A Metagenomic Study of Antibiotic Resistance Across Diverse Soil Types and Geographical Locations

Stephanie Pillay[1], Yasin Tepeli[1], Paul van Lent[1], Thomas Abeel[1,2]

[1] Delft Bioinformatics Lab, Delft University of Technology, Delft, the Netherlands

[2] Infectious Disease and Microbiome Program, Broad Institute of MIT and Harvard, Cambridge, USA

Correspondence: Thomas Abeel (T.Abeel@tudelft.nl)

Abstract

Background: Soil naturally harbours antibiotic resistant bacteria and is considered to be a reservoir for antibiotic resistance. The overuse of antibiotics across human, animal, and environmental sectors has intensified this issue leading to an increased acquisition of antibiotic resistant genes by bacteria in soil. Various biogeographical factors, such as soil pH, temperature, and pollutants, play a role in the spread and emergence of antibiotic resistance in soil. In this study, we utilised publicly available metagenomic datasets from four different soil types (rhizosphere, urban, natural, and rural areas) sampled from nine distinct geographic locations to explore the patterns of antibiotic resistance in soils from different regions.

Results: *Bradyrhizobium* was predominant in vegetation soil types regardless of soil pH and temperature. ESKAPE pathogen *Pseudomonas aeruginosa* was prevalent in rural soil samples. Antibiotic resistance gene families such as 16s rRNA with mutations conferring resistance to aminoglycoside antibiotics, OXA β-lactamase, ANT(3''), and the RND and MFS efflux pump gene were identified in all soil types, with their abundances influenced by anthropogenic activities, vegetation, and climate in different geographical locations. Plasmids were more abundant in rural soils and were linked to aminoglycoside resistance. Integrons and integrative elements identified were associated with commonly used and naturally occurring antibiotics, showing similar abundances across different soil types and geographical locations.

Conclusion: Antimicrobial resistance in soil may be driven by anthropogenic activities and biogeographical factors, increasing the risk of bacteria developing resistance and leading to higher morbidity and mortality rates in humans and animals.

Keywords: Metagenomics, Soil types, Antibiotic resistance, Mobile genetic elements, Biogeographical factors

Introduction

Antimicrobial resistance (AMR) has contributed to approximately 4.95 million deaths worldwide (Ramtahal *et al.*, 2022). The continuous use of antibiotics in clinical and environmental sectors such as animals, soil, and water has led to the development of resistant bacteria, which can spread between humans, animals, and the environment (Ramtahal *et al.*, 2022). As a result, antibiotic resistance has been identified as a major driver of mortality and one of the most critical environmental issues (Wang *et al.*, 2021).

Soil is a large reservoir of microbial diversity and a majority of antibiotics used in clinical and non-clinical sectors have been isolated from soil microorganisms (Wang *et al.*, 2021). Interestingly, environmental bacteria in soil possess antibiotic resistance genes (ARGs) pre-dating the discovery of antibiotics which ensured their survival in the natural environment (Song *et al.*, 2021, Ordine *et al.*, 2023). Despite the natural presence of ARGs in the soil microbiome, anthropogenic activity has impacted the intrinsic resistome. Fertilisation of crops, irrigation, excessive use of xenobiotics in crops, antibiotic use in livestock production and deforestation can alter the microbial community in the soil and disseminate ARGs throughout the environment (Ordine *et al.*, 2023). This makes soil a reservoir for both intrinsic and acquired antibiotic resistance due to the mixture of ARGs from indigenous microbes and those introduced by human activities (Song *et al.*, 2021, Wang *et al.*, 2021).

Biogeographical patterns can also influence antibiotic resistant bacteria and ARGs in the soil (Song *et al.*, 2021). Factors such as pH, temperature, moisture, and nutrients can affect ARG profiles and microbial composition. Soils with a neutral pH, low temperature, high moisture content and nutrient-rich will have an increased abundance and diversity of bacteria as well as a diverse range of ARGs (Wu *et al.*, 2023). These factors promote the spread of antibiotic resistance by mobile genetic elements (MGEs) which enables non-pathogenic and pathogenic bacteria to acquire resistance increasing the risk of bacterial infections in humans and animals (Han *et al.*, 2022).

Additionally, the soil microbiome and ARGs can differ across soil types. Natural and pristine soil is largely unaffected or undisturbed by human activity or external sources, serving as a reference point for understanding microbial communities and ARGs with little anthropogenic selection pressure (Van Goethem *et al.*, 2018). The rhizosphere soil surrounds the plant roots and is influenced by root secretions and agricultural practices. Urban soil encompasses anthropogenic soil near forests, parks, gardens, and residential areas, while rural soil includes

non-urban areas like small villages, towns, and settlements. Soil with more human activity is expected to contain a mixture of environmental and pathogenic bacteria; and resistance genes that confer resistance to clinically relevant antibiotics, heavy metals, biocides and disinfecting agents (Cytryn, 2013). It should be noted that similar soil types from different geographical locations will not necessarily have similar microbial communities or ARGs as agricultural activities, travel, environmental contamination, antibiotic usage, soil management practices, population and socioeconomic conditions differ between countries (Manyi-Loh *et al.*, 2018). These factors have complex and interrelated effects on antibiotic resistance in soil.

Since soil encompasses different environmental conditions with distinct microbial environments, it is important to understand how these factors interact and contribute to AMR to develop effective mitigation strategies (Manyi-Loh *et al.*, 2018, Wu *et al.*, 2023). In this study, we analysed metagenomic sequences from four different soil types: natural, urban, rhizosphere, and rural soil collected from nine different geographical locations i.e., South Africa, Singapore, China, Israel, Botswana, Chile, Germany, El Salvador, and Antarctica. We aim to determine the differences in microbial composition between each soil type sampled from these various geographical locations. Furthermore, we investigate the differences in antibiotic resistance genes and mobile genetic elements (plasmids, integrons and integrative elements) identified in each soil type with regards to geographical location.

Methods

Data sources

Whole metagenomic datasets were obtained from NCBI SRA (https://www.ncbi.nlm.nih.gov/sra). These datasets represented various soil types: natural soil (NS), urban soil (US), rhizosphere soil (RHS) and rural soil (RS) from different geographical locations (South Africa, Singapore, China, Israel, Botswana, Chile, Germany, El Salvador, and Antarctica) sampled over the last 10 years. Only metagenomic sequences that were sequenced using Illumina, had a read base count over 1e+09 and an average read length over 150bp were included. Detailed information about the metagenomes retrieved from the database is included in Supplementary Table 1 and presented in summarized form in Table 1.

96 Table 1: Summary of included studies, respective sample source, location and sample size. Study identifiers
97 start with the soil abbreviation: natural soil (NS), urban soil (US), rhizosphere soil (RHS) and rural soil (RS).

Study	Sample source	Location	Number of samples	Citation
NS1	Deep forest	China	3	(Zheng *et al.*, 2021)
NS2	Mountain	China	5	(Liao *et al.*, 2022)
NS3	Desert	Israel	12	(Bay *et al.*, 2020)
NS4	Desert	Chile	11	(Hwang *et al.*, 2021)
NS5	Antarctic	Antarctica	27	(Franco *et al.*, 2022)
US1	Forest park, residential areas, hospital areas	China	10	(Zheng *et al.*, 2021)
US2	Road verge, park	China	10	(Liao *et al.*, 2022)
US3	Nature reserve	Germany	8	(Mantri *et al.*, 2021)
RHS1	Greenhouse	Singapore	13	(Bandla *et al.*, 2020)
RHS2	Soybean farm	South Africa	12	(Babalola *et al.*, 2022)
RHS3	Peanut and maize farm	China	21	(Dong *et al.*, 2022)
RS1	Rural village	El Salvador	25	(Pehrsson *et al.*, 2016)
RS2	Rural village	Botswana	1	(Brooks *et al.*, 2023)

98

99 ## Bioinformatic analysis

100 Quality control of reads was conducted using FastQC v.0.11.9 (Andrews, 2010) and filtered with Trimmomatic
101 v.0.39 (Bolger *et al.*, 2014). Reads were trimmed for quality and adapter contamination using Trimmomatic with
102 the following parameters specified: *ILLUMINACLIP: adapters.fa:2:30:10 LEADING:3 TRAILING:3 MINLEN:30*
103 *SLIDINGWINDOW: 4:20*. The microbiome was analysed using Kraken2 v.2.1.0 (Wood *et al.*, 2019) with the
104 minikraken2 microbial database (2019, 8GB). Species confirmation and abundance estimation were performed
105 with Bracken v.2.6.2 (Lu *et al.*, 2017). The relative abundances of bacterial genera were used to determine the

106 microbial populations in each soil type. Statistical significance of the microbiome between studies in a soil type
107 was assessed using the non-parametric Friedman's ANOVA with SPSS software v.29 (IBM SPSS Statistics).
108 Differences in the microbiome were considered statistically significant at $p < 0.05$. The Beta diversity of the
109 microbiome among soil types was visualized using non-metric multidimensional scaling (NMDS) analysis with
110 Bray-Curtis dissimilarity in the R package vegan v.2.6-4.

111 The filtered reads were *de novo* assembled using metaSPAdes v.3.15.2 (Nurk *et al.*, 2017) on meta mode with
112 default parameters. The assembled metagenomes were annotated to determine the antibiotic resistance gene
113 families and drug classes using the Comprehensive Antimicrobial Resistance Database Resistance gene identifier
114 (CARD - RGI) v.3.1.2 with default settings including loose matches and the -low_quality -clean options (Jia *et al.*,
115 2016). The version of the database was consistent throughout the analysis. Moreover, the studies in each soil
116 type were compared by analysing the relative abundance of annotated antibiotic resistance genes. This
117 comparison was based on normalizing the data using the total number of datasets in each study indicating their
118 respective proportions.

119 *De novo* metagenomic assembled contigs were aligned to detect integrons and integrative elements by aligning
120 them to the INTEGRALL database (Moura *et al.*, 2009) and the ICEberg database (Liu *et al.*, 2019). Alignments
121 were done using BWA-mem v.0.7.10 with default parameters (Li & Durbin, 2009) which generated a SAM file.
122 To filter out soft and hard clipped reads and undesirable alignments, the SAM files were processed using the
123 Samclip tool with default parameters to remove alignments that could generate downstream issues. The
124 resulting SAM file was then converted to a BAM file using SAMtools version v.1.9 (Li & Durbin, 2009). Contigs
125 that aligned with databases were considered integrons and integrative conjugative elements. Contigs were
126 classified as plasmid-originating using Plasclass v.0.1.1 (Pellow *et al.*, 2020), with a minimum length threshold of
127 > 1000 and a probability threshold of > 0.75 for contigs to be considered plasmid-originating. To determine if
128 the detected plasmids were linked to antibiotic resistance gene families, the plasmid-originating contigs were
129 compared to previously identified contigs carrying antibiotic resistance genes and matched to corresponding
130 AMR drug classes.

Results and discussion

Microbial communities are dependent on their habitat.

To identify the bacterial communities within different types of soil from different locations (Supplementary Table 1), we performed taxonomic classification. Soil hosts a diverse range of microbial communities influenced by abiotic and biotic characteristics, microbial abundances, activity, and community composition. Consequently, there is no "typical" soil microbiome, and bacterial relative abundances vary by soil type (Fierer, 2017).

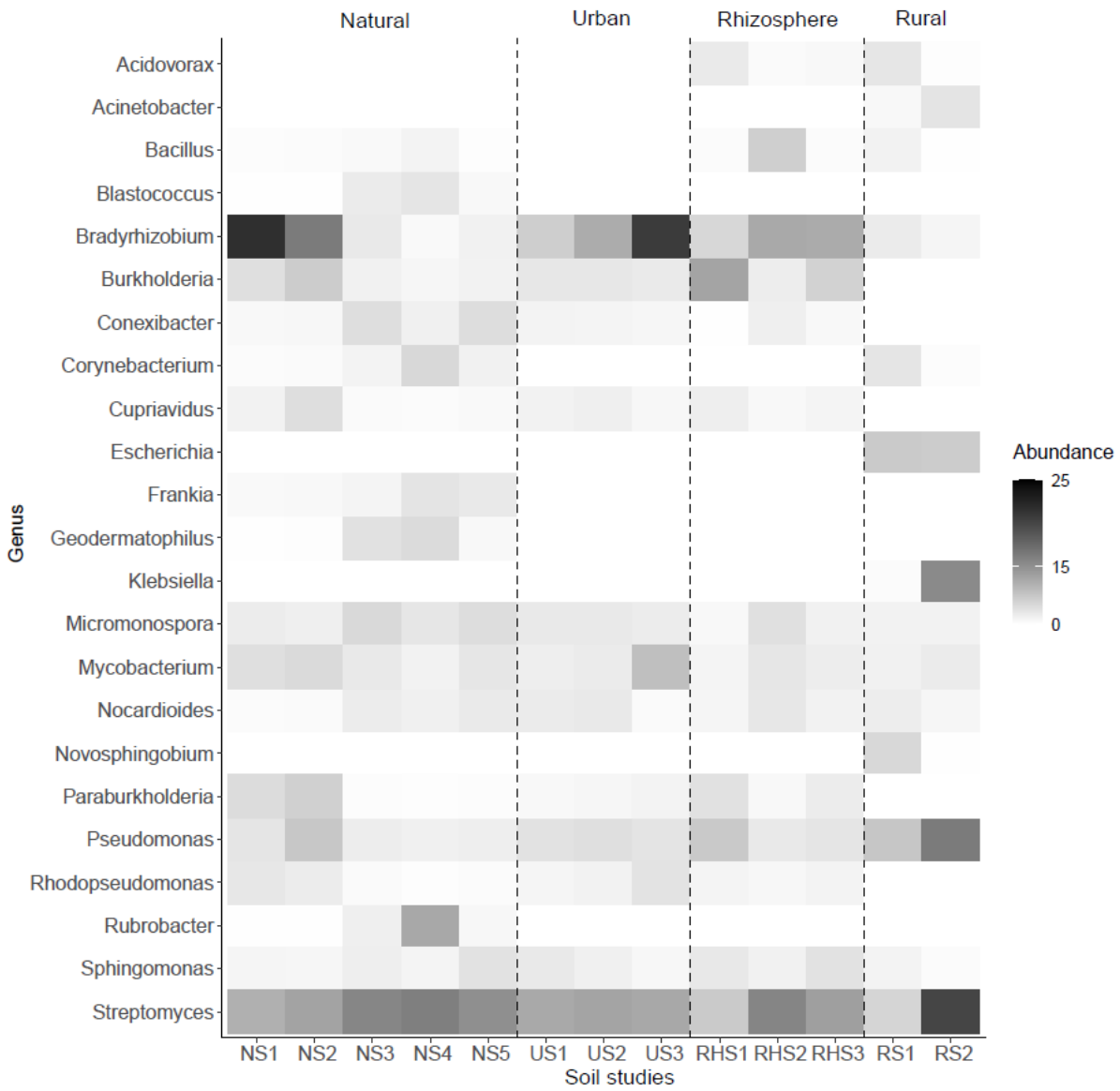


Figure 1: Heatmap showing the relative abundances of bacterial genera over 2% in each study from each soil type. The y-axis indicates the genera, and the x-axis represents the soil studies (Table 1). Each soil type is divided by the dashed black line and the abundances of bacterial genera are indicated by the intensity of grey.

141 Bacteria in natural soils are influenced by their habitat and environmental conditions (Supplementary Table 2).
142 In Figure 1, *Bradyrhizobium* dominates the deep forest areas (NS1 and NS2), whereas bacteria like *Rubrobacter*,
143 known for its thermophilic traits, are found in desert-like conditions (NS4), as confirmed by Connon *et al.* (2007)
144 Pajares & Bohannan (2016) and Zheng *et al.* (2021). The microbial communities detected were statistically
145 different between each of the natural studies indicating environmental influence ($p < 0.05$). Climate, pH,
146 temperature and rainfall may all influence the abundances and types of bacteria found soil as discussed by Chase
147 *et al.*, (2021).
148
149 *Streptomyces* was prevalent across all urban studies; however, the overall microbial community differed
150 significantly between these urban studies ($p < 0.05$) (Figure 1 and Supplementary Table 3). As expected, higher
151 abundances of *Bradyrhizobium*, *Mycobacterium*, and *Rhodopseudomonas* were observed in urban soil obtained
152 from the nature reserve (US3) compared to other urban soil sources. These bacteria thrive in areas with more
153 vegetation and reduced anthropogenic activity, such as nature reserves, compared to residential and forest park
154 areas (Wong *et al.*, 2014, Gorovtsov *et al.*, 2020, Glushakova *et al.*, 2021).
155
156 Plant growth-promoting bacteria such as *Streptomyces*, *Burkholderia*, *Pseudomonas*, and *Bradyrhizobium* were
157 dominant across the different rhizosphere soil studies (Figure 1 and Supplementary Table 4). Overall, these
158 microbial communities were different in each of the rhizosphere soil studies ($p < 0.05$). These bacteria also assist
159 in protecting plants against pathogens by producing antibiotics (Kashyap et al., 2019; de Sousa et al., 2022; Liu
160 et al., 2022). *Bradyrhizobium*, which aids in nitrogen fixation and plant growth, is found at an 8% abundance in
161 RHS1, 2% in RHS2 and 4% in RHS3 (Fierer, 2017). Soil in RHS1 and RHS3 consisted of crops supplemented with
162 nitrogen-phosphorus-potassium (NPK) fertiliser (Supplementary Table 1). Despite this, these studies showed a
163 higher abundance of *Bradyrhizobium* compared to RHS2, which was not supplemented. This contradicts previous
164 studies suggesting that *Bradyrhizobium* will be present in a low abundance when nitrogen fertilisers are used
165 (Okubo *et al.*, 2013, Yan *et al.*, 2017).

166 Lastly, in rural soil studies ($p < 0.05$), *Pseudomonas*, *Escherichia*, *Streptomyces*, and *Klebsiella* were prevalent,
167 including the ESKAPE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter*
168 *baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species) pathogens *Klebsiella pneumonia* and

169 *Pseudomonas aeruginosa* (Figure 1 and Supplementary Tables 5 and 6 respectively). The high prevalence of
170 these pathogens in rural areas is attributed to anthropogenic factors, such as the presence of domestic animals,
171 open defecation, and inadequate sanitation (Brooks et al., 2023). These conditions contribute to antibiotic
172 resistance hotspots in rural areas, as domestic animals spread faecal matter, and open sewage drains and
173 insufficient sanitation increases the selection pressure for antibiotic resistance (Nadimpalli *et al.*, 2020).

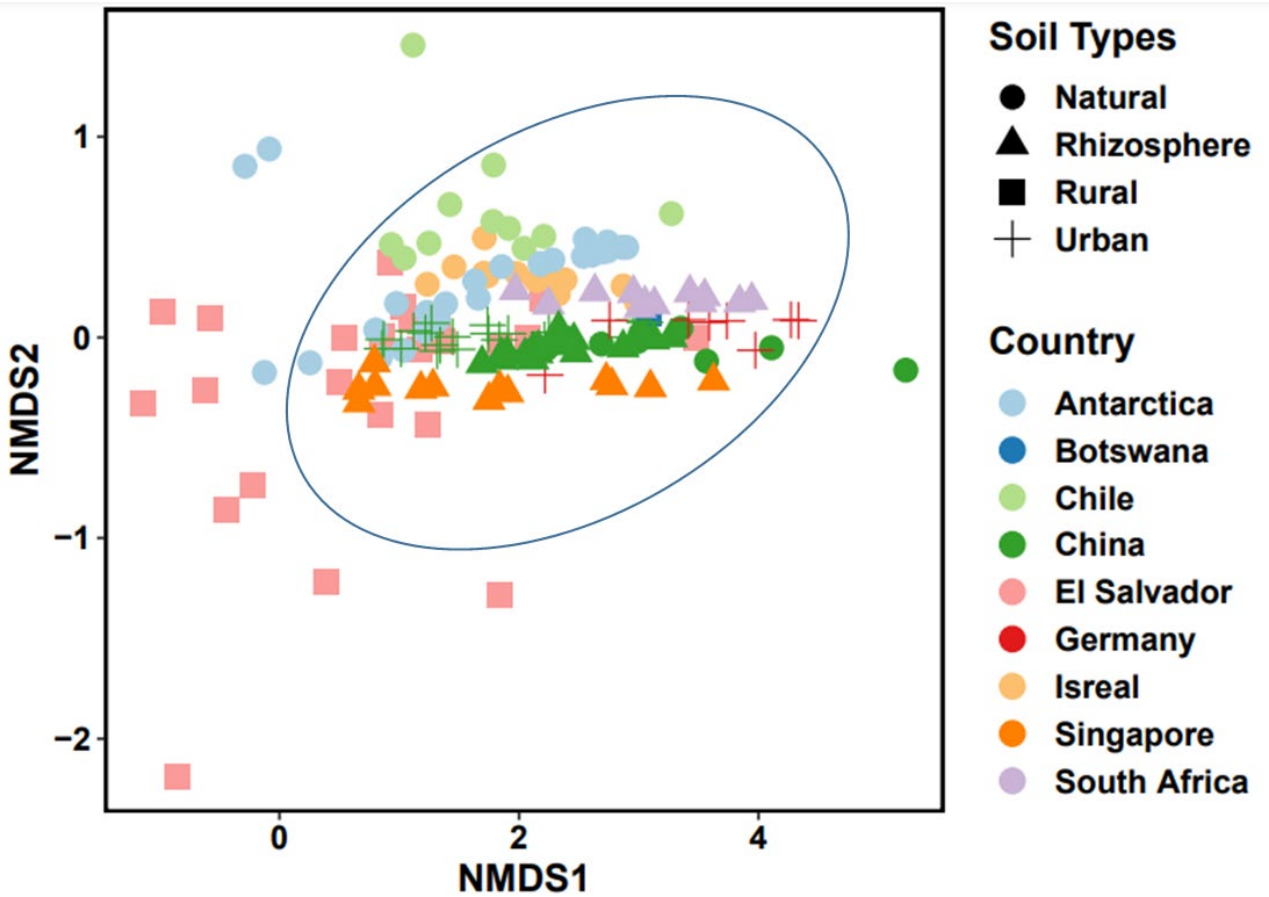
174 Overall, these results demonstrate that the bacterial composition and abundance within each soil type may be
175 influenced by a myriad of factors, including environmental conditions, nutrients, temperature, oxygen
176 availability, and pH types within the specific geographical location (Pajares & Bohannan, 2016, Fierer, 2017, de
177 Sousa *et al.*, 2022). Understanding the composition of microbial communities is critical for comprehending soil
178 health and potential risks associated with antibiotic resistance in various environments.

179 Natural, urban and rhizosphere soil types have similar microbial communities.

180 To assess the differences between microbial communities, present in different types of soil from different
181 geographical locations (Table 1), we analysed the beta-diversity. Additionally, we aimed to determine if the
182 geographical locations influence the microbial communities in each soil type. The beta-diversity quantifies the
183 "distance" between microbial communities using the Bray-Curtis index and can be visualized by non-metric
184 multidimensional scaling (NMDS). A smaller distance between samples from different countries and soil types

185 indicates a greater similarity in microbial composition. Figure 2 shows the NMDS plots for each type of soil from

186 their respective countries.



187 Figure 2: Non-metric multidimensional scaling (NMDS) visualising the dissimilarity (Beta-diversity) between

188 microbial communities from different soil types from different countries (Table 1). Each soil type is represented

189 by a different shape (natural = circle, rhizosphere = triangle, rural = square, urban = cross) and each country or

190 region is colour-coded (Antarctica = light blue, Botswana = dark blue, Chile = light green, China = dark green, El

191 Salvador = pink, Germany = red, Israel = salmon, Singapore = orange, South Africa = lilac). The samples that have

192 smaller distances between them are circled in blue.

193 Figure 2 shows that most samples cluster together regardless of the soil type or geographical location. Natural,

194 rhizosphere, urban, and many rural soil samples are closely grouped, suggesting similar microbial communities

195 across these soil types. This similarity may be related to vegetation and varying levels of anthropogenic activity

196 (Pajares & Bohannan, 2016, Zheng *et al.*, 2021). However, some rural samples from El Salvador differ which

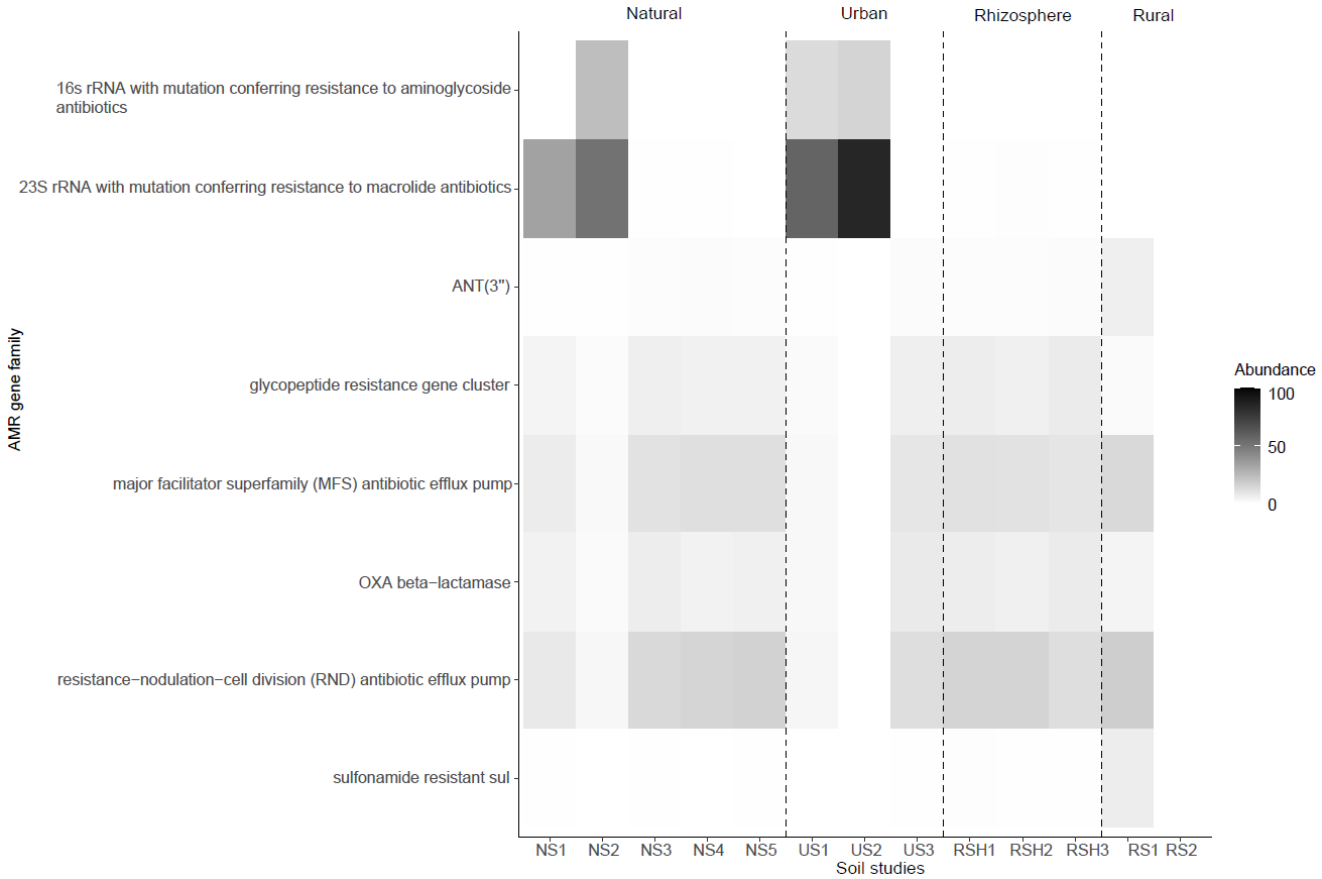
197 could be due to human activity or soil around latrine toilets and clothes washing areas. This can limit plant-

198 promoting bacteria and support enteric pathogens (Pehrsson *et al.*, 2016, Brooks *et al.*, 2023). Samples from

199 specific studies like RS1 (El Salvador), NS2 (China), NS4 (Chile) and NS5 (Antarctica) are further apart, indicating
200 variations in the microbial composition. Such differences within the same study may result from spatial
201 variability in the soil environment, even when sampling sites are only a few centimetres apart (Fierer, 2017).

202 Anthropogenic activities, vegetation, and climate play a role in the selection and prevalence 203 of antibiotic resistance genes.

204 To understand the connection between AMR gene families and the different types of soil, we examined the
205 presence and abundance of AMR gene families in the assembled metagenomes (contigs) from each study within
206 their respective soil type (Table 1). While AMR gene families are naturally present in soil environments, external
207 factors like livestock and human activities can influence their types and abundance (Hernández *et al.*, 2023).



208 Figure 3: Heatmap showing the abundance of AMR gene families present over 5% in each study within each soil
209 type. The y-axis indicates the AMR gene families, and the x-axis represents the soil studies (Table 1). Each soil
210 type is divided by a dashed black line and the abundances of AMR gene families are indicated by the intensity of
211 grey.

212 Overall, more than 0.001% of contigs contained AMR gene families in each study. We identified five AMR gene
213 families that were abundant (Figure 3). Two of which are associated with acquired resistance: 23S rRNA, which
214 confers resistance to macrolides, and 16S rRNA, which confers resistance to aminoglycosides. Intrinsic resistance
215 gene families include the major facilitator superfamily (MFS) and resistance-nodulation-cell division (RND)
216 antibiotic efflux pumps, and the OXA beta-lactamase gene family, which is associated with acquired and intrinsic
217 resistance (Poirel *et al.*, 2010, Garcia *et al.*, 2022). These gene families confer resistance to macrolides, and beta-
218 lactams including carbapenems, cephalosporins, and penams (Supplementary Table 7).

219 Figure 3 shows that the 23S rRNA mutation conferring resistance to macrolide antibiotics were more prevalent
220 in urban soils as compared to the natural, rhizosphere and rural soils. Specifically, in US2 from China, 85% of the
221 detected AMR gene families were the 23S rRNA gene with this mutation, with other urban studies like US1 had
222 58% abundance. In contrast, natural soil studies such as NS1 and NS2 had lower abundances of 33% and 52%,
223 respectively. Urban soil samples were collected from areas with high human activity (forest parks, residential
224 zones, and road verges) which have greater antibiotic pollution and selective pressures promoting the
225 proliferation of antibiotic resistant bacteria, as detailed by Osbiston *et al.* (2021).

226 The MFS and RND efflux pump gene families were detected across all types of soil except for US2 and RS2 (Figure
227 3). Efflux pumps are key for intrinsic resistance, providing bacteria with a baseline level of resistance by actively
228 expelling a broad range of antibiotics from their cells (Garcia *et al.*, 2022). In our study, RS1 exhibited a high
229 abundance of these efflux pump gene families, with 17% for RND and 13% for MFS (Figure 3 and Supplementary
230 Table 7). The presence of these efflux pump gene families in rural soils may also be influenced by factors such
231 as animal feedlots, domestic sewage, and human-excrement-irrigated vegetable fields (Cheng *et al.*, 2020).
232 Additionally, vegetation, flora and fauna influences the presence of efflux pump gene families, as discussed by
233 Martinez *et al.* (2009) and Pasqua *et al.* (2019).

234 Lastly, we found the OXA beta-lactamase gene family present in all rhizosphere and natural soil studies, except
235 US2 and RS2, with a high abundance in US3 (7%) and RHS3 (7%) (Figure 3 and Supplementary Table 7). The OXA
236 beta-lactamase gene family has been shown to confer both acquired and intrinsic resistance and can be found
237 in all soil types; from primitive natural soils with little to no anthropogenic activity to urban environments
238 (Djenadi *et al.*, 2018, Han *et al.*, 2022). Previous research also suggests that the climate affects the abundance

239 of ARGs, with beta-lactam resistance genes being more common in warmer areas (Li *et al.*, 2022). However, our
240 study found similar AMR gene families across all soil types, regardless of temperature.

241 Plasmids linked to aminoglycoside resistance are common in all soil types.

242 To investigate the presence of plasmids and their connection to antibiotic drug classes in different soil types, we
243 analysed the percentage of contigs classified as plasmids in each study and their association with antibiotic drug
244 classes. Although AMR is a natural occurrence in soil, human activity has led to increased contamination with
245 antibiotics and ARGs in the soil. Soil plasmids can transfer these ARGs from one bacterial host to another
246 integrating into plant and animal cells and spreading AMR (Meng *et al.*, 2022).

247 Firstly, less than 1% of contigs were classified as plasmids in each study from every soil type. Further analysis
248 linking detected plasmids to AMR drug classes revealed that, except RHS2, RHS3, RS2 and US2, all studies had
249 more than 0.01% of plasmids associated with AMR drug classes. Notably, RS1 had 1.8% of plasmids linked to
250 AMR. A high percentage of plasmids is common in rural communities due to unregulated access to antibiotics,
251 limited clean water and human and animal activities. These conditions increase the risk of exposure to plasmids
252 and ARGs from effluents, contaminated soils, and waste, enhancing plasmid accumulation and horizontal gene
253 transfer compared to natural and urban soils (Pehrsson *et al.*, 2016, Brooks *et al.*, 2023).

254 Secondly, we examined the association between plasmids and specific antibiotic drug classes (Supplementary
255 Table 8). Overall, all studies from different soil types, except RHS2, RHS3, US2 and RS2, contained plasmids
256 associated with aminoglycoside, carbapenem; cephalosporin; penam, glycopeptide and peptide drug classes.
257 Details on the abundances of plasmids linked to AMR drug classes and the individual AMR gene families can be
258 found in Supplementary Table 8.

259 Plasmids encoding naturally occurring antibiotics were identified in natural and urban soil studies. Among the
260 natural soil types, NS1 showed a high abundance of plasmids associated with aminoglycosides (13%) and
261 carbapenem; cephalosporin; penam (16%) drug classes. These high abundances were consistent with other
262 natural studies (Supplementary Table 8). Similar to NS1, US1 had a high abundance of plasmids associated with
263 aminoglycosides (13%), carbapenem; cephalosporin; penam (25%) along with fluoroquinolones (13%),
264 glycopeptide (25%), macrolide (13%) and tetracycline (13%) drug classes. Additionally, US3 had plasmids linked
265 to similar antibiotic drug classes: aminoglycosides (16%), carbapenem; cephalosporin; penam (11%),

266 glycopeptide (12%) and peptides (9%). In our study, the natural and urban studies, sampled from forests and
267 deserts for natural soils and residential areas and forest parks for urban soils, showed higher abundances of
268 plasmids associated with naturally occurring antibiotics such as aminoglycosides, glycopeptides, and beta-
269 lactams (Zhao *et al.*, 2019, Meng *et al.*, 2022). These abundances may also be influenced by heavy metals and
270 other contaminants in the soil types as noted by Zhao *et al.* (2019).

271 Amended rhizosphere soil (RHS1) showed a high abundance of plasmids associated with AMR drug classes.
272 Specifically, aminoglycoside (17%), carbapenem; cephalosporin; penam (8%), glycopeptide (10%) and
273 sulphonamide (10%) resistance plasmids were detected in rhizosphere soils. Although plasmids were detected
274 in RHS2 and RHS3, none were associated with previously identified ARGs. Amended soils, such as rhizosphere
275 soils and natural soils are nutrient-rich environments that can facilitate the spread of AMR through plasmids
276 (van Elsas & Bailey, 2002, Perry & Wright, 2013).

277 Lastly in rural soils, RS2, a single sample study, did not contain any plasmids associated with AMR. However,
278 RS1 had a high abundance of plasmids linked to aminoglycoside (24%), carbapenem; cephalosporin; penam (9%)
279 and sulphonamide (39%) resistance. This is consistent with a study from a rural village in India that found
280 resistance to carbapenems, cephalosporins, penams, sulphonamides, tetracyclines, and quinolones, with *E. coli*
281 carrying plasmids for resistance to quinolones, cephalosporins, and colistin. This study suggested that the local
282 antibiotic use patterns supports the persistence of plasmids with ARGs (Purohit *et al.*, 2017).

283 While antibiotics and ARGs naturally occur in soil, their transmission to different environments through plasmids
284 is concerning, especially in environments with increased anthropogenic activity. This contributes to the spread
285 of AMR to both pathogenic and non-pathogenic bacteria, posing a significant threat to the natural environment
286 and human health (Meng *et al.*, 2022).

287

288 AMR genes associated with integrons that are highly abundant were selected due to the 289 constant use of antibiotics.

290 To identify the presence of integrons and their association with AMR gene families, we classified integrons from
291 the metagenomic contigs present in each soil study. Furthermore, we linked the classified integrons with AMR
292 gene families to determine the differences between soil studies. Integrons are mobile genetic elements that

293 capture and express ARGs, particularly among gram-negative pathogenic bacteria. Integrons are present in all
294 environments and can serve as markers for tracing sources of pollution (Gillings, 2014).

295 We used the assembled metagenomic contigs to determine the percentage of classified integrons
296 (Supplementary Table 9). In all soil types studied, less than 0.001% of contigs were identified as integrons. The
297 integrons that were found were classified into their respective classes of 1, 2 and 3 (Supplementary Table 9).

298 Class 1 integrons made up a higher percentage (> 11%) of the integrons we found between all soil types from all
299 studies. Class 3 integrons constitutes a range of 2% to 5% of integrons found while class 2 integrons had a lower
300 percentage of 1% to 2%. The remainder of the integrons were unclassified. These high abundances of class 1
301 and class 3 in all types of soil regardless of country are expected as class 1 and 3 integrons are found in
302 *Proteobacteria* in soil environments whereas class 2 integrons are commonly found in marine environments.
303 Overall, integrons are found in diverse environments which include forest soil, desert soil, Antarctic soil and
304 plant surfaces (Gillings, 2014).

305 In our study, we identified class 1, 2 and 3 integrons and their association with AMR genes (Supplementary Table
306 10). We found a high abundance of class 1 integrons associated with disinfecting agents and sulphonamide
307 resistance in all types of soil. The high abundance of the *qacE* gene with class 1 integrons can be traced back to
308 the use of quaternary ammonium compounds which were first used as hospital disinfecting agents in 1930 and
309 have become a part of the class 1 integron, known to be highly abundant in soils (Gillings, 2014, Cheng *et al.*,
310 2020). Similarly, sulphonamides were the first true antibiotic to be used in the 1930s and have been selected for
311 and can be commonly found in class 1 integrons (Gillings, 2014).

312 A high abundance of class 2 integrons was associated with aminoglycoside resistance (*aadB*) which ranged from
313 15% in the RS1 to 75% in NS3 and NS4, and 83% in RHS3 and US1 (Supplementary Table 10). Aminoglycoside
314 antibiotics were originally isolated from soil bacteria and commonly used in agriculture, increasing their
315 presence in soil environments, potentially affecting plant-soil microbial communities. They also enter soils
316 through human waste, municipal wastewater systems, and clinical use. These direct and indirect exposures
317 contributes to the detection of aminoglycoside resistance genes in various soil types, often associated with class
318 1 and class 2 integrons (Blau *et al.*, 2018, Coates *et al.*, 2022). Previous studies have shown that the *aadB*
319 aminoglycoside resistance gene is frequently associated with class 1, 2, and 3 integrons and is present in

320 *Pseudomonas aeruginosa*, *Salmonella* spp., *Acinetobacter baumannii*, *Klebsiella* spp., and *Escherichia coli* which
321 are present in our study (Sabbagh *et al.*, 2021). Additionally, Jones *et al.*, (2005) showed that the *aadB* gene,
322 associated with class 1 integrons, had a close association with the bla_{SHV} gene conferring resistance to penicillin
323 and cephalosporins. This association was not seen in our study. Lastly, class 3 integrons were predominantly
324 associated with the *qacE* gene, which provides resistance to disinfectants. Over 55% of class 3 integrons in all
325 soil studies were linked to the *qacE* gene, with 100% in NS4, 97% in RHS2, and 97% in US3. Similarly to class 1
326 integrons, the *qacE* gene are commonly found in class 3 integrons as it is considered to be a conserved segment
327 and had been selected for in the past (Sabbagh *et al.*, 2021).

328 Integrative and mobilizable elements were identified in each type of soil.

329 Finally, we used the assembled metagenomic contigs to determine the percentage of integrative elements.
330 Integrative elements consist of integrative and conjugative elements (ICE), integrative and mobilizable elements
331 (IME) and cis-integrative and mobilizable elements (CIME) that are self-transmittable and can carry and spread
332 ARGs to other bacterial hosts (Delavat *et al.*, 2017).

333 We found that all the studies across all soil types had less than 0.1% of contigs classified as integrative elements.
334 ICE elements were the most prevalent integrative element compared to IMEs and CIMEs. In the RS2 study, 100%
335 of the integrative elements were CIMEs, while other studies had less than 20% CIMEs. The abundance of ICEs in
336 all studies across all soil types was over 70%, with no ICE elements found in RS2.

337 The most prominent ICE families among all studies in all soil types were *Tn4371* (>12%), *SXT/R391* (>5%) and
338 *ICEclc* (>8%) (Supplementary Table 12). These ICE families are commonly found in soil and aid in the degradation
339 of pollutants (Obi *et al.*, 2018, Hirose *et al.*, 2021, Roman *et al.*, 2021). Additionally, we identified different SGI1
340 IME families present in the different studies within the different types of soil (Supplementary Table 13). SGI1
341 has been characterized as a *Salmonella* genomic island that can spread multi-drug resistance to human and
342 animal pathogens (Cummins *et al.*, 2020). Bacteria carrying these integrative elements have a higher potential
343 for spreading infections thus facilitating the spread of AMR to other environments (Delavat *et al.*, 2017). This
344 underscores the importance of continuously monitoring mobile genetic elements in different soil types.

345 # Conclusion

346 We analysed publicly available metagenomic data to investigate the microbial population, antibiotic resistance
347 genes and mobile genetic elements in rhizosphere, urban, natural, and rural soils sampled from South Africa,
348 Singapore, China, Israel, Botswana, Chile, Germany, El Salvador, and Antarctica to understand the spread of
349 antibiotic resistance in different soil environments.

350 Our study revealed that plant-promoting bacteria such as *Bradyrhizobium*, dominates soil rich in vegetation and
351 nutrients such as the rhizosphere and natural soil regardless of geographic location. Urban soils contain a mix of
352 plant-promoting bacteria and pollutant-degrading bacteria, such as *Arthrobacter*, while rural village soils
353 harbour a combination of environmental bacteria and opportunistic pathogens like *E. coli.* Bacteria in the soil
354 are dependent on physiological (temperature, mixing of soil profiles, water change, soil compaction), chemical
355 (heavy metal, soil pH, polyaromatic hydrocarbons) and biological (artificial vegetation, faecal contamination)
356 factors. As such, more pathogenic bacteria especially those on the ESKAPE pathogen list are dominant in areas
357 with high human activity. These bacteria can carry antibiotic resistant genes that may spread to domestic and
358 food animals, vegetation, water sources and humans leading to untreatable infections in all sectors.

359 Antibiotic resistant gene families detected in this study i.e., 23S rRNA with mutation conferring resistance to
360 macrolide antibiotics, major facilitator superfamily (MFS) antibiotic efflux pump, resistance-nodulation-cell
361 division (RND) antibiotic efflux pump, OXA beta-lactamase, ANT(3'') and 16s rRNA with mutation conferring
362 resistance to aminoglycoside antibiotics in soil. These genes favour the development of multi-drug resistance,
363 posing a threat to antibiotic effectiveness. While antibiotic resistant gene families such as the RND and MFS
364 efflux pump gene families occur naturally in soil, anthropogenic activity, vegetation, and climate may play a role
365 in the emergence of AMR and the acquisition of ARGs. Additionally, plasmids associated with AMR gene families
366 like OXA beta-lactamase facilitates the spread of resistance from soil to various environments like water, humans
367 or animals.

368 Integrons detected in this study were linked to commonly used antibiotics in all soil types, suggesting that the
369 overuse of antibiotics contributes to the spread and emergence of antibiotic resistant bacteria. These bacteria
370 may also possess adaptive strategies provided by integrative elements, *SXT/R391, Tn4371, ICEclc* and IMEs which

can potentially carry accessory genes i.e., ARGs. Integrative elements allow bacteria in the soil to stabilise and adapt to environmental conditions and human activity as well as favouring gene transfer and the spread of AMR.

To fully understand the emergence and spread of AMR in the environment, factors such as geographical location, biogeographical patterns, and soil type must be considered. By analysing MGEs and ARGs in diverse soil types from different countries, we can gain insights into how AMR spreads and adapt strategies to manage it effectively. Future research should focus on collecting more rural soil data and considering additional factors such as antibiotic usage, residues, industrial practices, and local regulations to address AMR and environmental pollution.

Declarations

Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Funding

This work is based on the research supported wholly/in part by the National Research Foundation of South Africa (Grant Numbers: 120192).

Authors' Contributions

SP and TA designed experiments. SP conducted all experiments, analysed data and wrote the manuscript. YIT and PL assisted in data analysis. SP, YIT, PL and TA proof-read and edited the manuscript.

References

Andrews S (2010) Babraham bioinformatics-FastQC a quality control tool for high throughput sequence data. *URL: https://www bioinformatics babraham ac uk/projects/fastqc*.

Babalola OO, Ajiboye TT & Ayangbenro AS (2022) Microbiome of Soybean (Glycine max L.) Rhizosphere from Free State, South Africa. *Microbiology Resource Announcements* **11**: e00288-00222.

Bandla A, Pavagadhi S, Sridhar Sudarshan A, Poh MCH & Swarup S (2020) 910 metagenome-assembled genomes from the phytobiomes of three urban-farmed leafy Asian greens. *Scientific Data* **7**: 278.

Bay SK, McGeoch MA, Gillor O, Wieler N, Palmer DJ, Baker DJ, Chown SL & Greening C (2020) Soil bacterial communities exhibit strong biogeographic patterns at fine taxonomic resolution. *Msystems* **5**: e00540-00520.

Blau K, Casadevall L, Wolters B, Van den Meersche T, Kreuzig R, Smalla K & Jechalke S (2018) Soil texture-depending effects of doxycycline and streptomycin applied with manure on the bacterial community composition and resistome. *FEMS microbiology ecology* **94**: fix145.

Bolger AM, Lohse M & Usadel B (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* **30**: 2114-2120.

Brooks K, Eze J, Onalenna O & Rahube TO (2023) Analysis of antibiotic resistance from a rural community and wastewater contaminated environment linked to human and animal activities. *Journal of Hazardous Materials Advances* 100232.

Chase AB, Weihe C & Martiny JB (2021) Adaptive differentiation and rapid evolution of a soil bacterium along a climate gradient. *Proceedings of the National Academy of Sciences* **118**: e2101254118.

Cheng J, Tang X & Liu C (2020) Occurrence and distribution of antibiotic resistance genes in various rural environmental media. *Environmental Science and Pollution Research* **27**: 29191-29203.

Coates J, Bostick KJ, Jones BA, Caston N & Ayalew M (2022) What is the impact of aminoglycoside exposure on soil and plant root-associated microbiota? A systematic review protocol. *Environmental Evidence* **11**: 1-9.

Connon SA, Lester ED, Shafaat HS, Obenhuber DC & Ponce A (2007) Bacterial diversity in hyperarid Atacama Desert soils. *Journal of Geophysical Research: Biogeosciences* **112**.

Cummins ML, Hamidian M & Djordjevic SP (2020) Salmonella genomic island 1 is broadly disseminated within gammaproteobacteriaceae. *Microorganisms* **8**: 161.

Cytryn E (2013) The soil resistome: The anthropogenic, the native, and the unknown. *Soil biology & biochemistry* **63**: 18-23.

de Sousa LP, Guerreiro-Filho O & Mondego JMC (2022) The Rhizosphere Microbiomes of Five Species of Coffee Trees. *Microbiology Spectrum* **10**: e00444-00422.

Delavat F, Miyazaki R, Carraro N, Pradervand N & van der Meer JR (2017) The hidden life of integrative and conjugative elements. *FEMS microbiology reviews* **41**: 512-537.

Djenadi K, Zhang L, Murray AK & Gaze WH (2018) Carbapenem resistance in bacteria isolated from soil and water environments in Algeria. *Journal of Global Antimicrobial Resistance* **15**: 262-267.

Dong Q, Zhao X, Zhou D, Liu Z, Shi X, Yuan Y, Jia P, Liu Y, Song P & Wang X (2022) Maize and peanut intercropping improves the nitrogen accumulation and yield per plant of maize by promoting the secretion of flavonoids and abundance of Bradyrhizobium in rhizosphere. *Frontiers in Plant Science* **13**: 957336.

Fierer N (2017) Embracing the unknown: disentangling the complexities of the soil microbiome. *Nature Reviews Microbiology* **15**: 579-590.

Franco AL, Adams BJ, Diaz MA, Lemoine NP, Dragone NB, Fierer N, Lyons WB, Hogg I & Wall DH (2022) Response of Antarctic soil fauna to climate-driven changes since the Last Glacial Maximum. *Global Change Biology* **28**: 644-653.

Garcia ÍR, de Oliveira Garcia FA, Pereira PS, Coutinho HDM, Siyadatpanah A, Norouzi R, Wilairatana P, de Lourdes Pereira M, Nissapatorn V & Tintino SR (2022) Microbial resistance: The role of efflux pump superfamilies and their respective substrates. *Life Sciences* **295**: 120391.

Gillings MR (2014) Integrons: past, present, and future. *Microbiology and Molecular Biology Reviews* **78**: 257-277.

Glushakova A, Lysak L, Belov A, Ivanova A, Lapygina E, Prokofieva T & Umarova A (2021) Local monitoring of saprotrophic bacterial complexes of urban soils in Syktyvkar in 2019 and 2020. *Moscow University Soil Science Bulletin* **76**: 84-88.

Gorovtsov A, Rajput V, Pulikova E, Gerasimenko A, Ivanov F, Vasilchenko N, Demidov A, Jatav H & Minkina T (2020) Soil Microbial Communities in Urban Environment. *Advances in Environmental Research; Nova Science Publisher: Hauppauge, NY, USA*.

Han B, Ma L, Yu Q, Yang J, Su W, Hilal MG, Li X, Zhang S & Li H (2022) The source, fate and prospect of antibiotic resistance genes in soil: A review. *Frontiers in Microbiology* **13**.

Hernández M, Roy S, Keevil CW & Dumont MG (2023) Identification of diverse antibiotic resistant bacteria in agricultural soil with H218O stable isotope probing combined with high-throughput sequencing. *Environmental Microbiome* **18**: 34.

Hirose J, Watanabe T, Futagami T, Fujihara H, Kimura N, Suenaga H, Goto M, Suyama A & Furukawa K (2021) A new ICE clc subfamily integrative and conjugative element responsible for horizontal transfer of biphenyl and

salicylic acid catabolic pathway in the PCB-degrading strain Pseudomonas stutzeri KF716. *Microorganisms* **9**: 2462.

Hwang Y, Rahlff J, Schulze-Makuch D, Schloter M & Probst AJ (2021) Diverse viruses carrying genes for microbial extremotolerance in the Atacama Desert hyperarid soil. *Msystems* **6**: 10.1128/msystems. 00385-00321.

Jia B, Raphenya AR, Alcock B, Waglechner N, Guo P, Tsang KK, Lago BA, Dave BM, Pereira S & Sharma AN (2016) CARD 2017: expansion and model-centric curation of the comprehensive antibiotic resistance database. *Nucleic acids research* gkw1004.

Jones LA, McIver CJ, Kim M-J, Rawlinson WD & White PA (2005) The aadB gene cassette is associated with bla SHV genes in Klebsiella species producing extended-spectrum β-lactamases. *Antimicrobial agents and chemotherapy* **49**: 794-797.

Li H & Durbin R (2009) Fast and accurate short read alignment with Burrows–Wheeler transform. *bioinformatics* **25**: 1754-1760.

Li Z, Sun A, Liu X, Chen Q, Bi L, Ren P, Shen J, Jin S, He J & Hu H (2022) Climate warming increases the proportions of specific antibiotic resistance genes in natural soil ecosystems. *Journal of Hazardous Materials* **430**: 128442-128442.

Liao H, Li H, Duan C-S, Zhou X-Y, An X-L, Zhu Y-G & Su J-Q (2022) Metagenomic and viromic analysis reveal the anthropogenic impacts on the plasmid and phage borne transferable resistome in soil. *Environment International* **170**: 107595.

Liu M, Li X, Xie Y, Bi D, Sun J, Li J, Tai C, Deng Z & Ou H-Y (2019) ICEberg 2.0: an updated database of bacterial integrative and conjugative elements. *Nucleic acids research* **47**: D660-D665.

Lu J, Breitwieser FP, Thielen P & Salzberg SL (2017) Bracken: estimating species abundance in metagenomics data. *PeerJ Computer Science* **3**: e104.

Mantri SS, Negri T, Sales-Ortells H, Angelov A, Peter S, Neidhardt H, Oelmann Y & Ziemert N (2021) Metagenomic sequencing of multiple soil horizons and sites in close vicinity revealed novel secondary metabolite diversity. *Msystems* **6**: 10.1128/msystems. 01018-01021.

Manyi-Loh C, Mamphweli S, Meyer E & Okoh A (2018) Antibiotic use in agriculture and its consequential resistance in environmental sources: potential public health implications. *Molecules* **23**: 795.

Martinez JL, Sánchez MB, Martínez-Solano L, Hernandez A, Garmendia L, Fajardo A & Alvarez-Ortega C (2009) Functional role of bacterial multidrug efflux pumps in microbial natural ecosystems. *FEMS microbiology reviews* **33**: 430-449.

Meng M, Li Y & Yao H (2022) Plasmid-mediated transfer of antibiotic resistance genes in soil. *Antibiotics* **11**: 525.

Moura A, Soares M, Pereira C, Leitão N, Henriques I & Correia A (2009) INTEGRALL: a database and search engine for integrons, integrases and gene cassettes. *Bioinformatics* **25**: 1096-1098.

Nadimpalli ML, Marks SJ, Montealegre MC, Gilman RH, Pajuelo MJ, Saito M, Tsukayama P, Njenga SM, Kiiru J & Swarthout J (2020) Urban informal settlements as hotspots of antimicrobial resistance and the need to curb environmental transmission. *Nature microbiology* **5**: 787-795.

Nurk S, Meleshko D, Korobeynikov A & Pevzner PA (2017) metaSPAdes: a new versatile metagenomic assembler. *Genome research* **27**: 824-834.

Obi CC, Vayla S, De Gannes V, Berres ME, Walker J, Pavelec D, Hyman J & Hickey WJ (2018) The integrative conjugative element clc (ICEclc) of Pseudomonas aeruginosa JB2. *Frontiers in microbiology* **9**: 1532.

Okubo T, Fukushima S, Itakura M, Oshima K, Longtonglang A, Teaumroong N, Mitsui H, Hattori M, Hattori R & Hattori T (2013) Genome analysis suggests that the soil oligotrophic bacterium Agromonas oligotrophica (Bradyrhizobium oligotrophicum) is a nitrogen-fixing symbiont of Aeschynomene indica. *Applied and environmental microbiology* **79**: 2542-2551.

Ordine JVW, De Souza GM, Tamasco G, Virgilio S, Fernandes AFT, Silva-Rocha R & Guazzaroni M-E (2023) Metagenomic insights for antimicrobial resistance surveillance in soils with different land uses in Brazil. *Antibiotics* **12**: 334.

Osbiston K, Oxbrough A & Fernández-Martínez LT (2021) Antibiotic resistance levels in soils from urban and rural land uses in Great Britain. *Access Microbiology* **3**.

Pajares S & Bohannan BJ (2016) Ecology of nitrogen fixing, nitrifying, and denitrifying microorganisms in tropical forest soils. *Frontiers in microbiology* **7**: 1045.

Pasqua M, Grossi M, Zennaro A, Fanelli G, Micheli G, Barras F, Colonna B & Prosseda G (2019) The varied role of efflux pumps of the MFS family in the interplay of bacteria with animal and plant cells. *Microorganisms* **7**: 285.

Pehrsson EC, Tsukayama P, Patel S, Mejía-Bautista M, Sosa-Soto G, Navarrete KM, Calderon M, Cabrera L, Hoyos-Arango W & Bertoli MT (2016) Interconnected microbiomes and resistomes in low-income human habitats. *Nature* **533**: 212-216.

507 Pellow D, Mizrahi I & Shamir R (2020) PlasClass improves plasmid sequence classification. *PLoS computational*
508 *biology* **16**: e1007781.
509 Perry JA & Wright GD (2013) The antibiotic resistance "mobilome": searching for the link between environment
510 and clinic. *Frontiers in microbiology* **4**: 138.
511 Poirel L, Naas T & Nordmann P (2010) Diversity, epidemiology, and genetics of class D β-lactamases.
512 *Antimicrobial agents and chemotherapy* **54**: 24-38.
513 Purohit MR, Chandran S, Shah H, Diwan V, Tamhankar AJ & Stålsby Lundborg C (2017) Antibiotic resistance in an
514 Indian rural community: a 'One-Health'observational study on commensal coliform from humans, animals, and
515 water. *International journal of environmental research and public health* **14**: 386.
516 Ramtahal MA, Amoako DG, Akebe AL, Somboro AM, Bester LA & Essack SY (2022) A Public Health Insight into
517 Salmonella in Poultry in Africa: A Review of the Past Decade: 2010–2020. *Microbial Drug Resistance* **28**: 710-733.
518 Roman VL, Merlin C, Baron S, Larvor E, Le Devendec L, Virta MP & Bellanger X (2021) Abundance and
519 environmental host range of the SXT/R391 ICEs in aquatic environmental communities. *Environmental Pollution*
520 **288**: 117673.
521 Sabbagh P, Rajabnia M, Maali A & Ferdosi-Shahandashti E (2021) Integron and its role in antimicrobial resistance:
522 A literature review on some bacterial pathogens. *Iranian Journal of Basic Medical Sciences* **24**: 136.
523 Song M, Song D, Jiang L, Zhang D, Sun Y, Chen G, Xu H, Mei W, Li Y & Luo C (2021) Large-scale biogeographical
524 patterns of antibiotic resistome in the forest soils across China. *Journal of Hazardous Materials* **403**: 123990.
525 van Elsas JD & Bailey MJ (2002) The ecology of transfer of mobile genetic elements. *FEMS Microbiology Ecology*
526 **42**: 187-197.
527 Van Goethem MW, Pierneef R, Bezuidt OK, Van De Peer Y, Cowan DA & Makhalanyane TP (2018) A reservoir of
528 'historical'antibiotic resistance genes in remote pristine Antarctic soils. *Microbiome* **6**: 1-12.
529 Wang F, Fu Y-H, Sheng H-J, Topp E, Jiang X, Zhu Y-G & Tiedje JM (2021) Antibiotic resistance in the soil ecosystem:
530 A One Health perspective. *Current Opinion in Environmental Science & Health* **20**: 100230.
531 Wong W-T, Tseng C-H, Hsu S-H, Lur H-S, Mo C-W, Huang C-N, Hsu S-C, Lee K-T & Liu C-T (2014) Promoting effects
532 of a single Rhodopseudomonas palustris inoculant on plant growth by Brassica rapa chinensis under low fertilizer
533 input. *Microbes and environments* **29**: 303-313.
534 Wood DE, Lu J & Langmead B (2019) Improved metagenomic analysis with Kraken 2. *Genome biology* **20**: 1-13.

535 Wu J, Wang J, Li Z, Guo S, Li K, Xu P, Ok YS, Jones DL & Zou J (2023) Antibiotics and antibiotic resistance genes in
536 agricultural soils: A systematic analysis. *Critical Reviews in Environmental Science and Technology* **53**: 847-864.
537 Yan J, Chen W, Han X, Wang E, Zou W & Zhang Z (2017) Genetic diversity of indigenous soybean-nodulating
538 rhizobia in response to locally-based long term fertilization in a Mollisol of Northeast China. *World Journal of*
539 *Microbiology and Biotechnology* **33**: 1-9.
540 Zhao Y, Cocerva T, Cox S, Tardif S, Su J-Q, Zhu Y-G & Brandt KK (2019) Evidence for co-selection of antibiotic
541 resistance genes and mobile genetic elements in metal polluted urban soils. *Science of the Total Environment*
542 **656**: 512-520.
543 Zheng Y, Yu S, Wang G, Xie F, Xu H, Du S, Zhao H, Sang X, Lu J & Jiang W (2021) Comparative microbial antibiotic
544 resistome between urban and deep forest environments. *Environmental Microbiology Reports*.
545 Zheng Y, Yu S, Wang G, Xie F, Xu H, Du S, Zhao H, Sang X, Lu J & Jiang W (2021) Comparative microbial antibiotic
546 resistome between urban and deep forest environments. *Environmental Microbiology Reports* **13**: 503-508.

547

548