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Understanding drivers of antibiotic resistance genes in High Arctic soil ecosystems

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ABSTRACT

Soils in tropical and temperate locations are known to be a sink for the genetic potential of anthropogenic-driven acquired antibiotic resistance (AR). In contrast, accumulation of acquired AR is less probable in most Polar soils, providing a platform for characterizing background resistance and establishing a benchmark for assessing AR spread. Here, high-throughput qPCR and geochemistry were used to quantify the abundance and diversity of both antibiotic resistance genes (ARGs) and selected mobile genetic elements (MGEs) across eight soil clusters in the Kongsfjorden region of Svalbard in the High Arctic. Relative ARG levels ranged by over two orders of magnitude (10^{-6} to 10^{-4} copies/16S rRNA gene copy), and showed a gradient of potential human and wildlife impacts across clusters as evidenced by altered geochemical conditions and increased "foreign" ARG abundances (i.e., allochthonous), including $bla_{\text{NDM-1}}$. Impacted clusters exhibited 100× higher total ARGs and MGEs in tandem with elevated secondary nutrients, especially available P that is typically low and limiting in Arctic soils. In contrast, ARGs in less-impacted clusters correlated strongly to local soil lithology. The most plausible source of exogenous P and allochthonous ARGs in this region is bird and other wildlife guano, disseminated either by local human wastes or via direct carriage and deposition. Regardless of pathway, accumulation of apparent allochthonous ARGs and MGEs in High Arctic soils is concerning, highlighting the importance of characterizing Arctic sites now to establish benchmarks for tracking AR spread around the world.

1. Introduction

Increasing antibiotic resistance (AR) is a global health crisis with growing evidence that society may soon enter an era where our most critical antibiotics cease to be effective (O'Neill, 2016). While efforts are underway to reduce AR via improved antibiotics stewardship, local resistance continues to expand in many parts of the world, often becoming global very quickly (Molton et al., 2013). Most worryingly, multidrug resistant genotypes are undergoing especially rapid geographical spread. For example, possible "superbugs" that express New Delhi metallo-β-lactamase-1 protein (coded by $bla_{\text{NDM-1}}$), first reported in India in 2008, have now become global (Khan et al., 2017). Furthermore, resistance to the last resort antibiotic colistin, which was only detected in 2015, is now found in over 50 countries on five continents (Wang et al., 2018). Despite such trends, we have limited understanding of the main drivers of AR spread, especially the relative role of the environmental pathways at local and global scales (Allen et al., 2010). This knowledge gap largely results from the myriad of anthropogenic impacts in the temperate world. In contrast, Polar Regions, which are less directly impacted by humans, may be a better location to characterize and benchmark AR more typical of the pre-antibiotic era.

Soils are a useful platform for tracking changes in AR. While soils are a known source of ancient AR (D'Costa et al., 2011), recent soil archive data from the Netherlands show the relative abundance of antibiotic resistance genes (ARGs) has significantly increased since the 1940s (Knapp et al., 2010). Such increases often reflect the input of genes due to human and other exposures (i.e., "allochthonous" ARGs) on top of "ancient" soil ARGs that were initially present (i.e., "autochthonous" ARGs). Although apparent allochthonous inputs are

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evident, it is almost impossible to distinguish between allochthonous- versus autochthonous-sourced ARGs in the populated world due to widespread pollution from humans, agriculture, and other sources (Knapp et al., 2011; Nesme and Simonet, 2015; Graham et al., 2016); therefore, Polar soil work may be better. For example, studies in Antarctica already have shown that "contaminant" ARGs from human activity are present (Hernández and González-Acuña, 2016; Wang et al., 2016) and few truly "pristine" locations exist (Van Goethem et al., 2018). In contrast, little is known about AR in High Arctic soils, with previous studies limited to permafrost, birds, and marine sediments (Sjölund et al., 2008; Perron et al., 2015; Tan et al., 2018). Importantly, previous Arctic soil AR work did not measure parallel geochemical conditions in detail, especially potentially bioavailable nutrients. This critical information is required to explain autochthonous ARG levels and diversity as well as to delineate possible drivers of ARGs in local soils.

Here, we identified the Kongsfjorden region on Svalbard as an ideal location for AR studies because it is on a remote island; it has no agriculture or industry; it has exceedingly small human populations; and annual temperatures cold enough to readily preserve DNA (Pietramellara et al., 2009). Further, the region provides a different picture of AR than previous Polar studies (e.g., Wang et al., 2016; Van Goethem et al., 2018) as Kongsfjorden never freezes due to Gulf currents, potentially receiving year-round allochthonous inputs. The region is not pristine because possible ARG sources do exist (Shariatinajafabadi et al., 2014), such as local wildlife and a small human settlement (Ny-Ålesund; population < 120); however, the region is sufficiently undisturbed to allow contrasts between sites physically close together to understand local and general drivers of AR spread.

In this study, high-throughput quantitative PCR (HT-qPCR) was used to quantify ARGs and mobile genetic elements (MGEs) from major antibiotic classes, nine transposase genes, and the class 1 integron-integrase gene (*int*I1) to characterize AR across Kongsfjorden. MGEs are noteworthy here because they are often associated with "acquired" resistance and found at higher levels in human or animal waste impacted environments (Ma et al., 2017). In particular, *int*I1 is a possible proxy for fecal pollution and anthropogenic impact (Gillings et al., 2015; Ma et al., 2017). Associated with ARG and MGE data, soil geochemical conditions also were quantified to contextualize possible autochthonous (e.g., local soil geochemistry) versus allochthonous (e.g., wildlife, human inputs) drivers of AR within the region.

2. Materials and methods

2.1. Sample collection and locations

Soils were cored from eight locations (called clusters) along Kongsfjorden in late August 2013 (Fig. 1, Supplementary Table S1). Forty cores were collected in quintuplicate per cluster from within 10 m × 10 m squares. Coring was quasi-random within the squares, although each cluster location was carefully chosen based on data from fieldwork in 2010 (Gray et al., 2014; McCann et al., 2016); potential extents of human exposure and wildlife activity (e.g., proximity to Ny-Ålesund and local wildlife congregation points); and also major soil types across the region. Soils ranged from glacial till (clusters BZ4, BZ5) to polar desert (clusters RH1, BR3, BR4) to organic tundra (clusters GS3, SL3, SL4), although some overlap in soil types did exist within each cluster.

Soil sampling was carried out on the top 10 cm of the soil profile using sterile plastic coring syringes with the luer-end removed. Soil coring avoided places with obvious evidence of wildlife feces and vegetation cover. On return to the Natural Environment Research Council (NERC) research field laboratory in Ny-Ålesund, cores were frozen (−4 °C) and subsequently transported to the UK or USA, where they were stored at −20 °C until further analysis.

2.2. Soil geochemistry

All soils were air dried and sieved to < 2 mm prior to geochemical analyses. Soil pH was determined according to ISO 10390:2005 and total organic carbon (TOC) according to ISO 10694 using a LECO CS244 Carbon analyzer (LECO Instrument Ltd., UK). Soil moisture was measured by drying to constant mass at 105 °C. Soil organic matter (SOM) was quantified using the modified loss-on-ignition method described by Nelson and Sommers (1996). Total P was characterized using dried and homogenized samples extracted with aqua regia (1:3 for HNO_3:HCl), which were refluxed for 3 h, filtered, and diluted in 1% HNO_3 before Inductively Coupled Plasma Mass Spectrometry (Agilent 7500ce ICP-MS) analysis. Available P was determined using US EPA Method 131 (US EPA, 2008). Total nitrogen was estimated by quantification of total kjeldhal nitrogen (TKN) according to standard methods (APHA, 2012).

Soil metals were characterized using dried and homogenized samples extracted with reverse aqua regia (3:1 for HNO_3:HCl) using a DigiPrep HT 100 digestion block for 10 h (ramp to 95 °C for 2 h and then held 8 h). Digested samples were then dried and re-suspended in 1% ultrapure HNO_3 (Optima, Fisher Scientific, USA), filtered, diluted further with 1% HNO_3, and analyzed by Inductively Coupled Plasma Optical Emission Spectroscopy (PerkinElmer, Optima 5300 DV). An ICP certified solution, Cal 2 (PerkinElmer, USA), was used for all calibrations.

US EPA Method 131 (US EPA, 2008) for characterizing mobility of inorganic species in solids was used to quantify operationally defined "bioavailable" metals. Subsamples consisting of 1.0 g homogenized dried soils were extracted with 0.2 M acetic acid at pH 4.9 for 2 h at 25 °C. Extraction fluids were filtered to 0.2 µm, acidified to 1% with ultrapure HNO_3 (Optima, Fisher Scientific, USA), and analyzed by ICP-OES, as described above.

2.3. DNA extraction and high-throughput quantitative PCR

DNA was extracted from 0.5 g of soil using the FastDNA Spin Kit for Soil (MP Biomedicals, UK), according to the manufacturer's instructions. DNA concentration was measured by spectrophotometric analysis using a NanoDrop 1000 (ThermoFisher Scientific). Following extraction, DNA was freeze-dried and shipped to the Institute of Urban Environment, Chinese Academy of Science (Xiamen), where HT-qPCR of ARGs was performed using SmartChip Real-Time PCR (Wafergen Inc., USA). A total of 296 primer sets were used to detect ARGs, transposases, integrase, and 16S rRNA genes, as previously described (Zhu et al., 2013, 2017).

For each primer set, three technical PCR replicates were performed alongside a non-template control. After the initial enzyme activation at 95 °C for 10 min, 40 cycles of the following program were used for amplification: denaturation at 95 °C for 30 s and annealing at 60 °C for 30 s. The melting process was generated automatically by Wafergen software. Amplifications with efficiency beyond the range (90–110%) were discarded for each primer set. The results of the HT-qPCR were analyzed by SmartChip qPCR software (V2.7.0.1). Samples with a CT > 31 were removed, which previous experience indicated to be probable false positives (i.e., CT = 31 was the detection limit). For quality control, results were considered positive when samples with three technical replicates were amplified. Gene copy numbers were calculated by the following formula (Looft et al., 2012): gene copy number = $10^{((31\text{-}Ct)/(10/3))}$. The normalized copy number of ARGs was the ratio of ARG copy number to 16S rRNA gene copy number. Absolute 16S rRNA gene abundances were measured separately, as reported previously (Zhu et al., 2013).

2.4. Statistical analysis

All basic data analyses were conducted using SPSS (Chicago, IL; v. 17.0) and Excel 2010 (Microsoft Office 2010, Microsoft Corp., USA).

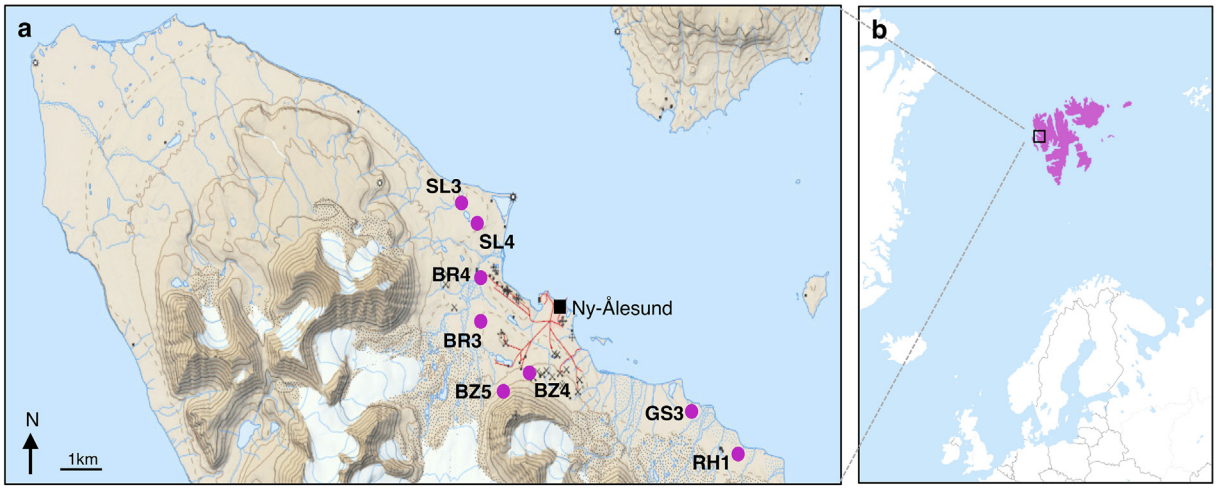

Fig. 1. Sampling locations of (a) the eight soil clusters in Kongsfjorden and (b) the geographical location of Svalbard, High Arctic. Topographic map has been adapted from the Norwegian Polar Institute.

Data were log-transformed where appropriate to ensure sample normality before analysis. Statistical significance was defined by 95% confidence intervals ($p < 0.05$). One core from the GS cluster was removed from all analysis because HT-qPCR amplification failed. Principal component analysis (PCA), distance-based linear modelling (DistLM), redundancy analysis (RDA) and principal coordinate analysis (PCoA) tests were conducted using PRIMER (PRIMER 7, V 7.0.13).

3. Results and discussion

3.1. Soil geochemistry and underlying lithology across soil clusters

The eight Kongsfjorden soil clusters were chosen based on possible AR sources/exposures and previous geochemical data (summarized in Supplementary Tables S2 and S3) on soils from the area (Gray et al., 2014; McCann et al., 2016). Differences among clusters were confirmed using a PCA that showed 67% of variations among soils are explained by lithological factors (e.g., pH, total Ca, total Al) or nutrient conditions (e.g., organic matter, TOC, available P). The eight clusters ranged from soils primarily defined by local lithological factors, such as BZ5 (glacial till) to soils primarily defined by available nutrients (e.g., P) and organic matter, such as SL3 (organic tundra) (Fig. 2). As will be shown, differences between "lithology-defined" and "organic-defined" clusters are helpful in explaining ARGs and MGEs found in clusters as well as possible roles of autochthonous versus allochthonous drivers of local soil ARGs.

Geochemical differences across clusters are consistent with previous reports on soils in the Kongsfjorden region, which indicate Ca and Mg levels are significant in defining local soil conditions (McCann et al., 2016). Ca and Mg are proxies for common carbonate minerals in Kongsfjorden (i.e., dolomite [$MgCa(CO_3)_2$] and calcite [$CaCO_3$]), which in turn influence soil pH conditions as well as microbial abundances and their associated community structure and diversity (Gray et al., 2014; McCann et al., 2016). As noted, although characteristic regional lithology is evident in most clusters, elevated available P and organic matter better define soils in some clusters (Fig. 2), which is important because very different clusters are sometimes close together (Fig. 1). This provides a useful palette for identifying drivers of ARGs and MGEs, given the similarity of background climate and other environmental factors.

3.2. ARGs and MGEs detected in High Arctic soils

In total, 131 ARGs were detected using HT-qPCR from all High

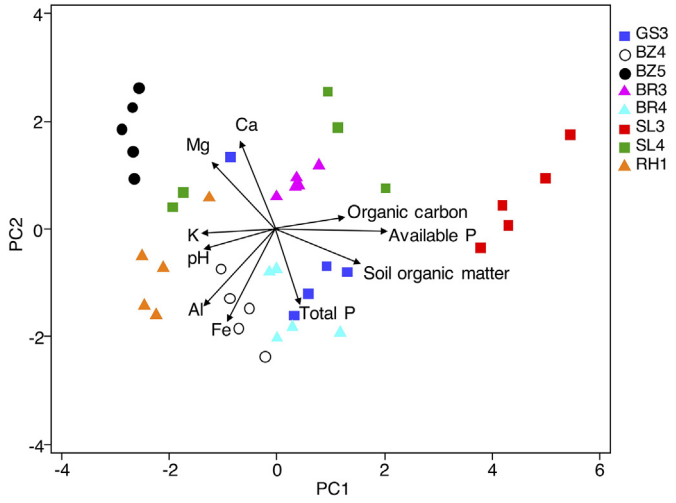

Fig. 2. Principal component analysis of the quintuplet cores in the High Arctic, which show characteristically different local soil geochemistry conditions among clusters, ranging from lithology-defined glacial till clusters (left; e.g. BZ5) to polar desert soil types (varied across center) to organic-defined tundra clusters (right; e.g. SL3). All variables were log transformed to fit a normal distribution.

Arctic soil cores, ranging from 19 ± 7 (in BZ5) to 89 ± 3 (in BR3) distinct ARGs per core (mean total ± standard error; n = 4 or 5), with an average of 66 ± 8 ARGs per core. Detected ARGs associated with nine major antibiotic classes, but predominately associated with aminoglycosides (33%), multidrug defense systems (e.g., efflux pump systems; 30%), MLSBs (Macrolide-Lincosamide-Streptogramin; 16%), and β-lactams (8%) (Supplementary Fig. S1a). The main resistance mechanism across soil cores were efflux pumps (42%) and antibiotic deactivation processes (42%), followed by specific cellular protection systems (12%) (Supplementary Fig. S1b). The most abundant ARG in all samples was aac(3)-VI (7.29×10^{-4} copies/16S rRNA gene copy), which is typically associated with aminoglycoside resistance, in particular gentamicin and sisomicin (Rather et al., 1993). This was followed by the acrA gene (6.21×10^{-4} copies/16S rRNA gene copy) and bla_{FOX} gene (3.82×10^{-4} copies/16S rRNA gene copy), which are often associated with β-lactam resistance in *E. coli* and *Klebsiella* (Gonzalez Leiza et al., 1994; Ma et al., 1995).

The relative abundance of ARGs among clusters varied by two orders of magnitude (Fig. 3). Total ARG values ranged from

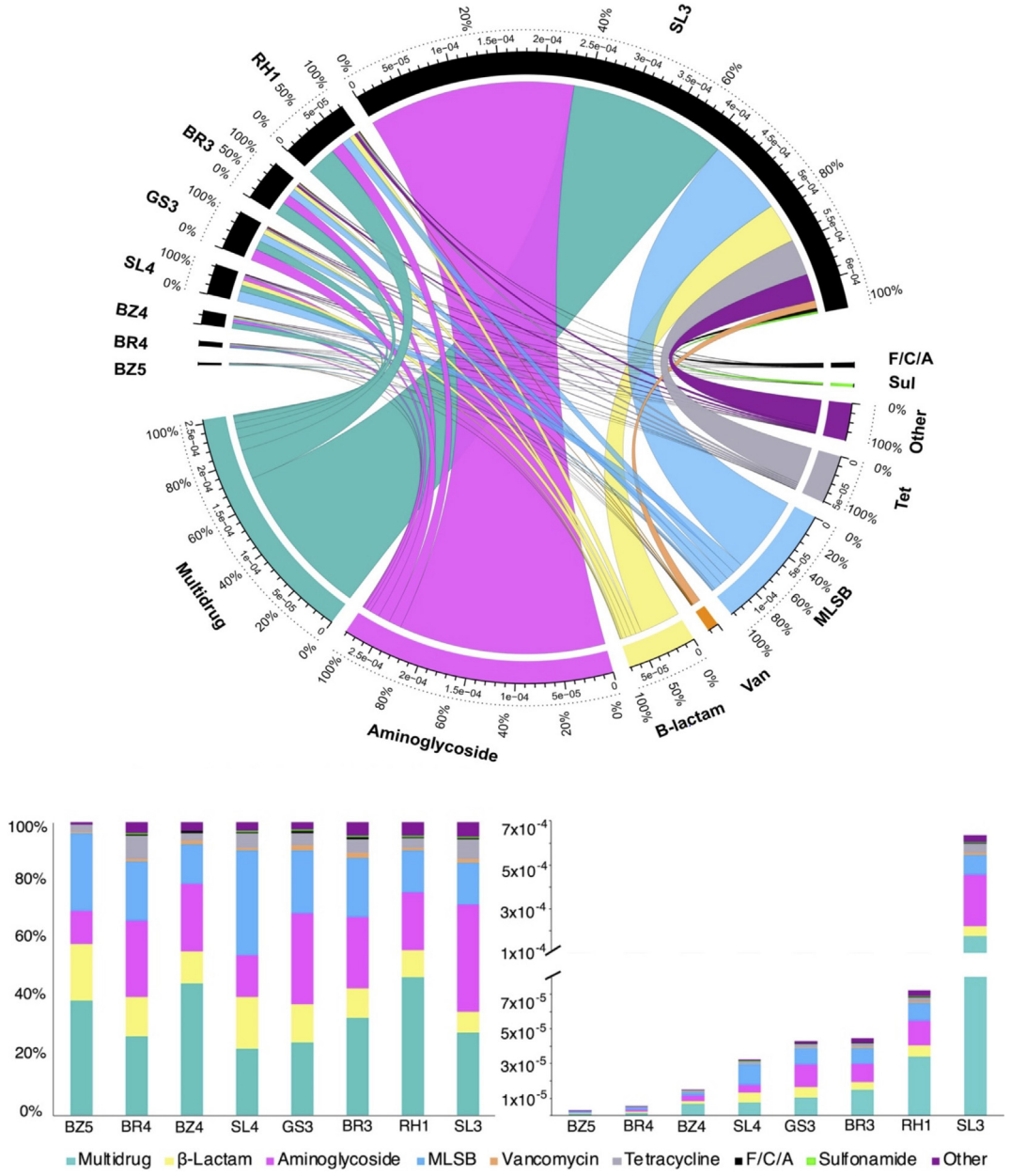

Fig. 3. Distribution and abundance of ARGs in the eight High Arctic clusters classified by antibiotic class and cluster location. The Chord diagram presents the abundance of ARGs in each sample that associate with each respective antibiotic class among the eight soil clusters (top). Presented below the diagram is the relative abundance of ARGs normalized to the total abundance of 16S rRNA gene (bottom right) and relative proportions of ARG classes in each cluster (bottom left). MLSB denotes Macrolide-Lincosamide-Streptogramin B. Other labels represent ARGs that do not have a direct antibiotic class. F/C/A denotes florcholoram-phenicol antibiotic class.

2.71×10^{-6} copies/16S rRNA gene copy in BZ5 to 6.35×10^{-4} copies/16S rRNA gene copy in SL3. Relative abundances of ARGs in Kongsfjorden soils were in agreement with reported levels in Arctic marine sediments, which ranged from 10^{-9} to 10^{-5} copies/16S rRNA gene copy (Tan et al., 2018), except for SL3, which had significantly higher abundances relative to other clusters (one-way ANOVA, $p \leq 0.05$). ARGs in eight clusters were relatively similar when sorted by antibiotic class (Fig. 3).

Multidrug resistance (MDR) genes were present and abundant in five out of eight clusters (BZ4, BZ5, BR3, BR4, RH1; Fig. 3), although MDR genes were dominated by non-specific mechanisms, particularly efflux pumps present in most microorganisms. Such differentiation is important because efflux systems are a common stress response to diverse bacterial stressors (e.g., heavy metals, metabolites, organic pollutants) and not exclusive to antibiotics (Blanco et al., 2016). As such, they likely represent evolutionary selection unrelated to the antibiotic era (i.e., they do not imply "superbugs") and are a common genetic trait in soil microorganisms (Van Goethem et al., 2018). In contrast, aminoglycoside and MLSB resistance genes were dominant in GS3, SL3, and SL4 (Fig. 3). Mechanisms for these antibiotic classes tend to be more drug specific, including modification of ribosomal binding sites or inactivation by specific enzymes (Garneau-Tsodikova and Labby, 2016), exemplifying "acquired" rather than basal AR within these soil clusters. PCoA, using Bray-Curtis distances of all ARGs detected (summarized in Supplementary Table S4), further confirmed that ARG composition within and between clusters differed, with SL3 and BZ5 forming separate and distinct groups from the other six clusters (Supplementary Fig. S2). Finally, PERMANOVA analysis suggested the

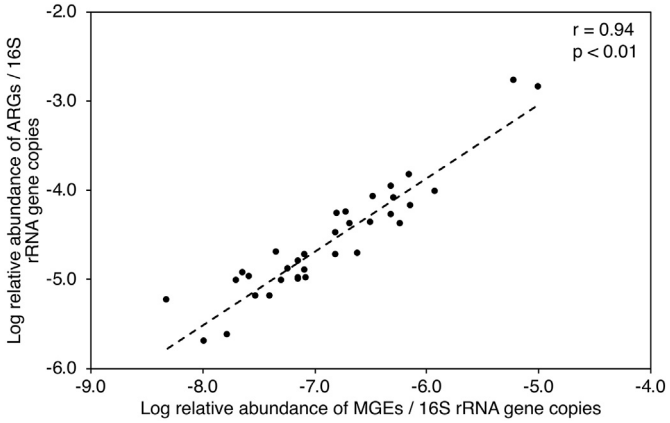

Fig. 4. Relationship between the log-transformed relative abundance of antibiotic resistance genes (ARGs) and mobile genetic elements (MGEs) in all High Arctic active layer soils from all sites.

eight clusters significantly differed in terms of ARG composition ($p < 0.001$), with pairwise comparisons showing all clusters contained significantly different ARG mosaics ($p > 0.03$).

MGEs were detected at seven out of the eight clusters and included eight transposases and *intI*1. BZ5 was the only cluster where none of the MGEs assessed were detected. The abundance of MGEs showed a significant correlation with ARGs across all cores (Pearson's r = 0.94, N = 39, $p < 0.01$; Fig. 4). Such a relationship between ARGs and MGEs has been reported previously (Tan et al., 2018), suggesting that MGE and ARG spread within Kongsfjorden are probably linked. Similar to ARG abundances, MGE levels also varied by two orders of magnitude, ranging from 1.77×10^{-8} in BZ4 to 3.38×10^{-6} copies/16S rRNA gene copy in SL3. Cluster SL3 showed clear MGE enrichment relative to the other seven clusters, although MGEs within SL3 cluster varied among individual cores (Fig. 5a). Presuming MGE levels are a possible proxy for anthropogenic impact (Gillings et al., 2015), this suggests different cores within the SL3 cluster had different levels of allochthonous inputs, such as differing fecal deposits.

3.3. Autochthonous versus allochthonous ARGs in High Arctic soils

To assess putative autochthonous versus allochthonous ARGs, the presence and absence of each ARG was compared across clusters. Of the 131 ARGs detected, 39 were found in all clusters, potentially representing autochthonous background ARGs (Supplementary Table S5). These represent 30% of all ARGs detected and are dominated by multidrug (38%) and β-lactam (24%) antibiotic classes, with the main resistance mechanism defined as efflux (50%). A noteworthy discovery was *pncA*, which was the fifth most abundant gene from across all clusters (1.91×10^{-4} copies/16S rRNA gene copy). This gene confers resistance to the drug Pyrazinamide, which is used in treating multiple-drug resistant tuberculosis. While recently highlighted as critically important to human medicine by the World Health Organization (Collignon et al., 2016), little research has been performed on the prevalence of this gene in either clinical or environmental settings. Finding this ARG in the High Arctic suggests this gene may be directly sourced from the natural environment. In addition, the $bla_{\text{CTX-M}}$ gene, which confers resistance to extended-spectrum β-lactamases (ESBLs) and can associate with carbapenem resistance, was common across all clusters. While it is known already that CTX-M ESBLs are distributed globally (Bevan et al., 2017), they have not been previously found in Polar soils.

Putative allochthonous ARGs, defined here as ARGs only found in some clusters, include a variety of ARGs associated with antibiotics critical to human health, including aminoglycosides, macrolides, carbapenems, penicillin, and cephalosporins (Supplementary Figs. S3–S5).

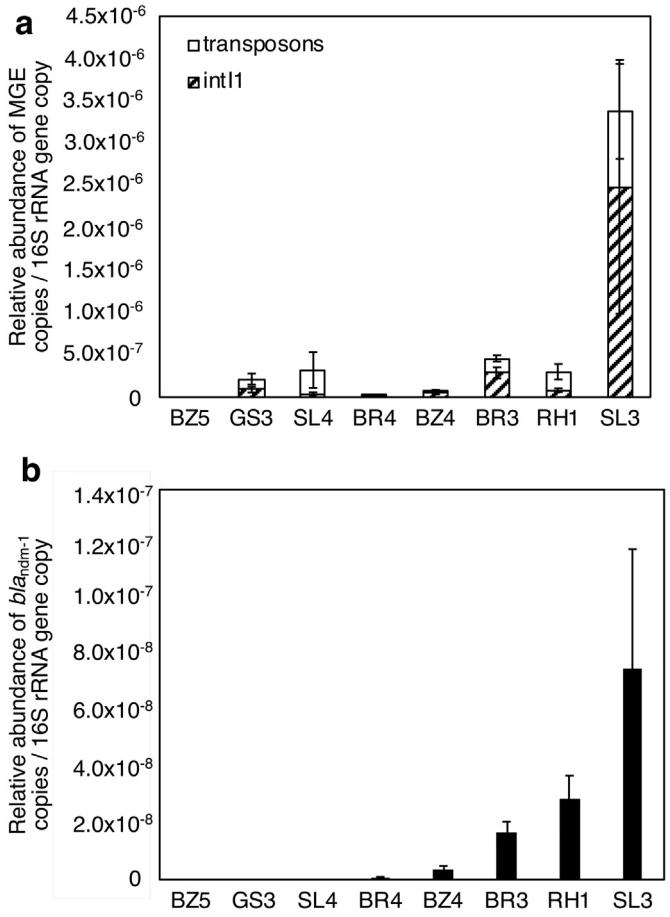

Fig. 5. Relative abundance of (a) mobile genetic elements (MGEs) and (b) $bla_{\text{NDM-1}}$ in all High Arctic active layer soils among soil clusters. Error bars represent the standard error of quintuplicate sampling replicates (n = 5) for all sites, except for GS3 where n = 4.

Of particular interest is $bla_{\text{NDM-1}}$, which was detected in five out of the eight clusters (Fig. 5), and to our knowledge has not been previously detected in the High Arctic. This finding has implications for global AR spread because $bla_{\text{NDM-1}}$ is clearly not "local," providing evidence of ARG migration at international scales. Further, the gene coding for FabK, which confers resistance to the synthetic biocide triclosan, was present in three out of the eight clusters (SL3, RH1, BR3). Triclosan pollution has been reported in High Arctic seawater (Kallenborn et al., 2008), but no previous information exists on this compound in Kongsfjorden. Exposure to triclosan itself in local Arctic soils is highly unlikely, which implies it is an allochthonous ARG in the soils.

Similar to total ARGs and MGEs, high and variable abundances of allochthonous ARGs were found in cluster SL3, but absent in BZ5. Levels of both $bla_{\text{NDM-1}}$ and FabK were approximately 100× greater in SL3 than in the other clusters. Indeed, relative abundances of $bla_{\text{NDM-1}}$ at SL3 were similar to levels found in environments impacted by hospital and urban wastewaters (Devarajan et al., 2016; Subirats et al., 2017).

3.4. Soil geochemistry, lithology, and ARGs

Multivariate RDA revealed 40% of the variation in the abundance of ARGs could be significantly explained by concentrations of available P, Ca, and Mg across all soils ($p < 0.01$), with clear distinctions between BZ5, which is lithology-defined and nutritionally deficient, and SL3, which has higher available P and organic matter (Fig. 6). Overall, Ca and Mg both displayed significant negative linear relationships with the

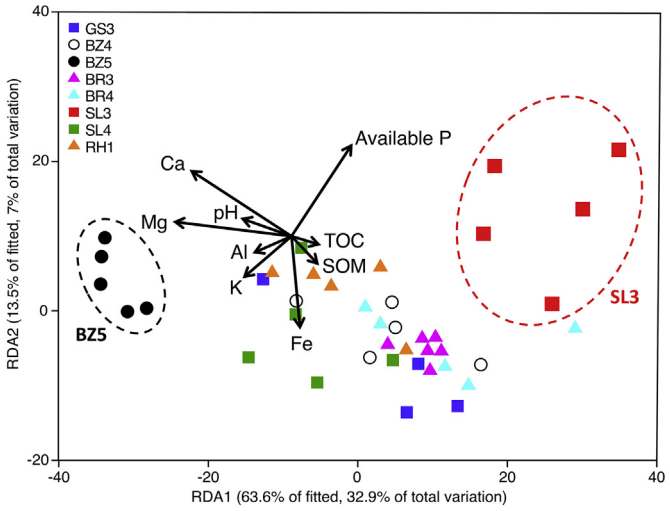

Fig. 6. Distance-based redundancy analysis (dbRDA) plot of the DistLM based on geochemical parameters fitted to the variation in ARG abundance. Vectors indicate direction of the parameter effect in the ordination plot. Soil clusters as noted.

relative abundance of ARGs ($p < 0.05$; Supplementary Table S6), which suggests that the more lithology-defined soil clusters harbor fewer AR genes. This is consistent with previous work in Kongsfjorden that showed Ca and Mg impact local soil pH, with pH displaying a significant, negative relationship with microbial abundances (Gray et al., 2014). A similar trend is seen here between soil pH and ARG abundances (Pearson's r = −0.351, $p = 0.029$, n = 39; Supplementary Table S6)—a relationship also observed elsewhere, including paddy soils in China (Xiao et al., 2016) and soils of Antarctica (Wang et al., 2016).

An inverse correlation between soil pH and microbial abundance has been previously explained by declining phosphorus availability as pH increases (Gray et al., 2014). Here, pH and ARG abundances also inversely correlate, but differences in available P among clusters is much greater than can be simply explained by differences the pH-dependent availability of autochthonous P (e.g., > 100× between BZ5 and SL3; 0.31 ± 0.01 and 44.4 ± 24.8 mg/kg, respectively). Further, SL3 has a large excess of SOM and TKN as well as higher moisture levels (see Supplementary Table S1), which cannot be explained by differences in pH. It is possible extraneous carbon and nutrients may have stimulated some local ARG enrichment. However, recent work showed that the in situ enrichment of ARGs in the soils is likely small compared with the scale of impact due to direct fecal inputs (Karkman et al., 2019).

In summary, SL3 exemplifies a location in the High Arctic with elevated allochthonous inputs, especially extraneous fecal matter, elevated available P, ARGs, and MGEs. The particular role of available P becomes apparent when one compares variations in total versus available P across clusters (Supplementary Table S6). Total P was much less variable across clusters (by 1.6×) and showed no statistical relationship with available P (Pearson's r = 0.081, $p = 0.8$, n = 12). Whereas, total soil P did significantly correlate with C, N, and Mg ($p < 0.05$, n = varied), suggesting total P is primarily defined by lithology. In contrast, available P did not correlate with any lithological variable (p always > 0.05), suggesting extraneous available P in the more organic-defined clusters primarily comes from ex-situ sources. This recognition is key to understanding drivers of AR in High Arctic soils; i.e., ARGs found in most soils are driven by basal lithology, but some soils are dominated by allochthonous inputs and are soils with the highest ARG and MGE abundances.

3.5. ARGs and MGEs in High Arctic soil ecosystems

High Arctic soil ecosystems are typically defined by very low nutrient availability, with P often being severely limiting (Wookey et al., 1995). As such, extraneous fecal matter from wildlife has been shown to be a dominant vector for P migration and deposition in the Arctic, strongly influencing soil geochemistry on local scales in places like Svalbard (Szymański et al., 2016; Zwolicki et al., 2016a,b). For example, the highest content of P in High Arctic soils has been directly related to fertilization from seabird colonies (Szymański et al., 2016), and it is now recognized that such colonies input a similar magnitude of P in global cycles, mostly in bioavailable forms (Otero et al., 2018).

In the Kongsfjorden area, extensive colonies of migratory and non-migratory birds exist, including herbivorous (pink-footed *Anser brachyrhynchus* and barnacle *Branta leucopsis* geese), planktivorous (little auk, *Alle alle*), and piscivorous colonies (mixed colony of Brunnich's guillemot, *Uria lomvia*, and black-legged kittiwake, *Rissa tridactyla*) (Zwolicki et al., 2016a,b). As such, elevated available P at SL3 is most likely attributable to related fecal matter. SL3 is adjacent to the largest freshwater lake near Ny-Ålesund, a place of wildlife congregation evidenced by varied scat along the lakeshore. Available P at SL3 is supplemented by feces from Arctic fox (*Vulpes lagopus*) that live in dens surrounded by green vegetation, which is in turn fertilized by discarded prey and scat from species attracted by food and fresh water (Gharajehdaghipour et al., 2016).

In addition to High Arctic seabirds being vectors of nutrients, ARGs also have been found in their guano (Sjölund et al., 2008; Hernandez et al., 2010; Hernández and González-Acuña, 2016). It is thought that migratory birds acquire ARGs from exposure during time spent in human-impacted places (Arnold et al., 2016). For example, many Kongsfjorden-nesting Barnacle geese overwinter in Scotland, and may acquire and transmit ARGs from the UK (Kolzsch et al., 2015). Locally, Arctic fox prey upon these nesting birds and scavenge human rubbish around the Kongsfjorden settlement. Further, seabirds congregate near the wastewater outfall from the research station into the bay, presenting another possible local AR dispersal pathway. Therefore, although we cannot explicitly link wildlife to elevated AR at SL3, stochastic and observational data imply a link between fecal inputs, available P, ARGs, and MGEs, probably mediated by birds and other wildlife.

In contrast to SL3, BZ5 had very low ARG abundances, no detected MGEs, and extremely low available nutrients, especially P. This is consistent with findings of Forsberg and colleagues (Forsberg et al., 2014), who showed MGEs associated with ARGs were rare in “unimpacted” soils. Further, ARGs at BZ5 strongly associate with local lithology, suggesting they are primarily autochthonous. In contrast, other clusters were impacted differentially by allochthonous drivers, with SL3 receiving the most acute inputs of ARGs and MGEs.

Taken together, these data show that Kongsfjorden soils are a useful platform for studying more elusive drivers of AR dissemination. Despite few obvious AR drivers in the area, an “ARG gradient” is evident across Kongsfjorden, suggesting differential “AR pollution”. The most remote soil clusters are dominated by autochthonous ARGs, signified by lithological control of soil ARGs (e.g., ARG correlations with metal levels) and a general lack of MGEs. In contrast, increasing “impact” is suggested by a progressive disconnection between detected ARGs and local lithology, presumably driven by allochthonous fecal inputs that overwhelm lithological factors. This observation helps explain why many studies on ARGs in soils have generated ambiguous results, despite well-known relationships between heavy metals and AR in bacteria. Results here suggest ambient soil ARGs might only correlate with lithology when the soil has almost no extraneous impact, or when nutrient, ARG, and MGE inputs co-correlate.

4. Conclusions

Antibiotic resistance genes from around the world are accumulating in even the most remote locations. While it is highly unlikely antibiotic use has had any direct effect on ARGs in places like Kongsfjorden (Ny-Ålesund does not even have an infirmary), ARG and MGE data from local soils suggest anthropogenic inputs originating from sources far away from the inlet (e.g., $bla_{\text{NDM-1}}$ in five clusters). This observation is noteworthy because this study used samples collected in 2013, less than three years after the first detection of $bla_{\text{NDM-1}}$ in surface seeps in urban India (Walsh et al., 2011). Although levels of $bla_{\text{NDM-1}}$ are comparatively localized in Kongsfjorden (with the exception of SL3) and pose no health threat, its detection reinforces how rapidly AR can globalize. Results here underscore the value of characterizing remote locations with minimal "impact", providing a baseline for quantifying the spread of AR around the world.

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Authors' contribution statement

The study was designed and developed by DWG, BC, JAR, and NDG; whereas, BC, JAR, and DWG performed the primary fieldwork. CCM and DWG analyzed the data and wrote the core manuscript with feedback from KEA, JAR, and the other authors. HT-qPCR analysis and data interpretation were performed by CMM, JQS, and YGZ.

Competing financial interests

The author(s) declare no competing financial interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2019.01.034.

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