophila
			Taphrina_sp
Basidiobolomycota	Basidiobolaceae	Basidiobolus	Basidiobolus_sp
Basidiomycota	Agaricaceae	Coprinus	foetidellus
			vosoustii
	Agaricales_fam_Incertae_sedis	Agaricales_gen_Incertae_sedis	Agaricales_sp
	Agaricomycetes_fam_Incertae_sedis	Agaricomycetes_gen_Incertae_sedis	Agaricomycetes_sp
	Agaricostilbales_fam_Incertae_sedis	Agaricostilbales_gen_Incertae_sedis	Agaricostilbales_sp
	Atheliaceae	Athelia	acrospora
			Athelia_sp
	Auriculariales_fam_Incertae_sedis	Auriculariales_gen_Incertae_sedis	Auriculariales_sp
		Oliveonia	Oliveonia_pauxilla
	Bolbitiaceae	Panaeolus	fimiputris
			papilionaceus
	Boletales_fam_Incertae_sedis	Hydnomerulius	pinastri
		Leucogyrophana	olivascens
	Buckleyzymaceae	Buckleyzyma	aurantiaca
	Byssocorticiaceae	Athelopsis	lembospora
	Camptobasidiaceae	Camptobasidium	Camptobasidium_sp
	Cantharellales_fam_Incertae_sedis	Cantharellales_gen_Incertae_sedis	Cantharellales_sp
	Ceratobasidiaceae	Ceratobasidiaceae_gen_Incertae_sedis	Ceratobasidiaceae_sp
		Ceratobasidium	Ceratobasidium_sp
		Ceratorhiza	rhizodes
		Thanatephorus	cucumeris
		Thanatephorus	Thanatephorus_sp
	Chionosphaeraceae	Ballistosporomyces	sasicola
		Kurtzmanomyces	Kurtzmanomyces_sp
	Chrysozymaceae	Bannozyma	yamatoana
		Chrysozyma	Chrysozyma_sp
		Chrysozymaceae_gen_Incertae_sedis	Chrysozymaceae_sp
		Oberwinklerozyma	silvestris
		Pseudohyphozyma	bogoriensis
		Slooffia	cresolica
		Slooffia	tsugae
	Classiculaceae	Classicula	sinensis
	Clathraceae	Camarophyllopsis	Camarophyllopsis_sp
		Clathrus	Clathrus_sp
		Clavaria	argillacea
			falcata

Phylum	Family	Genus	Species
			griseolilacina
			Clavaria_sp
			sphagnicola
		Clavariaceae_gen_Incertae_sedis	Clavariaceae_sp
		Clavulinopsis	corniculata
			fusiformis
			helvola
			laeticolor
			luteoalba
			Clavulinopsis_sp
		Ramariopsis	crocea
			flavescens
			Ramariopsis_sp
	Colacogloeaceae	Colacogloea	Colacogloea_sp
		Coleosporium	solidaginis
	Coniophoraceae	Coniophora	Coniophora_sp
	Corticiaceae	Laetisaria	Laetisaria_fuciformis
		Limonomyces	roseipellis
			Limonomyces_sp
	Cortinariaceae	Cortinarius	spilomeus
	Cryptococcaceae	Cryptococcus	watticus
	Cyphellaceae	Rectipilus	Rectipilus_sp
	Cystobasidiaceae	Cystobasidium	fimetarium
			minuta
			slooffiae
		Occultifur	externus
			kilbournensis
			mephitis
			Occultifur_sp
	Cystobasidiomycetes_fam_Incertae_sedis	Cystobasidiomycetes_gen_Incertae_sedis	Cystobasidiomycetes_sp
	Cystofilobasidiaceae	Cystofilobasidium	capitatum
	Entolomataceae	Clitopilus	baronii
			passeckerianus
			Clitopilus_sp
		Entocybe	nitida
		Entoloma	brunneosericeum
			conferendum
			cuspidiferum
			foliomarginatum
			lampropus
			longistriatum
			paraconferendum

Phylum	Family	Genus	Species
			pleopodium
			porphyrogriseum
			Entoloma_sp
		Inocephalus	murrayi
		Rhodocybe	cistetorum
	Entylomataceae	Entyloma	magnusii
	Eocronartiaceae	Eocronartium	Eocronartium_sp
	Erythrobasidiaceae	Erythrobasidium	hasegawae
	Erythrobasidiales_fam_Incertae_sedis	Erythrobasidiales_gen_Incertae_sedis	Erythrobasidiales_sp
	Exobasidiaceae	Exobasidium	rostrupii
	Filobasidiaceae	Filobasidium	globosum
			magnum
			Filobasidium_sp
			wieringae
		Goffeauzyma	agrionensis
		Goffeauzyma	gastrica
		Heterocephalacria	Heterocephalacria_sp
		Naganishia	diffluens
	Filobasidiales_fam_Incertae_sedis	Filobasidiales_gen_Incertae_sedis	Filobasidiales_sp
	Ganodermataceae	Ganoderma	applanatum
	Geastraceae	Nidulariopsis	iowensis
	Glomosporiaceae	Thecaphora	Thecaphora_sp
	GS29_fam_Incertae_sedis	GS29_gen_Incertae_sedis	GS29_sp
	Hydnaceae	Sistotrema	oblongisporum
			Sistotrema_sp
	Hydnodontaceae	Brevicellicium	Brevicellicium_sp
		Gliophorus	Gliophorus_sp
			irrigatus
			laetus
		Humidicutis	marginata
		Hygrocybe	cantharellus
			chlorophana
			flavescens
			helobia
			insipida
			miniata
			turunda
		Luellia	cystidiata
		Trechispora	caucasica
			invisitata
			Trechispora_sp
			stellulata

Phylum	Family	Genus	Species
			stevensonii
			verruculosa
	Hygrophoropsidaceae	Hygrophoropsis	aurantiaca
			Hygrophoropsis_sp
	Hymenochaetales_fam_Incertae_sedis	Hymenochaetales_gen_Incertae_sedis	Hymenochaetales_sp
	Hyphodermataceae	Hyphoderma	Hyphoderma_sp
	Kriegeriaceae	Phenoliferia	Phenoliferia_sp
	Kriegeriales_fam_Incertae_sedis	Kriegeriales_gen_Incertae_sedis	Kriegeriales_sp
	Leucosporidiaceae	Leucosporidium	creatinivorum
			fragarium
			golubevii
			Leucosporidium_sp
	Leucosporidiales_fam_Incertae_sedis	Leucosporidiales_gen_Incertae_sedis	Leucosporidiales_sp
	Lyophyllaceae	Sagaranella	Sagaranella_sp
	Marasmiaceae	Marasmius	curreyi
	Microbotryales_fam_Incertae_sedis	Microbotryales_gen_Incertae_sedis	Microbotryales_sp
	Microbotryomycetes_fam_Incertae_sedis	Curvibasidium	cygneicollum
		Microbotryomycetes_gen_Incertae_sedis	Microbotryomycetes_sp
	Microsporomycetaceae	Microsporomycetaceae_gen_Incertae_sedis	Microsporomycetaceae_sp
	Microstromatales_fam_Incertae_sedis	Pseudomicrostroma	phylloplanum
	Mrakiaceae	Mrakia	aquatica
			Mrakia_sp
	Mycenaceae	Mycena	abramsii
			albidolilacea
			filopes
			galericulata
			griseoviridis
			leptocephala
			metata
			pura
			rubromarginata
			sanguinolenta
			Mycena_sp
			stylobates
	Omphalotaceae	Collybiopsis	hasanskyensis
		Gymnopus	earleae
			impudicus
		Marasmiellus	paspali
		Mycetinis	scorodonius
		Rhodocollybia	Rhodocollybia_butyracea_fasema
	Phallaceae	Mutinus	ravenelii
			Mutinus_sp

Phylum	Family	Genus	Species
	Phallales_fam_Incertae_sedis	Phallales_gen_Incertae_sedis	Phallales_sp
	Piskurozymaceae	Piskurozyma	fildesensis
			silvicola
			Piskurozyma_sp
			taiwanensis
		Solicoccozyma	terricola
			zizaniae
	Platygloeales_fam_Incertae_sedis	Platygloeales_gen_Incertae_sedis	Platygloeales_sp
	Podoscyphaceae	Hypochnicium	Hypochnicium_sp
	Polyporales_fam_Incertae_sedis	Polyporales_gen_Incertae_sedis	Polyporales_sp
	Psathyrellaceae	Coprinellus	brevisetulosus
			curtus
		Coprinopsis	babosiae
			filamentifera
			pseudofriesii
			radiata
			Coprinopsis_sp
			villosa
		Parasola	misera
		Psathyrella	fimiseda
	Pucciniaceae	Puccinia	graminis
			striiformis
	Pucciniastraceae	Pucciniastraceae_gen_Incertae_sedis	Pucciniastraceae_sp
	Rhynchogastremataceae	Papiliotrema	fusca
	Rickenellaceae	Peniophorella	pallida
	Russulales_fam_Incertae_sedis	Russulales_gen_Incertae_sedis	Russulales_sp
	Sakaguchiaceae	Sakaguchia	dacryoidea
			Sakaguchia_sp
	Schizoporaceae	Lyomyces	Lyomyces_sp
	Serendipitaceae	Serendipita	Serendipita_sp
		Serendipita	herbamans
		Serendipita	vermifera
		Serendipitaceae_gen_Incertae_sedis	Serendipitaceae_sp
	Sphaerobolaceae	Sphaerobolus	ingoldii
			Sphaerobolus_sp
	Spiculogloeales_fam_Incertae_sedis	Meniscomyces	layueensis
	Sporidiobolaceae	Rhodotorula	graminis
			kratochvilovae
			mucilaginosa
			sphaerocarpa
		Sporidiobolaceae_gen_Incertae_sedis	Sporidiobolaceae_sp
		Sparidiabalus	salmonicolor

Phylum	Family	Genus	Species
		Sporobolomyces	roseus
	Sporidiobolales_fam_Incertae_sedis	Sporidiobolales_gen_Incertae_sedis	Sporidiobolales_sp
	Stephanosporaceae	Cristinia	helvetica
			Cristinia_sp
		Lindtneria	Lindtneria_sp
	Strophariaceae	Agrocybe	pediades
		Deconica	phillipsii
		Galerina	marginata
			Galerina_sp
			venenata
		Hypholoma	capnoides
			lateritium
		Protostropharia	semiglobata
		Psilocybe	semilanceata
	Symmetrosporaceae	Symmetrospora	symmetrica
			gracilis
	Thelephorales_fam_Incertae_sedis	Thelephorales_gen_Incertae_sedis	Thelephorales_sp
	Tilletiariaceae	Tilletiaria	anomala
	Trechisporales_fam_Incertae_sedis	Trechisporales_gen_Incertae_sedis	Trechisporales_sp
	Tremellales_fam_Incertae_sedis	Tremellales_gen_Incertae_sedis	Tremellales_sp
	Tremellodendropsidales_fam_Incertae_sedis	Tremellodendropsidales_gen_Incertae_sedis	Tremellodendropsidales_sp
	Tremellomycetes_fam_Incertae_sedis	Tremellomycetes_gen_Incertae_sedis	Tremellomycetes_sp
	Tricholomataceae	Ampulloclitocybe	clavipes
		Clitocybe	dealbata
		Delicatula	integrella
		Flagelloscypha	minutissima
			Flagelloscypha_sp
		Lachnella	Lachnella_sp
		Leucoinocybe	Leucoinocybe_sp
		Ripartites	Ripartites_sp
	Tubulicrinaceae	Butlerelfia	eustacei
	Typhulaceae	Typhula	Typhula_sp
	Ustilaginaceae	Farysia	Farysia_acheniorum
		Kalmanozyma	Kalmanozyma_fusiformata
		Moesziomyces	aphidis
			bullatus
		Sporisorium	graminicola
			Sporisorium_sp
		Tranzscheliella	yupeitaniae
		Ustilago	kummeri
			Ustilago_sp
	Ustilentylomataceae	Bauerago	Bauerago_sp

Phylum	Family	Genus	Species
		Ustilentyloma	graminis
			Ustilentyloma_sp
Calcarisporiellomycota	Calcarisporiellaceae	Calcarisporiella	Calcarisporiella_sp
Chytridiomycota	Alphamycetaceae	Betamyces	Betamyces_sp
	Chytridiomycota_fam_Incertae_sedis	Chytridiomycota_gen_Incertae_sedis	Chytridiomycota_sp
	Chytriomycetaceae	Rhizidium	Rhizidium_sp
	GS13_fam_Incertae_sedis	GS13_gen_Incertae_sedis	GS13_sp
	Powellomycetaceae	Fimicolochytrium	jonesii
			Fimicolochytrium_sp
		Geranomyces	variabilis
		Powellomyces	Powellomyces_sp
		Powellomycetaceae_gen_Incertae_sedis	Powellomycetaceae_sp
	Rhizophlyctidaceae	Rhizophlyctis	rosea
			Rhizophlyctis_sp
	Rhizophlyctidales_fam_Incertae_sedis	Rhizophlyctidales_gen_Incertae_sedis	Rhizophlyctidales_sp
	Rhizophydiaceae	Rhizophydiaceae_gen_Incertae_sedis	Rhizophydiaceae_sp
	Rhizophydiales_fam_Incertae_sedis	Rhizophydiales_gen_Incertae_sedis	Rhizophydiales_sp
	Sonoraphlyctidaceae	Sonoraphlyctis	ranzonii
			Sonoraphlyctis_sp
	Spizellomycetaceae	Spizellomyces	pseudodichotomus
	Spizellomycetales_fam_Incertae_sedis	Spizellomycetales_gen_Incertae_sedis	Spizellomycetales_sp
Fungi_phy_Incertae_sedis	Fungi_fam_Incertae_sedis	Fungi_gen_Incertae_sedis	Fungi_sp
Glomeromycota	Acaulosporaceae	Acaulospora	lacunosa
	Archaeosporaceae	Archaeospora	ecuadoriana
			trappei
	Archaeosporales_fam_Incertae_sedis	Archaeosporales_gen_Incertae_sedis	Archaeosporales_sp
	Claroideoglomeraceae	Claroideoglomus	Claroideoglomus_sp
	Glomeraceae	Glomeraceae_gen_Incertae_sedis	Glomeraceae_sp
		Rhizoglomus	vesiculiferum
		Rhizophagus	intraradices
			irregularis
			Rhizophagus_sp
	Glomeromycota_fam_Incertae_sedis	Glomeromycota_gen_Incertae_sedis	Glomeromycota_sp
Monoblepharomycota	Harpochytriaceae	Harpochytrium	Harpochytrium_sp
	Monoblepharidales_fam_Incertae_sedis	Monoblepharidales_gen_Incertae_sedis	Monoblepharidales_sp
Mortierellomycota	Mortierellaceae	Dissophora	globulifera
		Entomortierella	beljakovae
		Gryganskiella	cystojenkinii
		Linnemannia	amoeboidea
			hyalina
			zychae
		Mortierella	alliacea

Phylum	Family	Genus	Species
			alpina
			angusta
			basiparvispora
			echinula
			elongatula
			exigua
			fluviae
			formicae
			gemmifera
			macrocystis
			pulchella
			sarnyensis
			Mortierella_sp
			tsukubaensis
			turficola
		Mortierellaceae_gen_Incertae_sedis	Mortierellaceae_sp
		Podila	humilis
			minutissima
	Mortierellales_fam_Incertae_sedis	Mortierellales_gen_Incertae_sedis	Mortierellales_sp
Mucoromycota	Cunninghamellaceae	Absidia	glauca
			Absidia_sp
		Cunninghamella	elegans
	Endogonales_fam_Incertae_sedis	Endogonales_gen_Incertae_sedis	Endogonales_sp
	GS22_fam_Incertae_sedis	GS22_gen_Incertae_sedis	GS22_sp
	Umbelopsidaceae	Umbelopsis	isabellina
			ramanniana
Olpidiomycota	Olpidiomycota_fam_Incertae_sedis	Olpidiomycota_gen_Incertae_sedis	Olpidiomycota_sp
Rozellomycota	GS05 fam Incertae sedis	GS05 gen Incertae sedis	GS05 sp