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

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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 possessed 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

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

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

87 Methods

88 Data sources

89 Whole metagenomic datasets were obtained from NCBI SRA (https://www.ncbi.nlm.nih.gov/sra). These
90 datasets represented various soil types: natural soil (NS), urban soil (US), rhizosphere soil (RHS) and rural soil
91 (RS) from different geographical locations (South Africa, Singapore, China, Israel, Botswana, Chile, Germany, El
92 Salvador, and Antarctica) sampled over the last 10 years. Only metagenomic sequences that were sequenced
93 using Illumina, had a read base count over 1e+09 and an average read length over 150bp were included.
94 Detailed information about the metagenomes retrieved from the database is included in Supplementary Table
95 1 and presented in summarized form in Table 1.

Table 1: Summary of included studies, respective sample source, location and sample size. Study identifiers 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)

Bioinformatic analysis

Quality control of reads was conducted using FastQC v.0.11.9 (Andrews, 2010) and filtered with Trimmomatic v.0.39 (Bolger *et al.*, 2014). Reads were trimmed for quality and adapter contamination using Trimmomatic with the following parameters specified: *ILLUMINACLIP: adapters.fa:2:30:10 LEADING:3 TRAILING:3 MINLEN:30 SLIDINGWINDOW: 4:20*. The microbiome was analysed using Kraken2 v.2.1.0 (Wood *et al.*, 2019) with the minikraken2 microbial database (2019, 8GB). Species confirmation and abundance estimation were

106 performed with Bracken v.2.6.2 (Lu *et al.,* 2017). The relative abundances of bacterial genera were used to
107 determine the microbial populations in each soil type. Statistical significance of the microbiome between
108 studies in a soil type was assessed using the non-parametric Wilcoxon rank sum test and Friedman's ANOVA
109 with SPSS software (IBM SPSS Statistics). Differences in the microbiome were considered statistically
110 significant at $p < 0.05$. The Beta diversity of the microbiome among soil types was visualized using non-metric
111 multidimensional scaling (NMDS) analysis with Bray-Curtis dissimilarity in the R package vegan v.2.6-4.

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

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

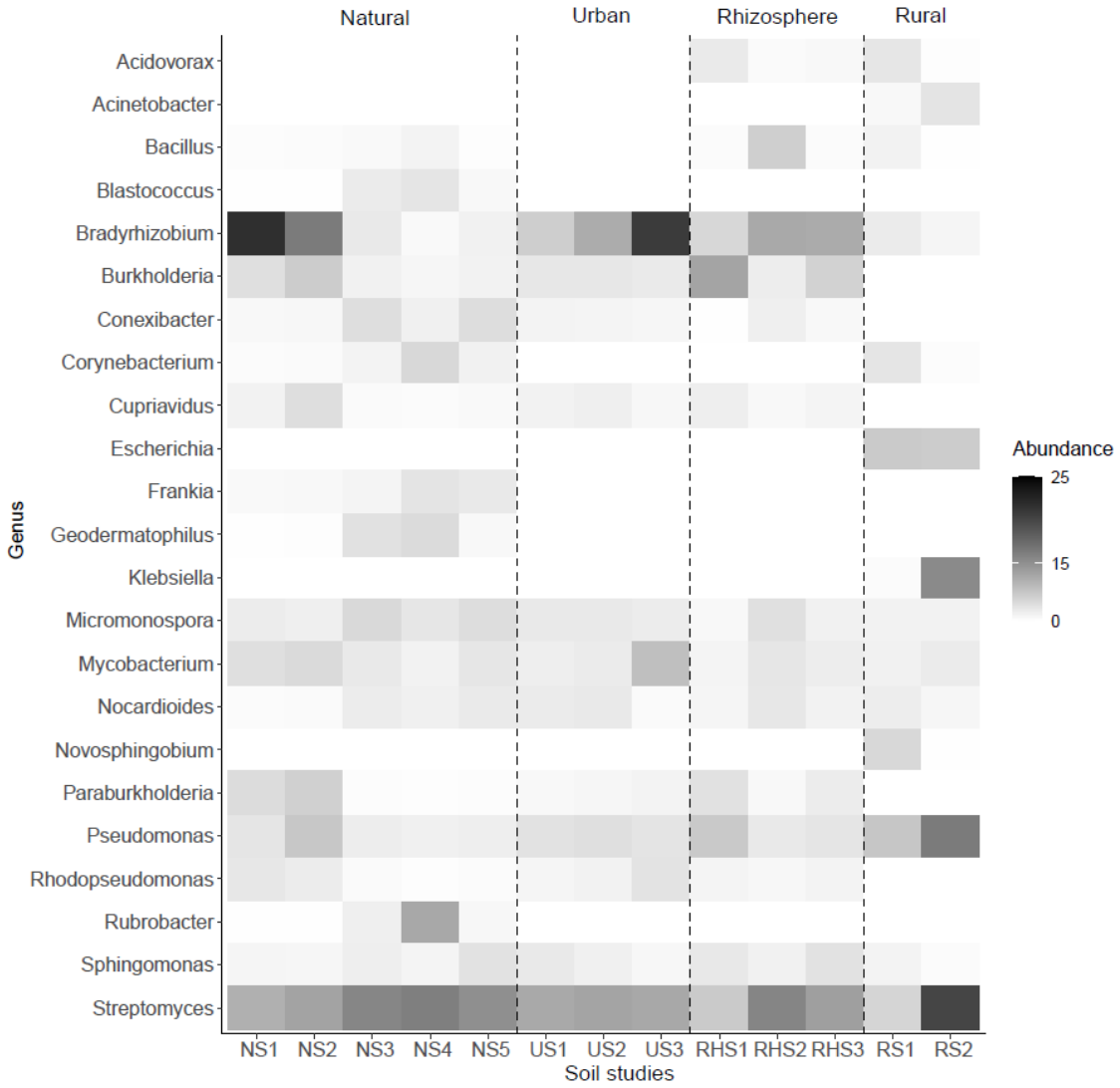


Figure 1: Heatmap showing the relative abundances of bacterial genera over 2% in each study from each soil type. The y-axis indicates the genera, and the x-axis represents the soil studies (Table 1). Each soil type is divided by the dashed black line and the abundances of bacterial genera are indicated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

Bacteria in natural soils are influenced by their habitat and environmental conditions (Supplementary Table 2). In Figure 1, *Bradyrhizobium* dominates the deep forest areas (NS1 and NS2), whereas bacteria like *Rubrobacter,* known for its thermophilic traits, are found in desert-like conditions (NS4), as confirmed by Connon *et al.* (2007) Pajares & Bohannan (2016) and Zheng *et al.* (2021). The microbial communities detected were statistically different between each of the natural studies indicating environmental influence ($p < 0.05$). Climate, pH, temperature and rainfall may all influence the abundances and types of bacteria found soil as discussed by Chase *et al.*, (2021).

Streptomyces was prevalent across all urban studies; however, the overall microbial community differed significantly between these urban studies ($p < 0.05$) (Figure 1 and Supplementary Table 3). As expected, higher abundances of *Bradyrhizobium*, *Mycobacterium,* and *Rhodopseudomonas* were observed in urban soil obtained from the nature reserve (US3). These bacteria thrive in areas with more vegetation and reduced anthropogenic activity, such as nature reserves, compared to residential and forest park areas (Wong *et al.*, 2014, Gorovtsov *et al.*, 2020, Glushakova *et al.*, 2021).

Plant growth-promoting bacteria such as *Streptomyces*, *Burkholderia*, *Pseudomonas,* and *Bradyrhizobium* were found in less than 12% abundance across different rhizosphere soil studies (Figure 1 and Supplementary Table 4). Overall, these microbial communities were different in each of the rhizosphere soil studies ($p < 0.05$). These bacteria also assist in protecting plants against pathogens by producing antibiotics (Kashyap et al., 2019; de Sousa et al., 2022; Liu et al., 2022). *Bradyrhizobium*, which aids in nitrogen fixation and plant growth, is found at an 8% abundance in RHS1, 2% in RHS2 and 4% in RHS3 (Fierer, 2017). Soil in RHS1 and RHS3 consisted of crops supplemented with nitrogen-phosphorus-potassium (NPK) fertiliser (Supplementary Table 1). Despite this, these studies showed a higher abundance of *Bradyrhizobium* compared to RHS2, which was not supplemented. This contradicts previous studies suggesting that *Bradyrhizobium* will be present in a low abundance when nitrogen fertilisers are used (Okubo *et al.*, 2013, Yan *et al.*, 2017).

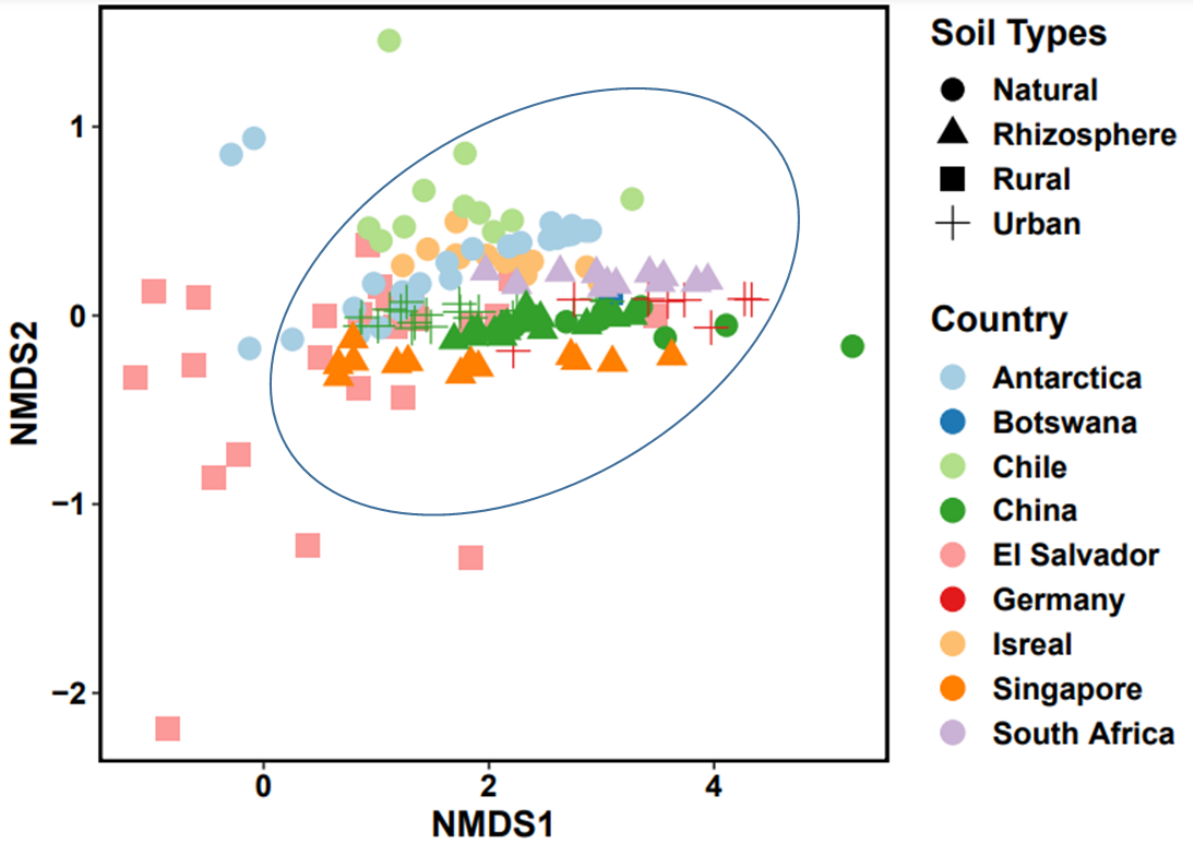
168 Lastly, in rural soil studies ($p < 0.05$), *Pseudomonas*, *Escherichia*, *Streptomyces*, and *Klebsiella* were prevalent,
169 including the ESKAPE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter*
170 *baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species) pathogens *Klebsiella pneumonia* and
171 *Pseudomonas aeruginosa* (Figure 1 and Supplementary Tables 5 and 6 respectively). The high prevalence of
172 these pathogens in rural areas is attributed to anthropogenic factors, such as the presence of domestic
173 animals, open defecation, and inadequate sanitation (Brooks et al., 2023). These conditions contribute to
174 antibiotic resistance hotspots in rural areas, as domestic animals spread faecal matter, and open sewage
175 drains and insufficient sanitation increases the selection pressure for antibiotic resistance (Nadimpalli *et al.*,
176 2020).

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

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

183 To assess the differences between microbial communities, present in different types of soil from different

184 geographical locations (Table 1), we analysed the beta-diversity. Additionally, we aimed to determine if the



185 geographical locations influence the microbial communities in each soil type. The beta-diversity quantifies the

186 "distance" between microbial communities using the Bray-Curtis index and can be visualized by non-metric

187 multidimensional scaling (NMDS). A smaller distance between samples from different countries and soil types

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

189 from their respective countries.

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

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

192 represented by a different shape (natural = circle, rhizosphere = triangle, rural = square, urban = cross) and

193 each country or region is colour-coded (Antarctica = light blue, Botswana = dark blue, Chile = light green, China

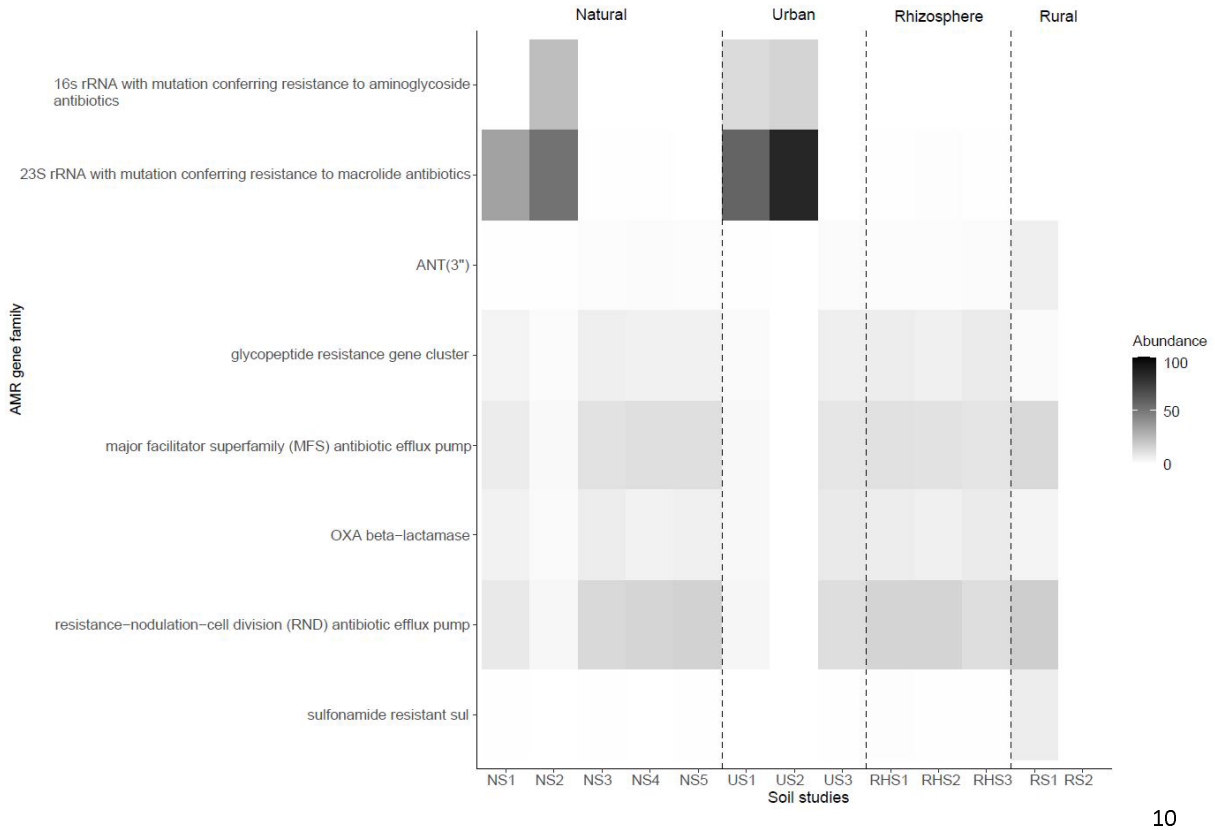
194 = dark green, El Salvador = pink, Germany = red, Israel = salmon, Singapore = orange, South Africa = lilac). The

195 samples that have smaller distances between them are circled in blue.

196 Figure 2 shows that most samples cluster together regardless of the soil type or geographical location. Natural,
197 rhizosphere, urban, and many rural soil samples are closely grouped, suggesting similar microbial communities
198 across these soil types. This similarity may be related to vegetation and varying levels of anthropogenic activity
199 (Pajares & Bohannan, 2016, Zheng *et al.*, 2021). However, some rural samples from El Salvador differ which
200 could be due to human activity or soil around latrine toilets and clothes washing areas. This can limit plant-
201 promoting bacteria and support enteric pathogens (Pehrsson *et al.*, 2016, Brooks *et al.*, 2023). Samples from
202 specific studies like RS1 (El Salvador), NS2 (China), NS4 (Chile) and NS5 (Antarctica) are further apart, indicating
203 variations in the microbial composition. Such differences within the same study may result from spatial
204 variability in the soil environment, even when sampling sites are only a few centimetres apart (Fierer, 2017).

205 Anthropogenic activities, vegetation, and climate play a role in the selection and prevalence 206 of antibiotic resistance genes.

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



211 2023).

212

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

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

224 Figure 3 shows that the 23S rRNA mutation conferring resistance to macrolide antibiotics were more prevalent
225 in urban soils as compared to the natural, rhizosphere and rural soils. Specifically, in US2 from China, 85% of
226 the detected AMR gene families were the 23S rRNA gene with this mutation, with other urban studies like US1
227 had 58% abundance. In contrast, natural soil studies such as NS1 and NS2 had lower abundances of 33% and
228 52%, respectively. Urban soil samples were collected from areas with high human activity (forest parks,
229 residential zones, and road verges) which have greater antibiotic pollution and selective pressures promoting
230 the proliferation of antibiotic resistant bacteria, as detailed by Osbiston *et al.* (2021).

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

237 *et al.*, 2020). Additionally, vegetation, flora and fauna influences the presence of efflux pump gene families, as
238 discussed by Martinez *et al.* (2009) and Pasqua *et al.* (2019).

239 Lastly, we found the OXA beta-lactamase gene family present in all rhizosphere and natural soil studies, except
240 US2 and RS2, with a high abundance in US3 (7%) and RHS3 (7%) (Figure 3 and Supplementary Table 7). The
241 OXA beta-lactamase gene family has been shown to confer both acquired and intrinsic resistance and can be
242 found in all soil types; from primitive natural soils with little to no anthropogenic activity to urban
243 environments (Djenadi *et al.*, 2018, Han *et al.*, 2022). Previous research also suggests that the climate affects
244 the abundance of ARGs, with beta-lactam resistance genes being more common in warmer areas (Li *et al.*,
245 2022). However, our study found similar AMR gene families across all soil types, regardless of temperature.

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

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

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

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

264 Plasmids encoding naturally occurring antibiotics were identified in natural and urban soil studies. Among the
265 natural soil types, NS1 showed a high abundance of plasmids associated with aminoglycosides (13%) and
266 carbapenem; cephalosporin; penam (16%) drug classes. These high abundances were consistent with other
267 natural studies (Supplementary Table 8). Similar to NS1, US1 had a high abundance of plasmids associated with
268 aminoglycosides (13%), carbapenem; cephalosporin; penam (25%) along with fluoroquinolones (13%),
269 glycopeptide (25%), macrolide (13%) and tetracycline (13%) drug classes. Additionally, US3 had plasmids linked
270 to similar antibiotic drug classes: aminoglycosides (16%), carbapenem; cephalosporin; penam (11%),
271 glycopeptide (12%) and peptides (9%). In our study, the natural and urban studies, sampled from forests and
272 deserts for natural soils and residential areas and forest parks for urban soils, showed higher abundances of
273 plasmids associated with naturally occurring antibiotics such as aminoglycosides, glycopeptides, and beta-
274 lactams (Zhao *et al.*, 2019, Meng *et al.*, 2022). These abundances may also be influenced by heavy metals and
275 other contaminants in the soil types as noted by Zhao *et al.* (2019).

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

282 Lastly in rural soils, RS2, a single sample study, did not contain any plasmids associated with AMR. However,
283 RS1 had a high abundance of plasmids linked to aminoglycoside (24%), carbapenem; cephalosporin; penam
284 (9%) and sulphonamide (39%) resistance. This is consistent with a study from a rural village in India that found
285 resistance to carbapenems, cephalosporins, penams, sulphonamides, tetracyclines, and quinolones, with *E. coli*
286 carrying plasmids for resistance to quinolones, cephalosporins, and colistin. This study suggested that the local
287 antibiotic use patterns supports the persistence of plasmids with ARGs (Purohit *et al.*, 2017).

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

292

293 AMR genes associated with integrons that are highly abundant were selected due to the

294 constant use of antibiotics.

295 To identify the presence of integrons and their association with AMR gene families, we classified integrons
296 from the metagenomic contigs present in each soil study. Furthermore, we linked the classified integrons with
297 AMR gene families to determine the differences between soil studies. Integrons are mobile genetic elements
298 that capture and express ARGs, particularly among gram-negative pathogenic bacteria. Integrons are present
299 in all environments and can serve as markers for tracing sources of pollution (Gillings, 2014).

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

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

310 In our study, we identified class 1, 2 and 3 integrons and their association with AMR genes (Supplementary
311 Tables 10 and 11). We found a high abundance of class 1 integrons associated with disinfecting agents and
312 sulphonamide resistance in all types of soil. The high abundance of the *qacE* gene with class 1 integrons can be
313 traced back to the use of quaternary ammonium compounds which were first used as hospital disinfecting
314 agents in 1930 and have become a part of the class 1 integron, known to be highly abundant in soils (Gillings,
315 2014, Cheng *et al.*, 2020). Similarly, sulphonamides were the first true antibiotic to be used in the 1930s and
316 have been selected for and can be commonly found in class 1 integrons (Gillings, 2014).

317 A high abundance of class 2 integrons was associated with aminoglycoside resistance (*aadB*) which ranged
318 from 15% in the RS1 to 75% in NS3 and NS4, and 83% in RHS3 and US1 (Supplementary Tables 10 and 11).

319 Aminoglycoside antibiotics were originally isolated from soil bacteria and commonly used in agriculture,
320 increasing their presence in soil environments, potentially affecting plant-soil microbial communities. They
321 also enter soils through human waste, municipal wastewater systems, and clinical use. These direct and
322 indirect exposures contributes to the detection of aminoglycoside resistance genes in various soil types, often
323 associated with class 1 and class 2 integrons (Blau *et al.*, 2018, Coates *et al.*, 2022). Previous studies have
324 shown that the *aadB* aminoglycoside resistance gene is frequently associated with class 1, 2, and 3 integrons
325 and is present in *Pseudomonas aeruginosa, Salmonella* spp., *Acinetobacter baumannii, Klebsiella* spp., and
326 *Escherichia coli* which are present in our study (Sabbagh *et al.*, 2021). Additionally, Jones *et al.*, (2005) showed
327 that the *aadB* gene, associated with class 1 integrons, had a close association with the bla_{SHV} gene conferring
328 resistance to penicillin and cephalosporins. This association was not seen in our study. Lastly, class 3 integrons
329 were predominantly associated with the *qacE* gene, which provides resistance to disinfectants. Over 55% of
330 class 3 integrons in all soil studies were linked to the *qacE* gene, with 100% in NS4, 97% in RHS2, and 97% in
331 US3. Similarly to class 1 integrons, the *qacE* gene are commonly found in class 3 integrons as it is considered to
332 be a conserved segment and had been selected for in the past (Sabbagh *et al.*, 2021).

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

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

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

342 The most prominent ICE families among all studies in all soil types were *Tn4371* (>12%), *SXT/R391* (>5%) and
343 *ICEclc* (>8%) (Supplementary Table 12). These ICE families are commonly found in soil and aid in the
344 degradation of pollutants (Obi *et al.*, 2018, Hirose *et al.*, 2021, Roman *et al.*, 2021). Additionally, we identified
345 different SGI1 IME families present in the different studies within the different types of soil (Supplementary

346 Table 13). SGI1 has been characterized as a *Salmonella* genomic island that can spread multi-drug resistance to
347 human and animal pathogens (Cummins *et al.,* 2020). Bacteria carrying these integrative elements have a
348 higher potential for spreading infections thus facilitating the spread of AMR to other environments (Delavat *et*
349 *al.,* 2017). This underscores the importance of continuously monitoring mobile genetic elements in different
350 soil types.

351 Conclusion

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

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

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

372 families like OXA beta-lactamase facilitates the spread of resistance from soil to various environments like
373 water, humans or animals.

374 Integrons detected in this study were linked to commonly used antibiotics in all soil types, suggesting that the
375 overuse of antibiotics contributes to the spread and emergence of antibiotic resistant bacteria. These bacteria
376 may also possess adaptive strategies provided by integrative elements, *SXT/R391, Tn4371, ICEclc* and IMEs
377 which can potentially carry accessory genes i.e., ARGs. Integrative elements allow bacteria in the soil to
378 stabilise and adapt to environmental conditions and human activity as well as favouring gene transfer and the
379 spread of AMR.

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

386 Declarations

387 Competing interests

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

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393 Authors' Contributions

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

396

397 # References

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

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

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

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

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

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

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

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

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

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

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

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

424 Cytryn E (2013) The soil resistome: The anthropogenic, the native, and the unknown. *Soil biology &*
425 *biochemistry* **63**: 18-23.
426 de Sousa LP, Guerreiro-Filho O & Mondego JMC (2022) The Rhizosphere Microbiomes of Five Species of Coffee
427 Trees. *Microbiology Spectrum* **10**: e00444-00422.
428 Delavat F, Miyazaki R, Carraro N, Pradervand N & van der Meer JR (2017) The hidden life of integrative and
429 conjugative elements. *FEMS microbiology reviews* **41**: 512-537.
430 Djenadi K, Zhang L, Murray AK & Gaze WH (2018) Carbapenem resistance in bacteria isolated from soil and
431 water environments in Algeria. *Journal of Global Antimicrobial Resistance* **15**: 262-267.
432 Dong Q, Zhao X, Zhou D, Liu Z, Shi X, Yuan Y, Jia P, Liu Y, Song P & Wang X (2022) Maize and peanut
433 intercropping improves the nitrogen accumulation and yield per plant of maize by promoting the secretion of
434 flavonoids and abundance of Bradyrhizobium in rhizosphere. *Frontiers in Plant Science* **13**: 957336.
435 Fierer N (2017) Embracing the unknown: disentangling the complexities of the soil microbiome. *Nature*
436 *Reviews Microbiology* **15**: 579-590.
437 Franco AL, Adams BJ, Diaz MA, Lemoine NP, Dragone NB, Fierer N, Lyons WB, Hogg I & Wall DH (2022)
438 Response of Antarctic soil fauna to climate-driven changes since the Last Glacial Maximum. *Global Change*
439 *Biology* **28**: 644-653.
440 Garcia ÍR, de Oliveira Garcia FA, Pereira PS, Coutinho HDM, Siyadatpanah A, Norouzi R, Wilairatana P, de
441 Lourdes Pereira M, Nissapatorn V & Tintino SR (2022) Microbial resistance: The role of efflux pump
442 superfamilies and their respective substrates. *Life Sciences* **295**: 120391.
443 Gillings MR (2014) Integrons: past, present, and future. *Microbiology and Molecular Biology Reviews* **78**: 257-
444 277.
445 Glushakova A, Lysak L, Belov A, Ivanova A, Lapygina E, Prokofieva T & Umarova A (2021) Local monitoring of
446 saprotrophic bacterial complexes of urban soils in Syktyvkar in 2019 and 2020. *Moscow University Soil Science*
447 *Bulletin* **76**: 84-88.
448 Gorovtsov A, Rajput V, Pulikova E, Gerasimenko A, Ivanov F, Vasilchenko N, Demidov A, Jatav H & Minkina T
449 (2020) Soil Microbial Communities in Urban Environment. *Advances in Environmental Research; Nova Science*
450 *Publisher: Hauppauge, NY, USA*.
451 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
452 antibiotic resistance genes in soil: A review. *Frontiers in Microbiology* **13**.

453 Hernández M, Roy S, Keevil CW & Dumont MG (2023) Identification of diverse antibiotic resistant bacteria in
454 agricultural soil with H218O stable isotope probing combined with high-throughput sequencing. *Environmental*
455 *Microbiome* **18**: 34.
456 Hirose J, Watanabe T, Futagami T, Fujihara H, Kimura N, Suenaga H, Goto M, Suyama A & Furukawa K (2021) A
457 new ICE clc subfamily integrative and conjugative element responsible for horizontal transfer of biphenyl and
458 salicylic acid catabolic pathway in the PCB-degrading strain Pseudomonas stutzeri KF716. *Microorganisms* **9**:
459 2462.
460 Hwang Y, Rahlff J, Schulze-Makuch D, Schloter M & Probst AJ (2021) Diverse viruses carrying genes for
461 microbial extremotolerance in the Atacama Desert hyperarid soil. *Msystems* **6**: 10.1128/msystems. 00385-
462 00321.
463 Jia B, Raphenya AR, Alcock B, Waglechner N, Guo P, Tsang KK, Lago BA, Dave BM, Pereira S & Sharma AN
464 (2016) CARD 2017: expansion and model-centric curation of the comprehensive antibiotic resistance database.
465 *Nucleic acids research* gkw1004.
466 Jones LA, McIver CJ, Kim M-J, Rawlinson WD & White PA (2005) The aadB gene cassette is associated with bla
467 SHV genes in Klebsiella species producing extended-spectrum β-lactamases. *Antimicrobial agents and*
468 *chemotherapy* **49**: 794-797.
469 Li H & Durbin R (2009) Fast and accurate short read alignment with Burrows–Wheeler transform.
470 *bioinformatics* **25**: 1754-1760.
471 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
472 proportions of specific antibiotic resistance genes in natural soil ecosystems. *Journal of Hazardous Materials*
473 **430**: 128442-128442.
474 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
475 anthropogenic impacts on the plasmid and phage borne transferable resistome in soil. *Environment*
476 *International* **170**: 107595.
477 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
478 integrative and conjugative elements. *Nucleic acids research* **47**: D660-D665.
479 Lu J, Breitwieser FP, Thielen P & Salzberg SL (2017) Bracken: estimating species abundance in metagenomics
480 data. *PeerJ Computer Science* **3**: e104.

481 Mantri SS, Negri T, Sales-Ortells H, Angelov A, Peter S, Neidhardt H, Oelmann Y & Ziemert N (2021)
482 Metagenomic sequencing of multiple soil horizons and sites in close vicinity revealed novel secondary
483 metabolite diversity. *Msystems* **6**: 10.1128/msystems. 01018-01021.
484 Manyi-Loh C, Mamphweli S, Meyer E & Okoh A (2018) Antibiotic use in agriculture and its consequential
485 resistance in environmental sources: potential public health implications. *Molecules* **23**: 795.
486 Martinez JL, Sánchez MB, Martínez-Solano L, Hernandez A, Garmendia L, Fajardo A & Alvarez-Ortega C (2009)
487 Functional role of bacterial multidrug efflux pumps in microbial natural ecosystems. *FEMS microbiology*
488 *reviews* **33**: 430-449.
489 Meng M, Li Y & Yao H (2022) Plasmid-mediated transfer of antibiotic resistance genes in soil. *Antibiotics* **11**:
490 525.
491 Moura A, Soares M, Pereira C, Leitão N, Henriques I & Correia A (2009) INTEGRALL: a database and search
492 engine for integrons, integrases and gene cassettes. *Bioinformatics* **25**: 1096-1098.
493 Nadimpalli ML, Marks SJ, Montealegre MC, Gilman RH, Pajuelo MJ, Saito M, Tsukayama P, Njenga SM, Kiiru J &
494 Swarthout J (2020) Urban informal settlements as hotspots of antimicrobial resistance and the need to curb
495 environmental transmission. *Nature microbiology* **5**: 787-795.
496 Nurk S, Meleshko D, Korobeynikov A & Pevzner PA (2017) metaSPAdes: a new versatile metagenomic
497 assembler. *Genome research* **27**: 824-834.
498 Obi CC, Vayla S, De Gannes V, Berres ME, Walker J, Pavelec D, Hyman J & Hickey WJ (2018) The integrative
499 conjugative element clc (ICEclc) of Pseudomonas aeruginosa JB2. *Frontiers in microbiology* **9**: 1532.
500 Okubo T, Fukushima S, Itakura M, Oshima K, Longtonglang A, Teaumroong N, Mitsui H, Hattori M, Hattori R &
501 Hattori T (2013) Genome analysis suggests that the soil oligotrophic bacterium Agromonas oligotrophica
502 (Bradyrhizobium oligotrophicum) is a nitrogen-fixing symbiont of Aeschynomene indica. *Applied and*
503 *environmental microbiology* **79**: 2542-2551.
504 Ordine JVW, De Souza GM, Tamasco G, Virgilio S, Fernandes AFT, Silva-Rocha R & Guazzaroni M-E (2023)
505 Metagenomic insights for antimicrobial resistance surveillance in soils with different land uses in Brazil.
506 *Antibiotics* **12**: 334.
507 Osbiston K, Oxbrough A & Fernández-Martínez LT (2021) Antibiotic resistance levels in soils from urban and
508 rural land uses in Great Britain. *Access Microbiology* **3**.

509 Pajares S & Bohannan BJ (2016) Ecology of nitrogen fixing, nitrifying, and denitrifying microorganisms in
510 tropical forest soils. *Frontiers in microbiology* **7**: 1045.
511 Pasqua M, Grossi M, Zennaro A, Fanelli G, Micheli G, Barras F, Colonna B & Prosseda G (2019) The varied role
512 of efflux pumps of the MFS family in the interplay of bacteria with animal and plant cells. *Microorganisms* **7**:
513 285.
514 Pehrsson EC, Tsukayama P, Patel S, Mejía-Bautista M, Sosa-Soto G, Navarrete KM, Calderon M, Cabrera L,
515 Hoyos-Arango W & Bertoli MT (2016) Interconnected microbiomes and resistomes in low-income human
516 habitats. *Nature* **533**: 212-216.
517 Pellow D, Mizrahi I & Shamir R (2020) PlasClass improves plasmid sequence classification. *PLoS computational*
518 *biology* **16**: e1007781.
519 Perry JA & Wright GD (2013) The antibiotic resistance "mobilome": searching for the link between
520 environment and clinic. *Frontiers in microbiology* **4**: 138.
521 Poirel L, Naas T & Nordmann P (2010) Diversity, epidemiology, and genetics of class D β-lactamases.
522 *Antimicrobial agents and chemotherapy* **54**: 24-38.
523 Purohit MR, Chandran S, Shah H, Diwan V, Tamhankar AJ & Stålsby Lundborg C (2017) Antibiotic resistance in
524 an Indian rural community: a 'One-Health'observational study on commensal coliform from humans, animals,
525 and water. *International journal of environmental research and public health* **14**: 386.
526 Ramtahal MA, Amoako DG, Akebe AL, Somboro AM, Bester LA & Essack SY (2022) A Public Health Insight into
527 Salmonella in Poultry in Africa: A Review of the Past Decade: 2010–2020. *Microbial Drug Resistance* **28**: 710-
528 733.
529 Roman VL, Merlin C, Baron S, Larvor E, Le Devendec L, Virta MP & Bellanger X (2021) Abundance and
530 environmental host range of the SXT/R391 ICEs in aquatic environmental communities. *Environmental*
531 *Pollution* **288**: 117673.
532 Sabbagh P, Rajabnia M, Maali A & Ferdosi-Shahandashti E (2021) Integron and its role in antimicrobial
533 resistance: A literature review on some bacterial pathogens. *Iranian Journal of Basic Medical Sciences* **24**: 136.
534 Song M, Song D, Jiang L, Zhang D, Sun Y, Chen G, Xu H, Mei W, Li Y & Luo C (2021) Large-scale biogeographical
535 patterns of antibiotic resistome in the forest soils across China. *Journal of Hazardous Materials* **403**: 123990.
536 van Elsas JD & Bailey MJ (2002) The ecology of transfer of mobile genetic elements. *FEMS Microbiology*
537 *Ecology* **42**: 187-197.

538 Van Goethem MW, Pierneef R, Bezuidt OK, Van De Peer Y, Cowan DA & Makhalanyane TP (2018) A reservoir of
539 'historical'antibiotic resistance genes in remote pristine Antarctic soils. *Microbiome* **6**: 1-12.
540 Wang F, Fu Y-H, Sheng H-J, Topp E, Jiang X, Zhu Y-G & Tiedje JM (2021) Antibiotic resistance in the soil
541 ecosystem: A One Health perspective. *Current Opinion in Environmental Science & Health* **20**: 100230.
542 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
543 effects of a single Rhodopseudomonas palustris inoculant on plant growth by Brassica rapa chinensis under
544 low fertilizer input. *Microbes and environments* **29**: 303-313.
545 Wood DE, Lu J & Langmead B (2019) Improved metagenomic analysis with Kraken 2. *Genome biology* **20**: 1-13.
546 Wu J, Wang J, Li Z, Guo S, Li K, Xu P, Ok YS, Jones DL & Zou J (2023) Antibiotics and antibiotic resistance genes
547 in agricultural soils: A systematic analysis. *Critical Reviews in Environmental Science and Technology* **53**: 847-
548 864.
549 Yan J, Chen W, Han X, Wang E, Zou W & Zhang Z (2017) Genetic diversity of indigenous soybean-nodulating
550 rhizobia in response to locally-based long term fertilization in a Mollisol of Northeast China. *World Journal of*
551 *Microbiology and Biotechnology* **33**: 1-9.
552 Zhao Y, Cocerva T, Cox S, Tardif S, Su J-Q, Zhu Y-G & Brandt KK (2019) Evidence for co-selection of antibiotic
553 resistance genes and mobile genetic elements in metal polluted urban soils. *Science of the Total Environment*
554 **656**: 512-520.
555 Zheng Y, Yu S, Wang G, Xie F, Xu H, Du S, Zhao H, Sang X, Lu J & Jiang W (2021) Comparative microbial
556 antibiotic resistome between urban and deep forest environments. *Environmental Microbiology Reports*.
557 Zheng Y, Yu S, Wang G, Xie F, Xu H, Du S, Zhao H, Sang X, Lu J & Jiang W (2021) Comparative microbial
558 antibiotic resistome between urban and deep forest environments. *Environmental Microbiology Reports* **13**:
559 503-508.

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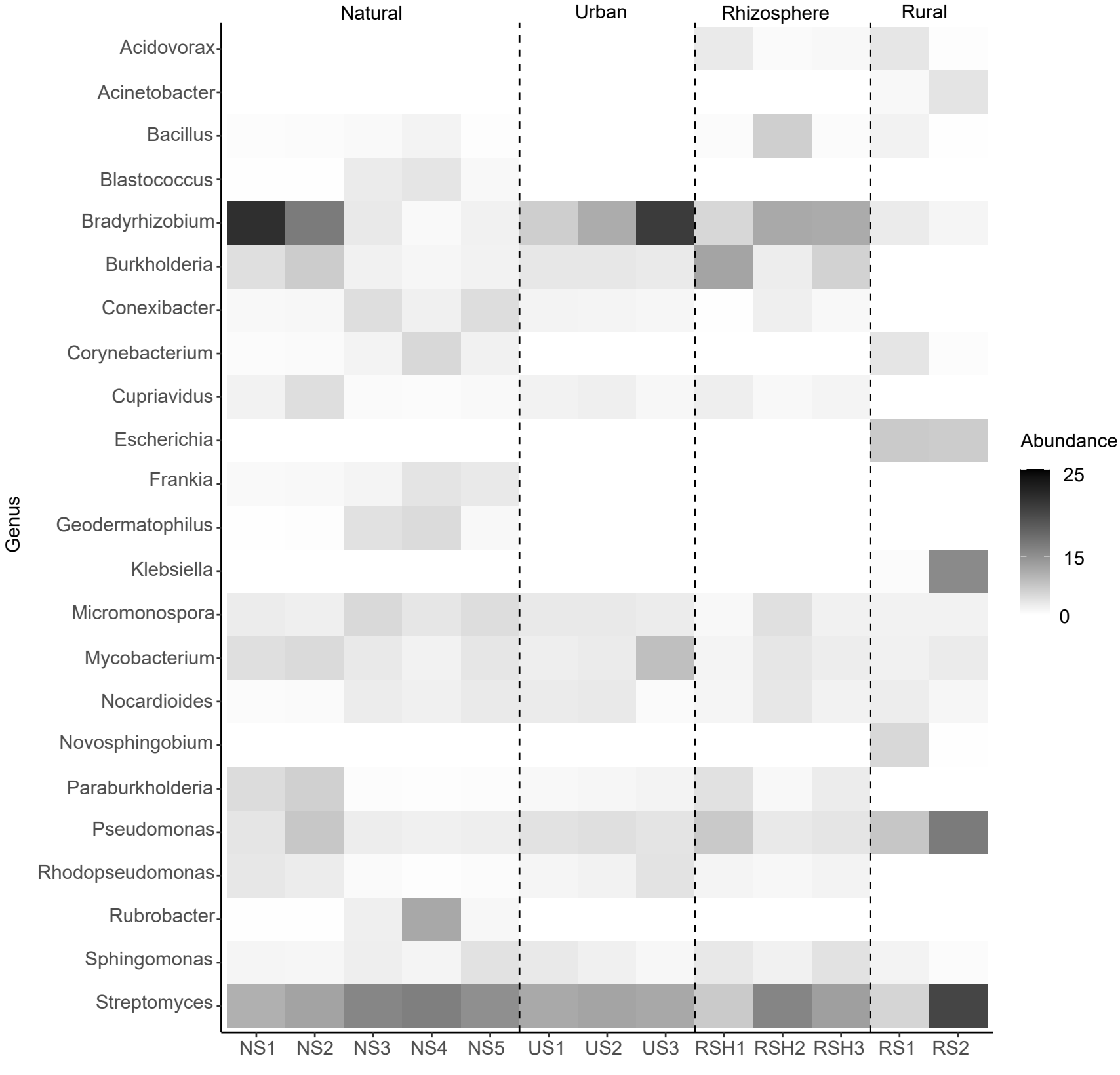
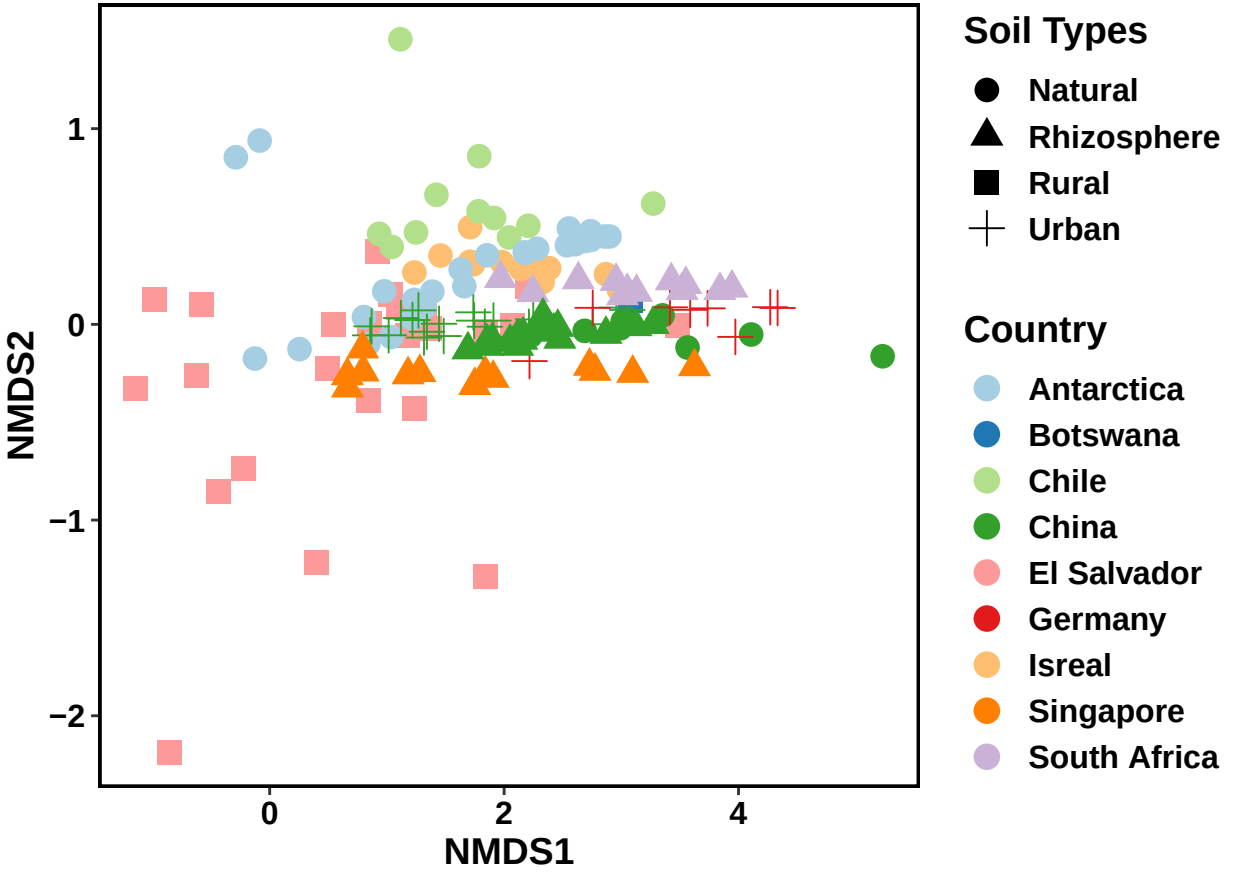
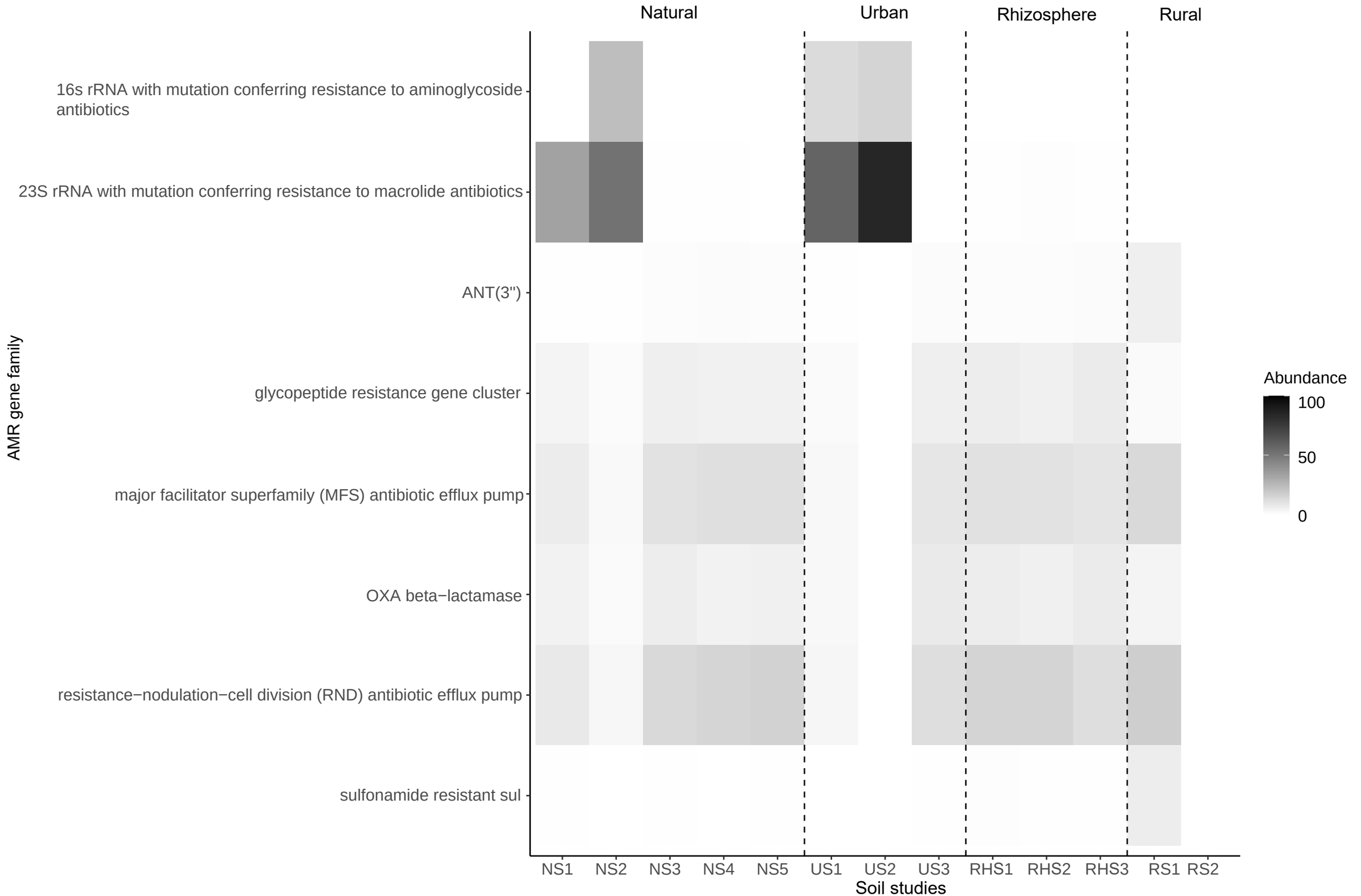

Natural
Urban
Rhizosphere
Rural
Genus
Acidovorax
Acinetobacter
Bacillus
Blastococcus
Bradyrhizobium
Burkholderia
Conexibacter
Corynebacterium
Cupriavidus
Escherichia
Frankia
Geodermatophilus
Klebsiella
Micromonospora
Mycobacterium
Nocardioides
Novosphingobium
Paraburkholderia
Pseudomonas
Rhodopseudomonas
Rubrobacter
Sphingomonas
Streptomyces
NS1
NS2
NS3
NS4
NS5
US1
US2
US3
RSH1
RSH2
RSH3
RS1
RS2
Abundance
25
15
0


Soil Types
Natural
Rhizosphere
Rural
Urban
Country
Antarctica
Botswana
Chile
China
El Salvador
Germany
Isreal
Singapore
South Africa
NMDS2
NMDS1
1
0
−1
−2
0
2
4


Natural
Urban
Rhizosphere
Rural
16s rRNA with mutation conferring resistance to aminoglycoside antibiotics
23S rRNA with mutation conferring resistance to macrolide antibiotics
ANT(3")
glycopeptide resistance gene cluster
major facilitator superfamily (MFS) antibiotic efflux pump
OXA beta−lactamase
resistance−nodulation−cell division (RND) antibiotic efflux pump
sulfonamide resistant sul
AMR gene family
NS1
NS2
NS3
NS4
NS5
US1
US2
US3
RHS1
RHS2
RHS3
RS1
RS2
Soil studies
Abundance
100
50
0