Science of the Total Environment 788 (2021) 147693

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Diversity of microbial communities and genes involved in nitrous oxide emissions in Antarctic soils impacted by marine animals as revealed by metagenomics and 100 metagenome-assembled genomes

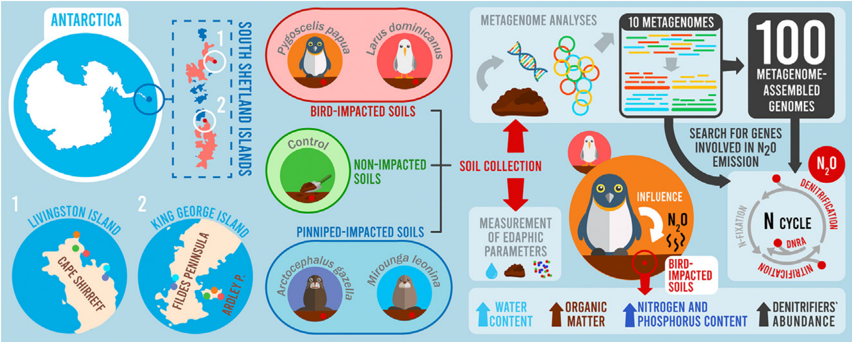
Lia Ramírez-Fernández [a], Luis H. Orellana [b,1], Eric R. Johnston [b,2], Konstantinos T. Konstantinidis [b,c], Julieta Orlando [a,*]

[a] Laboratorio de Ecología Microbiana, Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile, Santiago, Chile
[b] School of Civil and Environmental Engineering, Georgia Institute of Technology, Atlanta, GA, USA
[c] School of Biological Sciences, Georgia Institute of Technology, Atlanta, GA, USA

HIGHLIGHTS

- Marine animals, birds and pinnipeds, enrich soil nutrients in ice-free coastal lands.
- We assessed microbial diversity by shotgun metagenomics and population genome binning.
- Animal-impacted soils from Antarctica have a novelty microbial diversity.
- *Rhodanobacter* is one of the most abundant genera in soils impacted by marine animals.
- Denitrification genes are highly abundant in bird-impacted soils from Antarctica.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 23 January 2021
Received in revised form 2 April 2021
Accepted 7 May 2021
Available online 12 May 2021

Editor: Frederic Coulon

Keywords:
King George Island
Livingston Island
Animal-impacted soils
Soil microbial diversity
Nitrous oxide emission
Rhodanobacter

ABSTRACT

Antarctic soils generally have low temperatures and limited availability of liquid water and nutrients. However, animals can increase the nutrient availability of ice-free areas by transferring nutrients from marine to terrestrial ecosystems, mainly through their excreta. In this study, we employed shotgun metagenomics and population genome binning techniques to study the diversity of microbial communities in Antarctic soils impacted by marine pinnipeds and birds relative to soils with no evident animal presence. We obtained ~285,000 16S rRNA gene-carrying metagenomic reads representing ~60 phyla and 100 metagenome-assembled genomes (MAGs) representing eight phyla. Only nine of these 100 MAGs represented previously described species, revealing that these soils harbor extensive novel diversity. *Proteobacteria*, *Actinobacteria*, and *Bacteroidetes* were the most abundant phyla in all samples, with *Rhodanobacter* being one of the most abundant genera in the bird-impacted soils. Further, the relative abundance of genes related to denitrification was at least double in soils impacted by birds than soils without animal influence. These results advance our understanding of the microbial populations and their genes involved in nitrous oxide emissions in ice-free coastal Antarctic soils impacted by marine animals and reveal novel microbial diversity associated with these ecosystems.

---

[*] Corresponding author at: Laboratorio de Ecología Microbiana, Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile. Las Palmeras 3425, Ñuñoa, Santiago, Chile.

E-mail address: jorlando@uchile.cl (J. Orlando).

[1] Present address: Laboratorio de Enteropatógenos, Programa de Microbiología y Micología, ICBM, Facultad de Medicina, Universidad de Chile, Chile.

[2] Present address: Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, TN, USA.

https://doi.org/10.1016/j.scitotenv.2021.147693

1. Introduction

Antarctic soils are among the driest, coldest, and most oligotrophic terrestrial environments on Earth (Cary et al., 2010). However, ice-free coastal areas are usually colonized by a wide variety of marine animals. The interaction between the animals and the terrestrial ecosystem allows the formation of soils with high amounts of nutrients relative to Antarctic soils unaffected by marine animals (Ugolini, 1972).

Marine animals, primarily pinnipeds and birds, transfer significant amounts of nutrients from the marine to the terrestrial ecosystem (Bokhorst et al., 2007) through their excreta, eggs, prey, and carcasses (Nie et al., 2012; Shravage et al., 2007). Depositions from marine vertebrates strongly influence the soil physicochemical properties (Liguang et al., 2004; Myrcha and Tatur, 1991; Schaefer et al., 2008), forming terrestrial ecosystems enriched in nutrients (Sun et al., 2002; Zhu et al., 2012, 2009). The impact of these depositions can be observed over a few kilometers inland and strongly influence terrestrial biodiversity (Bokhorst et al., 2019). These soils are described as the largest carbon and nitrogen reservoirs in Antarctic terrestrial ecosystems (Simas et al., 2007). Nitrogen is added to the soils mainly by urine depositions, enriched in uric acid and urea from seabirds and pinnipeds, respectively, although uric acid waste is often barely detected in penguins rookery deposits because it is rapidly degraded to ammonium (Lindeboom, 1984).

Recent studies have reported that marine animals influence edaphic factors such as pH, organic matter, phosphorus and nitrogen contents, and the diversity and composition of soil microbial communities in Antarctic soils (Guo et al., 2018; Kim et al., 2012; Ramírez-Fernández et al., 2019; Wang et al., 2015; Yew et al., 2018). For instance, our team has previously shown that animal-impacted soils in Cape Shirreff (Livingston Island, Maritime Antarctica) were dominated by bacteria belonging to the *Gammaproteobacteria* class, particularly the genus *Rhodanobacter*, followed by *Bacteroidetes*, *Actinobacteria*, and *Chloroflexi* phyla. In contrast, soils without animal influence were dominated by members of the *Acidobacteria* and *Gemmatimonadetes* phyla (Ramírez-Fernández et al., 2019). However, these previous 16S rRNA gene-based surveys result in limited information at the species and subspecies levels as well as the functional gene content of the sampled microbial communities (Cole et al., 2010; Yarza et al., 2014).

One of the most relevant impacts of marine animals in Antarctica is the higher N_2O emissions from the soil of their colonies in coastal terrestrial ecosystems (Bao et al., 2018; Neufeld et al., 2015; Zhu et al., 2013, 2012, 2009). N_2O is an ozone-depleting greenhouse gas, with an effect ~200–300 times higher than CO_2 (Khalil et al., 2002), which can be produced abiotically as well as by different microbial nitrogen cycling pathways: denitrification, nitrification, and dissimilatory nitrate reduction to ammonium (DNRA) (Butterbach-Bahl et al., 2013). High N_2O emissions have been documented in penguin-, seal- and skua-impacted soils in Antarctica (Neufeld et al., 2015; Zhu et al., 2013). These emissions are comparable with environments considered as hotspots of N_2O emissions, such as artic cryoturbated peat soil (Repo et al., 2009), farmyards used by livestock (Misselbrook et al., 2001), and heavily fertilized agricultural systems and tropical forests (Pérez et al., 2001; Xing and Zhu, 1997). However, the genomes encoding these nitrogen cycling genes, their taxonomy, and their spatial distribution in Antarctic soils remain elusive.

Despite this apparent relationship between animal presence and increased N_2O emissions, few studies have investigated the abundance and diversity of the microbial pathways involved in N_2O emissions from animal-impacted Antarctic terrestrial ecosystems. For example, Jung et al. (2011) found that nitrogen addition altered the abundance of microbial nitrogen cycling genes, significantly increasing the abundance of the *nirS* and *nirK* nitrate reductase genes of the denitrification pathway. Similarly, denitrification rates and the abundance of *nirS* and *nirK* genes were found to be higher in animal-impacted Antarctic soils than tundra soils (Dai et al., 2020). Furthermore, ammonia-oxidizing bacteria predominated over archaea in animal-impacted soils, suggesting a more important role for bacteria in the nitrogen-loss pathways in Antarctic soils (Wang et al., 2019). Finally, although DNRA is a common pathway found in soil metagenomes (Nelson et al., 2016; Rütting et al., 2011), it is still considered one of the least understood nitrogen cycle processes (Kuypers et al., 2018). To the best of our knowledge, no previous studies have evaluated the effects of animal presence on the DNRA gene abundance in Antarctic soils.

Climate change models predict warmer temperatures and higher weather variability in Antarctica, with increased precipitation and altered freeze-thaw patterns (Hoegh-Guldberg et al., 2018). Indeed, the glacier retreat in the Antarctic Peninsula has accelerated (Konrad et al., 2018), which is also evident in its adjacent islands. These islands in the Antarctic Peninsula region are reported as belonging to different climate zones than the rest of the continent, with higher temperatures and precipitation levels (Wagner et al., 2018). These characteristics make the islands to be highly relevant ecosystems to study the impact of climate change. The increased population sizes of penguins and pinnipeds on the islands due to the expansion of ice-free lands on one hand (Clucas et al., 2016; Siniff et al., 2008; Watanabe et al., 2020), and the rising of the nutrient available to soil microorganisms through the freeze-thaw cycles on the other hand (Prieme and Christensen, 2001; Teepe et al., 2001), are expected to stimulate the microbial N_2O emissions.

In this study, we employed shotgun metagenome sequencing to provide novel insights into the taxonomy and functional gene diversity of the most abundant and prevalent microbial populations in Antarctic coastal soils. Moreover, the genomes of the predominant microbial populations were recovered as metagenome-assembled genomes (MAGs) through assembly and binning techniques (Parks et al., 2017; Tyson et al., 2004), allowing us to link specific gene functions to individual populations. The specific questions that this study aimed to address are: what is the relative importance (abundance) of the pathways potentially involved in N_2O emissions (denitrification, nitrification, or DNRA) in animal-impacted soils vs. non-impacted soils; and which are the specific taxa that carry out these pathways and their distribution in the sampled sites. To this end, we compared the diversity and distribution of animal-impacted and non-impacted soil microbial communities from ice-free regions of two islands in the South Shetland Archipelago in Maritime Antarctica, Livingston Island and King George Island, with emphasis on microbial populations involved in nitrogen cycling pathways related to N_2O emissions.

2. Material and methods

2.1. Sampling sites description and samples collection

Animal-impacted soil samples were collected during the austral summer of 2016 (52^{nd} Antarctic Scientific Expedition coordinated by the Instituto Antártico Chileno) at two sampling sites: i) King George Island (KGI), specifically at Fildes Peninsula and Ardley Peninsula, and ii) Livingston Island (LI), specifically at Cape Shirreff. These areas are categorized as "Antarctic Special Protected Areas" (ASPA) (ASPA-125, 2009; ASPA-149, 2009; ASPA-150, 2009). KGI is the largest island of the South Shetland Archipelago, with a surface of 1400 km^2 and only 5% of ice-free areas. At the southeast of KGI, Fildes Peninsula and Ardley Peninsula are connected by an isthmus. LI is the second-largest island of the South Shetland Archipelago, with a surface of 828 km^2 and 16% of ice-free areas, including Cape Shirreff.

During the summer sampling season, at both sampling sites, the temperature ranged from −1 °C to 1 °C, and the average precipitation was 56 mm and 62 mm for KGI and LI, respectively (Kummu et al., 2018). Soils from Fildes Peninsula and Ardley Peninsula are classified as turbic cryosols with slightly acidic to neutral pH (Michel et al., 2014), while those from Cape Shirreff are porous and consist mainly of volcanic ash and scoria (Smellie et al., 1996).

In KGI, the pinniped-impacted soil samples were collected from a Northwest beach from Fildes Peninsula, where we found small to medium colonies of elephant-seals and fur-seals (62°11′00″S 58°59′17″W). The bird-impacted soil samples were collected in a North beach from Ardley Peninsula (62°12′34″S 58°56′00″W), which is characterized by a high abundance of penguins of different species (*Pygoscelis* spp.) and other birds like kelp gulls. In LI, the bird-impacted soil samples were collected in the North region of Cape Shirreff called Bahamonde Beach (62°29′51″S 60°43′00″W), specifically in areas occupied by penguin and kelp gull colonies. Meanwhile, closest to Modulo Beach (62°32′17″S 60°41′30″W), the pinniped-impacted samples were collected from areas occupied by colonies of seals and sea-lions. Further, three soil samples without the visible impact of plants or animals were collected in both KGI (62°12′41″S 58°56′22″W) and LI (62°31′17″S 60°42′59″W) (Fig. 1).

We established three 1 m × 1 m squares as close as possible to the animal colonies and with no more than 5 m between adjacent squares for each sampling site. Inside each square, we randomly collected five soil samples. Although there was a mixture of animals at each sampling point, the samples were named according to the animal species more commonly observed in the area. Therefore, bird-impacted soil samples were called Ld (*Larus dominicanus*) and Pp (*Pygoscelis papua*), and pinniped-impacted soil samples were called Ml (*Mirounga leonina*) and Ag (*Arctocephalus gazella*). All names also included the initials of the sampling location, KGI (King George Island) and LI (Livingston Island), in addition to the initials of the prevailing animal at the time of sampling. The soil samples from the *M. leonina* settlement in King George Island (Ml_KGI) were collected further away from the animal colony than the other samples since it was not safe to get closer to the animals during the sampling. The samples with no visible presence of plants or animals were designed as Ct (Control) and collected at least 1 km apart from the animal colonies to reduce the likelihood of animal influence.

Sterile shovels and tubes were used for sampling the top 10 cm of soil, and the samples were stored in hermetically sealed bags and maintained at 4 °C for about two weeks. In the laboratory, the samples were homogenized, sieved through a 2 mm mesh, and stored at −20 °C until further analyses. For edaphic measurements, we combined the five samples from each square; therefore, three biological replicates (sampling squares) were available from each sample type. For metagenomics analysis, we combined the three biological replicates for each sample type, giving a total of 10 sequenced samples. Further, two technical replicate samples from the same DNA extraction of sample Pp_LI were independently sequenced for validation purposes and quality control.

2.2. Edaphic factors

For each soil sample, the following edaphic factors were measured: pH, water content (WC), organic matter (OM), nitrogen content in ammonium ($N\text{-}NH_4^+$), nitrogen content in nitrate ($N\text{-}NO_3^-$), and bioavailable phosphorus content (P-Bray) (Sadzawka et al., 2004). The pH was determined potentiometrically from 1:10 (*w*/*v*) soil in KCl 1 M extracts using a pH electrode connected to a pH 500 meter (Oakton Instruments, Vernon Hills, IL, USA). The water content (WC) was determined from the mass of soil samples before (moist mass, MM) and after (dry mass, DM)

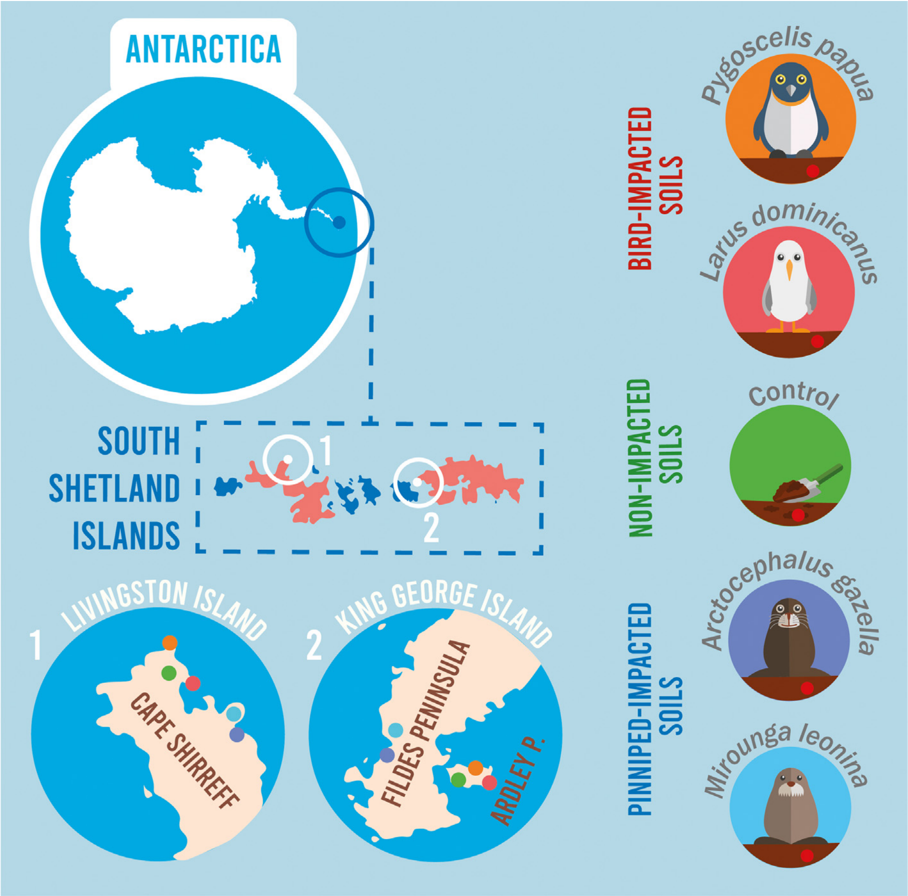


Fig. 1. Location of the sampling sites at South Shetland Archipelago, Antarctica. Samples were collected at Fildes Peninsula and Ardley Peninsula in King George Island and Cape Shirreff in Livingston Island. Colored points show sampling sites from where samples of animal-impacted soils and soils without animal influence were taken. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

drying at 65 °C for 24 h according to WC = ((MM-DM)/DM) ∗ 100. The organic matter content (OM) was determined from the mass of the dry soil samples calcined at 400 °C for 16 h (calcined mass, CM) multiplied by a factor of 0.8 according to OM = (((DM-CM)/DM) ∗ 100) ∗ 0.8. The nitrogen content in ammonium (N-NH_4^+) was determined from 1:10 (*w*/*v*) soil in water extracts using a selective ion electrode connected to an Ion 510 meter (Oakton Instruments, Vernon Hills, IL, USA). The nitrogen content in nitrate (N-NO_3^-) was determined by a colorimetric method involving electrophilic aromatic substitution (nitration) between nitronium and salicylate (Yang et al., 1988), and measuring its absorbance at 410 nm using an Epoch microplate reader (BioTek, Winooski, VT, USA). The phosphorus was extracted using the Bray 1 method (Bray and Kurtz, 1945) and quantified by the absorbance at 880 nm of the phospho-antimonyl-molybdenum blue complex using an Epoch microplate reader (BioTek, Winooski, VT, USA).

2.3. DNA extraction and shotgun sequencing

DNA was extracted from 0.25 g of soil from each sample using the PowerSoil™ DNA Isolation kit (previously MoBio, now Qiagen, Hilden, Germany) according to the manufacturer's instructions. The DNA was eluted in 50 μl TE buffer (10 mM Tris-HCl [pH 8.0], 1 mM EDTA) and stored at −20 °C until analysis. The DNA concentration was determined by the Pico Green dsDNA assay (Invitrogen, Carlsbad, CA, USA) using a Victor 3 fluorometer (PerkinElmer, Billerica, MA, USA), and the DNA quality was determined electrophoretically in a 1% (w/v) agarose gel running for 30 min at 100 V. The library for DNA sequencing was prepared using the TruSeq Nano DNA Kit (Illumina, San Diego, CA, USA) according to the manufacturer's instructions. The libraries were run in two lanes of Illumina HiSeq 2500 with the 2 × 150 bp paired-end format. Sequencing was performed by Macrogen Inc. (Seoul, South Korea). The reads, assemblies, and MAGs obtained from this study were submitted to the European Nucleotide Archive (ENA) database with the study accession number PRJEB34461.

2.4. Short reads assembly and analysis

The sequences were trimmed using the scripts of the enveomics collection (Rodriguez-R and Konstantinidis, 2016). Sequences with quality equal or higher of 20 Phred score for each base position were trimmed using the SolexaQA software (Cox et al., 2010). Only trimmed sequences longer than 50 bp were considered for further analysis. The reads were assembled using IDBA_UD v1.1.1 and default settings (Peng et al., 2012). Nonpareil 2.0 (Rodriguez-R and Konstantinidis, 2014a) was used with default settings to estimate the coverage of the microbial community achieved by the sequencing effort applied.

The recovery and identification of 16S rRNA gene fragments were performed using Parallel-META v2.1 (Su et al., 2012). Subsequently, the sequences were assigned to the SILVA database v1.1.1 (Quast et al., 2013) using the closed-reference picking strategy as implemented by QIIME 1.9.1 (Caporaso et al., 2010) and, subsequently, the matching reference sequences were clustered in operational taxonomic units (OTUs) at a nucleotide identity level ≥ 97% using UCLUST (Edgar, 2010).

2.5. MAGs recovery and analysis

Contigs longer than 2000 bp were used for genome binning using MaxBin v2.1.1 (Wu et al., 2014). It is important to mention that no dereplication and no co-assembly were performed, but each metagenome was binned on its own. Taxonomic classification and novelty of each MAG were determined by the genome-aggregate average amino-acid identity (AAI) analysis against the genomes in the NCBI RefSeq database using the Microbial Genomes Atlas (MiGA) webserver (Rodriguez-R et al., 2018a). The *p*-value of the taxonomic assignment was estimated from the empirical distribution observed of AAI values among all the reference genomes at the same taxonomic level. Taxonomic assignment using a $p \leq 0.01$ as the threshold was used for most analysis. Completeness, contamination, and overall quality (completeness – 5× contamination) were determined using the "HMM.essential.rb" script of the enveomics collection. The relative abundance of MAGs of each sample was estimated as the truncated average sequencing depth (TAD80) according to Rodriguez-R et al. (2020) using the matching reads from a competitive search using Magic-BLAST (Boratyn et al., 2019) (95% minimum nucleotide identity and >100 bp minimum read length for a read match) normalized by genome equivalents (Nayfach and Pollard, 2015). This approach allows, on one hand, the removal of the top and bottom 10% positions in terms of sequencing depth to exclude highly conserved or mobile regions of the genome, and on the other hand, to minimize the influence of differences in average genome size between samples by normalizing by genome equivalents. For identification of MAGs representing the same genomospecies (gsp), we performed Average Nucleotide Identity (ANI) pairwise comparations between all recovered MAG with the "ani.rb" script of the enveomics collection. MAGs with >95% ANI and sharing at least 30% of their gene content were assigned to the same genomospecies (Konstantinidis and Tiedje, 2005).

The recruitment of reads against MAG #52 (see Results section) to assess intra- and inter-population sequence diversity was performed as previously described (Konstantinidis and Delong, 2008). Briefly, short reads from the metagenomes were searched against each MAG using megablast v2.2.28 (-e-value $1e^{-11}$, -max_target_seqs 250). Matches ≥75% nucleotide identity and ≥70 bp were retained for the generation of the recruitment plot. In addition, we examined the relative abundance of MAG #52 in other available metagenomes from distant places using the same approach. For this, we used agricultural soil metagenomes affected by annual nitrogen fertilization in the USA (Orellana et al., 2014) and ice-free soils with low organic matter from East Antarctica (Ji et al., 2017).

2.6. Identification of protein-encoding genes and nitrogen cycling pathways

Protein-encoding genes were identified in non-binned contigs longer than 500 bp or MAG sequences using Prodigal (Hyatt et al., 2010) with default parameters. The functional annotation of the resulting amino acid sequences was performed based on a Diamond v0.7.9.58 search (Buchfink et al., 2014), with the Blastx searching option and default parameters, against the Swiss-Prot database (Uniprot-Consortium, 2015). The output was filtered for the best match only when a bit score higher than 60 was obtained. The predicted genes were further functionally annotated using the SEED database subsystem categories (Overbeek et al., 2005).

The identification of genes in the metagenomic reads was performed using the trimmed reads as the query for Blastx, with default parameters, against a curated *in-house* database. In particular, the previously identified and verified reference sequences used in ROCker models (Orellana et al., 2017) for the bacterial ammonium monooxygenase (*amoA*), hydroxylamine oxidoreductase (*hao*), nitrate reductase (*narG*), nitrite reductase (*nirK*), nitrous oxide reductase (*nosZ*), and nitrite reductase (*nrfA*), along with the gene for the β subunit of the RNA polymerase (*rpoB*) were used as the *in-house* protein databases. The resulting blastx output from the search of metagenomic reads against the *in-house* database was filtered using ROCker models (Orellana et al., 2017). The relative abundance of each gene was determined as genome equivalents by calculating the ratio between normalized target reads (read counts divided by median protein length) against the number of identified *rpoB* reads (read counts divided by median *rpoB* protein length), which is typically a single-copy gene in bacterial genomes. Gene databases and ROCker models used are available through http://enve-omics.ce.gatech.edu/rocker/.

2.7. Statistical analyses

Statistically significant differences between the samples in terms of edaphic factors, diversity indices, and relative abundance of nitrogen cycle genes measurements were determined by pairwise comparisons using Dunn's test of multiple comparisons (Dunn, 1964) following a Kruskal-Wallis test with p-values adjusted with the Benjamini-Hochberg method in R v3.2.3 (R Core Team, 2019). A raw (not-normalized) counts table was used to perform an internal normalization, where geometric mean is calculated for each functional subsystem category and OTU, to assess the significance of differences in their abundances between animal-impacted samples vs. samples without animal influence using the DESeq2 package (Love et al., 2014) in R v3.4.2.

The distances among the whole metagenomes (read-level) were calculated using the software MASH (v1.0.2), with default settings (Ondov et al., 2016). MASH distances were plotted in a non-metric multidimensional scaling (NMDS) ordination plot using the function metaMDS from the vegan package (Oksanen, 2017) in R v3.4.2. Subsequently, the function envfit was used to fit the environmental factors (median values for each type of soil sample) onto the ordination, calculating the significance of the fitted vectors using 1000 permutations in the Monte Carlo test (Robinson, 2007). Finally, pairwise comparisons between MASH distances were made using PERMANOVA analysis with the function adonis.pair.

3. Results

3.1. Description of the edaphic factors and metagenomes from Antarctic soils

The large variation (i.e., standard deviation) in values of the measured edaphic factor among the samples indicated high spatial heterogeneity (Table 1). Despite the high variation, we observed significant higher values (Kruskal-Wallis test, Dunn *posthoc* test) when comparing bird-impacted soils with pinniped-impacted soils and soils without animal influence for water content ($p = 9.4\text{e}-4$ and $p = 6.6\text{e}-04$, respectively), organic matter content ($p = 3.4\text{e}-4$ and $p = 8.5\text{e}-04$, respectively) and phosphorus content ($p = 3.9\text{e}-3$ and $p = 6.1\text{e}-05$, respectively). Further, nitrogen content as nitrate was higher in bird-impacted soils than in soils without animal influence ($p = 4.5\text{e}-3$), and nitrogen content as ammonium was significantly different in the three types of soil samples (Table 1).

In total, ten metagenomes, representing pooled samples from each sampling site (i.e., three replicate samples pooled), were obtained in this study; two technical replicates from Pp_LI sample were also independently sequenced for quality control. Metagenomes ranged in size from 50 to 80 million reads per sample (7.7 to 12.1 Gbp), with an average read length of ~145 bp after quality trimming. An average of ~8% of the reads from each metagenomic dataset was recruited by MAGs (Supplementary material Table A1), indicating, as probably expected, highly diverse microbial communities (see also next section).

We compared microbial communities from soils impacted by different types of animals and from different islands, taking into account the (measured) environmental parameters that could drive microbial community diversity patterns. In the NMDS analysis, based on MASH distances of whole communities/metagenomes, animal-impacted soils were separated from soils without animal influence (except for sample Ml_KGI, data not shown). However, the differences in MASH distances among microbial communities from soils without animal influence, pinniped-impacted soils, and bird-impacted soils were not statistically significant according to PERMANOVA (Supplementary material Table A2), presumably due to the relatively small number of samples. Furthermore, the microbial composition variation was not significantly explained by the measured edaphic variables according to a Monte Carlo permutation test (Supplementary material Table A3).

3.2. Taxonomic diversity of microorganisms in animal-impacted Antarctic soils

A total of 284,625 16S rRNA gene sequence fragments were identified in all metagenomes and clustered into 16,028 OTUs (97% nucleotide sequence identity threshold) using closed-reference OTU picking (~23% of these reads remained unassigned to OTUs at the threshold used). Based on these OTUs, differences in microbial community diversity between animal-impacted soils and soils without animal influence were not statistically significant (Supplementary material Table A4). Consistent with these results, no statistically significant differences in whole-community diversity index (Nd) or coverage by sequencing were observed between the samples based on Nonpareil (Supplementary material Table A1). This tool employs the level of redundancy among reads to estimate the coverage of the microbial community obtained by a metagenomic dataset and provides an estimate of community diversity (Rodriguez-R et al., 2018b). Based on Nonpareil curves (Supplementary material Fig. A1) and coverage values (Supplementary material Table A1), we obtained an average coverage of 0.33 for soils without animal influence (i.e., at the sequencing effort applied, there is ~30% likelihood that any additional sequence obtained will be redundant with sequences already observed), and an average coverage of 0.55 in pinniped-impacted soils and 0.63 in bird-impacted soils. As mentioned above, these coverage values were not statistically different, presumably due to large sample heterogeneity and a relatively low number of samples compared but allowed for robust comparisons of features (e.g., OTUs, genes) among the metagenomic datasets (Rodriguez-R and Konstantinidis, 2014b).

Regarding the taxonomic composition based on 16S rRNA gene OTUs, *Proteobacteria* (~32%), *Bacteroidetes* (~26%), and *Actinobacteria* (~20%) were consistently the phyla with the highest relative abundance in all soil samples (Fig. 2), but their abundances were not statistically

Table 1

Edaphic factors measurements in soil samples with and without animal impact.

Type of soil sample	Sample	pH		WC		OM (%)		N-NO_3^- (μg/g)		N-NH_4^+ (μg/g)		P (μg/g)	
Soils w/o animal influence	Ct_LI	5.31 ± 0.10	a	13.04 ± 1.85	a	2.61 ± 0.52	a	15.20 ± 2.37	a	0.00 ± 0.00	a	14.16 ± 2.61	a
	Ct_KGI	6.25 ± 0.19		16.09 ± 2.43		1.84 ± 1.00		15.63 ± 0.70		0.00 ± 0.04		0.78 ± 3.64	
Pinniped-impacted soils	Ag_LI	4.50 ± 0.75	a	9.78 ± 2.85	a	1.68 ± 0.49	a	108.83 ± 21.99	ab	0.10 ± 0.12	b	155.29 ± 65.48	a
	Ag_KGI	4.44 ± 0.11		22.35 ± 6.57		1.95 ± 1.55		21.93 ± 19.93		0.10 ± 0.06		18.13 ± 1.42	
	Ml_LI	6.34 ± 0.20		19.54 ± 23.62		3.52 ± 3.81		46.27 ± 20.66		12.59 ± 8.53		437.22 ± 122.91	
	Ml_KGI	4.80 ± 0.26		24.39 ± 5.40		1.93 ± 0.60		37.51 ± 57.81		10.07 ± 5.19		13.63 ± 4.47	
Bird-impacted soils	Ld_LI	4.06 ± 0.04	a	54.41 ± 14.66	b	8.24 ± 0.66	b	138.32 ± 75.69	b	0.27 ± 0.12	c	259.00 ± 95.52	b
	Ld_KGI	6.46 ± 0.44		49.25 ± 22.42		13.13 ± 0.77		561.67 ± 140.26		280.54 ± 62.12		1830.79 ± 169.27	
	Pp_LI	7.06 ± 0.29		42.86 ± 3.19		13.91 ± 5.45		13.29 ± 422.85		231.93 ± 113.92		1746.38 ± 179.17	
	Pp_KGI	3.89 ± 0.34		44.93 ± 36.02		12.75 ± 7.51		153.08 ± 48.63		26.10 ± 31.30		585.69 ± 136.19	

Values represent the median ± standard deviation among the replicate soil samples in each series. The number of replicates was 6 for soils without animal influence and 12 for soils with animal influence. Different letters indicate statistically significant differences in each column, comparing the different types of soil samples ($p \leq 0.05$, Kruskal Wallis, Dunn *posthoc* test with Benjamini-Hochberg correction).

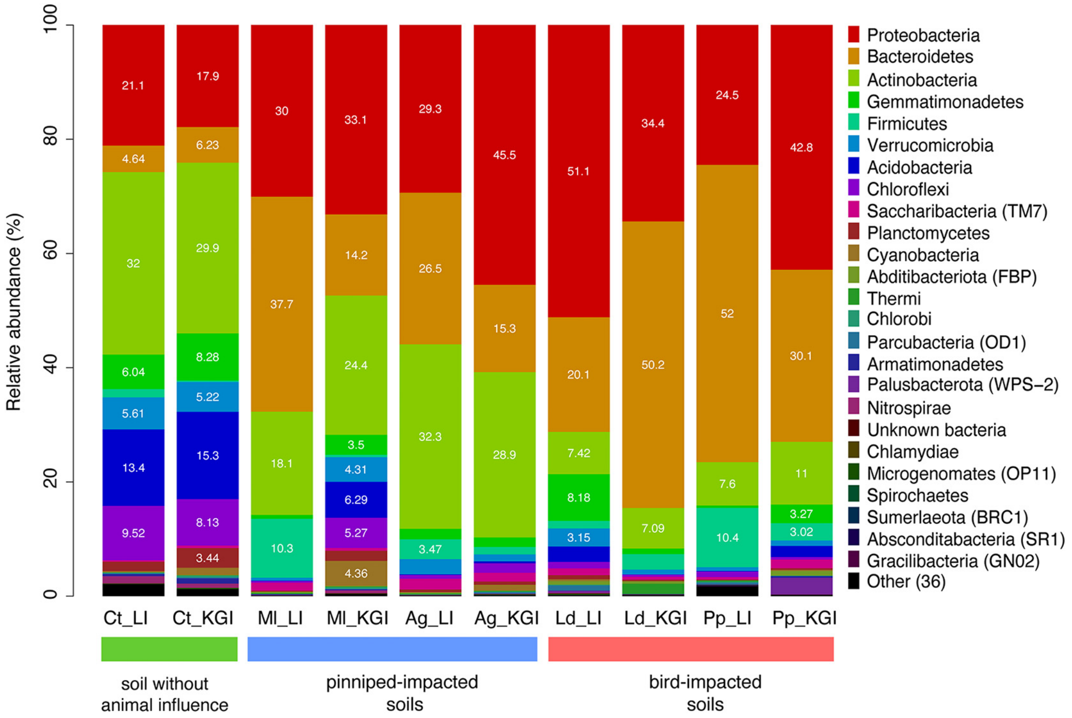


Fig. 2. Bar plot of the structure of the microbial communities from the Antarctic soil metagenomes. The relative abundance of the 16S rRNA gene-based OTUs classified at the phylum level is shown. Each color represents a different phylum (see figure key). The blocks below indicate the type of sample soil: in blue, pinniped-impacted soils; in red, bird-impacted soils; in green, soil samples without animal influence. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

significantly different among the soil samples. To identify significant differences in the abundance of individual OTUs in the different soil types, we employed DESeq2 ($p \le 0.05$; after Bonferroni correction) (Love et al., 2014). We found 132 and 29 OTUs to be significantly more abundant in soils without animal influence than in bird-impacted soils and pinniped-impacted soils, respectively. Notably, the 29 OTUs found in the latter comparison were also found in the former comparison (Supplementary material Table A5). Specifically, we observed OTUs assigned to families *Gaiellaceae* and *Nitrososphaeraceae* to be statistically significantly more abundant in soils without animal influence than in pinniped-impacted soils and bird-impacted soils. Additionally, we found 8 OTUs significantly more abundant in bird-impacted soils than soils without animal influence (Supplementary material Table A5), among them OTUs assigned to genera *Rhodanobacter*, *Dokdonella*, and *Candidatus* Aquiluna.

3.3. Functional diversity in animal-impacted Antarctic soils

Protein-coding genes from assembled contig were classified at the subsystem level of the hierarchical annotation scheme of SEED to evaluate the functional gene diversity of the sampled microbial communities. On average, 303,335 genes were annotated per assembled metagenome, representing between 46% and 51% of the total predicted genes (Supplementary material Table A6). The most abundant broad categories were cofactors, vitamins, prosthetic groups and pigments, protein metabolism, and metabolisms for carbohydrates, amino acids, and derivatives. Using DESeq2 (Love et al., 2014), we found that the abundance of genes for 223 of the total 1667 (13.3%) subsystem categories was significantly different ($p < 1e{-}03$; after Bonferroni correction) between animal-impacted soils and soils without animal influence. The subsystem categories with the most significant differences between soils without animal influence, pinniped-impacted soils, and bird-impacted soils, in pairwise comparisons, are shown in Supplementary material Table A7. From this set, the three subsystem categories most significantly abundant in soils without animal influence were (i) “A Gram-positive cluster that relates ribosomal protein L28P to a set of uncharacterized proteins”, (ii) “ABC transporter oligopeptide (TC 3. A.1.5.1)” and (iii) “CBSS-350688.3.peg.1509”, which includes a peptidase, a ribosomal protein, components of the riboflavin metabolism and a polyribonucleotide nucleotidyltransferase. Conversely, the three categories most significantly abundant in animal-impacted soils (both pinnipeds and birds) compared to soils without animal influence were (i) “N-ATPase”, (ii) “Murein hydrolase regulation and cell death”, and (iii) “Flagellar motility”. In addition, in soils without animal influence, we found the highest fold changes for categories related to “Ribosome SSU eukaryotic and archaeal” and “Ribosome LSU eukaryotic and archaeal” when compared to pinniped-impacted soils (6.15 and 5.91, respectively), and for categories related to “Coenzyme F420” comparing to bird-impacted soils (2.77 each one). Specifically, for the nitrogen cycle, we found that reads carrying denitrification genes were 2.12-fold ($p = 2.6e{-}07$) and 1.7-fold ($p = 0.011$) more abundant in bird-impacted soils and pinniped-impacted soils than in soils without animal influence, respectively. Also, reads carrying urea decomposition genes were 3.10-fold ($p = 6.6e{-}03$) more abundant in pinniped-impacted soils than in bird-impacted soils.

3.4. Recovery of metagenomes-assembled genomes (MAGs)

From the ten metagenomes, a total of 100 metagenome-assembled genomes (MAGs) were recovered. The MAGs had average estimated completeness of 83.9%, contamination of 4.3%, and quality of 63%, while the fraction of shared proteins used in the AAI calculation was higher than 40% of the total protein sequences in the genome for almost all MAG to MAG comparisons (Supplementary material Table A8). Based on their best AAI match against available (classified) isolate genomes (Rodriguez-R et al., 2018a), 50 MAGs were classified to phylum *Proteobacteria* (26 *Beta*-, 22 *Gamma*-, one *Alpha*-, one *Delta*-), 22 to *Actinobacteria*, 15 to *Bacteroidetes*, six to *Firmicutes*, four to *Gemmatimonadetes*, and one to *Deinococcus-Thermus*, one to *Saccharibacteria*, and one to *Verrucomicrobia*. MAGs related to

Rhodanobacter denitrificans, and *Candidatus* Tremblaya princeps were the most frequently detected MAGs across samples, with seven MAGs representatives each of these two species, followed by *Dokdonella koreensis* with six MAGs (Fig. 3).

In concordance with their lower estimated coverage, the metagenomes Ct_LI, Ct_KGI and Ml_KGI, yielded fewer MAGs (four, four, and one, respectively) than other animal-impacted metagenomes (approximately 10 MAGs per sample). The analysis based on AAI values against the closest related (classified) genome showed that out of the 100 obtained MAGs, two likely represented new families, 18 new genera (of a previously described family), 71 new species, and only nine were related to previously described species.

3.5. Nitrogen cycling pathways related to N_2O emissions in animal-impacted Antarctic soils

We assessed the relative abundance of nitrogen cycle genes related to pathways potentially involved in N_2O emission based on both unassembled metagenomic short reads and metagenome-assembled genomes (MAGs). Based on the analysis of the unassembled metagenomic short reads, we found that denitrification genes (*narG*, *nirK*, and *nosZ*) were statistically significant ($p < 0.05$, Kruskal-Wallis test, Dunn *posthoc* test) more abundant (relative abundance) than those of nitrification (*amoA* and *hao*) and DNRA (*nrfA*) pathways (Supplementary material Table A9). In particular, nitrite reductase (*nirK*) and nitrous oxide reductase (*nosZ*) genes were more abundant in bird-impacted soils than soils without animal influence. On average, the number of genome equivalents, i.e., the fraction of the total sampled cells that are expected to carry the gene of interest assuming the gene is single-copy per genome, was 6.4-fold higher for *nirK* genes ($p = 0.0395$) and 9.1-fold higher for *nosZ* genes ($p = 0.0301$) in bird-impacted soils than soils without animal influence (Supplementary material Table A10). Besides, we found a significant positive correlation ($r = 0.67$, $p = 0.0342$) between the soil nitrate content and the *nosZ* gene relative abundance; however, no significant correlations were found between nitrate content and the abundance of other denitrification genes (data not shown).

For DNRA, we found the *nrfA* gene, which encodes a periplasmic nitrite reductase, in seven MAGs, all assigned to the *Bacteroidetes* phylum. This gene was found in 2/37 of the MAGs obtained from pinniped-impacted metagenomes and 5/55 from bird-impacted ones. Concerning nitrification, five MAGs carried genes of this pathway, and only MAG #38 related to *Nitrosospira lacus* (69.1% AAI), obtained from bird-impacted soils, included an ammonia monooxygenase (*amoA*) gene (Fig. 3). Finally, regarding denitrification, only three MAGs included all necessary genes for the complete pathway (i.e., reductions from NO_3^- to N_2); two from pinniped-impacted soils and one from bird-impacted soils. Specifically, these MAGs were #2, #48, #91 and were related to *Pandoraea thiooxydans* (51.4% AAI), *Pseudomonas stutzeri* (64.95% AAI) and *Oblitimonas alkaliphila* (63.17% AAI), respectively. Most MAGs (89/100) included only some of the denitrification genes and, thus, they were likely incomplete denitrifiers, although the possibility of gene missing due to genome incompleteness cannot be excluded in some cases. For example, the *narG* gene (coding for nitrate reductase) was present in 16/37 of the MAGs from pinniped-impacted soils and 27/55 of the MAGs from bird-impacted soils, and was the most abundant nitrogen cycle gene, often present in more than one copy in several MAGs (Fig. 3). More MAGs contained *nirS* than *nirK* genes (coding nitrite reductase): 5/37 of the MAGs from pinniped-impacted soils and 9/55 of the MAGs from bird-impacted soils carried *nirS* gene compared to 0/37 and 3/55 of the MAGs from the same metagenomes carrying *nirK* gene. The *norB* (coding nitric oxide reductase) and *nosZ* (coding nitrous oxide reductase) genes were similarly present in bird-impacted soils (13/55 and 11/55 of the MAGs, respectively) and pinniped-impacted soils (8/37 and 7/37 of the MAGs, respectively). Six out of the total seven of our MAGs identified as close relatives of *Rhodanobacter* included at least one gene involved in denitrification. Three of them encoded only the *nosZ* gene among the denitrification genes (Supplementary material Fig. A2). Notably, no denitrification genes were detected in MAGs obtained from soils without animal influence (Fig. 3).

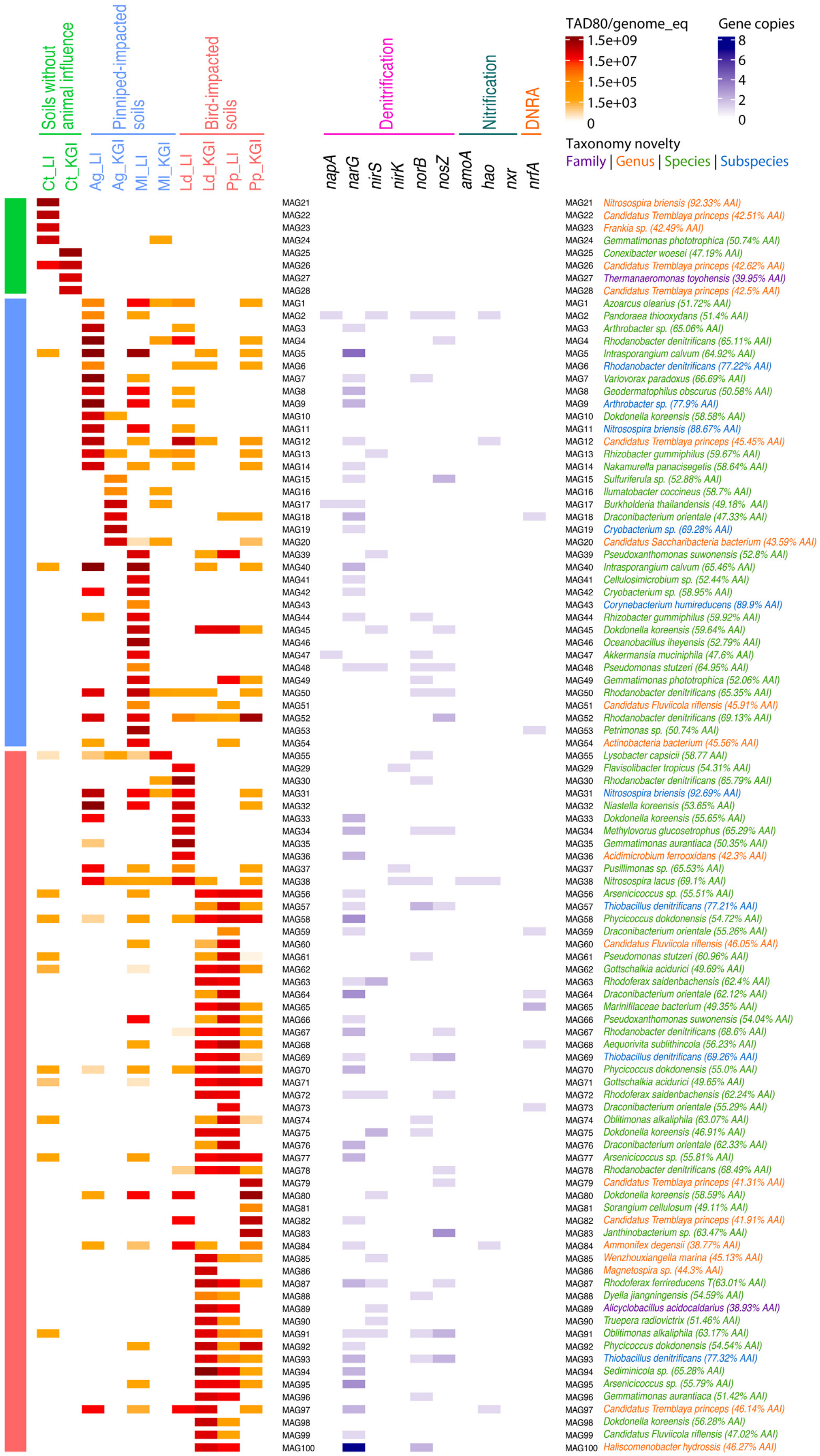
3.6. Geographic distribution of the MAGs

The recovery of MAGs allowed us to evaluate their geographic distribution in our metagenomes and other available metagenomes. To assess the prevalence of each MAG in our samples, a heatmap that shows MAGs' relative abundance (i.e., TAD80 values derived from read recruitment plots) in each metagenome was built (Fig. 3). We also determined the genomospecies, or gsp, that each MAG was assignable to, i.e., species that can be differentiated from each other using the 95% ANI genomic-level threshold (Konstantinidis and Tiedje, 2005) (Supplementary material Table A8). Our results showed that, in general, most MAGs were detected only in one sample and represented different gsp, revealing a high overall heterogeneity across samples. Among the 100 MAGs, we found 83 different gsp, with 13 gsp represented by more than one MAG. Interestingly, for the animal-impacted soils from different islands, separated by approximately 100 km, we were able to find the same MAG population (>95% ANI) for several gsp (Supplementary material Fig. A3). For example, in samples from bird-impacted soils (Pp_KGI, Pp_LI, Ld_KGI), we recovered MAGs related to *Candidatus* Tremblaya princeps (MAG #12 and MAG #97 belonging to the gsp #12), *Arsenicicoccus* sp. (MAG #56, MAG #77 and MAG #95 belonging to the gsp #54), *Gottschalkia acidurici* (MAG #62 and MAG #71 belonging to the gsp #60), *Rhodoferax saidenbachensis* (MAG #63, MAG #72 and MAG #87 belonging to the gsp #61) and *Phycicoccus dokdonensis* (MAG #58, MAG #70 and MAG #92 belonging to the gsp #56).

Rhodanobacter was one of the most abundant genera in our samples; approximately 2% of all 16S rRNA gene-carrying reads were grouped in 11 OTUs assigned to this genus. Seven assembled MAGs were related to *Rhodanobacter*, and 10% of all reads recruited by obtained MAGs were related to this genus. Almost all these MAGs represented distinct gsp, except for MAG #67 and MAG #78 that belong to the same gsp #64.

Assessing the diversity among metagenomic reads mapped on their respective MAG can reveal the level of sequence diversity (or clonality level) within each population (Meziti et al., 2019). Typically, reads with greater than 95% identity represented sequence-discrete populations in our data, consistent with previous findings from other habitats (Caro-Quintero and Konstantinidis, 2012; Jain et al., 2018; Konstantinidis and Delong, 2008; Olm et al., 2020). Reads with less than 95% identity were relatively rare and, thus, presumably represent different co-occurring populations or hypervariable regions of the genome (Caro-Quintero and Konstantinidis, 2012; Konstantinidis and Delong, 2008).

Specifically, for one of the most dominant genera in our samples, we observed the following patterns when we used its representative MAG #52, related to *Rhodanobacter denitrificans* (69.13% AAI), to perform read recruitment plots for each metagenome (Supplementary material Fig. A4). In almost all samples, even coverage across the entire MAG sequence was observed, revealing a homogenous sequence-discrete population (nucleotide identity ≥ 95%) and the presence of related co-occurring population(s) (nucleotide identity < 95%). Livingston Island samples showed both populations present in similar relative abundance compared with King George Island samples; the latter samples (i.e., Ag_KGI, Ml_KGI, and Ct_KGI) showed a sequence-discrete population of relatively low abundance (Supplementary material Fig. A4). On the other hand, we observed poor read recruitment (sequencing depth lower than 0.1×) and non-homogenous coverage across the entire MAG #52 when examining metagenomes obtained in other studies (Ji et al., 2017; Orellana et al., 2018) (Supplementary material Fig. A5), indicating the MAG recovered here was locally abundant only.


Soils without animal influence
Pinniped-impacted soils
Bird-impacted soils
Ct_LI
Ct_KGI
Ag_LI
Ag_KGI
Ml_LI
Ml_KGI
Ld_LI
Ld_KGI
Pp_LI
Pp_KGI
Denitrification
Nitrification
DNRA
napA
narG
nirS
nirK
norB
nosZ
amoA
hao
nxr
nrfA
TAD80/genome_eq
1.5e+09
1.5e+07
1.5e+05
1.5e+03
0
Gene copies
8
6
4
2
0
Taxonomy novelty
Family | Genus | Species | Subspecies
MAG21 Nitrosospira briensis (92.33% AAI)
MAG22 Candidatus Tremblaya princeps (42.51% AAI)
MAG23 Frankia sp. (42.49% AAI)
MAG24 Gemmatimonas phototrophica (50.74% AAI)
MAG25 Conexibacter woesei (47.19% AAI)
MAG26 Candidatus Tremblaya princeps (42.62% AAI)
MAG27 Thermanaeromonas toyohensis (39.95% AAI)
MAG28 Candidatus Tremblaya princeps (42.5% AAI)
MAG1 Azoarcus olearius (51.72% AAI)
MAG2 Pandoraea thiooxydans (51.4% AAI)
MAG3 Arthrobacter sp. (65.06% AAI)
MAG4 Rhodanobacter denitrificans (65.11% AAI)
MAG5 Intrasporangium calvum (64.92% AAI)
MAG6 Rhodanobacter denitrificans (77.22% AAI)
MAG7 Variovorax paradoxus (66.69% AAI)
MAG8 Geodermatophilus obscurus (50.58% AAI)
MAG9 Arthrobacter sp. (77.9% AAI)
MAG10 Dokdonella koreensis (58.58% AAI)
MAG11 Nitrosospira briensis (88.67% AAI)
MAG12 Candidatus Tremblaya princeps (45.45% AAI)
MAG13 Rhizobacter gummiphilus (59.67% AAI)
MAG14 Nakamurella panacisegetis (58.64% AAI)
MAG15 Sulfuriferula sp. (52.88% AAI)
MAG16 Ilumatobacter coccineus (58.7% AAI)
MAG17 Burkholderia thailandensis (49.18% AAI)
MAG18 Draconibacterium orientale (47.33% AAI)
MAG19 Cryobacterium sp. (69.28% AAI)
MAG20 Candidatus Saccharibacteria bacterium (43.59% AAI)
MAG39 Pseudoxanthomonas suwonensis (52.8% AAI)
MAG40 Intrasporangium calvum (65.46% AAI)
MAG41 Cellulosimicrobium sp. (52.44% AAI)
MAG42 Cryobacterium sp. (58.95% AAI)
MAG43 Corynebacterium humireducens (89.9% AAI)
MAG44 Rhizobacter gummiphilus (59.92% AAI)
MAG45 Dokdonella koreensis (59.64% AAI)
MAG46 Oceanobacillus iheyensis (52.79% AAI)
MAG47 Akkermansia muciniphila (47.6% AAI)
MAG48 Pseudomonas stutzeri (64.95% AAI)
MAG49 Gemmatimonas phototrophica (52.06% AAI)
MAG50 Rhodanobacter denitrificans (65.35% AAI)
MAG51 Candidatus Fluviicola riflensis (45.91% AAI)
MAG52 Rhodanobacter denitrificans (69.13% AAI)
MAG53 Petrimonas sp. (50.74% AAI)
MAG54 Actinobacteria bacterium (45.56% AAI)
MAG55 Lysobacter capsicii (58.77 AAI)
MAG29 Flavisolibacter tropicus (54.31% AAI)
MAG30 Rhodanobacter denitrificans (65.79% AAI)
MAG31 Nitrosospira briensis (92.69% AAI)
MAG32 Niastella koreensis (53.65% AAI)
MAG33 Dokdonella koreensis (55.65% AAI)
MAG34 Methylovorus glucosetrophus (65.29% AAI)
MAG35 Gemmatimonas aurantiaca (50.35% AAI)
MAG36 Acidimicrobium ferrooxidans (42.3% AAI)
MAG37 Pusillimonas sp. (65.53% AAI)
MAG38 Nitrosospira lacus (69.1% AAI)
MAG56 Arsenicicoccus sp. (55.51% AAI)
MAG57 Thiobacillus denitrificans (77.21% AAI)
MAG58 Phycicoccus dokdonensis (54.72% AAI)
MAG59 Draconibacterium orientale (55.26% AAI)
MAG60 Candidatus Fluviicola riflensis (46.05% AAI)
MAG61 Pseudomonas stutzeri (60.96% AAI)
MAG62 Gottschalkia acidurici (49.69% AAI)
MAG63 Rhodoferax saidenbachensis (62.4% AAI)
MAG64 Draconibacterium orientale (62.12% AAI)
MAG65 Marinifilaceae bacterium (49.35% AAI)
MAG66 Pseudoxanthomonas suwonensis (54.04% AAI)
MAG67 Rhodanobacter denitrificans (68.6% AAI)
MAG68 Aequorivita sublithincola (56.23% AAI)
MAG69 Thiobacillus denitrificans (69.26% AAI)
MAG70 Phycicoccus dokdonensis (55.0% AAI)
MAG71 Gottschalkia acidurici (49.65% AAI)
MAG72 Rhodoferax saidenbachensis (62.24% AAI)
MAG73 Draconibacterium orientale (55.29% AAI)
MAG74 Oblitimonas alkaliphila (63.07% AAI)
MAG75 Dokdonella koreensis (46.91% AAI)
MAG76 Draconibacterium orientale (62.33% AAI)
MAG77 Arsenicicoccus sp. (55.81% AAI)
MAG78 Rhodanobacter denitrificans (68.49% AAI)
MAG79 Candidatus Tremblaya princeps (41.31% AAI)
MAG80 Dokdonella koreensis (58.59% AAI)
MAG81 Sorangium cellulosum (49.11% AAI)
MAG82 Candidatus Tremblaya princeps (41.91% AAI)
MAG83 Janthinobacterium sp. (63.47% AAI)
MAG84 Ammonifex degensii (38.77% AAI)
MAG85 Wenzhouxiangella marina (45.13% AAI)
MAG86 Magnetospira sp. (44.3% AAI)
MAG87 Rhodoferax ferrireducens T(63.01% AAI)
MAG88 Dyella jiangningensis (54.59% AAI)
MAG89 Alicyclobacillus acidocaldarius (38.93% AAI)
MAG90 Truepera radiovictrix (51.46% AAI)
MAG91 Oblitimonas alkaliphila (63.17% AAI)
MAG92 Phycicoccus dokdonensis (54.54% AAI)
MAG93 Thiobacillus denitrificans (77.32% AAI)
MAG94 Sediminicola sp. (65.28% AAI)
MAG95 Arsenicicoccus sp. (55.79% AAI)
MAG96 Gemmatimonas aurantiaca (51.42% AAI)
MAG97 Candidatus Tremblaya princeps (46.14% AAI)
MAG98 Dokdonella koreensis (56.28% AAI)
MAG99 Candidatus Fluviicola riflensis (47.02% AAI)
MAG100 Haliscomenobacter hydrossis (46.27% AAI)

4. Discussion

4.1. Edaphic factors and microbial community diversity are more affected by bird-impacted soils than pinniped-impacted soils and soils without animal influence

We found that water content, organic matter content, and phosphorus content were higher in bird-impacted soils than pinniped-impacted soils and soils without animal influence, and nitrogen content was higher in bird-impacted soils than soils without animal influence. Consistently, in our previous study (Ramírez-Fernández et al., 2019), we found higher nutrient content in soils impacted by penguins than soils impacted by Antarctic fur seals and soils without animal influence. Similar trends were also reported in previous studies, where penguins produce higher alterations in soil physicochemical properties than seals (Ma et al., 2013; Wang et al., 2015). The great influence of penguins is most likely related to the fact that their colonies are more crowded than the pinniped ones. Although it has been described that intense activity of penguins in ice-free areas leads to acidic pH in ornithogenic soils (Simas et al., 2007), we found a wide range of pH, between neutral to slightly acidic in our penguin- (and other animals) impacted soils. This range, however, is concordant with values found in soils impacted by penguins and seals in Antarctica (Ramírez-Fernández et al., 2019; Wang et al., 2015).

Despite the significant differences in these edaphic parameters, overall microbial community diversity was not statistically significantly different among soil samples, presumably due to the relatively small number of datasets obtained. However, several individual OTUs and functional subsystem categories were significantly different in abundance between animal-impacted soils and soils without animal influence. The relatively higher amount of nutrients in bird-impacted soils than soils without animal influence would allow the proliferation of specific microbial taxa capable of tolerating or using these nutrients, resulting in overall reduced microbial diversity in these soils. Indeed, we recover, on average, 63% of the microbial community complexity from bird-impacted soils, 50% from pinniped-impacted soils, and 32% from soils without animal influence when we applied similar sequencing efforts in all samples. In agreement with these results, the diversity analysis of these OTUs, based on the Shannon or Simpson_1-D indices, showed that soils without animal influence generally had higher diversity, followed by pinniped- and bird-impacted soils.

Although the measured edaphic variables did not significantly explain the microbial composition variation, we found that pH and ammonium, nitrate, and phosphorus contents represented the highest loading values in the ordination based on the distance between metagenomes. Concordantly, these edaphic factors have been previously reported as the main drivers of the diversity of soil microbial communities in animal-impacted soils from Antarctic ecosystems (Ramírez-Fernández et al., 2019; Siciliano et al., 2014; Wang et al., 2015). Further, it has been reported that Antarctic marine animals create nutrient gradients even beyond their colonies (>1000 m), and their presence is associated with terrestrial biodiversity hotspots (Bokhorst et al., 2019).

4.2. Denitrification genes are highly abundant in Antarctic soils impacted by marine birds

Multiple microbial pathways lead to N_2O emissions, including ammonia (hydroxylamine) oxidation, heterotrophic denitrification, and DNRA (dissimilatory nitrate reduction to ammonium) (Hallin et al., 2018). Nitrification-related pathways and heterotrophic denitrification have been established as the most predominant sources of N_2O emissions from soils (Hu et al., 2015). In several previous studies, high N_2O emissions in Antarctic soils impacted by animals, especially penguins, were reported (Zhu et al., 2009, 2013; Neufeld et al., 2015; Wang et al., 2019). In our study, we found that denitrification genes had higher relative abundance than genes related to nitrification and DNRA, and that *nirK* and *nosZ* genes were more significantly abundant in bird-impacted soils than soils without animal influence. Therefore, assuming that DNA abundance reflects activity in ecosystems that experience stable, long-term perturbations such as these Antarctic soils (Orellana et al., 2019), we hypothesize that incomplete microbial denitrification, rather than nitrification and DNRA, is the primary source of the previously measured N_2O emissions in these Antarctic soils (Zhu et al., 2009, 2013; Neufeld et al., 2015; Wang et al., 2019). However, somehow inconsistent with this interpretation, the *nosZ* gene, responsible for reducing N_2O to N_2, was highly abundant in our animal-impacted datasets. A possible explanation that could reconcile these findings is that even though there is a high relative abundance of *nosZ* genes in bird-impacted soils, this gene could be not being transcribed or translated adequately or could be active only under specific conditions, e.g., warm temperatures are typically required for its activity (Domeignoz-Horta et al., 2016; Liu et al., 2014). Another possibility is that even though a high amount of metabolically active NosZ enzyme is produced, the N_2O emissions could be higher than N_2O consumption rates, or N_2O emissions originate from abiotic processes. Testing these hypotheses in the future will be necessary for a complete understanding of these greenhouse gas emissions from these Antarctic soils. The data (genes and genomes/MAGs) provided here should facilitate such studies in the future.

In our soils without animal influence, we did not detect any genes related to nitrogen cycling pathways. Concordantly, Nelson et al. (2016) found the highest frequencies of nitrogen pathway genes in nutrient-enriched environments such as tropical forests and human-dominated (pasture, lawn, and agriculture) soils; in contrast, the lowest frequencies were observed in cold deserts. Consistently, the low contents of organic matter and nitrogen found in soils without animal influence presumably explain the non-detection, or detection at low frequencies, of genes related to denitrification, nitrification, and DNRA in the metagenomes or MAGs obtained from these soils. Conversely, in our animal-impacted soils, seven MAGs were found to carry the *nrfA* gene involved in DNRA, all belonging to the phylum *Bacteroidetes*. DNRA is an under-studied nitrogen cycling process in soils compared to other major nitrogen transformation pathways. However, in a recent study, Nelson et al. (2016) found an unexpectedly high frequency of bacteria with DNRA capabilities in soils. Also, DNRA genes have been detected across a wide range of bacterial clades (Welsh et al., 2014). Regarding nitrification, only the MAG #38, identified as *Nitrosospira lacus* (69.1% AAI), contained the complete nitrification pathway. This bacterium has been described as a psychrotolerant ammonia-oxidizer capable of growth over a wide pH range (Urakawa et al., 2015). Even though a representative of this genus has been isolated from Antarctica (Palleroni, 1952), no sequences have been reported from polar regions. Thus, the MAG reported here is the first genome representative of this genus from Antarctic soils to the best of our knowledge. Finally, denitrification is an anaerobic process carried out by diverse bacterial taxa, which could possess the complete (nitrate to nitrogen gas) or incomplete (modular) denitrification pathway. However, incomplete denitrification appears to be more common than the complete

Fig. 3. Heatmaps of the relative abundance of MAGs and nitrogen cycle genes in the Antarctic soil metagenomes. The left panel shows the MAG relative abundance in each sample calculated as truncated average sequencing depth (TAD80) normalized by genome equivalents. The right panel shows the number of copies of genes involved in denitrification (*napA*, *narG*, *nirS nirK*, *norB*, and *nosZ*), nitrification (*amoA*, *hao*, and *nrx*), and DNRA (*nrfA*) from MAGs. The left blocks indicate the type of sample from where each MAG was recovered: in blue, pinniped-impacted soils; in red, bird-impacted soils; in green, soil samples without animal influence. The taxonomic novelty of each MAG is indicated by the corresponding color (see figure key on top) in the name of the closest relative bacteria found in the NCBI database. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

pathway in available isolate genomes and metagenomes (Graf et al., 2014; Sanford et al., 2012). Consistently, we found that 89/92 of the MAGs obtained from animal-impacted soils, representing diverse bacterial phyla, carried an incomplete denitrification pathway; only the remaining three MAGs belonging to the class *Betaproteobacteria* had the complete denitrification pathway. While some of the former results may be due to incomplete MAGs (as opposed to real gene absence), our results collectively suggested that, in bird-impacted soils, denitrification is a highly abundant and predominantly modular process carried out by diverse bacterial species. Therefore, it appears that a diverse microbial guild is responsible for N_2O emissions related to denitrification in these Antarctic soils.

4.3. *Rhodanobacter* is the most abundant genus in Antarctic soils impacted by marine birds

The taxonomic affiliations of the assembled MAGs were, in general, consistent with the 16S rRNA gene-based taxonomic community composition. For instance, phyla *Proteobacteria*, *Actinobacteria*, and Bacteroidetes were highly represented among the MAGs, consistent with their high relative abundance based on 16S rRNA gene-carrying metagenomic reads. On the other hand, we found ~23% of the 16S rRNA gene-carrying read sequences to be taxonomically unassigned, and our analysis of MAGs showed that 91 of the total MAGs obtained represented a species not previously taxonomically described based on isolates, and they all presumably represent yet uncultured bacteria. These results indicate a high degree of novelty of the microbial communities in our Antarctic soil samples. Consistently, in a previous reanalysis of 16S rRNA gene sequences from 37 studies representing both cultivation-dependent and -independent Antarctic soil surveys (Lambrechts et al., 2019), 85.6% of bacterial sequences represented yet uncultured genera. Only three out of the 100 recovered MAGs had close relatives in the database with a high percentage of similarity (>80% AAI). Specifically, MAG #1 and MAG #31 (gsp1) were related to *Nitrosospira briensis* C128 NZ CP012371^T with 92.33% AAI and 92.69% AAI, respectively. This bacterium is an ammonia-oxidizer that has been isolated from an acid agricultural soil, and its gene inventory supports a chemolithotrophic metabolism with implications for ecosystem function in soil environments (Rice et al., 2016). Even though we did not find genes related to the nitrogen cycle in these two *Nitrosospira* MAGs, representatives of the *Nitrosospira* genus have been found to be related to N_2O emissions in soils amended with inorganic nitrogen fertilizers (Lourenço et al., 2018), indicating that the corresponding nitrogen pathway genes were missed during genome binning in this case due to the ~70% completeness (or this gsp is not involved in N_2O emissions). Finally, MAG #43 was related to *Corynebacterium humireducens* with 89.9% AAI. This species was isolated from a microbial fuel cell continuously fed with artificial wastewater and was described as a novel halotolerant and alkaliphilic bacterium capable of reducing humic acids (Wu et al., 2011). Besides, it was reported that *Corynebacterium* strains reduce nitrate, nitrite, and nitric oxide to N_2O under anaerobic conditions (Renner and Becker, 1970).

Rhodanobacter was the genus with the highest number of MAGs related to a (known) genus (65.11% to 77.22% AAI). Specifically, *Rhodanobacter* was one of the most abundant genera in our bird-impacted soils; approximately 2% of all the 16S rRNA gene-carrying reads were assigned to this genus. Most of the *Rhodanobacter* MAGs recovered from our samples represent distinct gsp and were present in both King George Island and Livingston Island. *Rhodanobacter* species are known for a combination of physiologic traits that allow growth under (or tolerate) diverse conditions such as circumneutral pH (4–8), high concentration of organic nutrients, nitrate, NaCl, and heavy metals (Prakash et al., 2021). Most of these conditions can be found in our Antarctic sampled soils impacted by animals (Ramírez-Fernández et al., 2019; Wang et al., 2019), which could stimulate the growth of *Rhodonobacter*. In fact, this genus was previously found at a high relative abundance in studies of animal-impacted Antarctic soils from the Ross Sea region (Aislabie et al., 2009), King George Island (Kim et al., 2012), and Livingston Island (Ramírez-Fernández et al., 2019). Also, *Rhodanobacter* has been found at high abundance in contaminated soils such as petroleum hydrocarbon-impacted soils in Macquarie Island from Australian sub-Antarctica (Van Dorst et al., 2016), crude oil-contaminated soils from the USA (Hamamura et al., 2006), gold mining contaminated soil from China (Guo et al., 2017), and nuclear legacy waste in Oak Ridge from the USA (Green et al., 2012). Thus, *Rhodanobacter* species appear to be versatile organisms and important members of soil microbial communities in various naturally or anthropogenically disturbed habitats, including our bird-impacted Antarctic soils, and could serve as model organisms for future studies of disturbed ecosystems.

Rhodanobacter may also be an essential clade involved in denitrification in acidic soils with high emissions of N_2O as the denitrification end-product (Van Den Heuvel et al., 2010). Analysis of the genomes of six publicly available *Rhodanobacter* isolate genomes revealed that all contained nearly complete denitrification pathways, including two copies of the nitrite reductase gene *nirK* (except *R. spathiphylli* carrying only a single copy) (Kostka et al., 2012). All denitrifying isolates contain most of the genes in the dissimilatory denitrification pathway, and non-denitrifying isolates lack several essential genes involved in nitrate respiration, such as nitrate reductase genes (i.e., *narG*, *narH*, *narJ*, and *narI*). Lycus et al. (2017) isolated six additional *Rhodanobacter* strains from low pH soils and found that three of them were unable to reduce N_2O at neither low nor high pH, yet they did carry the *nosZ* gene. However, one of the *Rhodanobacter* isolates did reduce N_2O at low pH. These findings show that *Rhodanobacter* strains differ in their regulation of denitrification and N_2O consumption in low pH soils. Therefore, it is challenging to infer if *Rhodanobacter* strains contribute to the reduction of N_2O in our animal-impacted soil samples, and more experimental work will be needed to test this hypothesis in the future.

5. Conclusions

Collectively, we found that in the Antarctic ice-free areas studied here, most of the measured edaphic factors, including nitrogen content, were higher in bird-impacted soils than soils without animal influence. Consistently, a high relative abundance of denitrification genes (i.e., *nirK* and *nosZ*) and denitrifying bacteria related to the *Rhodanobacter* genus were observed in these soils. Sequences and genomes (MAGs) reported here should facilitate future studies of these soil microbial communities and their activities by providing, for example, reference sequences for qPCR or other targeted molecular approaches. Finally, the results reported here advance our understanding of the potential influence of animal presence over soil microbial populations and nitrogen cycling genes potentially involved in N_2O emissions in Antarctic terrestrial ecosystems.

Funding

This work was financially supported by the Instituto Antártico Chileno [project INACH DT_10_15 to Lia Ramírez-Fernández]. Additionally, this research was supported, in part, by the US National Science Foundation [award 1831582 to Konstantinos T. Konstantinidis] and the Agencia Nacional de Investigación y Desarrollo [project CONICYT-PIA ACT172065, http://www.antarcticgenomics.cl, to Julieta Orlando].

CRediT authorship contribution statement

Lia Ramírez-Fernández: Conceptualization, Formal analysis, Investigation, Resources, Writing – original draft, Visualization, Project administration, Funding acquisition. **Luis H. Orellana:** Formal analysis, Writing – review & editing. **Eric R. Johnston:** Formal analysis, Writing – review & editing. **Konstantinos T. Konstantinidis:** Formal analysis, Resources, Writing – review & editing, Visualization, Supervision. **Julieta Orlando:** Conceptualization, Formal analysis, Resources, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors would like to sincerely thank the INACH personnel at Escudero Antarctic Research Station for logistic facilities and Renato Borras for the Cape Shirreff, Livingston Island, sampling collection. Also, the authors want to thank Karla Veas for her collaboration in the elaboration of the graphical abstract.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.147693.

References

Aislabie, J., Jordan, S., Ayton, J., Klassen, J.L., Barker, G.M., Turner, S., 2009. Bacterial diversity associated with ornithogenic soil of the Ross Sea region, Antarctica. Can. J. Microbiol. 55, 21–36. https://doi.org/10.1139/w08-126.

ASPA-125, 2009. Antarctic Specially Protected Area No. 125: Peninsula Fildes, King George Island (May 25th Island): Revised Management Plan.

ASPA-149, 2009. Antarctic Specially Protected Area No. 149: Cape Shirreff and San Telmo Island, Livingston Island, South Shetland Islands: Revised Management Plan.

ASPA-150, 2009. Antarctic Specially Protected Area No. 150: Ardley Island, Maxwell Bay, King George Island (May 25th Island): Revised Management Plan.

Bao, T., Zhu, R., Li, X., Ye, W., Cheng, X., 2018. Effects of Multiple Environmental Variables on Tundra Ecosystem Respiration in Maritime Antarctica. https://doi.org/10.1038/s41598-018-30263-6.

Bokhorst, S., Huiskes, A., Convey, P., Aerts, R., 2007. External nutrient inputs into terrestrial ecosystems of the Falkland Islands and the Maritime Antarctic region. Polar Biol. 30, 1315–1321. https://doi.org/10.1007/s00300-007-0292-0.

Bokhorst, S., Convey, P., Aerts, R., 2019. Nitrogen inputs by marine vertebrates drive abundance and richness in Antarctic terrestrial ecosystems. Curr. Biol. 29. https://doi.org/10.1016/j.cub.2019.04.038.

Boratyn, G.M., Thierry-Mieg, J., Thierry-Mieg, D., Busby, B., Madden, T.L., 2019. Magic-BLAST, an accurate RNA-seq aligner for long and short reads. BMC Bioinforma. 20, 405. https://doi.org/10.1186/s12859-019-2996-x.

Bray, R.H., Kurtz, L.T., 1945. Determination of total, organic, and available forms of phosphorus in soils. Soil Sci. 59, 39–45. https://doi.org/10.1097/00010694-194501000-00006.

Buchfink, B., Xie, C., Huson, D.H., 2014. Fast and sensitive protein alignment using DIAMOND. Nat. Methods 12, 59–60. https://doi.org/10.1038/nmeth.3176.

Butterbach-Bahl, K., Baggs, E.M., Dannenmann, M., Kiese, R., Zechmeister-Boltenstern, S., 2013. Nitrous oxide emissions from soils: how well do we understand the processes and their controls? Philos. Trans. R. Soc. B Biol. Sci. 368, 20130122. https://doi.org/10.1098/rstb.2013.0122.

Caporaso, J., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F., Costello, E., Fierer, N., Gonzalez-Peña, A., Goodrich, J., Gordon, J., Al, E., 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods 7, 1–12. https://doi.org/10.1038/nmeth.f.303.

Caro-Quintero, A., Konstantinidis, K.T., 2012. Bacterial species may exist, metagenomics reveal. Environ. Microbiol. 14, 347–355. https://doi.org/10.1111/j.1462-2920.2011.02668.x.

Cary, S.C., McDonald, I.R., Barrett, J.E., Cowan, D.A., 2010. On the rocks: the microbiology of Antarctic Dry Valley soils. Nat. Rev. Microbiol. 8, 129–138. https://doi.org/10.1038/nrmicro2281.

Clucas, G.V., Younger, J.L., Kao, D., Rogers, A.D., Handley, J., Miller, G.D., Jouventin, P., Nolan, P., Gharbi, K., Miller, K.J., Hart, T., 2016. Dispersal in the sub-Antarctic: king penguins show remarkably little population genetic differentiation across their range. BMC Evol. Biol. https://doi.org/10.1186/s12862-016-0784-z.

Cole, J.R., Konstantinidis, K., Farris, R.J., Tiedje, J.M., 2010. Microbial diversity and phylogeny: extending from rRNAs to genomes. In: Liu, W.-T., Jansson, J.K. (Eds.), Environmental Molecular Microbiology. Caister Academic Press, U.K., pp. 1–19.

Cox, M.P., Peterson, D.A., Biggs, P.J., 2010. SolexaQA: at-a-glance quality assessment of Illumina second. BMC Bioinforma. 11, 485. https://doi.org/10.1016/j.ophtha.2011.10.010.

Dai, H.T., Zhu, R. Bin, Sun, B.W., Che, C.S., Hou, L.J., 2020. Effects of sea animal activities on tundra soil denitrification and *nirS*- and *nirK*-encoding denitrifier community in Maritime Antarctica. Front. Microbiol. 11. https://doi.org/10.3389/fmicb.2020.573302.

Domeignoz-Horta, L.A., Putz, M., Spor, A., Bru, D., Breuil, M.C., Hallin, S., Philippot, L., 2016. Non-denitrifying nitrous oxide-reducing bacteria - an effective N_2O sink in soil. Soil Biol. Biochem. 103, 376–379. https://doi.org/10.1016/j.soilbio.2016.09.010.

Dunn, O.J., 1964. Multiple comparisons using rank sums. Technometrics 6, 241–252. https://doi.org/10.1080/00401706.1964.10490181.

Edgar, R.C., 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26, 2460–2461. https://doi.org/10.1093/bioinformatics/btq461.

Graf, D.R.H., Jones, C.M., Hallin, S., 2014. Intergenomic comparisons highlight modularity of the denitrification pathway and underpin the importance of community structure for N_2O emissions. PLoS One 9, 1–20. https://doi.org/10.1371/journal.pone.0114118.

Green, S.J., Prakash, O., Jasrotia, P., Overholt, W.A., Cardenas, E., Hubbard, D., Tiedje, J.M., Watson, D.B., Schadt, C.W., Brooks, S.C., Kostka, J.E., 2012. Denitrifying bacteria from the genus *Rhodanobacter* dominate bacterial communities in the highly contaminated subsurface of a nuclear legacy waste site. Appl. Environ. Microbiol. 78, 1039–1047. https://doi.org/10.1128/AEM.06435-11.

Guo, G., Tian, F., Ding, K., Wang, L., Liu, T., Yang, F., 2017. Effect of a bacterial consortium on the degradation of polycyclic aromatic hydrocarbons and bacterial community composition in Chinese soils. Int. Biodeterior. Biodegrad. 123, 56–62. https://doi.org/10.1016/j.ibiod.2017.04.022.

Guo, Y., Wang, N., Li, G., Rosas, G., Zang, J., Ma, Y., Liu, J., Han, W., Cao, H., 2018. Direct and indirect effects of penguin feces on microbiomes in Antarctic ornithogenic soils. Front. Microbiol. 9, 552. https://doi.org/10.3389/fmicb.2018.00552.

Hallin, S., Philippot, L., Löffler, F.E., Sanford, R.A., Jones, C.M., 2018. Genomics and ecology of novel N_2O-reducing microorganisms. Trends Microbiol. 26, 43–55. https://doi.org/10.1016/j.tim.2017.07.003.

Hamamura, N., Olson, S.H., Ward, D.M., Inskeep, W.P., 2006. Microbial population dynamics associated with crude-oil biodegradation in diverse soils. Appl. Environ. Microbiol. 72, 6316–6324. https://doi.org/10.1128/AEM.01015-06.

Hoegh-Guldberg, O., J, D., T, M., B, M., B, S., C, I., D, A., D, R., Ebi, K., Engelbrecht, F., Guiot, J., Hijioka, Y., Mehrotra, S., Payne, A., Seneviratne, S.I., Thomas, A., Warren, R., Zhou, G., 2018. Chapter 3: impacts of 1.5 °C global warming on natural and human systems. Global Warming of 1.5 °C an IPCC Special Report on the Impacts of Global Warming of 1.5 °C Above Pre-Industrial Levels and Related Global Greenhouse Gas Emission Pathways, in the Context of Strengthening the Global Response to the Threat of Climate Change.

Hu, H.W., Chen, D., He, J.Z., 2015. Microbial regulation of terrestrial nitrous oxide formation: understanding the biological pathways for prediction of emission rates. FEMS Microbiol. Rev. 39, 729–749. https://doi.org/10.1093/femsre/fuv021.

Hyatt, D., Chen, G., LoCascio, P., Land, M., Larimer, F., Hauser, L., 2010. Prodigal: prokaryotic gene recognition and translation initiation site identification. BMC Bioinforma. 11, 119. https://doi.org/10.3389/fgene.2015.00348.

Jain, C., Rodriguez-R, L.M., Phillippy, A.M., Konstantinidis, K.T., Aluru, S., 2018. High throughput ANI analysis of 90K prokaryotic genomes reveals clear species boundaries. Nat. Commun. 9, 5114. https://doi.org/10.1038/s41467-018-07641-9.

Ji, M., Greening, C., Vanwonterghem, I., Carere, C.R., Bay, S.K., Steen, J.A., Montgomery, K., Lines, T., Beardall, J., Van Dorst, J., Snape, I., Stott, M.B., Hugenholtz, P., Ferrari, B.C., 2017. Atmospheric trace gases support primary production in Antarctic desert surface soil. Nature 552, 400–403. https://doi.org/10.1038/nature25014.

Jung, J., Yeom, J., Kim, J., Han, J., Lim, H.S., Park, H., Hyun, S., Park, W., 2011. Change in gene abundance in the nitrogen biogeochemical cycle with temperature and nitrogen addition in Antarctic soils. Res. Microbiol. 162, 1018–1026. https://doi.org/10.1016/j.resmic.2011.07.007.

Khalil, M.A.K., Rasmussen, R.A., Shearer, M.J., 2002. Atmospheric nitrous oxide: patterns of global change during recent decades and centuries. Chemosphere 47. https://doi.org/10.1016/S0045-6535(01)00297-1.

Kim, O.S., Chae, N., Lim, H.S., Cho, A., Kim, J.H., Hong, S.G., Oh, J., 2012. Bacterial diversity in ornithogenic soils compared to mineral soils on King George Island, Antarctica. J. Microbiol. 50, 1081–1085. https://doi.org/10.1007/s12275-012-2655-7.

Konrad, H., Shepherd, A., Gilbert, L., Hogg, A.E., McMillan, M., Muir, A., Slater, T., 2018. Net retreat of Antarctic glacier grounding lines. Nat. Geosci. 11. https://doi.org/10.1038/s41561-018-0082-z.

Konstantinidis, K.T., Delong, E.F., 2008. Genomic patterns of recombination clonal divergence and environment in marine microbial populations. ISME J. 2, 1052–1065. https://doi.org/10.1038/ismej.2008.62.

Konstantinidis, K.T., Tiedje, J.M., 2005. Towards a genome-based taxonomy for prokaryotes. J. Bacteriol. 187, 6258–6264. https://doi.org/10.1128/JB.187.18.6258-6264.2005.

Kostka, J.E., Green, S.J., Rishishwar, L., Prakash, O., Katz, L.S., Mariño-Ramírez, L., King Jordan, I., Munk, C., Ivanova, N., Mikhailova, N., Watson, D.B., Brown, S.D., Palumbo, A.V., Brooks, S.C., 2012. Genome sequences for six Rhodanobacter strains, isolated from soils and the terrestrial subsurface, with variable denitrification capabilities. J. Bacteriol. 194, 4461–4462. https://doi.org/10.1128/JB.00871-12.

Kummu, M., Taka, M., Guillaume, J.H.A., 2018. Gridded global datasets for gross domestic product and human development index over 1990–2015. Sci. Data https://doi.org/10.1038/sdata.2018.4.

Kuypers, M.M.M., Marchant, H.K., Kartal, B., 2018. The microbial nitrogen-cycling network. Nat. Rev. Microbiol. 16, 263–276. https://doi.org/10.1038/nrmicro.2018.9.

Lambrechts, S., Willems, A., Tahon, G., 2019. Uncovering the uncultivated majority in Antarctic soils: toward a synergistic approach. Front. Microbiol. 10, 1–19. https://doi.org/10.3389/fmicb.2019.00242.

Liguang, S., Renbin, Z., Xuebin, Y., Xiaodong, L., Zhouqing, X., Yuhong, W., 2004. A geochemical method for the reconstruction of the occupation history of a penguin colony in the maritime Antarctic. Polar Biol. 27, 670–678. https://doi.org/10.1007/s00300-004-0635-z.

Lindeboom, H.J., 1984. The nitrogen pathway in a penguin rookery. Ecology 65, 269–277. https://doi.org/10.2307/1939479.

Liu, B., Frostegård, Å., Bakken, L.R., 2014. Impaired reduction of N_2O to N_2 in acid soils is due to a posttranscriptional interference with the expression of nosZ. MBio 5, e01383-14. https://doi.org/10.1128/mBio.01383-14.

Lourenço, K.S., Cassman, N.A., Pijl, A.S., van Veen, J.A., Cantarella, H., Kuramae, E.E., 2018. Nitrosospira sp. govern nitrous oxide emissions in a tropical soil amended with residues of bioenergy crop. Front. Microbiol. https://doi.org/10.3389/fmicb.2018.00674.

Love, M.I., Huber, W., Anders, S., 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 15, 550. https://doi.org/10.1186/s13059-014-0550-8.

Lycus, P., Bøthun, K.L., Bergaust, L., Shapleigh, J.P., Bakken, L.R., Frostegård, Å., 2017. Phenotypic and genotypic richness of denitrifiers revealed by a novel isolation strategy. ISME J. 11, 2219–2232. https://doi.org/10.1038/ismej.2017.82.

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. https://doi.org/10.1007/s00300-013-1355-z.

Meziti, A., Tsementzi, D., Rodriguez-R, L.M., Hatt, J.K., Karayanni, H., Kormas, K.A., Konstantinidis, K.T., 2019. Quantifying the changes in genetic diversity within sequence-discrete bacterial populations across a spatial and temporal riverine gradient. ISME J. 13, 767–779. https://doi.org/10.1038/s41396-018-0307-6.

Michel, R.F.M., Schaefer, C.E.G.R., López-Martínez, J., Simas, F.N.B., Haus, N.W., Serrano, E., Bockheim, J.G., 2014. Soils and landforms from Fildes Peninsula and Ardley Island, Maritime Antarctica. Geomorphology https://doi.org/10.1016/j.geomorph.2014.03.041.

Misselbrook, T.H., Webb, J., Chadwick, D.R., Ellis, S., Pain, B.F., 2001. Gaseous emissions from outdoor concrete yards used by livestock. Atmos. Environ. 35, 5331–5338. https://doi.org/10.1016/S1352-2310(01)00289-8.

Myrcha, A., Tatur, A., 1991. Ecological role of the current and abandoned penguin rookeries in the land environment of the maritime Antarctic. Polish Polar Res. 12, 3–24.

Nayfach, S., Pollard, K.S., 2015. Average genome size estimation improves comparative metagenomics and sheds light on the functional ecology of the human microbiome. Genome Biol. 16, 51. https://doi.org/10.1186/s13059-015-0611-7.

Nelson, M.B., Martiny, A.C., Martiny, J.B.H., 2016. Global biogeography of microbial nitrogen-cycling traits in soil. Proc. Natl. Acad. Sci. 113, 8033–8040. https://doi.org/10.1073/pnas.1601070113.

Neufeld, Â.D.H., de Godoi, S.G., Pereira, A.B., Bayer, C., Schünemann, A.L., Victoria, F. de C., de Albuquerque, M.P., Camargo, E., Vieira, F.C.B., 2015. Methane and nitrous oxide fluxes in relation to vegetation covers and bird activity in ice-free soils of Rip Point, Nelson Island, Antarctica. Polar Res. 34, 1–12. https://doi.org/10.3402/polar.v34.23584.

Nie, Y., Liu, X., Sun, L., Emslie, S.D., 2012. Effect of penguin and seal excrement on mercury distribution in sediments from the Ross Sea region, East Antarctica. Sci. Total Environ. 433, 132–140. https://doi.org/10.1016/j.scitotenv.2012.06.022.

Oksanen, J., 2017. Vegan: Ecological Diversity (R Packag. Version 2.4-4).

Olm, M.R., Crits-Christoph, A., Diamond, S., Lavy, A., Matheus Carnevali, P.B., Banfield, J.F., 2020. Consistent metagenome-derived metrics verify and delineate bacterial species boundaries. mSystems 5, e00731-19. https://doi.org/10.1128/msystems.00731-19.

Ondov, B.D., Treangen, T.J., Melsted, P., Mallonee, A.B., Bergman, N.H., Koren, S., Phillippy, A.M., 2016. Mash: fast genome and metagenome distance estimation using MinHash. Genome Biol. 17, 132. https://doi.org/10.1186/s13059-016-0997-x.

Orellana, L.H., Rodriguez-R, L.M., Higgins, S., Chee-Sanford, J.C., Sanford, R.A., Ritalahti, K.M., Löffler, F.E., Konstantinidis, K.T, 2014. Detecting nitrous oxide reductase (nosZ) genes in soil metagenomes: method development and implications for the nitrogen cycle. MBio 5 (3), e01193–e011914. https://doi.org/10.1128/mBio.01193-14.

Orellana, L.H., Rodriguez-R, L.M., Konstantinidis, K.T., 2017. ROCker: accurate detection and quantification of target genes in short-read metagenomic data sets by modeling sliding-window bitscores. Nucleic Acids Res. 45, e14. https://doi.org/10.1093/nar/gkw900.

Orellana, L., Chee-Sanford, J., Sanford, R.A., Löffler, F.E., Konstantinidis, K.T., 2018. Year-round shotgun metagenomes reveal stable microbial communities in agricultural soils and novel ammonia oxidizers responding to fertilization. Appl. Environ. Microbiol. 84. https://doi.org/10.1128/AEM.01646-17.

Orellana, L.H., Hatt, J.K., Iyer, R., Chourey, K., Hettich, R.L., Spain, J.C., Yang, W.H., Chee-Sanford, J.C., Sanford, R.A., Löffler, F.E., Konstantinidis, K.T., 2019. Comparing DNA, RNA and protein levels for measuring microbial dynamics in soil microcosms amended with nitrogen fertilizer. Sci. Rep. 9. https://doi.org/10.1038/s41598-019-53679-0.

Overbeek, R., Begley, T., Butler, R.M., Choudhuri, J.V., Chuang, H.Y., Cohoon, M., de Crécy-Lagard, V., Diaz, N., Disz, T., Edwards, R., Fonstein, M., Frank, E.D., Gerdes, S., Glass, E.M., Goesmann, A., Hanson, A., Iwata-Reuyl, D., Jensen, R., Jamshidi, N., Krause, L., Kubal, M., Larsen, N., Linke, B., McHardy, A.C., Meyer, F., Neuweger, H., Olsen, G., Olson, R., Osterman, A., Portnoy, V., Pusch, G.D., Rodionov, D.A., Rül;ckert, C., Steiner, J., Stevens, R., Thiele, I., Vassieva, O., Ye, Y., Zagnitko, O., Vonstein, V., 2005. The subsystems approach to genome annotation and its use in the project to annotate 1000 genomes. Nucleic Acids Res. 33, 5691–5702. https://doi.org/10.1093/nar/gki866.

Palleroni, N., 1952. *Nitrosospira* en tierras de la Antartida Argentina. Revista de la Facultad de Ciencias Agrarias 2, p. 46. https://bdigital.uncu.edu.ar/6901.

Parks, D.H., Rinke, C., Chuvochina, M., Chaumeil, P.A., Woodcroft, B.J., Evans, P.N., Hugenholtz, P., Tyson, G.W., 2017. Recovery of nearly 8,000 metagenome-assembled genomes substantially expands the tree of life. Nat. Microbiol. 2, 1533–1542. https://doi.org/10.1038/s41564-017-0012-7.

Peng, Y., Leung, H.C.M., Yiu, S.M., Chin, F.Y.L., 2012. IDBA-UD: a de novo assembler for single-cell and metagenomic sequencing data with highly uneven depth. Bioinformatics 28, 1420–1428. https://doi.org/10.1093/bioinformatics/bts174.

Pérez, T., Trumbore, S., Tyler, S., Matson, P., Ortiz-Monasterio, T., Griffith, D., 2001. Identifying the agricultural imprint on the global N:O budget using stable isotopes. J. Geophys. Res. 106, 9869–9878. https://doi.org/10.1029/2000JD900809.

Prakash, O., Green, S.J., Singh, P., Jasrotia, P., Kostka, J.E., 2021. Stress-related ecophysiology of members of the genus Rhodanobacter isolated from a mixed waste contaminated subsurface. Front. Environ. Sci. Eng. 15. https://doi.org/10.1007/s11783-020-1315-0.

Prieme, A., Christensen, S., 2001. Natural perturbations, drying-wetting and freezing-thawing cycles, and the emission of nitrous oxide, carbon dioxide and methane from farmed organic soils. Soil Biol. Biochem. 33, 2083–2091. https://doi.org/10.1016/S0038-0717(01)00140-7.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O., 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 41, 590–596. https://doi.org/10.1093/nar/gks1219.

R Core Team, 2019. R: A Language and Environment for Statistical Computing. R Found. Stat. Comput.

Ramírez-Fernández, L., Trefault, N., Carú, M., Orlando, J., 2019. Seabird and pinniped shape soil bacterial communities of their settlements in Cape Shirreff, Antarctica. PLoS One 14, 1–22. https://doi.org/10.1371/journal.pone.0209887.

Renner, E.D., Becker, G.E., 1970. Production of nitric oxide and nitrous oxide during denitrification by Corynebacterium nephridii. J. Bacteriol. https://doi.org/10.1128/jb.101.3.821-826.1970.

Repo, M.E., Susiluoto, S., Lind, S.E., Jokinen, S., Elsakov, V., Biasi, C., Virtanen, T., Martikainen, P.J., 2009. Large nitrous oxide emissions from cryoturbated peat soil in tundra. Nat. Geosci. 2, 189–192. https://doi.org/10.1038/ngeo434.

Rice, M.C., Norton, J.M., Valois, F., Bollmann, A., Bottomley, P.J., Klotz, M.G., Laanbroek, H.J., Suwa, Y., Stein, L.Y., Sayavedra-Soto, L., Woyke, T., Shapiro, N., Goodwin, L.A., Huntemann, M., Clum, A., Pillay, M., Kyrpides, N., Varghese, N., Mikhailova, N., Markowitz, V., Palaniappan, K., Ivanova, N., Stamatis, D., Reddy, T.B.K., Ngan, C.Y., Daum, C., 2016. Complete genome of Nitrosospira briensis C-128, an ammonia-oxidizing bacterium from agricultural soil. Stand. Genom. Sci. https://doi.org/10.1186/s40793-016-0168-4.

Robinson, A., 2007. Randomization, bootstrap and Monte Carlo methods in biology. J. R. Stat. Soc. A 170, 856. https://doi.org/10.1111/j.1467-985x.2007.00485_5.x.

Rodriguez-R, L.M., Konstantinidis, K.T., 2014a. Nonpareil: a redundancy-based approach to assess the level of coverage in metagenomic datasets. Bioinformatics 30, 629–635. https://doi.org/10.1093/bioinformatics/btt584.

Rodriguez-R, L.M., Konstantinidis, K.T., 2014b. Estimating coverage in metagenomic data sets and why it matters. ISME J. 8, 2349–2351. https://doi.org/10.1038/ismej.2014.76.

Rodriguez-R, L.M., Konstantinidis, K.T, 2016. The enveomics collection: a toolbox for specialized analyses of microbial genomes and metagenomes. PeerJ Preprints 4, e1900v1. https://doi.org/10.7287/peerj.preprints.1900v1.

Rodriguez-R, L.M., Gunturu, S., Harvey, W.T., Rosselló-Mora, R., Tiedje, J.M., Cole, J.R., Konstantinidis, K.T., 2018a. The Microbial Genomes Atlas (MiGA) webserver: taxonomic and gene diversity analysis of Archaea and Bacteria at the whole genome level. Nucleic Acids Res. 46, W282–W288. https://doi.org/10.1093/nar/gky467.

Rodriguez-R, L.M., Gunturu, S., Tiedje, J.M., Cole, J.R., Konstantinidis, K.T., 2018b. Nonpareil 3: fast estimation of metagenomic coverage and sequence diversity. mSystems 10, e00039-18. https://doi.org/10.1128/msystems.00039-18.

Rodriguez-R, L.M., Tsementzi, D., Luo, C., Konstantinidis, K.T., 2020. Iterative subtractive binning of freshwater chronoseries metagenomes identifies over 400 novel species and their ecologic preferences. Environ. Microbiol. 22, 3394–3412. https://doi.org/10.1111/1462-2920.15112.

Rütting, T., Boeckx, P., Müller, C., Klemedtsson, L., 2011. Assessment of the importance of dissimilatory nitrate reduction to ammonium for the terrestrial nitrogen cycle. Biogeosciences 8, 1779–1791. https://doi.org/10.5194/bg-8-1779-2011.

Sadzawka, A., Grez, R., Mora, M.L., 2004. Métodos de análisis recomendados para los suelos chilenos. Comisión de normalización y acreditación sociedad chilena de la ciencia del suelo. Instituto de Investigaciones Agropecuarias, Santiago, Chile Serie Actas INIA N°34.

Sanford, R.A., Wagner, D.D., Wu, Q., Chee-Sanford, J.C., Thomas, S.H., Cruz-Garcia, C., Rodriguez, G., Massol-Deya, A., Krishnani, K.K., Ritalahti, K.M., Nissen, S., Konstantinidis, K.T., Loffler, F.E., 2012. Unexpected nondenitrifier nitrous oxide reductase gene diversity and abundance in soils. Proc. Natl. Acad. Sci. 109, 19709–19714. https://doi.org/10.1073/pnas.1211238109.

Schaefer, C.E.G.R., Simas, F.N.B., Gilkes, R.J., Mathison, C., da Costa, L.M., Albuquerque, M.A., 2008. Micromorphology and microchemistry of selected Cryosols from maritime Antarctica. Geoderma 144, 104–115. https://doi.org/10.1016/j.geoderma.2007.10.018.

Shravage, B.V., Dayananda, K.M., Patole, M.S., Shouche, Y.S., 2007. Molecular microbial diversity of a soil sample and detection of ammonia oxidizers from Cape Evans, Mcmurdo Dry Valley, Antarctica. Microbiol. Res. 162, 15–25. https://doi.org/10.1016/j.micres.2006.01.005.

Siciliano, S.D., Palmer, A.S., Winsley, T., Lamb, E., Bissett, A., Brown, M.V., van Dorst, J., Ji, M., Ferrari, B.C., Grogan, P., Chu, H., Snape, I., 2014. Soil fertility is associated with fungal and bacterial richness, whereas pH is associated with community composition in polar soil microbial communities. Soil Biol. Biochem. 78. https://doi.org/10.1016/j.soilbio.2014.07.005.

Simas, F.N.B., Schaefer, C.E.G.R., Melo, V.F., Albuquerque-Filho, M.R., Michel, R.F.M., Pereira, V.V., Gomes, M.R.M., da Costa, L.M., 2007. Ornithogenic cryosols from Maritime Antarctica: phosphatization as a soil forming process. Geoderma 138, 191–203. https://doi.org/10.1016/j.geoderma.2006.11.011.

Siniff, D.B., Garrott, R.A., Rotella, J.J., Fraser, W.R., Ainley, D.G., 2008. Opinion: projecting the effects of environmental change on Antarctic seals. Antarct. Sci. 20. https://doi.org/10.1017/S0954102008001351.

Smellie, J.L., Pallàs, R., Sàbat, F., Zheng, X., 1996. Age and correlation of volcanism in central Livingston Island, South Shetland Islands: K-Ar and geochemical constraints. J. S. Am. Earth Sci. https://doi.org/10.1016/0895-9811(96)00012-0.

Su, X., Xu, J., Ning, K., 2012. Parallel-META: efficient metagenomic data analysis based on high-performance computation. BMC Syst. Biol. 6, S16. https://doi.org/10.1186/1752-0509-6-S1-S16.

Sun, L., Zhu, R., Xie, Z., Xing, G., 2002. Emissions of nitrous oxide and methane from Antarctic Tundra: role of penguin dropping deposition. Atmos. Environ. 36, 4977–4982. https://doi.org/10.1016/S1352-2310(02)00340-0.

Teepe, R., Brumme, R., Beese, F., 2001. Nitrous oxide emissions from soil during freezing and thawing periods. Soil Biol. Biochem. 33, 1269–1275. https://doi.org/10.1016/S0038-0717(01)00084-0.

Tyson, G.W., Chapman, J., Hugenholtz, P., Allen, E.E., Ram, R.J., Richardson, P.M., Solovyev, V.V., Rubin, E.M., Rokhsar, D.S., Banfield, J.F., 2004. Community structure and metabolism through reconstruction of microbial genomes from the environment. Nature 428, 37–43. https://doi.org/10.1038/nature02340.

Ugolini, F., 1972. Ornithogenic soils of Antarctica. Antarct. Terr. Biol. 20, 181–193.

Uniprot-Consortium, 2015. UniProt: a hub for protein information. Nucleic Acids Res. 43, D204–D212. https://doi.org/10.1093/nar/gku989.

Urakawa, H., Garcia, J.C., Nielsen, J.L., Le, V.Q., Kozlowski, J.A., Stein, L.Y., Lim, C.K., Pommerening-Röser, A., Martens-Habbena, W., Stahl, D.A., Klotz, M.G., 2015. Nitrosospira lacus sp. nov., a psychrotolerant, ammonia-oxidizing bacterium from sandy lake sediment. Int. J. Syst. Evol. Microbiol. 65, 242–250. https://doi.org/10.1099/ijs.0.070789-0.

Van Den Heuvel, R.N., Van Der Biezen, E., Jetten, M.S.M., Hefting, M.M., Kartal, B., 2010. Denitrification at pH 4 by a soil-derived *Rhodanobacter*-dominated community. Environ. Microbiol. 12, 3264–3271. https://doi.org/10.1111/j.1462-2920.2010.02301.x.

Van Dorst, J.M., Hince, G., Snape, I., Ferrari, B.C., 2016. Novel culturing techniques select for heterotrophs and hydrocarbon degraders in a subntarctic soil. Sci. Rep. 6, 1–13. https://doi.org/10.1038/srep36724.

Wagner, M., Trutschnig, W., Bathke, A.C., Ruprecht, U., 2018. A First Approach to Calculate BIOCLIM Variables and Climate Zones for Antarctica. https://doi.org/10.1007/s00704-017-2053-5.

Wang, N.F., Zhang, T., Zhang, F., Wang, E.T., He, J.F., Ding, H., Zhang, B.T., Liu, J., Ran, X. Bin, Zang, J.Y., 2015. Diversity and structure of soil bacterial communities in the Fildes Region (maritime Antarctica) as revealed by 454 pyrosequencing. Front. Microbiol. 6, 1–11. https://doi.org/10.3389/fmicb.2015.01188.

Wang, Q., Zhu, R., Zheng, Y., Bao, T., Hou, L., 2019. Effects of sea animal colonization on the coupling between dynamics and activity of soil ammonia-oxidizing bacteria and archaea in maritime Antarctica. Biogeosciences 16. https://doi.org/10.5194/bg-16-4113-2019.

Watanabe, Y.Y., Ito, K., Kokubun, N., Takahashi, A., 2020. Foraging behavior links sea ice to breeding success in Antarctic penguins. Sci. Adv. https://doi.org/10.1126/sciadv.aba4828.

Welsh, A., Chee-Sanford, J.C., Connor, L.M., Löffler, F.E., Sanford, R.A., 2014. Refined NrfA phylogeny improves PCR-based nrfA gene detection. Appl. Environ. Microbiol. 80, 2110–2119. https://doi.org/10.1128/AEM.03443-13.

Wu, C.Y., Zhuang, L., Zhou, S.G., Li, F.B., He, J., 2011. Corynebacterium humireducens sp. nov., an alkaliphilic, humic acid-reducing bacterium isolated from a microbial fuel cell. Int. J. Syst. Evol. Microbiol. https://doi.org/10.1099/ijs.0.020909-0.

Wu, Y.-W., Tang, Y.-H., Tringe, S.G., Simmons, B.A., Singer, S.W., 2014. MaxBin: an automated binning method to recover individual genomes from metagenomes using. Microbiome 2, 4904–4909. https://doi.org/10.1073/pnas.1402564111.

Xing, G., Zhu, Z., 1997. Preliminary studies on N_2O emission fluxes from upland soils and paddy soils in China. Nutr. Cycl. Agroecosyst. 49, 17–22. https://doi.org/10.1023/a:1009775413487.

Yang, J.E., Kim, J.J., Skogley, E.O., Schaff, B.E., 1988. A simple spectrophotometric determination of nitrate in water, resin, and soil extracts. Soil Sci. Soc. Am. J. 62, 1108–1115. https://doi.org/10.2136/sssaj1998.03615995006200040036x.

Yarza, P., Yilmaz, P., Pruesse, E., Glöckner, F.O., Ludwig, W., Schleifer, K.H., Whitman, W.B., Euzéby, J., Amann, R., Rosselló-Móra, R., 2014. Uniting the classification of cultured and uncultured bacteria and archaea using 16S rRNA gene sequences. Nat. Rev. Microbiol. 12, 635–645. https://doi.org/10.1038/nrmicro3330.

Yew, W.C., Pearce, D.A., Dunn, M.J., Adlard, S., Alias, S.A., Samah, A.A., Convey, P., 2018. Links between bacteria derived from penguin guts and deposited guano and the surrounding soil microbiota. Polar Biol. 41, 269–281. https://doi.org/10.1007/s00300-017-2189-x.

Zhu, R., Liu, Y., Ma, E., Sun, J., Xu, H., Sun, L., 2009. Nutrient compositions and potential greenhouse gas production in penguin guano, ornithogenic soils and seal colony soils in coastal Antarctica. Antarct. Sci. 21, 427. https://doi.org/10.1017/S0954102009990204.

Zhu, R., Chen, Q., Ding, W., Xu, H., 2012. Impact of seabird activity on nitrous oxide and methane fluxes from High Arctic tundra in Svalbard, Norway. J. Geophys. Res. Biogeosci. 117, 1–16. https://doi.org/10.1029/2012JG002130.

Zhu, R., Liu, Y., Xu, H., Ma, D., Jiang, S., 2013. Marine animals significantly increase tundra N_2O and CH_4 emissions in maritime Antarctica. J. Geophys. Res. Biogeosci. 118, 1773–1792. https://doi.org/10.1002/2013JG002398.