A metagenomic survey of global antimicrobial resistance

By

Aaron Berkman

(1831650)

MSc Dissertation

Submitted in fulfilment of the requirements for the degree

Master of Science

In

Molecular and Cell Biology

In the faculty of science, University of the Witwatersrand, Johannesburg, South Africa

Supervisor:

Professor Pieter De Maayer

4 February 2025

Declaration

I, Aaron Berkman (1831650), am a student registered for the degree of Master of Science (by dissertation) in the academic year 2025.

I hereby declare the following:

- ☐ I am aware that plagiarism (the use of someone else's work without their permission and/or without acknowledging the original source) is wrong.
- ☐ I confirm that the work submitted for assessment for the above degree is my own unaided work except where explicitly indicated otherwise.
- ☐ I have not submitted this work before for any other degree or examination at this or any other University.
- ☐ I have followed the required conventions in referencing the thoughts and ideas of others.
- ☐ I understand that the University of the Witwatersrand may take disciplinary action against me if there is a belief that this is not my own unaided work or that I have failed to acknowledge the source of the ideas or words in my writing.

Signed: 

Date: 4 February 2025

Abstract

Antimicrobial resistance (AMR) poses a major global health threat, yet understanding of environmental reservoirs remains limited. This study analysed 1,758 metagenomes to assess microbial species and antimicrobial resistance gene (ARG) diversity across global soil and aquatic environments. Using Kraken2 for taxonomic classification and DeepARG for ARG identification, coupled with diversity analyses, this research revealed distinct patterns in microbial and antibiotic resistance gene profiles. Microbial communities showed similar overall diversity between soil and aquatic environments (Shannon indices: 5.409 and 5.410 respectively) but exhibited distinct structural patterns. Urban wastewater demonstrated the highest microbial diversity in aquatic settings (Shannon index: 5.43), while cold biomes showed highest diversity in soils (Shannon index: 5.63). ARG profiles revealed different patterns, with soil environments showing higher median diversity (4.68) compared to aquatic environments (4.52). Notably, anthropogenic soils exhibited the highest ARG diversity (Shannon index: 5.71) despite reduced microbial diversity, highlighting human activity's role in ARG proliferation. The soil core resistome was substantially larger than aquatic (179 vs 113 ARGs), with 101 shared ARGs between environments. ESKAPE pathogens were most prevalent in human-impacted environments, reaching 8.32% in groundwater and 8.23% in temperate soils. This comprehensive analysis reveals how environmental conditions and human activities distinctly shape microbial communities and their associated resistomes. These findings underscore the urgent need for integrated AMR surveillance beyond clinical settings and highlight critical intervention points for mitigating the spread of antibiotic resistance, particularly in agricultural and urban environments where human activities substantially influence resistance patterns.

Keywords: antimicrobial resistance; environmental resistome; ESKAPE pathogens; metagenomics; microbial diversity; core resistome; environmental reservoirs; anthropogenic impact; soil microbiome; aquatic microbiome

Preface

Antimicrobial resistance (AMR) is a rapidly escalating global health threat, fuelled by the dissemination of antibiotic resistance genes (ARGs) across diverse environments. While clinical settings are often at the centre of AMR discussions, an equally critical challenge exists in environmental reservoirs—soil and water—that act as critical reservoirs of resistance. These environments facilitate complex microbial interactions, providing opportunities for ARGs to emerge, persist and spread. This study harnesses metagenomic analyses to explore ARG prevalence and diversity in soil and aquatic ecosystems worldwide, aiming to understand how these natural reservoirs contribute to AMR on a global scale.

Chapter 1 provides a brief introduction to the topic, a problem statement and the aims and objectives of this research. Chapter 2 demonstrates a comprehensive review of the current literature on AMR, emphasising the importance of environmental ARG reservoirs. This chapter covers the fundamental mechanisms of antimicrobial resistance, the impact of anthropogenic activities on resistance gene distribution and the role of soil and water environments in sustaining and amplifying antimicrobial resistance. It also highlights the limitations of traditional culture-based AMR studies and the transformative potential of metagenomics in providing a broader, more accurate perspective of global AMR patterns.

Chapter 3 describes the methodology that combines taxonomic profiling through Kraken2 and resistance gene profiling using DeepARG with statistical analyses across diverse soil and aquatic biomes. Chapter 4 presents the findings from 1,758 metagenomes and compares ARG diversity, ESKAPE pathogen presence and core resistome structures across soil and aquatic samples worldwide. The findings reveal important ARG diversity patterns influenced by anthropogenic and environmental factors, with human-impacted regions, such as urban wastewater and agricultural soils, showing elevated ARG diversity and ESKAPE pathogen presence.

Finally, chapter 5 concludes by addressing the limitations and implications of these findings for AMR management, stressing the need for a global, integrative approach to monitoring environmental resistomes – one that broadens the scope of AMR research beyond clinical confines. This work aims to provide essential insights and benchmarks that can guide future research and policies, enabling a more comprehensive strategy in the ongoing fight against AMR.

Acknowledgements

I would like to extend my deepest gratitude to my supervisor, Professor Pieter De Maayer, for his unwavering mentorship and guidance throughout my academic journey, beginning with my Honours year in 2023 and continuing through the completion of this thesis. His expertise, patience and encouragement have been invaluable to my growth as a researcher and have profoundly shaped both my skills and understanding of this field. I am sincerely thankful for his support and commitment to my success.

I would also like to express my appreciation to the University of the Witwatersrand and the Postgraduate Merit Award (PMA) program for the generous financial support that enabled me to pursue this degree.

Table of Contents

List of figures

List of tables

List of abbreviations

AMR - Antimicrobial resistance

ARDB - Antibiotic Resistance Genes Database

ARG - Antibiotic resistance gene

AST - Antimicrobial susceptibility testing

CARD - Comprehensive Antibiotic Resistance Database

CRKP - Carbapenem-resistant *Klebsiella pneumoniae*

ECDC - European Centre for Disease Prevention and Control

EFSA - The European Food Safety Authority

ESBL – Extended-spectrum-beta-lactamase

ESKAPE - *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter* species

GLASS - Global Antimicrobial Surveillance System

GRCh38 - Genome Reference Consortium Human Build 38

HGT - Horizontal gene transfer

ICU - Intensive care unit

IQR - Interquartile range

LCA - Lowest common ancestor (in taxonomic classification)

MBC - Minimum bactericidal concentration

MDR - Multi-drug resistant

MG-RAST - Metagenomics Rapid Annotation using Subsystems Technology

MIC - Minimum inhibitory concentration

MLS - Macrolide-lincosamide-streptogramin

MRSA - Methicillin-resistant *Staphylococcus aureus*

NCBI - National Center for Biotechnology Information

PCR – Polymerase chain reaction

PCoA - Principal coordinate analysis

PDR - Pan drug-resistant

PLSC - Partial least squares correlation

qPCR - Quantitative polymerase chain reaction

RefSeq - Reference Sequence Database

Spp. - Species

WHO – World Health Organization

WGS – Whole genome sequencing

WMS - Whole metagenome sequencing

WWTP - Wastewater treatment plant

XDR - Extensively drug-resistant

Chapter 1: Introduction

Infectious diseases remain one of the leading causes of mortality worldwide, with bacterial infections posing a major threat to global health (Murray *et al.,* 2022). While antibiotics have revolutionised the treatment of bacterial infections since their introduction in the early 20th century, their effectiveness is increasingly compromised by the emergence and spread of antimicrobial resistance (AMR) (Uddin *et al.,* 2021). This growing crisis continues to undermine many advances in modern medicine, all of which rely on effective antibiotics to prevent and treat infections (Marshall & Levy, 2011; Murray *et al.,* 2022).

While the clinical impact of AMR is well documented, there is growing recognition that the crisis extends far beyond hospital walls (Barathe *et al.,* 2024). Environmental reservoirs - particularly soil and water systems - play crucial roles in the emergence, persistence, and spread of antimicrobial resistance (Cycoń *et al.,* 2019; Aguilar-Salazar *et al.,* 2023; Barathe *et al.,* 2024). These environments serve as conduits where resistance genes can be exchanged between diverse bacterial populations through horizontal gene transfer (Hutinel *et al.,* 2021). Agricultural practices, wastewater discharge, and other human activities introduce antibiotics and resistant bacteria into these environments, creating selective pressures that promote the maintenance and dissemination of resistance (Manyi-Loh *et al.,* 2018; Häder *et al.,* 2020; Barathe *et al.,* 2024).

Traditional approaches to AMR surveillance have focused primarily on clinical isolates, providing an important but incomplete picture of resistance patterns (Pillay *et al.,* 2022). The advent of high-throughput sequencing technologies, particularly metagenomics, has created unprecedented opportunities to study AMR in complex environmental communities. By analysing all genetic material present in environmental samples, metagenomic approaches can reveal the full spectrum of resistance genes present in these reservoirs, including those carried by unculturable organisms that constitute the majority of environmental bacteria (Bengtsson-Palme *et al.,* 2017; Pillay *et al.,* 2022).

1.1 Problem statement

Despite the transformative potential of metagenomic approaches, current AMR surveillance strategies remain heavily focused on traditional culture-based techniques and clinical settings (Sirijatuphat *et al.,* 2018). This limited scope reflects a broader pattern of inadequate stewardship that emerged following the antibiotic golden era of the 1970s, when complacency led to widespread antibiotic misuse (Uddin *et al.,* 2021). The resulting rise of multidrug-resistant (MDR),

extensively drug-resistant (XDR), and pandrug-resistant (PDR) pathogens now poses an urgent global health challenge (Magiorakos *et al.,* 2012). Even the WHO's Global Antimicrobial Surveillance System (GLASS) continues to rely primarily on culture-based analyses of clinical isolates, neglecting the complex environmental reservoirs where resistance emerges and spreads (Sirijatuphat *et al.,* 2018; Pillay *et al.,* 2022).

This study directly addresses these surveillance limitations by demonstrating the power of metagenomics for comprehensive AMR monitoring. Through analysis of publicly available metagenomes from diverse soil and aquatic environments globally, this research surveys and compares resistomes to reveal the full extent of the AMR threat across different settings. By moving beyond traditional single-organism approaches, this study uncovers the intricate networks driving AMR dissemination and provides crucial insights to inform more effective surveillance and control strategies.

1.2 Aims and Objectives

1.2.1 Aim

The aim of this research was to determine the influence of environmental, geographical, and human factors on the prevalence and distribution of ARGs in various non-clinical environments. This investigation intended to reveal how different conditions and environments affect the spread of ARGs and the extent of human impact on these processes.

1.2.2 Objectives

1. Data acquisition: Obtain metagenomes from the MG-RAST database, focusing on metagenomes from a diverse set of environments.

2. Quality control: Conduct thorough quality control on the metagenomic data to ensure a high-quality and valid analysis.

3. Taxonomic analysis: Determine the taxonomic composition of each metagenome.

4.Resistome analysis: Identify and analyse the ARGs present in each metagenome, exploring the diversity and structure of the resistomes.

5. Comparative analysis: Compare the resistomes across different environmental samples, linking differences to taxonomic distributions, environmental conditions, and assessing human influence.

Chapter 2: Antimicrobial resistance in the age of metagenomics

2.1 A revolution in healthcare: The introduction of antibiotics and antimicrobials

The discovery of antimicrobials represented a substantial advancement in therapeutic strategies for the treatment of infections, shifting medicinal approaches from supportive care to targeted healthcare (Aminov, 2010). Antimicrobials boast a rich history, with many origins in traditional medicines before the first man-made drugs (Aminov, 2010). For instance, artemisinin, an effective antimicrobial and antimalarial drug was used for millennia by practitioners of traditional Chinese medicine, before it was integrated into mainstream medicine in the twentieth century (Cui & Su, 2009). It was only in 1910, under the supervision of Paul Ehrlich, that arsphenamine, the first laboratory-created antimicrobial was synthesised (Nicolaou & Rigol, 2018). In addition to arsphenamine, subsequent man-made drugs such as sulphonamides were routinely used to treat bacterial infections (Landecker, 2019). These developments inspired Ehrlich to envision a "magic bullet" – a selective drug that would target specific pathogenic microorganisms. (Nicolaou & Rigol, 2018; Landecker, 2019).

While the earlier work with antimicrobials laid the foundations, the discovery of naturally-derived antibiotics revolutionised healthcare (Aminov, 2010). Penicillin was discovered in 1928 by Alexander Fleming (Gaynes, 2017). However, it was not until 1943 that penicillin was successfully produced and distributed (Gaynes, 2017). The following years saw the synthesis of an array of novel antibiotic classes (Table 1) in the "golden age" of antibiotic development (Chopra *et al.,* 2002). With antibiotics becoming widespread, common drivers of mortality, such as bacteria that cause pneumonia and tuberculosis, became treatable (Iseman, 2002). This saw the mortality rate due to infectious diseases reach an all-time low in the 1980s (Armstrong *et al.,* 1999).

Table 1. Overview of Antibiotic Classes Discovered from the 1910s to 2020s. A timeline of the discovery of novel antibiotic classes from the 1910s to the 2020s.

Decade	Antibiotic Class	Example	Target in Bacterial Cell	Reference
1910s	Arsphenamine	Salvarsan, Compound 606	Bacterial enzymes in *Treponema pallidum*	(Abraham, 1948; Landecker, 2019)
1930s	Sulphonamides	Sulfanilamide	Inhibits folic acid synthesis	(Ovung & Bhattacharyya, 2021)
1920s-1940s	Penicillins	Penicillin G	Inhibits cell wall synthesis	(Gaynes, 2017)
1940s	Aminoglycosides	Streptomycin	Inhibits protein synthesis by binding to ribosomal RNA	(Doi *et al.,* 2016)
1940s	Tetracyclines	Chlortetracycline	Inhibits protein synthesis by preventing tRNA attachment to the ribosome	(Nelson & Levy, 2011)
1950s	Macrolides	Erythromycin	Inhibits protein synthesis by binding to the ribosome	(Dinos, 2017)
1950s	Glycopeptides	Vancomycin	Inhibits cell wall synthesis by binding to peptidoglycan precursors	(Blaskovich *et al.,* 2018)
1960s	Quinolones	Nalidixic acid	Inhibits DNA gyrase, preventing DNA replication	(Pham *et al.,* 2019)
1970s	Cephalosporins	(Multiple Generations)	Inhibits cell wall synthesis by binding to penicillin-binding proteins	(Hewitt, 1973)
2010s	Malacidins	Malacidin A	Binds to lipid II, disrupting cell wall synthesis	(Hover *et al.,* 2018)
2020s	Thiadiazol	Halicin	Disrupts bacterial membrane potential	(Stokes *et al.,* 2020)

Although antibiotic discovery continues, concerns have grown about the long-term success of these drugs (Chopra *et al.,* 2002). Indeed, from the 1950s the success rates of many antibiotics saw a concerning decline (Levy & Marshall, 2004). In response, treatment strategies shifted to using multiple antibiotics and combination therapies (Kerantzas & Jacobs, 2017). Still, the long-term effectiveness of antibiotics remained a concern as the microbial pathogens began to become less susceptible towards antimicrobial treatments - a phenomenon known as antimicrobial resistance (AMR) (Levy & Marshall, 2004; Aminov, 2010).

Alarmingly, after the golden era of antibiotic discovery in the mid-20th century, no novel antibiotic classes were identified for several decades (Uddin *et al.,* 2021). The lack of new antibiotic development has worsened the AMR crisis. (Uddin *et al.,* 2021). Recent advancements in artificial intelligence and machine learning have bolstered the search for new antibiotics (Stokes *et al.,* 2020). For instance, the discovery of Halicin, an antibiotic effective against several drug-resistant bacteria, was facilitated by AI-driven models that screened millions of compounds in silico (Stokes *et al.,* 2020). Another recent discovery is Malacidin, a calcium-dependent antibiotic identified from soil metagenomes, which has shown effectiveness against multidrug-resistant pathogens (Hover *et al.,* 2018). However, it is important to note that neither Halicin nor Malacidin has yet been approved for human use (Hover *et al.,* 2018; Stokes *et al.,* 2020). Despite these promising discoveries, the pace of antibiotic development remains slow, and the threat of AMR continues to escalate (Skender, 2024). Left unchecked, it is estimated that by 2050, AMR will cost the global economy $1 trillion annually, driving millions into extreme poverty and increasing mortality rates (Dadgostar, 2019; Wang *et al.,* 2019). The World Health Organization (WHO) projects that by 2050, infectious disease outbreaks, particularly those due to hard-to-treat bacterial infections, will continue to overwhelm communities and global economies, affecting over four hundred million people and driving the most vulnerable populations into severe poverty (Kotwani *et al.,* 2021; Murray *et al.,* 2022). The threat of infectious disease, both emerging and persistent, underscores the urgent need for effective antimicrobial treatments. As the crisis deepens, it is essential to revisit the early warnings and initial observations of antimicrobial resistance, which continue to inform our approach.

2.2 Early insights into antimicrobial resistance

AMR was not a sudden phenomenon. Evidence of antibiotic resistance emerged early, with laboratory observations of penicillin-resistant bacteria before the widespread distribution of the drug (Lobanovska & Pilla, 2017). These concerns were expressed by Alexander Fleming during his 1945 Nobel lecture – warning of the dangers of antibiotic overuse, which could accelerate the development of resistance, particularly in the human body (Magalhães *et al.,* 2021). In support, Barber and Rozwadowska-Dowzenko (1948) noted that prior to 1944 very few penicillin-resistant bacterial strains had been encountered. In the following years, leading to 1948, the rate of penicillin-resistant bacteria, particularly those isolated from the clinical setting, drastically increased (Barber & Rozwadowska-Dowzenko, 1948). While penicillin-resistant bacterial infections continued to rise, many medical practitioners were not sufficiently alarmed and believed that while this had complicated treatment strategies in certain hospitals, it was likely not the case for the global community (Forbes, 1949).

While the discovery of novel antibiotic classes promised medical breakthroughs – their overuse fuelled the alarming emergence of antibiotic-resistant bacterial strains (Uddin *et al.,* 2021). The rapid development of these drugs in a short timeframe led to the dramatic expansion of antibiotic distribution. The consequences were two-fold. On the one hand, this resulted in unnecessary, broad-spectrum and excessive antibiotic prescriptions – a practice that has been maintained to date (Ventola, 2015). On the other hand, it led to the expansion of antibiotics into the agricultural setting (Manyi-Loh *et al.,* 2018). Today, antibiotic use in agricultural sectors is at an all-time high and is projected to increase in line with growing demands for food (Reardon, 2023). The excessive use of antibiotics in both clinical and agricultural settings has driven a marked increase in antibiotic-resistant bacteria (Murray *et al.,* 2022). The widespread and continued contamination of various environments with antibiotics and antibiotic-resistant bacteria further elevates the risk of human, animal and plant exposure to antibiotic-resistant bacteria (Ventola, 2015; Manyi-Loh *et al.,* 2018), presenting a major threat to both human health and food security (Mulchandani *et al.,* 2023).

2.3 The emergence of multidrug-resistant pathogens

Today, the AMR crisis is worse than ever due to the emergence of multidrug-resistant (MDR), extensively drug-resistant (XDR), and pandrug-resistant (PDR) bacterial strains (Falagas & Karageorgopoulos, 2008). The most adopted definitions describe MDR bacteria as those

displaying non-susceptibility to at least three antibiotic classes (Magiorakos *et al.,* 2012). For example, methicillin-resistant *Staphylococcus aureus* (MRSA) and multidrug-resistant *Enterobacteriaceae* that pose serious treatment challenges (Tiwari *et al.,* 2008; Bassetti *et al.,* 2015). XDR strains demonstrate stronger resistance, remaining susceptible to antibiotics from only one or two classes (Magiorakos *et al.,* 2012). Examples include extensively drug-resistant strains of *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, both often found in hospital settings (Willmann *et al.,* 2015; Mirzaei *et al.,* 2020). Finally, PDR strains are resistant to all treatments. Oxacillinase (OXA)-48-positive carbapenem-resistant *Klebsiella pneumoniae* (CRKP) fall into this category, rendering many last-resort antibiotics ineffective (Magiorakos *et al.,* 2012; Havan *et al.,* 2023). There has been a general increase in the number of these MDR, XDR and PDR taxa on a global level, posing a considerable threat to patient care (Karam *et al.,* 2016). While many factors have contributed to this crisis, the fundamental issue is clear – the overuse of antibiotics provides a selective pressure that favours resistant phenotypes (Levy & Marshall, 2004).

Hospitals, especially high-care units such as ICUs, are hotspots for the spread of antibiotic resistance genes (ARGs). With high infection rates and antibiotic use, they have become reservoirs of MDR, XDR, and PDR microorganisms, hindering effective treatment (Karam *et al.,* 2016). Moreover, the threat of these hospital-derived pathogens extends beyond hospital doors. Antibiotics and MDR microorganisms in hospital wastewater contaminate surrounding communities, exposing them to harmful pathogens (Talat *et al.,* 2023).

The continued emergence of MDR, XDR and PDR strains presents a major global health burden. In 2019 it is estimated that 4.95 million deaths were linked to AMR (Murray *et al.,* 2022). Furthermore, AMR disproportionately impacts low-income countries, harming the most vulnerable populations, with the highest AMR-related mortality rate in Sub-Saharan Africa (Murray *et al.,* 2022). Allowed to continue unchecked the AMR crisis will undoubtedly worsen. Continued surveillance of AMR and the ARGs driving resistance is required to further understand this crisis (Sirijatuphat *et al.,* 2018). Targeted clinical surveillance remains vital; however, the global scope of the AMR crisis shows limitations in this approach. To grasp the interplay between human, animal, and environmental health, broader surveillance strategies are required (Pillay *et al.,* 2022).

2.4 The environmental spread of antimicrobial resistance

Beyond the clinical setting, the excessive use of antibiotics in agriculture presents an often-overlooked reservoir of antimicrobial-resistant microorganisms (Marshall & Levy, 2011). Antibiotics are routinely used in livestock not only to treat infections but also as a prophylactic measure to prevent disease and as growth promoters to enhance production efficiency (Low *et al.,* 2021). This widespread use exerts selective pressure on bacterial populations in animals, leading to the emergence and proliferation of resistant strains (Manyi-Loh *et al.,* 2018). Humans may acquire these resistant bacteria through direct interaction with animals, ingestion of meat that has been contaminated or even by handling animal waste (Van Boeckel *et al.,* 2015; Jin *et al.,* 2023). Well-documented examples of this include the spread of *Escherichia coli* and *Salmonella* spp., both of which are common in livestock and can be resistant to multiple antibiotics used in animal husbandry (Hunduma *et al.,* 2023). These bacteria can enter the human food chain through improperly cooked meat or cross-contamination during food preparation (Manyi-Loh *et al.,* 2018; Jin *et al.,* 2023). The implications of this are concerning, as infections caused by these resistant strains are difficult to treat leading to prolonged illness and increased mortality (Murray *et al.,* 2022).

The environmental impact of agricultural practices extends beyond the direct transmission of resistant bacteria to humans (Manyi-Loh *et al.,* 2018). The use of antibiotics in agriculture also results in the contamination of soil and water sources (Kumar *et al.,* 2005). Manure and sludge, rich with antibiotics and antibiotic-resistant bacteria, are frequently used as fertilisers, introducing these contaminants into the environment (Kumar *et al.,* 2005). This practice promotes the spread of antimicrobial resistance genes among soil bacteria, which can then be transferred to human pathogens through horizontal gene transfer (HGT) (Hutinel *et al.,* 2021). Water sources, including rivers, lakes, and groundwater, can also become contaminated through runoff from agricultural lands and leaching from animal waste storage facilities (Manyi-Loh *et al.,* 2018). This contamination is not confined to rural areas; urban water systems are also affected, particularly where wastewater treatment processes are inadequate (Pazda *et al.,* 2019).

Urban environments contribute to the environmental spread of AMR in several ways (Hendriksen *et al.,* 2019). Wastewater from hospitals, households, and industrial sources often contains antibiotics and resistant bacteria, which can persist even after conventional wastewater treatment

(Raza *et al.,* 2021). These resistant bacteria and ARGs can then be released into natural water bodies, where they mix with other environmental bacteria, facilitating the spread of resistance (Hutinel *et al.,* 2021).

The spread of AMR in aquatic environments is particularly concerning due to the interconnectedness of water systems (Reddy *et al.,* 2022). Rivers and streams can carry resistant bacteria and ARGs over long distances, potentially affecting diverse and large populations (Reddy *et al.,* 2022; Aguilar-Salazar *et al.,* 2023). The spread of resistance through aquatic environments is further exacerbated by global trade and travel, which can introduce resistant strains to new regions and contribute to the global dissemination of AMR (Mason *et al.,* 2016; Hendriksen *et al.,* 2019). The presence of ARGs in marine environments has also been documented, particularly in areas near wastewater discharge, highlighting the role of human activity in spreading resistance - even in remote locations (Cuadrat *et al.,* 2020).

The implications of environmental AMR spread are far-reaching (Murray *et al.,* 2022). The contamination of food, water, and the broader environment with resistant bacteria poses a direct threat to human health, particularly in regions with limited access to healthcare and effective antibiotics (Murray *et al.,* 2022). Given the complexity of AMR spread in the environment, there is an imperative need for surveillance of AMR (Pillay *et al.,* 2022). The evolution of AMR research and surveillance has adapted to these challenges, with traditional laboratory techniques evolving alongside modern molecular methods to track and understand the spread of resistance in both clinical and environmental contexts.

2.5 The evolution of AMR research and surveillance: Traditional laboratory techniques in AMR research

Traditionally, AMR surveillance was implemented using the paper disk method (Vincent *et al.,* 1944). A paper disk was immersed in an antimicrobial agent at a single concentration. The disk was placed onto an agar plate containing the target bacterium and the growth of bacterial colonies around the disk was used to judge the resistance to the selected antimicrobial agent (Vincent *et al.,* 1944). Bauer *et al.* (1966) introduced an improved, highly reproducible and standardised paper disk method used in most clinical laboratories. The Kirby-Bauer assay tests multiple concentrations of the antimicrobial against a standardised concentration of the bacterial pathogen (0.5 McFarland standard), with standardised incubation periods and growth media (Mueller-

Hinton) (Bauer *et al.,* 1966). Along with these technical standardisations, a table measuring inhibition zones to the nearest millimetre was introduced. This allowed microbiologists to accurately classify bacteria as resistant, intermediately resistant or susceptible to a particular antimicrobial agent (Bauer *et al.,* 1966). This table is frequently updated according to the bacterial taxonomy and antimicrobial compound allowing for the accurate and reproducible surveillance of AMR on the global level (Matuschek *et al.,* 2014). The principles of standardisation established by the Kirby-Bauer assay laid the groundwork for other antimicrobial susceptibility testing (AST) techniques. Researchers such as Gavan and Town (1970) further refined these methods by developing the broth microdilution assay, which measures the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of an antimicrobial compound in liquid culture. Today, this approach is widely used in laboratories for AMR surveillance (Rodríguez-Melcón *et al.,* 2021).

While essential for reproducibility in AMR research, traditional culture-based techniques have notable limitations (Su *et al.,* 2019). For instance, Kirby-Bauer assays and similar AST methods are both time-consuming and inherently isolate-focused. While classifying the AMR status of isolated microorganisms is critical, these methods fail to elucidate the underlying mechanism of resistance, necessitating the use of more modern molecular techniques (Su *et al.,* 2019).

2.6 Molecular techniques in AMR research

The polymerase chain reaction (PCR) revolutionised AMR research, enabling the rapid (within hours) and sensitive detection of antibiotic resistance genes (ARGs) (Anjum *et al.,* 2017). This has considerably advanced the molecular characterisation of ARGs and AMR networks - the complex ways in which ARGs spread and evolve within and between bacterial populations (Galhano *et al.,* 2021). Today, PCR-based approaches are standard for AMR surveillance. They are in active use by the European Center for Disease Control (ECDC) as well as the European Food Safety Authority (EFSA) (Anjum *et al.,* 2017). However, PCR approaches are limited by our dependence on existing knowledge – screening cannot be carried out for unknown resistance mechanisms. For example, an assay designed solely for penicillinases would fail to detect a strain harbouring an extended-spectrum beta-lactamase (ESBL) gene (Cason *et al.,* 2022). Although advancements such as multiplex PCR, quantitative PCR (qPCR), and real-time RT-PCR have provided increased

sensitivity, precision and speed, they do not overcome this inherent limitation of PCR-based surveillance (Anjum *et al.,* 2017).

Beyond PCR, other molecular techniques have been implemented for the detection of AMR. DNA microarrays are routinely used to detect ARGs with the same level of accuracy as PCR (Frye *et al.,* 2010). This approach uses complementary DNA probes designed to hybridise with known resistance genes (Frye *et al.,* 2010). Microarrays offer an advantage over standard PCR by allowing simultaneous screening for multiple resistance genes, producing a large volume of results (Frye *et al.,* 2010). Despite this advantage, this technique ultimately suffers from the same constraints as PCR – a reliance on known resistance genes (Batchelor *et al.,* 2008; Frye *et al.,* 2010). This inherent constraint in both approaches underscores the need for novel AMR surveillance methods capable of detecting both known and unknown AMR mechanisms.

2.7 The power of genomics in understanding AMR diversity

While traditional molecular techniques remain essential tools for AMR research, the advent of Whole Genome Sequencing (WGS) has allowed for the comprehensive characterisation of AMR mechanisms in microorganisms (Waddington *et al.,* 2022). That is, beyond the simple detection of ARGs in a PCR or microarray approach, WGS provides insight into the genomic context of the ARGs, their potential to spread via mobile genetic elements such as plasmids, and the evolutionary relationships between antibiotic-resistant strains (Khezri *et al.,* 2020). Paired with the development of more affordable and effective sequencing technologies and a growing arsenal of bioinformatic tools, the scope of these analyses continues to expand (Waddington *et al.,* 2022). The ability to sequence entire genomes has led to the discovery of novel, uncharacterised ARGs – even in previously well-studied microorganisms (Deekshit & Srikumar, 2022). For instance, Kim and co-workers used WGS to identify novel gentamicin resistance mechanisms in *Salmonella* species (Kim *et al.,* 2020).

The availability of high-resolution genomic data and the ability to identify novel ARGs has revolutionised outbreak investigations, allowing researchers to survey and source the transmission of MDR strains (Roberts *et al.,* 2021). Growing databases of genomic information have further given rise to novel drug targets (Köser *et al.,* 2014; Roberts *et al.,* 2021). Comparing the genomes of resistant and non-resistant strains enables the detection of specific mutations or gene acquisitions that confer resistance (Roberts *et al.,* 2021). For instance, comparative genomic

analyses have revealed that resistance to fluoroquinolones in *Escherichia coli* is linked to mutations in the *gyrA* and *parC* genes, which alter the target site of the antibiotic, reducing its efficacy (Gruger *et al.,* 2004). Moreover, WGS has been used to identify the *vanA* and *vanB* gene clusters responsible for vancomycin resistance in Enterococcus faecium and *Enterococcus faecalis*. These genes, often carried on plasmids, allow the bacteria to alter their peptidoglycan precursors, reducing vancomycin binding affinity (Arias & Murray, 2012). Comparative genomics has revealed that methicillin resistance in *Staphylococcus aureus* is enabled by the *mecA* gene, which encodes an altered penicillin-binding protein (PBP2a) with reduced affinity for beta-lactam antibiotics (Ballhausen *et al.,* 2014). These insights not only improve our understanding of resistance mechanisms but also inform the development of novel antimicrobial agents by targeting these specific genetic changes (Roberts *et al.,* 2021).

Initially, sequencing technologies were prohibitively expensive, limiting their application to specialised research settings (Mardis, 2008). However, advancements in next-generation sequencing (NGS) and high-throughput methodologies have substantially reduced costs, making sequencing more accessible (Xiao & Zhou, 2020). Since its introduction, WGS has become more affordable - broadening the scope of WGS studies beyond clinical settings (Oniciuc *et al.,* 2018). This affordability has enabled studies that explore the transmission and persistence of AMR across various environments, illustrating how clinical, agricultural, and environmental reservoirs contribute to the AMR crisis (Pillay *et al.,* 2022). For instance, recent studies have used WGS to track the spread of antibiotic-resistant bacteria from livestock to humans through food products and the environment (Lu *et al.,* 2022; Pillay *et al.,* 2022). These studies highlight the interconnectedness of different reservoirs in the spread of AMR and underscore the importance of a holistic approach to surveillance.

While a powerful tool for AMR surveillance, microorganism-by-microorganism WGS approaches have limitations when it comes to fully understanding the complexity of microbial communities. Focusing solely on individual microorganisms may miss the broader context of how resistance genes are expressed and interact within entire microbial communities (Oniciuc *et al.,* 2018; Pillay *et al.,* 2022). Therefore, a comprehensive understanding of AMR requires a move beyond individual microorganisms to consider the entire resistome within complex microbial ecosystems.

2.8 Metagenomics: A paradigm shift in understanding AMR ecology

Traditional culture-based techniques in AMR research have notable limitations. Primarily, their reliance on culturing restricts insights to a small fraction of culturable microorganisms, excluding the vast majority of "microbial dark matter"—those microorganisms that cannot be cultured and yet dominate many environments (Solden *et al.,* 2016). These unculturable microorganisms are now recognised as potential AMR reservoirs, as they may harbour antimicrobial resistance genes that can be transferred to pathogenic bacteria (Solden *et al.,* 2016; Pillay *et al.,* 2022). The networks of uncultivable bacteria can drive the AMR crisis through HGT or other uncharacterised ecological interactions (Solden *et al.,* 2016; Ku *et al.,* 2021; Pillay *et al.,* 2022).

Whole metagenome sequencing (WMS) is a powerful tool in genomics and microbial ecology that allows for the analysis of entire microbial communities without the need to culture bacteria—thereby addressing these limitations and providing insights into both culturable and non-culturable microorganisms (Oniciuc *et al.,* 2018; Pillay *et al.,* 2022). By directly sampling environmental DNA, WMS enables the analysis of the taxonomic composition and functional potential of entire microbial communities and the identification of all ARGs (the resistome) within any community (Ko *et al.,* 2022).

Applied to the AMR crisis – metagenomics offers a promising tool for a comprehensive analysis and surveillance of AMR that considers how complex microbial communities collectively influence global AMR trends. By expanding surveillance from the individual microorganism to the community level, the flow of ARGs can be traced through diverse environments such as hospitals, agricultural soils, water sources, and oceans (Bueno *et al.,* 2021; Pillay *et al.,* 2022). This knowledge is crucial for identifying not only established ARGs and AMR hotspots but also the uncharacterised reservoirs where unexpected resistance threats may emerge (Djordjevic *et al.,* 2024).

While metagenomic-based approaches are to date the most promising tools for thorough AMR surveillance, fully realising their potential presents many challenges. For instance, sequencing platforms are required to be sufficiently robust to capture all the microorganisms in a sample (Bengtsson-Palme *et al.,* 2017). Furthermore, downstream approaches including genome assembly, taxonomic classification, and functional annotation are not standardised and tend to produce highly variable results depending on the bioinformatics tool used to achieve each outcome

(Quince *et al.,* 2017). The absence of a standardised bioinformatic pipeline for AMR-focused metagenomics presents a major hurdle for in-depth and precise AMR surveillance (Bengtsson-Palme *et al.,* 2017).

Despite these challenges, metagenomics represents a transformative shift in AMR surveillance. Its ability to uncover historically overlooked reservoirs of AMR and trace the global flow of ARGs provides an unparalleled tool for comprehensive AMR surveillance (Bengtsson-Palme *et al.,* 2017; Pillay *et al.,* 2022).

2.8.1 Applications of metagenomics beyond AMR research

While metagenomics has proven invaluable in AMR research, its applications extend across numerous fields. In environmental science, metagenomic approaches have transformed our understanding of ecosystem function and biodiversity. The Global Ocean Sampling Expedition represents a landmark example of metagenomic application in marine research, revealing unprecedented microbial diversity in ocean environments and discovering many previously unknown genes that have enhanced our understanding of marine ecosystem functioning (Yooseph *et al.,* 2007). Similarly, in terrestrial environments, metagenomic studies have illuminated the complex relationships between soil microbial communities and critical ecosystem processes such as carbon cycling and agricultural productivity (Delgado-Baquerizo *et al.,* 2022).

The field of human microbiome research has particularly benefited from metagenomic approaches. The Human Microbiome Project utilised metagenomics to characterise microbial communities across different body sites, fundamentally changing our understanding of human-microbe interactions in health and disease (Turnbaugh *et al.,* 2007). These investigations have provided important insights into various conditions, from inflammatory bowel disease to mental health disorders, demonstrating the far-reaching implications of microbial community composition for human health (Lynch & Pedersen, 2016).

In biotechnology, metagenomics has emerged as a powerful tool for bioprospecting - the discovery of novel enzymes and bioactive compounds (Kodzius & Gojobori, 2015). Metagenomic screening of deep-sea sediments has led to the identification of cold-adapted enzymes with potential industrial applications, particularly in sectors requiring low-temperature bioprocessing (Ferrer *et al.,* 2019). The exploration of soil metagenomes has revealed novel biosynthetic gene clusters

involved in antibiotic production, offering new possibilities for drug development (Hover *et al.,* 2018)

2.8.2 Key concepts in metagenomic analyses

Understanding several fundamental concepts is essential for interpreting metagenomic data and its implications for AMR research. Within environmental samples, the microbiome represents the complete genetic arsenal of all microorganisms inhabiting a specific environment, providing comprehensive insights into community composition and functional potential (D'Costa *et al.,* 2006). Within this broader genomic context, the resistome comprises a specific subset - the collection of genes that confer antimicrobial resistance, whether these genes are currently active or latent (Pillay *et al.,* 2022).

In analysing both microbiomes (typically referring to the taxonomic composition) and resistomes (referring to genes conferring resistance), one can differentiate between core and accessory elements. The core components comprise members (species or genes) that are consistently present across samples from a given environment type, representing the stable, essential elements of that ecosystem (Hellewell *et al.,* 2024). These core elements often perform fundamental functions necessary for ecosystem stability (Segerman, 2012). In contrast, the accessory elements include more variable components that may reflect local adaptations or transient environmental pressures (Segerman, 2012; Hellewell *et al.,* 2024). This distinction helps researchers understand both the fundamental and dynamic aspects of microbial communities, particularly in the context of antimicrobial resistance.

2.9 ESKAPE pathogens in environmental and clinical settings

The term "ESKAPE" encompasses six bacterial pathogens that have emerged as major threats to public health due to their antimicrobial resistance profiles and their alarming ability to acquire antimicrobial resistance (Denissen *et al.,* 2022; Venkateswaran *et al.,* 2023). This group includes *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species. These organisms warrant special attention because they demonstrate remarkable adaptability in developing resistance mechanisms (Denissen *et al.,* 2022; Aguilar-Salazar *et al.,* 2023).

Recent research has revealed concerning patterns in how these pathogens interact with antimicrobial compounds. *Enterococcus faecium* exhibits increasing resistance to glycopeptide

antibiotics, particularly in healthcare settings where these drugs serve as last-line treatments (Venkateswaran *et al.,* 2023). *Staphylococcus aureus* populations have evolved complex resistance mechanisms, with methicillin-resistant strains now widespread in both hospital and community environments (Antonov *et al.,* 2015). *Klebsiella pneumoniae* demonstrates remarkable genomic plasticity, readily acquiring new resistance genes through horizontal transfer (Hirvonen *et al.,* 2021). *Acinetobacter baumannii* combines intrinsic and acquired resistance mechanisms, particularly concerning in healthcare facilities where it persists on surfaces (Mirzaei *et al.,* 2020). *Pseudomonas aeruginosa* utilises complex efflux systems and enzymatic mechanisms to resist multiple antibiotic classes (Alcalde-Rico *et al.,* 2016). *Enterobacter* species show rapid and concerning resistance development during treatment, complicating therapeutic approaches (Abedinzadeh *et al.,* 2023).

Environmental surveillance has detected these pathogens beyond clinical settings, particularly in urban wastewater, surface waters, and anthropogenically impacted soils (Aguilar-Salazar *et al.,* 2023). Their presence in environmental reservoirs suggests complex transmission networks between clinical and environmental settings, highlighting the need for comprehensive surveillance approaches that consider both domains (Denissen *et al.,* 2022). Understanding these transmission patterns and their implications for public health requires integrative approaches that combine traditional surveillance with modern metagenomic techniques.

2.10 Landmark metagenomic AMR studies and current approaches

The transformative potential of metagenomics in AMR research has been demonstrated by several large-scale studies (Hemamalini *et al.,* 2022; Pillay *et al.,* 2022). These multi-institutional efforts have laid a strong foundation for understanding the complexities of the global resistome. For instance, the TARA Oceans Project, although not a dedicated AMR effort, meticulously collected thousands of samples across the world's oceans, enabling researchers to map the ocean resistome (Sunagawa *et al.,* 2020). These analyses revealed not only an extensive diversity of resistance genes but also the presence of AMR hotspots, particularly in regions with high human activity, such as coastal areas near major cities and shipping lanes, where the relative abundance of ARGs was notably higher compared to open ocean regions (Cuadrat *et al.,* 2020; Sunagawa *et al.,* 2020). Another landmark effort, the consortium-led MetaSUB Project, sampled metagenomes from mass transit systems around the world (Mason *et al.,* 2016). The analysis of these metagenomes

uncovered surprising similarities in the resistomes of distant cities, particularly in terms of the core ARGs associated with common pathogens found in urban environments. This core urban resistome was consistent across geographically distant locations, indicating that urban environments share a common set of resistance genes. Moreover, the study highlighted the potential role of human travel and global trade in the spread of AMR, as resistomes in cities with high levels of international connectivity showed greater similarities (Danko *et al.,* 2020).

Wastewater metagenomic studies are increasingly being employed to elucidate complex interactions between clinical, agricultural and environmental resistomes (Pillay *et al.,* 2022; Talat *et al.,* 2023). Wastewater acts as a key convergence of human, animal, and environmental influences, making metagenomic surveys of wastewater a crucial tool for identifying AMR trends and emerging threats in a broader, multi-input context. That is, wastewater collects ARGs from various sources, including hospitals, farms, and residential areas, thus providing a comprehensive snapshot of the resistomes circulating within a community and the potential for ARGs to spread across different environments (Pillay *et al.,* 2022; Raza *et al.,* 2021). Metagenomic endeavours, such as the Global Sewage Surveillance Project, have set out to use wastewater metagenomics to create a global map of AMR, pinpointing areas of concern and potential intervention points (Munk *et al.,* 2022). This effort demonstrated clear geographic variations in the abundance and types of ARGs, highlighting the influence of local factors such as antibiotic usage patterns, healthcare infrastructure, and environmental management practices (Munk *et al.,* 2022). However, the project also identified a core set of ARGs present across all locations, emphasising the global nature of the AMR crisis. Potential intervention points identified by this project include targeted improvements in wastewater treatment processes, stricter regulation of antibiotic usage, and enhanced surveillance in regions identified as AMR hotspots (Munk *et al.,* 2022).

While these landmark studies have played a crucial role in our understanding of AMR dynamics and spread, there are still several limitations. The focus on specific, singular environments (oceans, urban systems, wastewater) serves as strong proxies for specific aspects of AMR spread (Pillay *et al.,* 2022). However, the AMR crisis is a global challenge that demands a broader and more integrated focus. Few studies directly compare resistomes across vastly diverse environments (Djordjevic *et al.,* 2024). This limits our understanding of how microorganisms within these ecological niches interact and how ARGs might move between these environments. This gap

underscores the need for a comprehensive approach that moves beyond isolated environmental studies.

2.11 A comprehensive metagenomic survey of soil and water environments

Soil and aquatic settings act as reservoirs and conduits for resistance genes, representing two critical domains for understanding the dissemination and spread of AMR on a global scale (Häder *et al.,* 2020; Pillay *et al.,* 2022; Zheng *et al.,* 2022). Soil environments harbour a vast and diverse microbial population, including many potential carriers of resistance genes (Zheng *et al.,* 2022). Furthermore, these microbial populations and their resistome are substantially influenced by human activities such as agriculture and wastewater irrigation (Ordine *et al.,* 2023). By analysing metagenomes derived from a diverse range of soil types, from pristine to heavily urbanised landscapes, the distribution of ARGs in soil environments can be mapped and the impact of human influence on the global resistome can be elucidated. Likewise, aquatic environments play a critical role in the dissemination of ARGs (Häder *et al.,* 2020). They act as conduits, facilitating the movement of resistant bacteria and resistance genes across vast distances through rivers, oceans, and groundwater (Häder *et al.,* 2020; Qin *et al.,* 2020). By analysing metagenomes derived from a comprehensive set of aquatic environments, from freshwater to marine and coastal waters, the flow of ARGs through the entire water cycle and the role of anthropogenic influences in the global spread of antimicrobial resistance can be determined. This global metagenomic approach moves beyond isolated snapshots to provide a comprehensive understanding of ARG distribution and movement.

Chapter 3: Materials and methods

3. Materials and methods

3.1 Data Acquisition and Preprocessing

A total of 1,863 publicly available metagenomes, both assembled and non-assembled, were obtained from the MG-RAST v4.0.3 server (Meyer *et al.,* 2008). MG-RAST is a comprehensive metagenome repository comprising 512,961 metagenomes and 2.287 billion sequences. MetaQUAST v5.2.0 (Mikheenko *et al.,* 2016) was used to assess the quality of these metagenomes focusing on metrics such as the number of contigs and the total base pair count, which reflects the overall size of each metagenome. These parameters were used for quality control of the metagenomes as outlined in section 2.5.

3.2 Taxonomic Classification

Taxonomic classification of the metagenomic contigs was performed using Kraken2 (Wood *et al.,* 2019). The standard Kraken2 database was utilised, which includes genomes from RefSeq encompassing non-redundant archaeal, bacterial, viral and the human (version GRCh38) genome sequences as well as plasmids and UniVec_Core sequences (Pruitt *et al.,* 2007; Wood *et al.,* 2019). This comprehensive database ensures accurate classification across a wide range of organisms, including potential contaminants such as human DNA.

Kraken 2 employs a k-mer-based approach combined with the lowest common ancestor (LCA) strategy to assign taxonomic labels to sequences. It constructs a database of k-mers from the reference genomes, and for each metagenomic read or contig, it searches for exact k-mer matches within this database. Using minimisers, the smallest k-mer in a sequence window, it substantially reduces computational memory requirements and enhances processing speed compared to traditional k-mer methods (Wood *et al.,* 2019). The taxonomic assignment is based on the LCA of all matched k-mers for a given sequence. Default parameters were applied for the Kraken 2 searches, with a k-mer length of 35 (k=35) and a minimiser length of 31 (ℓ=31) (Wood *et al.,* 2019).

3.3 Antibiotic Resistance Gene (ARG) Identification

Identification of ARGs within the metagenomes was conducted using DeepARG-SS v1.0.2 (Arango-Argoty *et al.,* 2018), which is optimised for short-read data. DeepARG-SS utilises a custom reference database, DeepARG-DB, that integrates three key databases: the Comprehensive

Antibiotic Resistance Database (CARD) (Jia *et al.,* 2017), the Antibiotic Resistance Genes Database (ARDB) (Liu & Pop, 2009), and UniProt (The Universal Protein Resource, 2008). CARD provides detailed information on antimicrobial resistance mechanisms and genes, facilitating accurate identification of known ARGs. ARDB offers a comprehensive catalogue of resistance genes, including both experimentally verified and computationally predicted entries. UniProt, a large protein database, aids in identifying intrinsic and acquired resistance mechanisms and potentially discovering novel resistance proteins. The integration of these databases into DeepARG-DB ensures extensive coverage of known resistance mechanisms.

DeepARG-SS employs a two-step approach for ARG identification. Initially, it performs a best-hit search against DeepARG-DB to identify potential ARGs within the metagenomic sequences. Following this, it employs a deep learning framework using neural networks trained on extensive ARG datasets to validate and refine the initial results (Arango-Argoty *et al.,* 2018). This step is particularly effective in detecting ARGs with lower sequence similarity to reference entries. A stringent probability threshold of ≥0.8 was applied to ensure high confidence in ARG classification and minimise false positives.

3.4 Data Normalisation

To account for variations in sequencing depth, library size, and assembly quality across the metagenomes, normalisation was performed. The total metagenome size and the number of contigs were obtained from the MetaQUAST output, while the number of bacterial and archaeal reads was derived from the Kraken2 output. A normalisation metric was calculated for each metagenome using the equation:

$$Normalisation\ metric = \frac{total\ metagenome\ size}{number\ of\ contigs\ in\ metagenome} \times (\#\ bacterial\ reads + \#\ archaeal\ reads)$$

This metric accounts for both the assembly quality and the abundance of prokaryotic reads. The count data from the DeepARG-SS reports (ARG counts) and the Kraken 2 reports (taxonomic counts) were then normalised using the equation:

$$Normalised\ count = \frac{ARG\ or\ taxonomic\ count}{Normalisation\ metric\ (metagenome\ specific)}$$

All subsequent analyses were conducted using these normalised counts.

3.5 Quality Control

Metagenomes were filtered based on their normalisation metric to ensure sufficient data quality and coverage. Only metagenomes with a normalised size ≥3,870,000 base pairs, the approximate size of an average bacterial genome (diCenzo & Finan, 2017), were retained for analysis. After normalisation and filtering, 1,758 metagenomes passed the quality control criteria, while 105 metagenomes with normalisation metrics below the threshold, were excluded.

The retained metagenomes originated from aquatic (n=829) and soil (n=929) environments on a global scale. Aquatic metagenomes were categorised into six biomes: coastal seawater (n=289), marine seawater (n=176), surface saline water (n=7), surface freshwater (n=87), groundwater (n=57), and urban wastewater (n=213). Soil metagenomes were classified into six biomes: anthropogenic (n=316), arid (n=24), cold (n=65), subtropical (n=180), temperate (n=206), and tropical (n=138) soils (Supplementary Table 1; Figure 1).

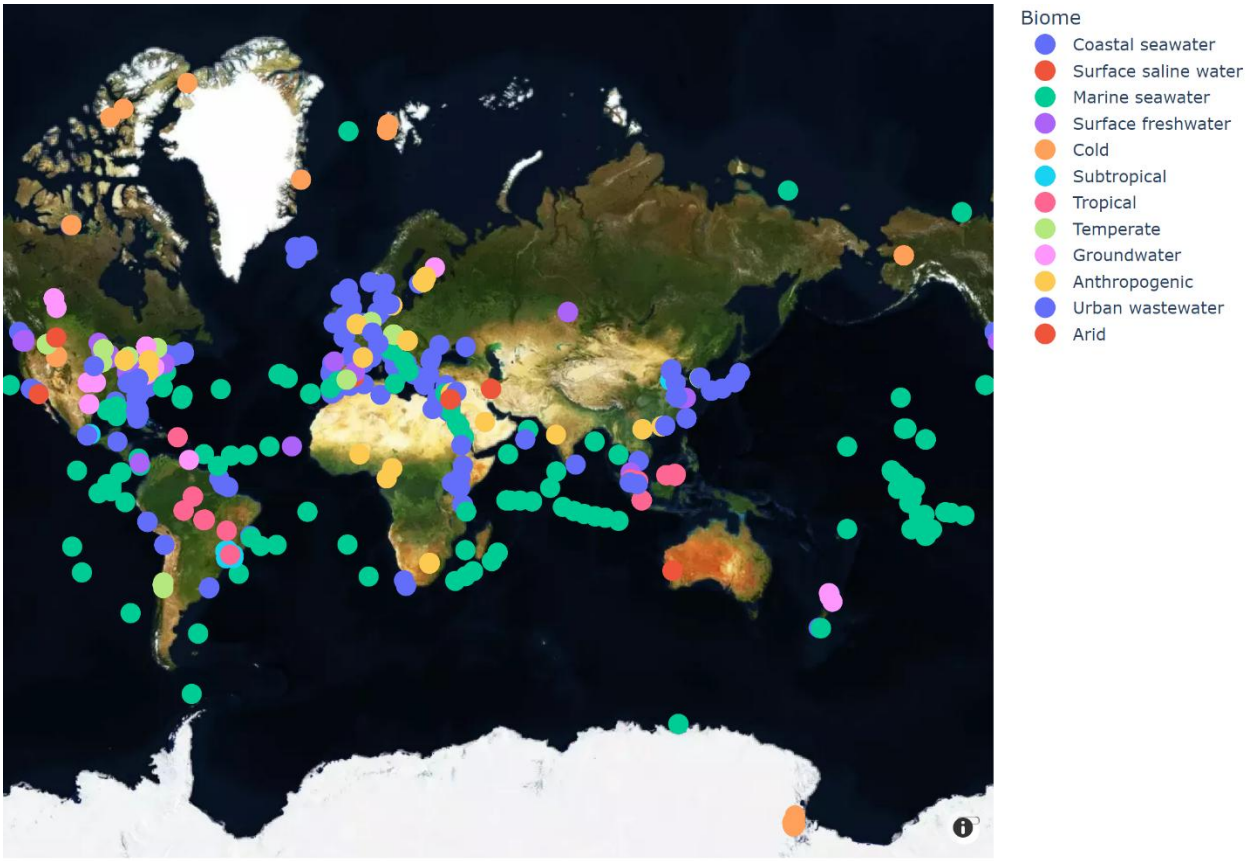


Figure 1. **Global map of metagenomes**. The global distribution of 1,758 metagenomes analysed in this study. Each dot on the map represents a sampling location, color-coded by biome as indicated in the legend. The dataset includes 829 metagenomes from aquatic settings and 929 metagenomes from soil environments. Map created with Mapbox.

3.6 Statistical Analysis

Statistical analyses were performed using both Python v3.12.2 and R v4.3.2. Data manipulation and computations were carried out using libraries that included NumPy (Harris *et al.,* 2020), SciPy (Virtanen *et al.,* 2020), pandas (Mckinney, 2011) and scikit-learn (Pedregosa *et al.,* 2011). Visualisations were generated using Matplotlib (Hunter, 2007) and Seaborn (Waskom, 2021). Custom scripts were developed for diversity analyses, correlation assessments, and regression modelling.

3.6.1 Alpha Diversity Analysis

Alpha diversity, representing the diversity within individual samples, was assessed for both microbial species and ARG profiles across different biomes (Kers & Saccenti, 2022). The Shannon

diversity index (H') (Shannon, 1948) was employed due to its sensitivity to both richness (the number of different species or ARGs) and evenness (the relative abundance of each species or ARG). This index provides insights into the complexity and diversity of microbial communities and their resistomes within each sample.

The Shannon diversity index was calculated using the formula:

$$H' = -\sum p_i * \ln(p_i)$$

where p_i is the proportional abundance of species/ARG.

To evaluate the reliability of the diversity estimates and account for potential variations due to sampling, a bootstrapping approach with 1,000 iterations per biome was implemented using the scikit-bio library (Pedregosa *et al.,* 2011). This resampling method provides confidence intervals for the diversity measures, enhancing the robustness of the analyses.

3.6.2 Beta Diversity Analysis and Principal Coordinate Analysis (PCoA)

Beta diversity analyses were conducted to investigate compositional differences between microbial communities across samples from different biomes. Beta diversity measures the extent of change in species or ARG composition between samples, reflecting ecological distances (Kers & Saccenti, 2022).

The Bray-Curtis dissimilarity metric (Bray & Curtis, 1957) was used, as it considers both the presence/absence and abundance of species or ARGs, providing a measure of community dissimilarity. Before computing the dissimilarity matrices, a logarithmic transformation with a pseudocount of 1 was applied to the normalised count data:

$$Transformed\ count = \log(Normalised\ count + 1)$$

This log transformation reduces the impact of highly abundant features, stabilises variance, and allows rarer species or ARGs to contribute more equally to the analysis. The addition of a pseudocount prevents undefined logarithms for zero counts, ensuring all data points are included.

Principal Coordinate Analysis (PCoA) (Gower, 1966) was performed on the Bray-Curtis dissimilarity matrices to visualise the high-dimensional data in a reduced-dimensional space. This ordination method helps in detecting patterns of similarity or dissimilarity among samples (Gower,

1966). Scatter plots of the PCoA scores were generated, with samples coloured according to their biome classification, to explore clustering patterns and potential ecological gradients.

3.6.3 Partial Least Squares Correlation (PLSC) Analysis

Partial Least Squares Correlation (PLSC) analysis (Sarstedt *et al.,* 2017) was employed to explore the relationship between microbial community composition and the prevalence of ARGs. PLSC is a multivariate technique that identifies latent variables (components or factors) explaining the maximum covariance between two datasets—in this case, species abundances and ARG abundances.

Pre-processed datasets containing species and ARG abundances were used as input for the PLSC model, implemented using the PLSRegression module from scikit-learn (Pedregosa *et al.,* 2011). The number of components was set to two (n_components=2) to capture the primary sources of covariance.

Sample scores from the PLSC model were visualised to examine potential associations within the reduced-dimensional space. Pearson's correlation coefficient was calculated to assess the strength and significance of the linear relationship between species and ARG scores. Bootstrapping procedures with 1,000 resamples were implemented to determine a 95% confidence interval for the correlation coefficient. Ordinary least squares (OLS) regression analysis (Seber & Lee, 2003) was performed to model the relationship and Fisher's Z-transformation (Fisher, 1915) was applied for corrected confidence interval estimation.

3.6.4 Core and Accessory Resistome Analysis

To distinguish between pervasive (core) and less common (accessory) ARGs, an analysis was conducted based on their occurrence across samples. A binary presence/absence matrix was derived from the normalised ARG abundance data.

Binomial tests were performed using SciPy (Virtanen *et al.,* 2020) to assess whether the observed presence of each ARG across samples was significantly higher than expected under a null hypothesis of equal probability of presence. The Benjamini-Hochberg method (Benjamini & Hochberg, 1995) was applied to control the false discovery rate during multiple testing, using the statsmodels library (Seabold & Perktold, 2010). ARGs with adjusted p-values below a threshold of 0.05 were designated as part of the global core resistome.

The same methodology was applied to determine the core resistome within each individual biome (local core resistome). For each biome, the accessory resistome was defined by filtering out both the global and local core ARGs. The occurrences of the remaining accessory ARGs were analysed, focusing on their predicted ARG classes. To account for potential variations in the total abundance of accessory ARGs across biomes, the proportions of each accessory ARG class within each biome were calculated, providing insights into biome-specific resistome profiles.

3.6.5 Phylum-level analysis

A phylum-level analysis was conducted to assess the relative abundance of microbial phyla across both soil and aquatic biomes. Phylum-level reads were extracted from the Kraken2 (Wood *et al.,* 2019) output for both datasets, followed by data normalisation. The soil and aquatic metagenome datasets were combined to enable cross-biome comparisons. For each metagenome, the normalised phylum-level abundance data was averaged across the respective biomes to generate biome-specific phylum profiles. These averages were calculated using custom Python scripts, and the results were saved to a combined dataset. A heatmap was generated to visualise the distribution of phyla across biomes using R's pheatmap package (Kolde, 2019).

In addition, a network graph was constructed to explore relationships between phyla and biomes. An undirected graph was generated, where biomes were represented as nodes and edges were weighted based on the average phylum-level abundance values. A Fruchterman-Reingold layout was employed to highlight clustering patterns (Fruchterman & Reingold, 1991). This approach facilitated visual exploration of phylum distribution and potential biome-specific associations.

3.6.6 ESKAPE pathogen identification and analysis

ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species) were identified using the Kraken2 (Wood *et al.,* 2019) classification results. ESKAPE pathogens are major contributors to the spread of AMR and pose a substantial health threat due to their ability to evade antibiotic treatments (Denissen *et al.,* 2022; Aguilar-Salazar *et al.,* 2023). Species-level reads were extracted using a custom Python script. The relative proportion of ESKAPE species in each biome was then calculated by normalising the species-level ESKAPE reads against the total number of species-level reads, using the normalisation metric.

The calculated proportions provided an estimate of ESKAPE prevalence compared to the total prokaryotic microbial community. These data were used for further analysis of ESKAPE distribution across different biomes and environments.

Chapter 4: Results and discussion

4.1 Results

4.1.1 Microbial species diversity in aquatic and soil environments

Microbial species of the aquatic and soil metagenomic datasets were analysed using Kraken2 for taxonomic classification of metagenomic reads. The resultant taxonomic reports were used to calculate Shannon diversity indices to assess alpha diversity.

In aquatic settings, urban wastewater exhibited the highest microbial diversity with a Shannon index of 5.43 and an average of 9,844.68 species per sample (Figure 2A). The interquartile range (IQR) for urban wastewater was relatively narrow, indicating that there is more consistent evenness in the microbial diversity across the urban wastewater samples. This rigid clustering around the median reflects a stable community structure across these samples. Groundwater showed a median Shannon index of 4.77 with a broader IQR, suggesting more variability in microbial diversity among different groundwater samples as well as an average of 9,074.84 species per sample.

Coastal seawater and surface freshwater recorded the second and third-highest Shannon indices of 5.10 and 4.90, respectively, reflecting a moderate but diverse range of species. Coastal seawater exhibited a relatively broad IQR, indicating that microbial diversity varies across samples. Marine seawater and surface saline water showed Shannon indices of 4.23 and 4.28. Marine water samples comprised on average 7,020.72 distinct microbial species per sample, 28.7% lower than urban wastewater.

In soil environments, cold biomes recorded the highest median Shannon diversity (5.63), indicating a rich and diverse microbial community and contained an average of 9,688.51 microbial species per sample (Figure 2B). Despite having the highest median diversity, cold soils showed a relatively compact IQR, suggesting that microbial diversity is consistent across samples in this biome. Temperate soils exhibited a lower median Shannon index of 4.99 but had the broadest IQR among all soil biomes. This indicates substantial variability in microbial diversity across samples in temperate regions.

Tropical soils, with a median Shannon index of 5.55, displayed a moderately wide IQR, reflecting some variability in microbial diversity, but still more uniform than temperate soils. Subtropical soils with a similar median Shannon diversity of 5.54 exhibited a relatively narrow IQR, indicating

that microbial diversity in subtropical environments tends to be more uniform across different samples.

Arid soils, with a Shannon index of 5.53, showed the narrowest IQR, indicating highly uniform microbial diversity across samples. Notably, anthropogenic soils had the lowest median Shannon diversity (4.92), with an average of 8,792.77 microbial species per sample, which is 9.25% lower than that observed for cold soils. The large IQR in these soils suggests variability in microbial diversity across samples.

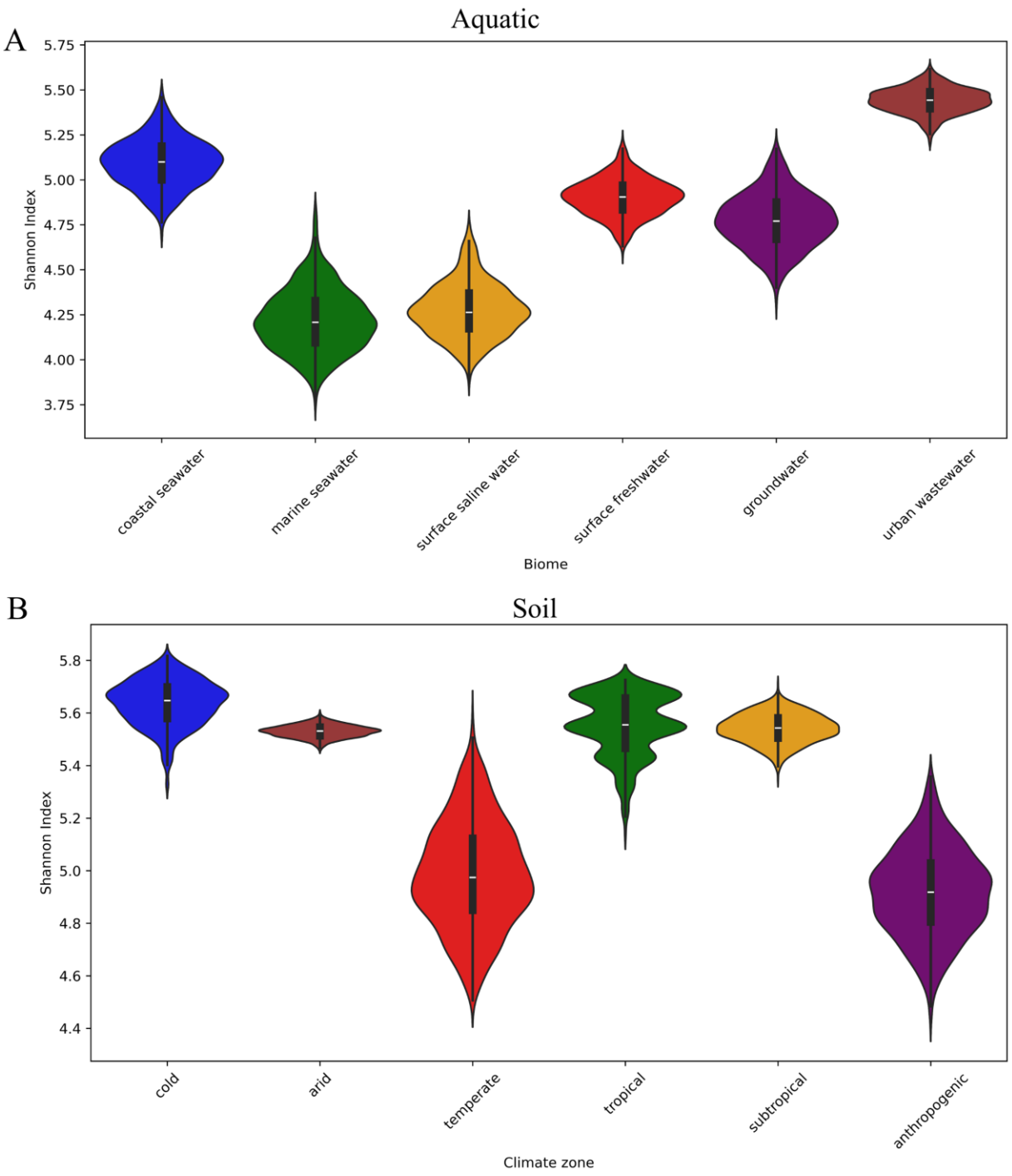


Figure 2. **Shannon diversity of microbial species in aquatic and soil biomes.** Violin plots depicting Shannon diversity indices for microbial species in aquatic (A) and soil (B) environments. Black bars represent the interquartile range, and white dots represent the medians.

Overall, the aquatic and soil biomes showed near-identical Shannon indices (5.409 for soil and 5.410 for aquatic metagenomes), suggesting similar microbial diversities for both sets of biomes

(Figure 3). However, it is important to note that there were structural differences in the microbial communities. Aquatic biomes displayed a broader distribution of Shannon diversity values, indicating more variability in microbial community composition across different samples, whereas soil biomes were more consistent, with tighter clustering around the median. This suggests that while aquatic environments may support highly diverse microbial communities, they are subject to greater fluctuations.

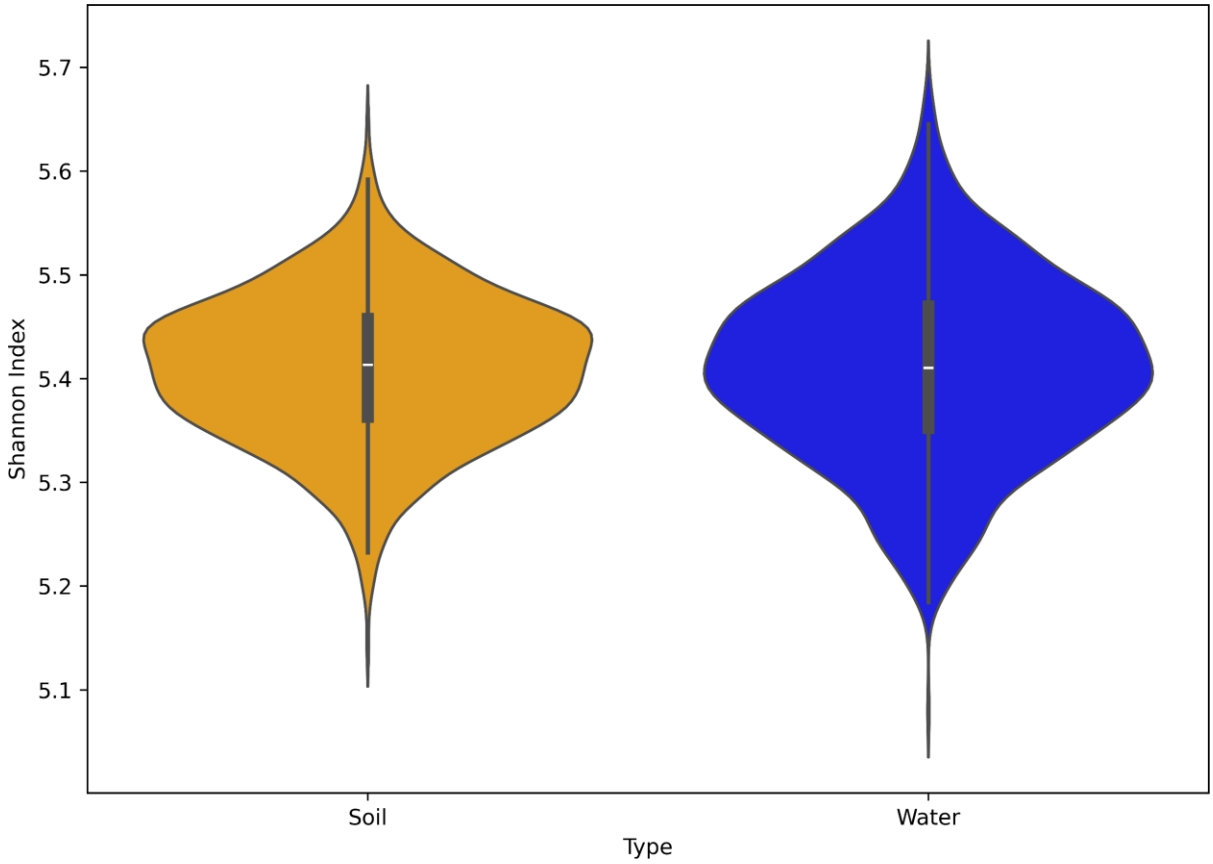


Figure 3. **Comparative analysis of Shannon diversity of microbial communities in soil and aquatic environments.** The Shannon diversity index in soil biomes (orange) was 5.409 and that of aquatic biomes (blue) was 5.410 demonstrating a nearly identical average Shannon diversity of microbial species. Black bars represent the interquartile range, and white dots show the median index values.

4.1.2 PCoA Analysis of Microbial Communities

To further investigate microbial species composition and community structure, a Principal Coordinate Analysis (PCoA) was performed using Bray-Curtis dissimilarity, a metric that captures

beta diversity by quantifying compositional differences between samples (Bray & Curtis, 1957). This analysis revealed distinct clustering patterns between the microbial communities of aquatic and soil environments. The data for this analysis were sourced from the Kraken2 reports for microbial species, with Bray-Curtis dissimilarity used to highlight the variability between different microbial populations.

In aquatic environments, coastal and marine seawater formed overlapping clusters distinct from other aquatic biomes (Figure 4A). Urban wastewater, groundwater and surface freshwater are clustered closely together. Surface saline water samples showed broad dispersion, though this biome had a relatively small sample size (n=7).

In soil environments, the PCoA demonstrated distinct clustering patterns based on biome (Figure 4B). Tropical soils formed a tight and distinct cluster indicating that microbial communities within this biome are relatively consistent in composition. This contrasts with the broader interquartile range (IQR) seen in the Shannon diversity of tropical soils (Figure 2B), suggesting that while overall diversity (richness and evenness) may vary between samples, the core microbial taxa remain stable.

Cold soils, on the other hand, showed a broader and more dispersed clustering in the PCoA plot. This suggests variability in microbial community composition across samples, despite the relatively limited distribution seen in the alpha diversity (Shannon index) between the samples. The consistency in alpha diversity (Figure 2B) points to stable microbial richness and evenness within each sample, while the spread in the PCoA indicates that the specific species present differ more substantially between cold soil samples.

Anthropogenic soils displayed a distinct but broadly dispersed cluster in the PCoA, which demonstrates both variability in microbial composition and the influence of human activity. The large IQR observed in the alpha diversity analyses (Figure 2B) suggests that microbial richness and evenness are also highly variable in these soils.

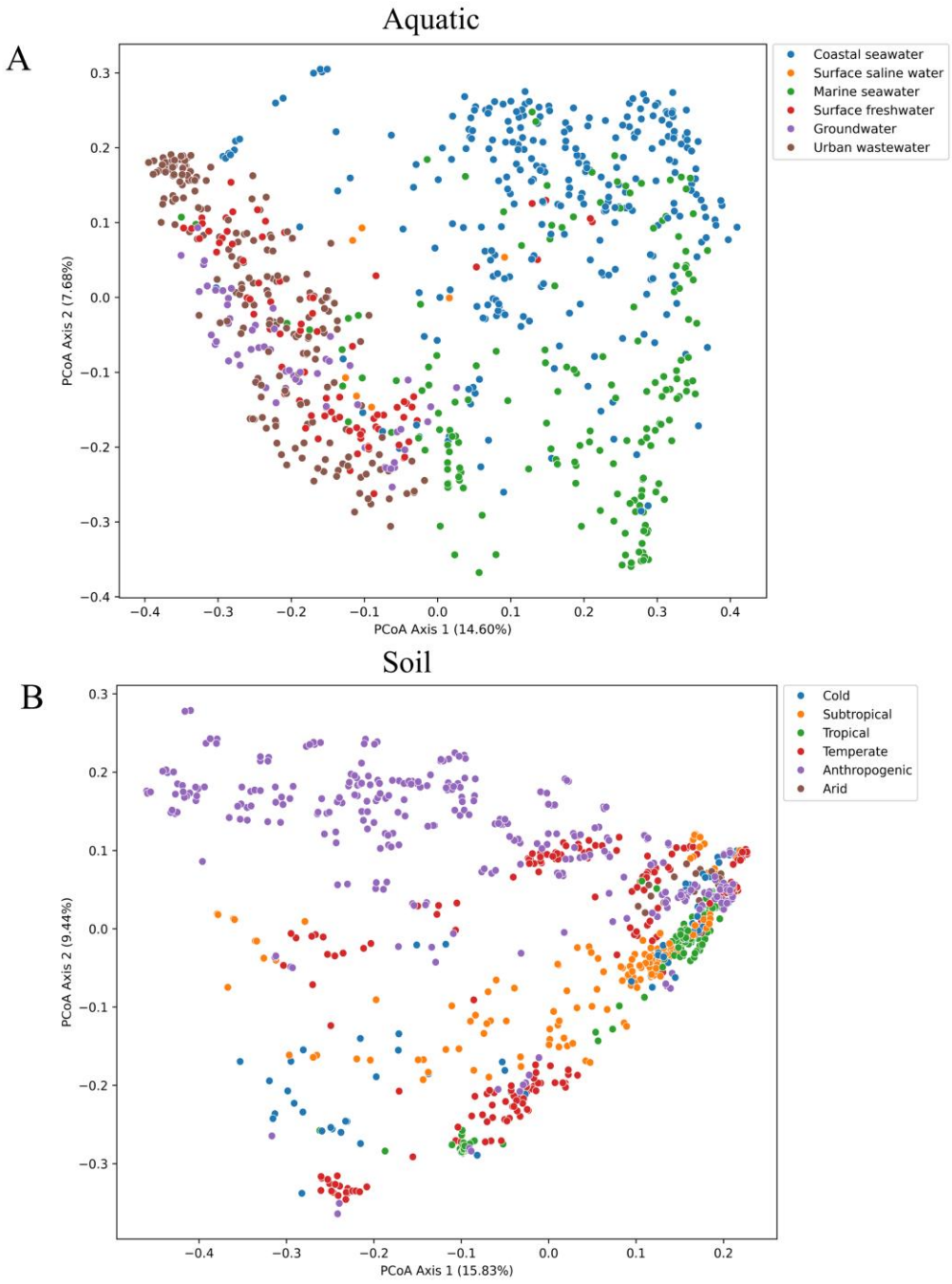


Figure 4. **Principal Coordinate Analysis (PCoA) of microbial communities in aquatic and soil biomes.** (A) Aquatic Biomes: the PCoA plot of microbial communities across aquatic environments, using Bray-Curtis dissimilarity, shows distinct clustering patterns, explaining 14.60% of variation on Axis 1 and 7.68% on Axis 2. (B) Soil Biomes: the PCoA plot of soil biomes explains 15.83% of variation on Axis 1 and 9.44% on Axis 2.

A combined PCoA plot (Figure 5) showed wider dispersion in aquatic samples emphasising the greater variability in microbial species composition in aquatic environments compared to soil. The soil samples demonstrated closer clustering, reflecting the more homogeneous nature of microbial communities in terrestrial environments.

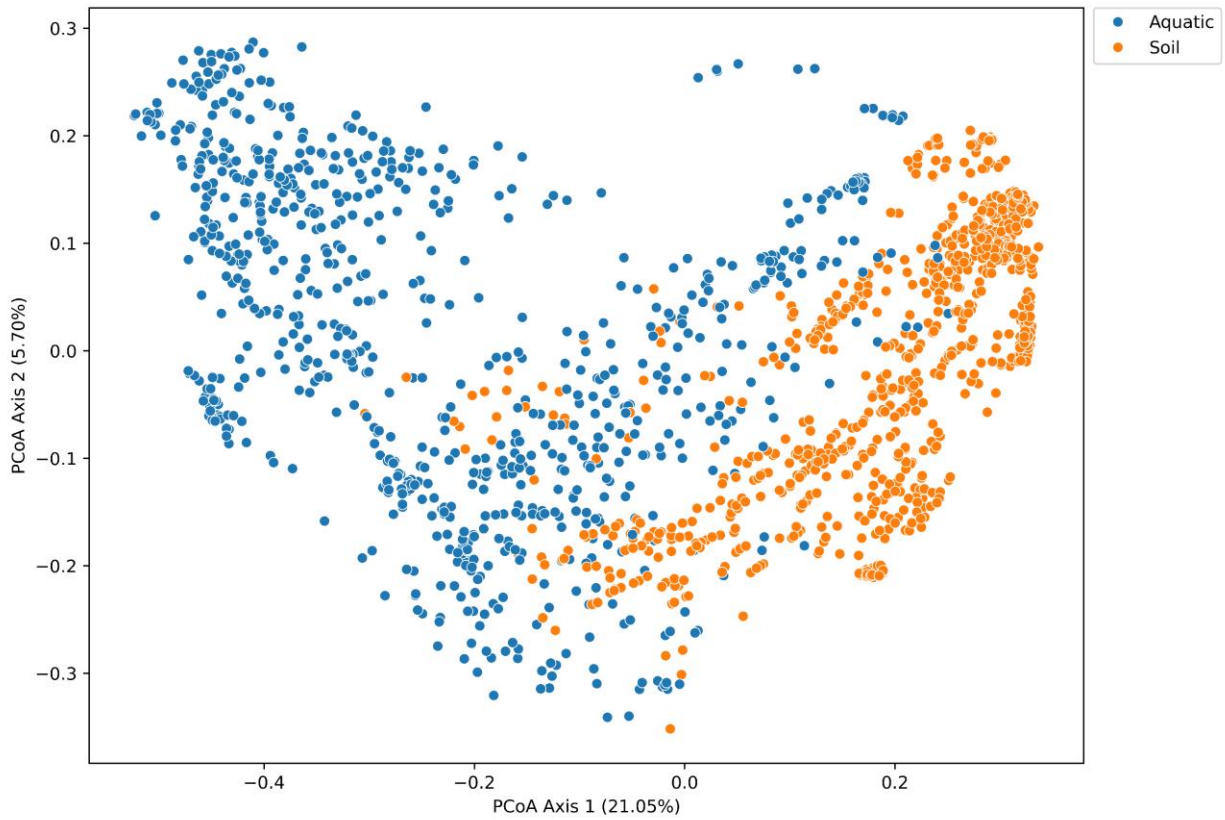


Figure 5. **Comparative PCoA of microbial community profiles in aquatic and soil biomes.** The PCoA plot, based on the Bray-Curtis dissimilarity of microbial community profiles in soil (orange) and aquatic (blue) biomes, highlights distinct compositional patterns across the two environments. The first two axes explain 21.05% (Axis 1) and 5.70% (Axis 2) of the variation.

4.1.3 Phylum-level analysis of microbial communities

Analysis of phylum-level abundance patterns revealed that aquatic environments harboured a broader range of phyla with higher relative abundances compared to soil environments (Figure 6A). This finding presents an interesting contrast to the species-level analysis, where both environment types showed nearly identical Shannon diversity indices (Shannon indices of 5.409 and 5.410, for soil and aquatic environments, respectively). This disparity suggests that while aquatic and soil environments maintain similar levels of species diversity, they achieve this

through different community structures - aquatic environments support a wider array of phyla, each with substantial populations, while soil environments may harbour more species diversity within a more restricted set of phyla.

The hierarchically clustered heatmap showed distinct abundance patterns across different biomes, with several phyla showing notably high abundances in urban wastewater (Polyangia) and coastal seawater (Nitrososphaerota). Pseudomonadota, Bacteroidota and Actinomycetota exhibited consistently high abundance across most aquatic biomes. Surface saline water showed unique abundance patterns for certain phyla including Euryarchaeota, Rhodothermota and Stenosarchaea.

The soil-specific heatmap (Figure 6B) revealed patterns initially obscured by the higher abundances in aquatic samples. Cold soils exhibited elevated abundances of Acidobacteriota, Verrucomicrobiota, and Cyanobacteria relative to other soil biomes. Anthropogenic soils showed lower phylum-level diversity except for Candidatus Micrarchaeota, Bacillota and Campylobacterota.

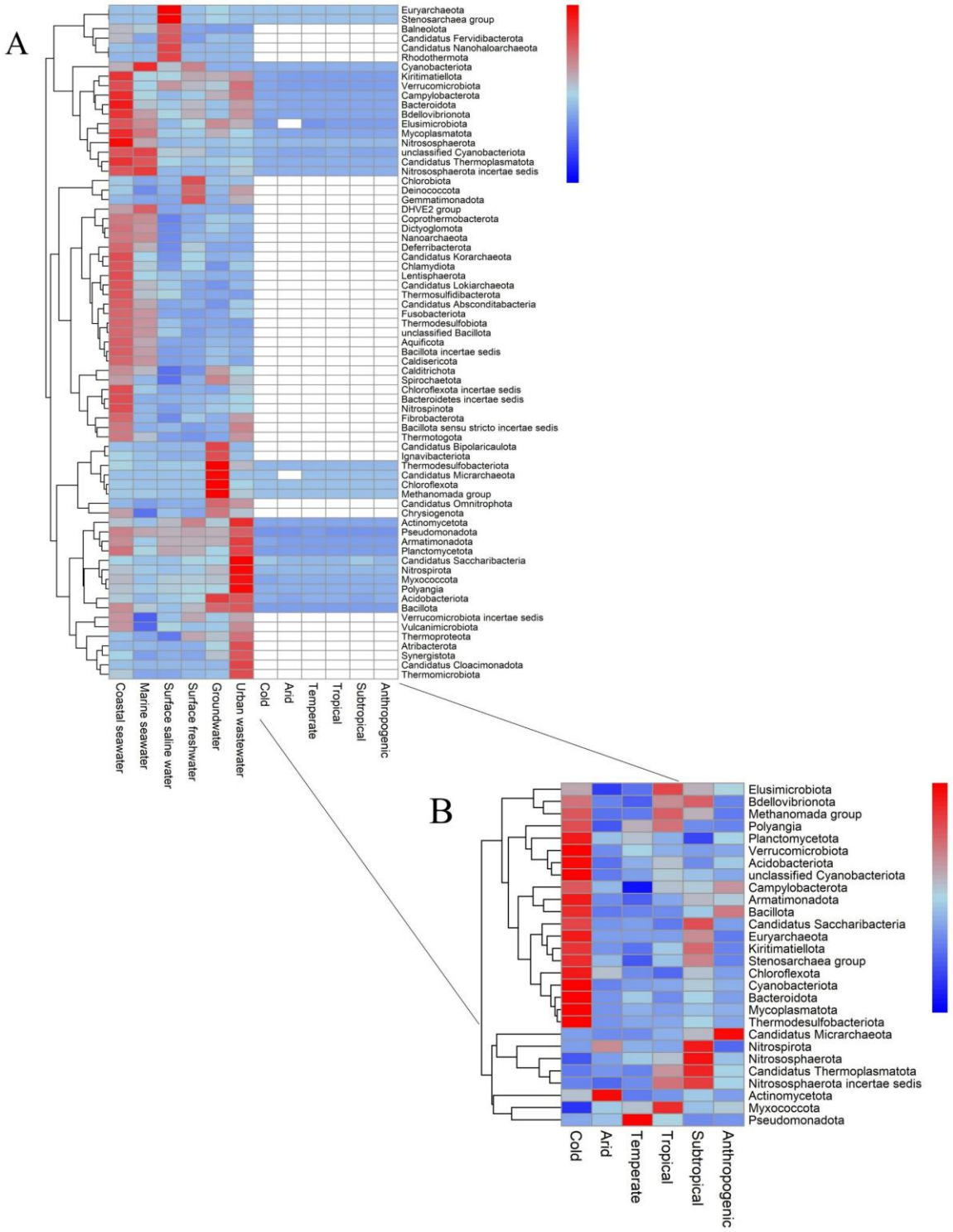


Figure 6**. Heatmap of phyla abundances across soil and aquatic biomes.** (A) A hierarchically clustered heatmap showing the relative abundance of microbial phyla across aquatic and soil biomes. (B) Additionally, soil biomes are individually analysed to reveal trends obscured by higher

abundances in the aquatic biomes. The colour scale represents standardised abundance values, with red indicating above-average abundance, light blue indicating average abundance and dark blue indicating below-average abundance. White cells indicate the absence of the phylum in that biome. The dendrogram shows hierarchical clustering of phyla based on abundance patterns

The network visualisation (Figure 7) provides additional insights into the relationships between microbial phyla and their environments. The graph utilises the Fruchterman-Reingold layout where closely related nodes cluster together, and the spatial arrangement reflects relationship strengths (Fruchterman & Reingold, 1991).

The clustering pattern in the network supports the heatmap findings regarding phylogenetic diversity. Aquatic biomes occupy central positions with numerous connections to diverse phyla, reflecting their role as reservoirs of phylogenetic diversity. The multiple connections radiating from these central nodes visualise the broader range of phyla present in aquatic environments. In contrast, soil biomes display fewer connections and are positioned more peripherally, indicating more specialised phylum associations. This spatial arrangement underscores the restricted phylogenetic diversity in soil environments. The network structure also highlights several phyla maintaining strong connections across multiple environments, such as Pseudomonadota, Bacteroidota and Stenosarchaea, demonstrating their ecological versatility.

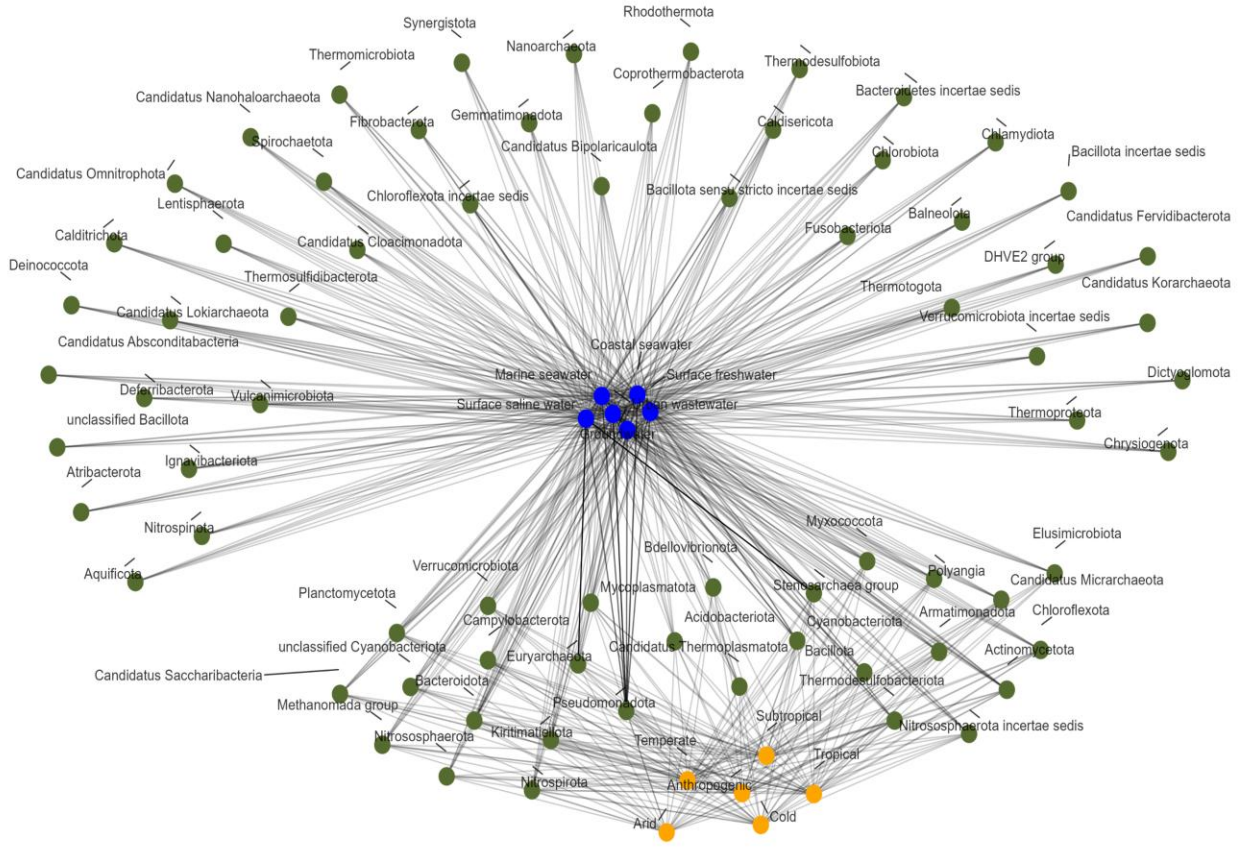


Figure 7. **Network visualisation of relationships between microbial phyla and environmental biomes.** Blue nodes represent aquatic biomes, orange nodes represent soil biomes and green nodes represent microbial phyla. Edge thickness corresponds to the strength of association between phyla and biomes. The visualisation employs the Fruchterman-Reingold layout algorithm, which positions nodes based on force-directed placement, resulting in naturally clustered communities where closely related nodes are positioned near each other.

4.1.4 ESKAPE pathogens across aquatic and soil biomes

The distribution of ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *and Enterobacter* species) was analysed using Kraken2 reports to calculate their relative abundance at the prokaryotic species level.

In aquatic biomes, the highest proportions of ESKAPE pathogens were observed in groundwater (8.32%), urban wastewater (3.14%), and surface freshwater (4.81%) (Figure 8). *Staphylococcus aureus* dominated these environments with proportions of 5.40% in groundwater, 1.95% in urban wastewater, and 3.25% in surface freshwater. *Klebsiella pneumoniae* showed the second-highest abundance at 2.45% in groundwater, 0.73% in urban wastewater, and 1.28% in surface freshwater.

Coastal and marine seawater biomes exhibited lower proportions of ESKAPE pathogens, with coastal seawater showing a total ESKAPE species proportion of 1.14%. Surface saline water contained the lowest proportions of ESKAPE species at 0.09%.

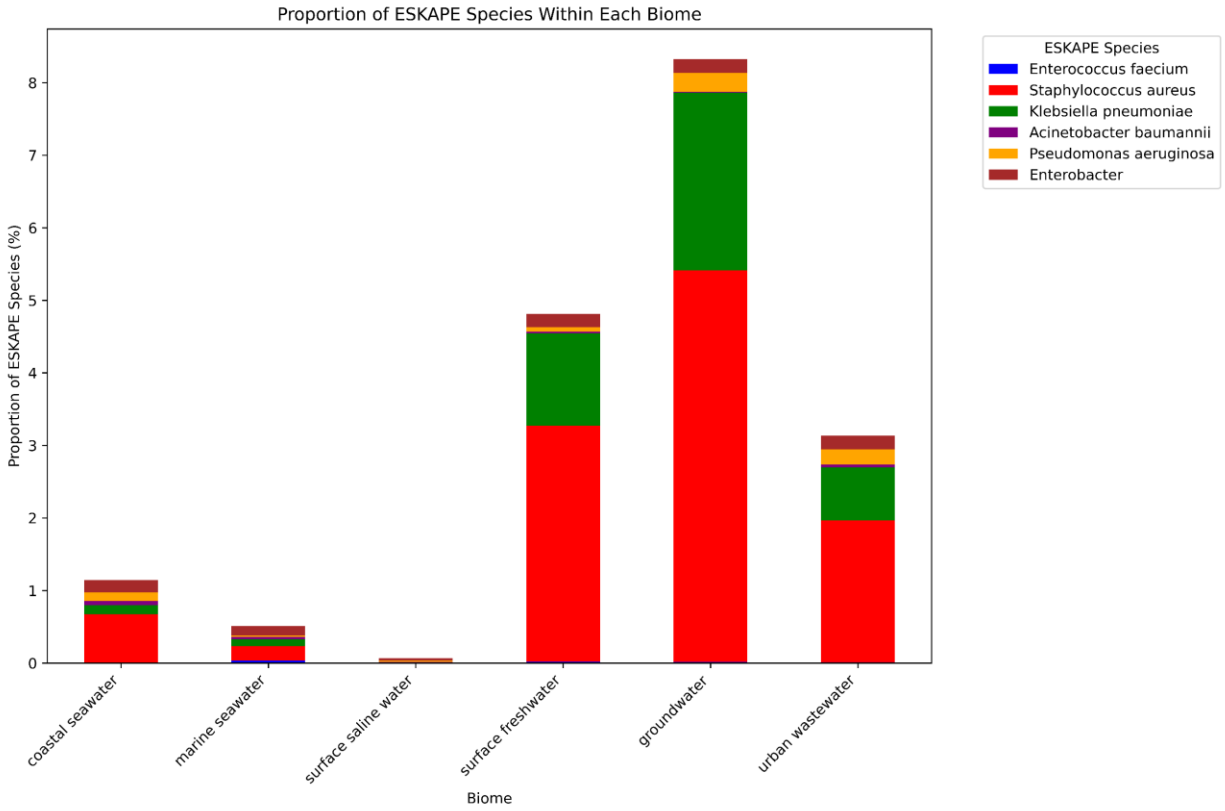


Figure 8. **Proportion of ESKAPE pathogen species in aquatic biomes.** This stacked bar chart illustrates the relative abundance of ESKAPE pathogens across the aquatic biomes, including coastal seawater, marine seawater, surface saline water, surface freshwater, groundwater, and urban wastewater. The proportions were calculated at the prokaryotic species level using Kraken2 reports.

In soil biomes (Figure 9), temperate soils harboured the highest proportion of ESKAPE species (8.23%), with *Klebsiella pneumoniae* and *Staphylococcus aureus* contributing 5.17% and 2.84%

respectively. Anthropogenic soils showed the dominance of *Enterobacter* spp. at 2.65%, contributing to a total ESKAPE proportion of 2.90%.

Remote soil biomes showed lower ESKAPE pathogen proportions. Cold soils exhibited a total ESKAPE proportion of 0.66%, with *Staphylococcus aureus* constituting 0.47%. Tropical soils had a total ESKAPE proportion of 0.26%, with *Klebsiella pneumoniae* reaching 0.13%.

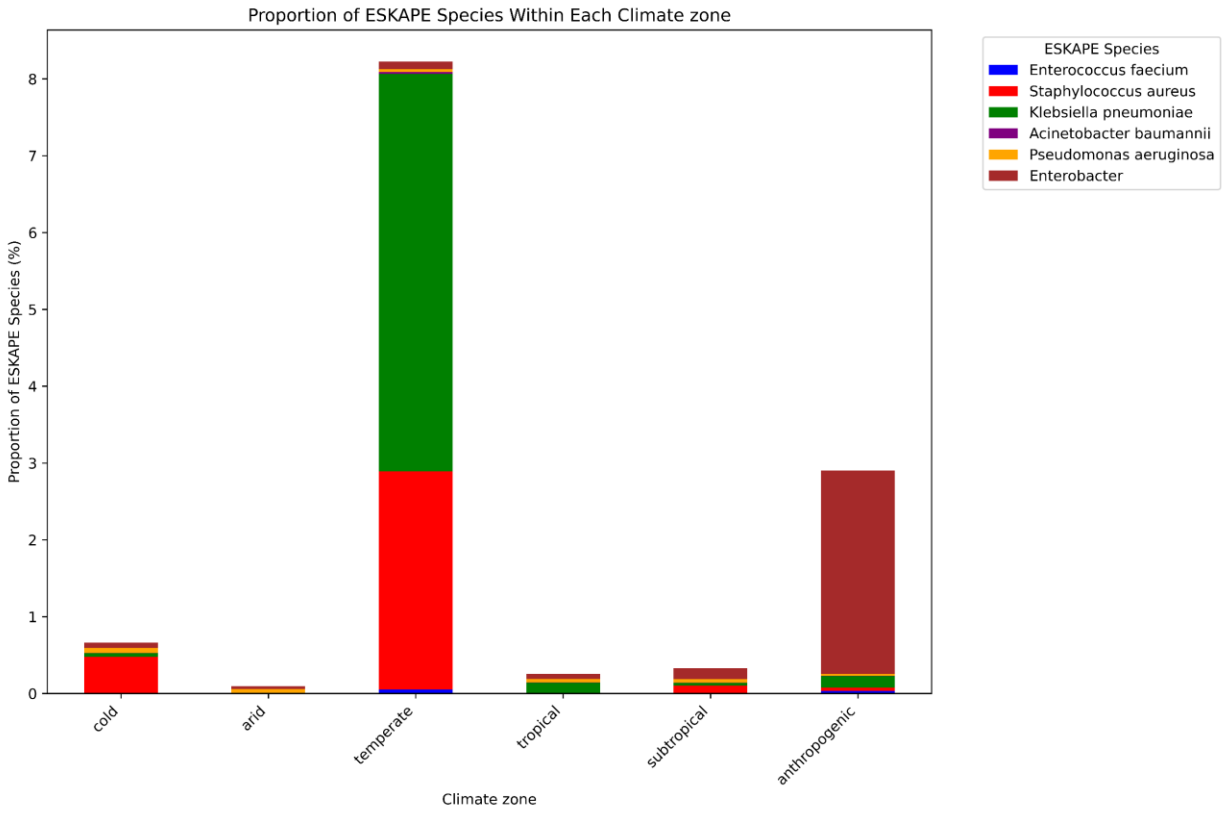


Figure 9. **Proportion of ESKAPE pathogen species in soil biomes.** This stacked bar chart shows the relative abundance of ESKAPE pathogens across various soil biomes, including temperate, anthropogenic, cold, arid, tropical, and subtropical soils. The proportions were calculated at the prokaryotic species level using Kraken2 reports.

4.1.5 ARG Diversity Analysis

The diversity of ARGs was analysed on the basis of both alpha and beta diversity, using Shannon diversity indices, PCoA and Partial Least Squares Correlation (PLSC) analysis. Data for ARG profiles were derived from DeepARG, with these analyses aimed at understanding how different environmental pressures shape resistomes across aquatic and soil biomes.

4.1.6 ARG diversity in aquatic and soil environments

In aquatic environments, a total of 733 distinct ARGs were detected across all samples. Urban wastewater exhibited the highest ARG diversity (Shannon index: 4.28, with an average of 239.61 distinct ARGs per sample) (Figure 10A), while surface saline water showed the lowest ARG diversity (Shannon index: 3.05, with an average of 52 distinct ARGs per sample). Although the ARG diversity is high among the urban wastewater samples, the IQR is moderate and smaller compared to other aquatic biomes, suggesting that the level of ARG diversity is relatively consistent across different urban wastewater samples. While community composition may vary, the overall richness and evenness of ARGs remain stable. Groundwater, similar to anthropogenic soils, displayed moderate ARG diversity (Shannon index: 4.19, with an average of 242.05 ARGs per sample) and had a comparatively smaller IQR. This indicates that, while individual groundwater samples may contain different ARG profiles, the level of diversity remains stable across samples.

In contrast, the other aquatic environments, surface freshwater, coastal seawater, marine seawater, and surface saline water, exhibited larger IQRs, indicating greater variability in the levels of ARG diversity between samples. Coastal and marine seawater had Shannon indices of 3.14 and 3.11, respectively, but the large IQRs suggest that, while the overall diversity is lower on average compared to the other aquatic environments, there is substantial variation in diversity between samples. Coastal seawater had an average of 104.96 ARGs per sample, while marine seawater had an average of 118.32 ARGs per sample.

In soil environments, a total of 721 distinct ARGs were detected across all samples. Anthropogenic soils displayed the highest ARG diversity (Shannon index: 5.71, with an average of 240.78 ARGs per sample) (Figure 10B), despite having lower microbial species diversity (Figure 2B). Subtropical soils, with an average of 216.36 ARGs per sample, and tropical soils, with an average of 217.34 ARGs per sample, followed with Shannon indices of 5.12 and 4.84, respectively. Notably, arid soils, despite exhibiting the lowest ARG diversity (Shannon index: 3.15), contained a relatively high average number of ARGs (239.13), suggesting that ARG abundance is dominated by a few ARG types, resulting in low evenness and therefore, a lower Shannon index in this environment.

Across all soil biomes, the IQRs for ARG diversity were consistently narrow (Figure 10B), indicating that the level of ARG diversity is relatively uniform across different samples within each biome. This uniformity does not imply that the same resistome is present in every sample, but rather that the richness and evenness of ARGs are consistent across the samples.

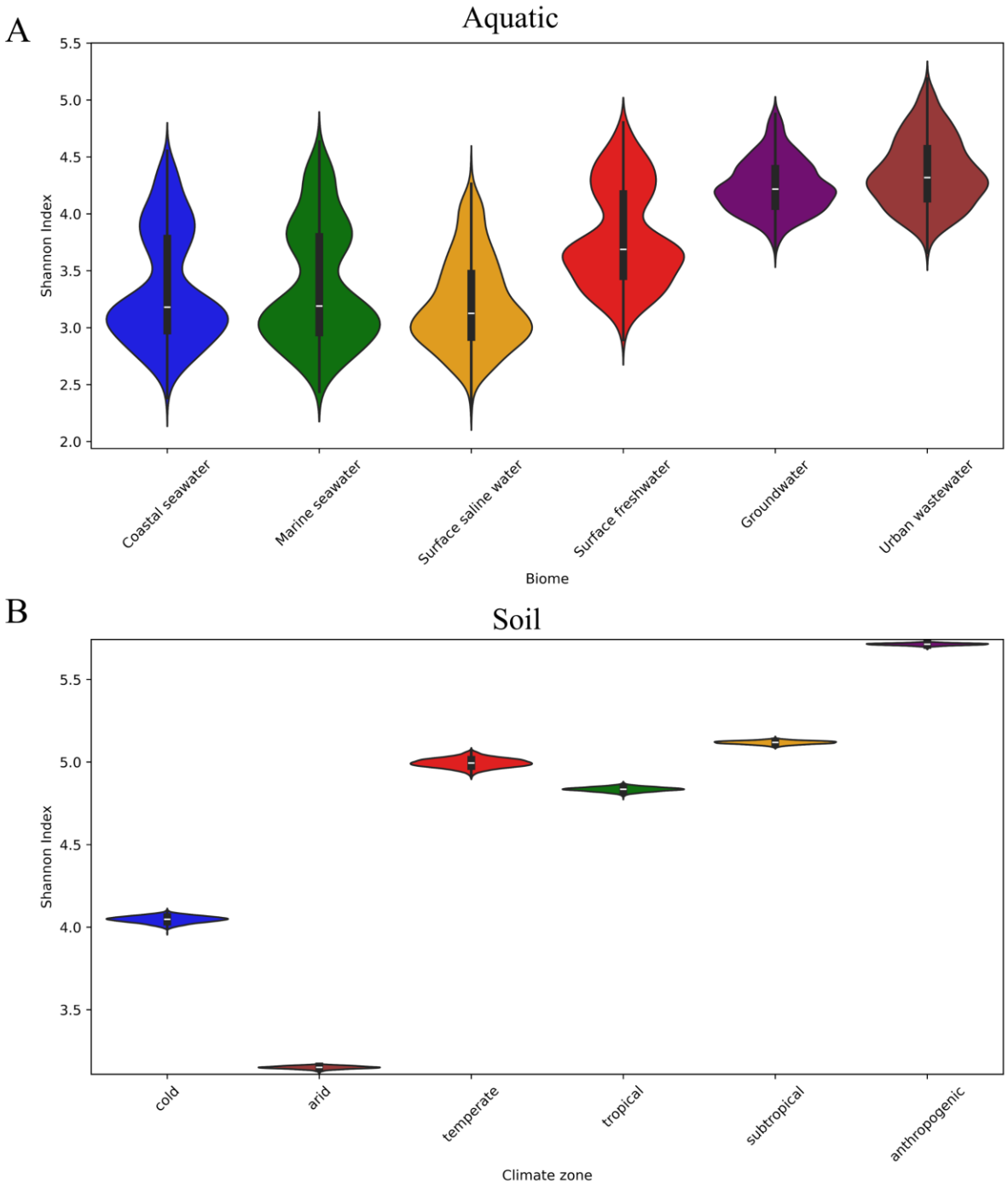


Figure 10**. Shannon diversity of ARGs in aquatic and soil biomes**. Violin plots depicting Shannon diversity indices for ARGs in aquatic (A) and soil (B) environments. Black bars represent medians, and white dots show median indices.

A comparison of the Shannon diversity indices of the soil and aquatic environments (Figure 11), demonstrated that soil samples exhibited a higher median index (4.68) compared to aquatic samples (4.52). This indicates that soil environments tend to support a more diverse set of ARGs overall. Additionally, the narrow IQR observed for soil samples suggests that the level of ARG diversity is more consistent across different soil environments. In contrast, aquatic environments showed broader variability in their Shannon diversity values, reflected by the wider IQR. This variability suggests that ARG diversity is more variable across aquatic samples. The broader range of ARG diversity in water samples indicates that aquatic environments, particularly those exposed to dynamic and changing conditions, harbour more variable ARG profiles, even though their overall diversity is lower than observed for the soil biomes.

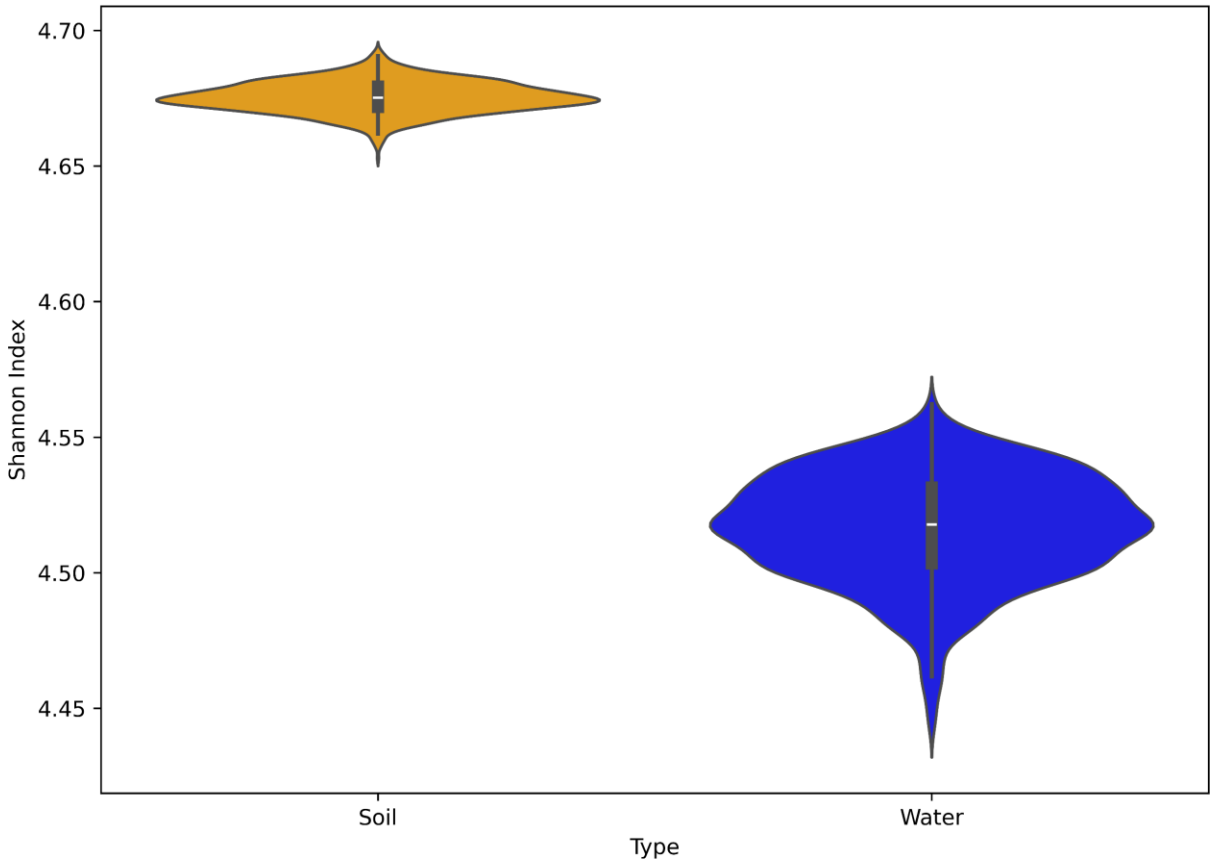


Figure 11. **Comparative Shannon diversity indices of ARGs in soil and aquatic environments.** This violin plot compares the Shannon diversity of ARGs between soil (orange) and aquatic (blue) environments. Soil samples exhibit a higher median Shannon diversity index of approximately

4.68, compared to aquatic samples with a median of 4.52. The black bars represent the interquartile range, with the white dots indicating the median index values for each environment.

4.1.7 Principal coordinate analyses of ARG diversity

To further explore the compositional differences in ARG profiles of the different aquatic and soil biomes, PCoA analyses were performed using Bray-Curtis dissimilarity. The ARG profiles were generated using data from DeepARG, and Bray-Curtis dissimilarity was employed to capture the variability in ARG composition across different biomes.

In aquatic environments, the PCoA plot revealed a broad dispersion of ARG profiles across most biomes, reflecting the dynamic nature of aquatic systems (Figure 12A). However, coastal and marine seawater formed distinct, well-defined clusters, suggesting more uniform ARG profiles.

In soil environments, the PCoA plot revealed clearer clustering patterns. Anthropogenic soils formed a distinct cluster, reflecting highly consistent ARG profiles across samples, correlating with the highest ARG Shannon diversity index (5.71) among the soil biomes. In contrast, subtropical, tropical, temperate, arid, and cold soils showed more overlap, particularly in the lower-right portion of the plot, suggesting that shared environmental pressures (Figure 12B).

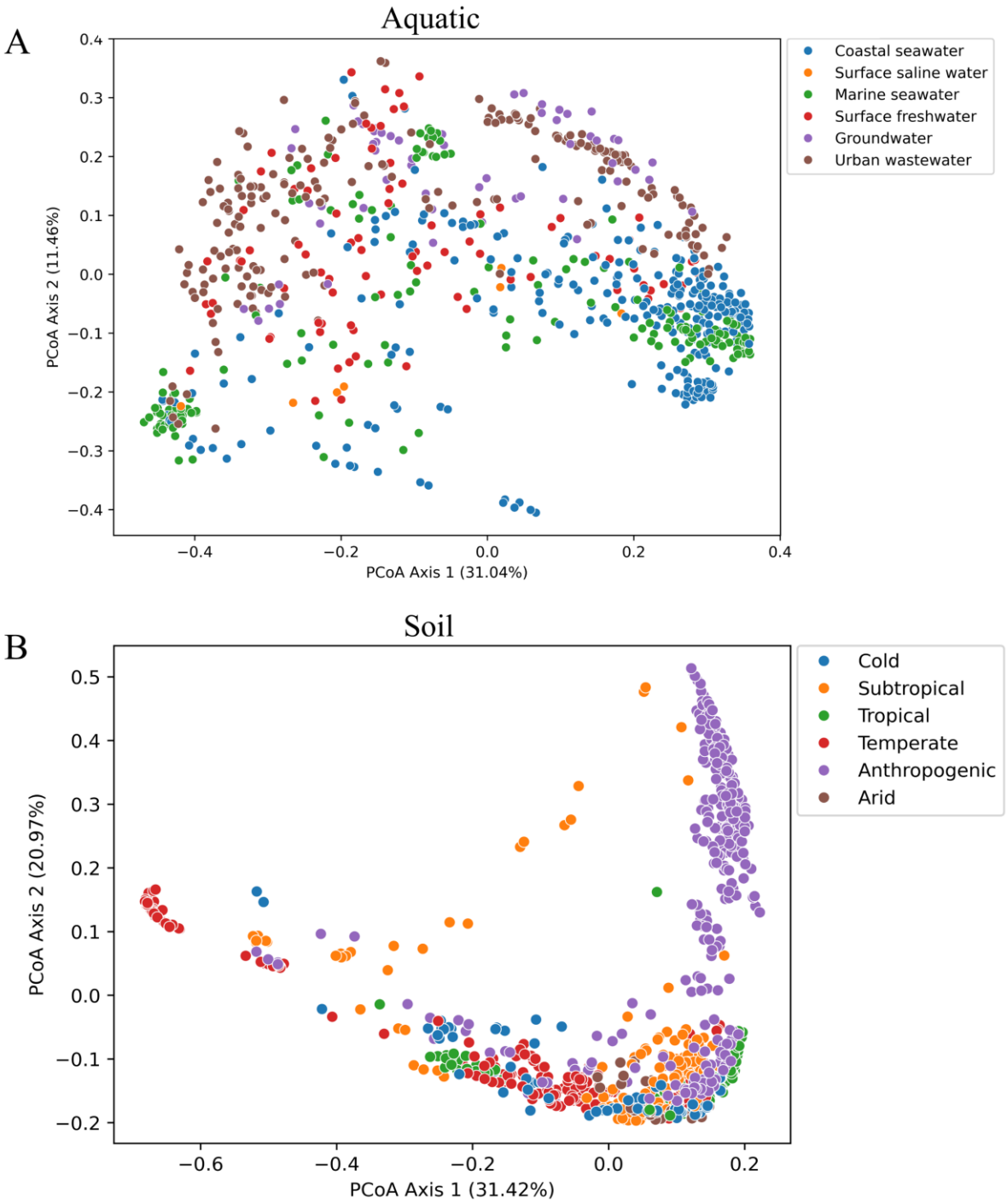


Figure 12**. Principal Coordinate Analysis (PCoA) of ARGs in aquatic and soil biomes.** (A) Aquatic Biomes: PCoA plot based on Bray-Curtis dissimilarity of ARG profiles in aquatic environments explains 31.04% of the variation on Axis 1 and 11.46% on Axis 2. (B) Soil Biomes: PCoA of soil biomes explains 31.42% of variation on Axis 1 and 20.97% on Axis 2.

The combined PCoA plot of ARG profiles for both soil and aquatic biomes (Figure 13) further underscores the distinct patterns of ARG diversity across these environments. Soil samples formed well-defined clusters along the PCoA axes, reflecting a more homogeneous resistome structure. Aquatic samples, by contrast, exhibited broader dispersion along the PCoA axes, highlighting the greater variability in ARG composition in these environments. Overall, the distinct separation between soil and aquatic ARG profiles in the PCoA plot emphasizes how different ecological pressures influence resistome compositions in these two environments, with soil biomes tending to support more stable and uniform ARG profiles, while aquatic biomes are characterized by greater variability.

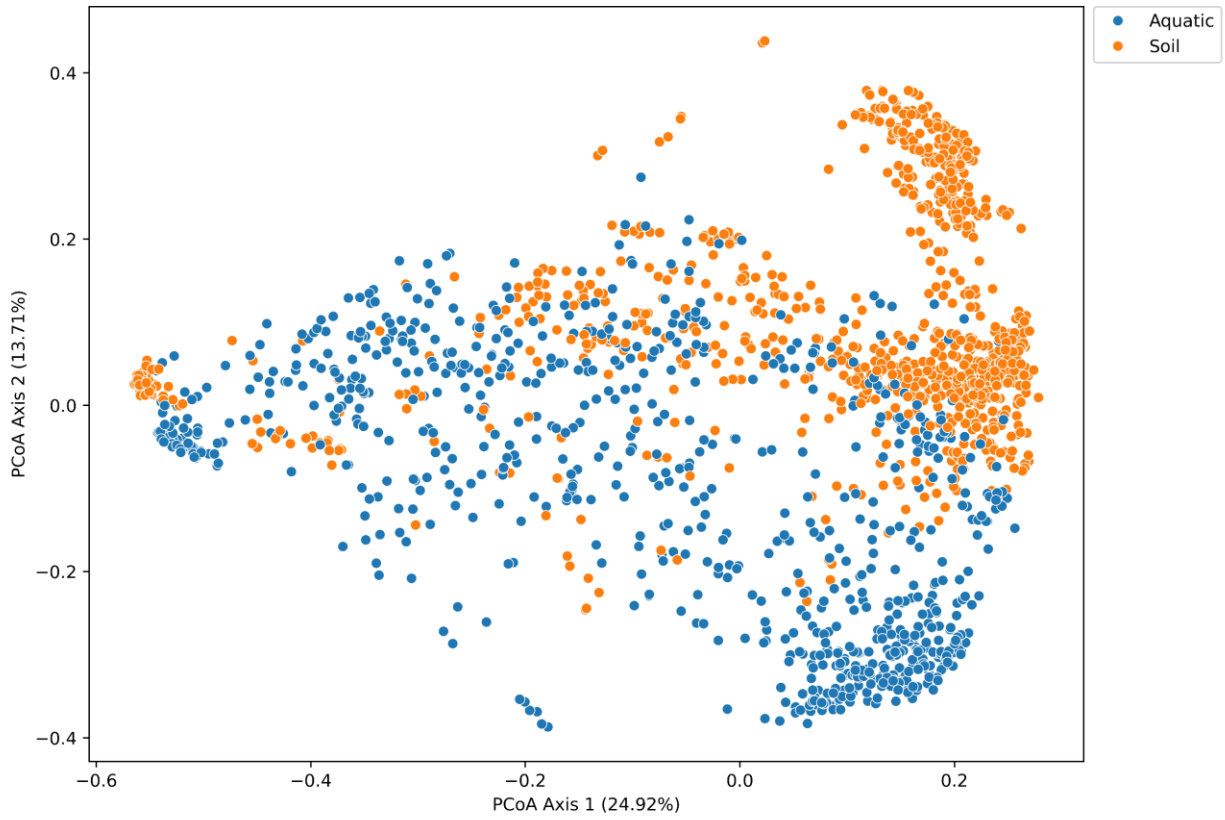


Figure 13. **Comparative PCoA of ARG profiles in aquatic and soil biomes.** PCoA plot based on Bray-Curtis dissimilarity of ARG profiles (log-transformed) in soil (orange) and aquatic (blue) environments, illustrating distinct resistome compositions. The first two axes explain 24.92% and 13.74% of the variation, respectively.

4.1.8 Partial least squares correlation analysis

A partial least squares correlation (PLSC) analysis was conducted to assess the relationship between microbial species abundance and ARG diversity. In aquatic environments, the PLSC confirmed a positive correlation ($r = 0.6936$, $p < 0.001$) (Figure 14A, B), indicating that environments with higher microbial species diversity tend to harbour a broader range of resistance genes. Similarly, in soil environments, a significant but weaker correlation was observed between microbial species abundance and ARG diversity ($r = 0.6423$, $p < 0.001$) (Figure 15A, B).

Outliers in both environments reveal the complexity of the relationship between microbial and ARG diversity. In aquatic biomes, coastal seawater emerged as an outlier, where high microbial diversity did not correspond to high ARG diversity (Figure 2A;10A). This indicates that factors other than microbial abundance, such as specific selective pressures or the composition of microbial communities, may shape ARG profiles more strongly in certain environments. In contrast, anthropogenic soils in terrestrial biomes exhibited the opposite pattern, with low microbial diversity but high ARG diversity (Figure 2B;10B).

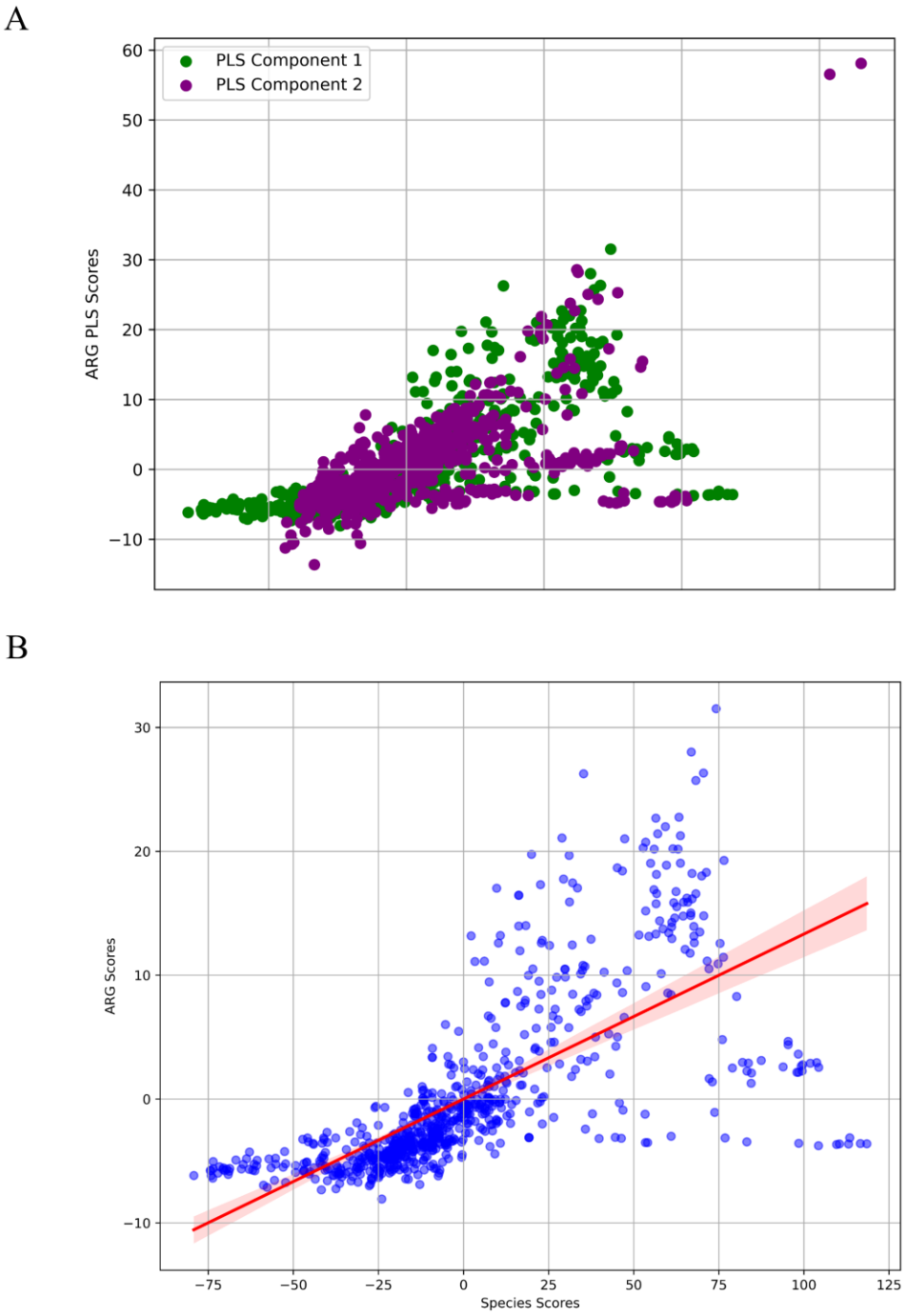
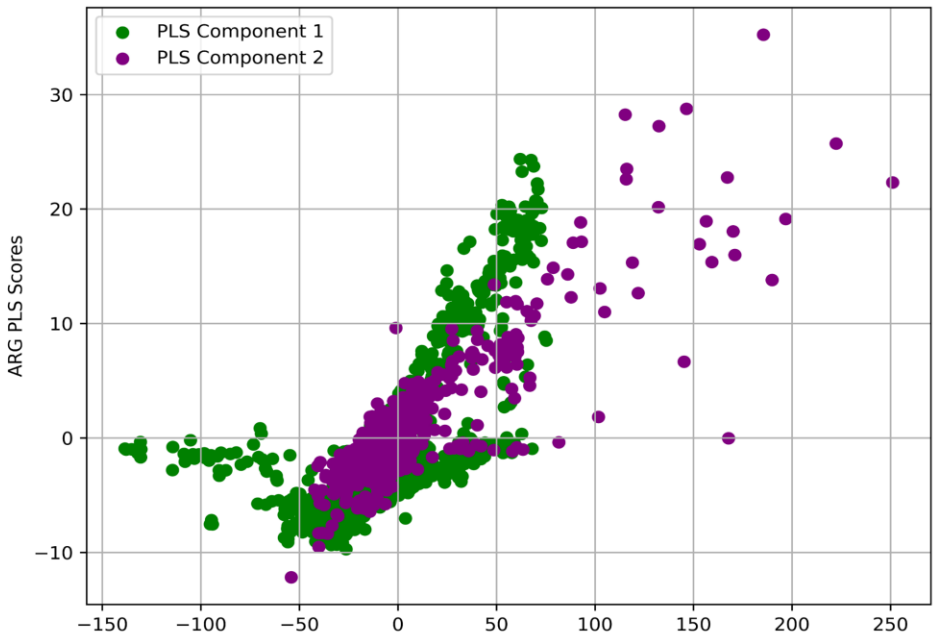


Figure 14. **(A) PLSC of species vs. ARG abundances in aquatic biomes.** The PLS scores are shown in purple and green respectively. (B) Regression plot demonstrating the positive linear relationship between microbial species diversity and ARG scores with a 95% confidence interval.

A



B

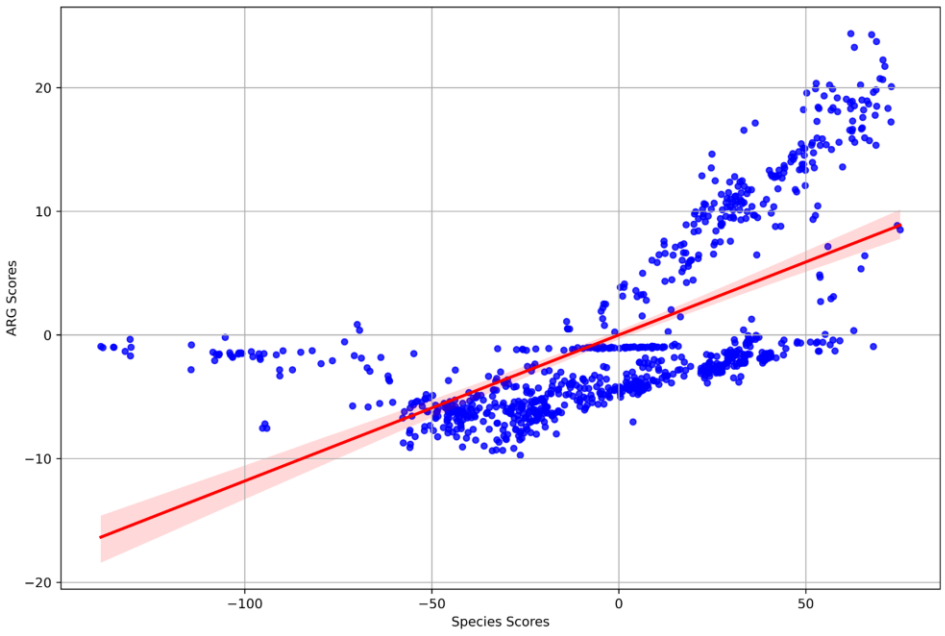


Figure 15. **(A) PLSC of Species vs. ARG Abundances in Soil Biomes.** The PLS scores are shown in purple and green respectively. (B) Regression plot demonstrating the positive linear relationship between microbial species diversity and ARG scores with a 95% confidence interval.

4.1.9 Core and accessory resistomes in aquatic and soil biomes

The core resistome consists of ARGs that are pervasive across samples within an environment, reflecting a baseline level of resistance that is consistently present (D'Costa *et al.,* 2006). In contrast, the accessory resistome includes ARGs with more variable distributions and abundances, often reflecting emerging resistance mechanisms or localised contaminations driven by dynamic environmental or anthropogenic influences (D'Costa *et al.,* 2006; Kim & Cha, 2021).

4.1.10 Core resistome analysis in aquatic and soil biomes

The core resistome was determined by a binomial test with adjusted p-values below 0.05 to identify those ARGs that were significantly abundant across samples. Rather than requiring a presence in all samples, a statistical threshold was set, identifying ARGs that occur in a high proportion of samples. This approach ensures that the core resistome reflects genes that are persistently present across samples at a frequency higher than expected by chance.

In aquatic environments, the core resistome comprised 113 ARGs (Figures 16, 17), with multidrug resistance (MDR) genes being the most abundant (40 genes). The *mexF* and *mexW* genes were present in 91.9% and 90.7% of samples respectively. The *ompR* gene appeared in 90.5% of aquatic metagenomes.

Tetracycline resistance genes *tetP* and *otrA* were represented in 91.9% and 90.5% of aquatic metagenomes respectively. The *macB* gene occurred in 97.3% of aquatic samples, while *mupA* was detected in 92.4% of samples.

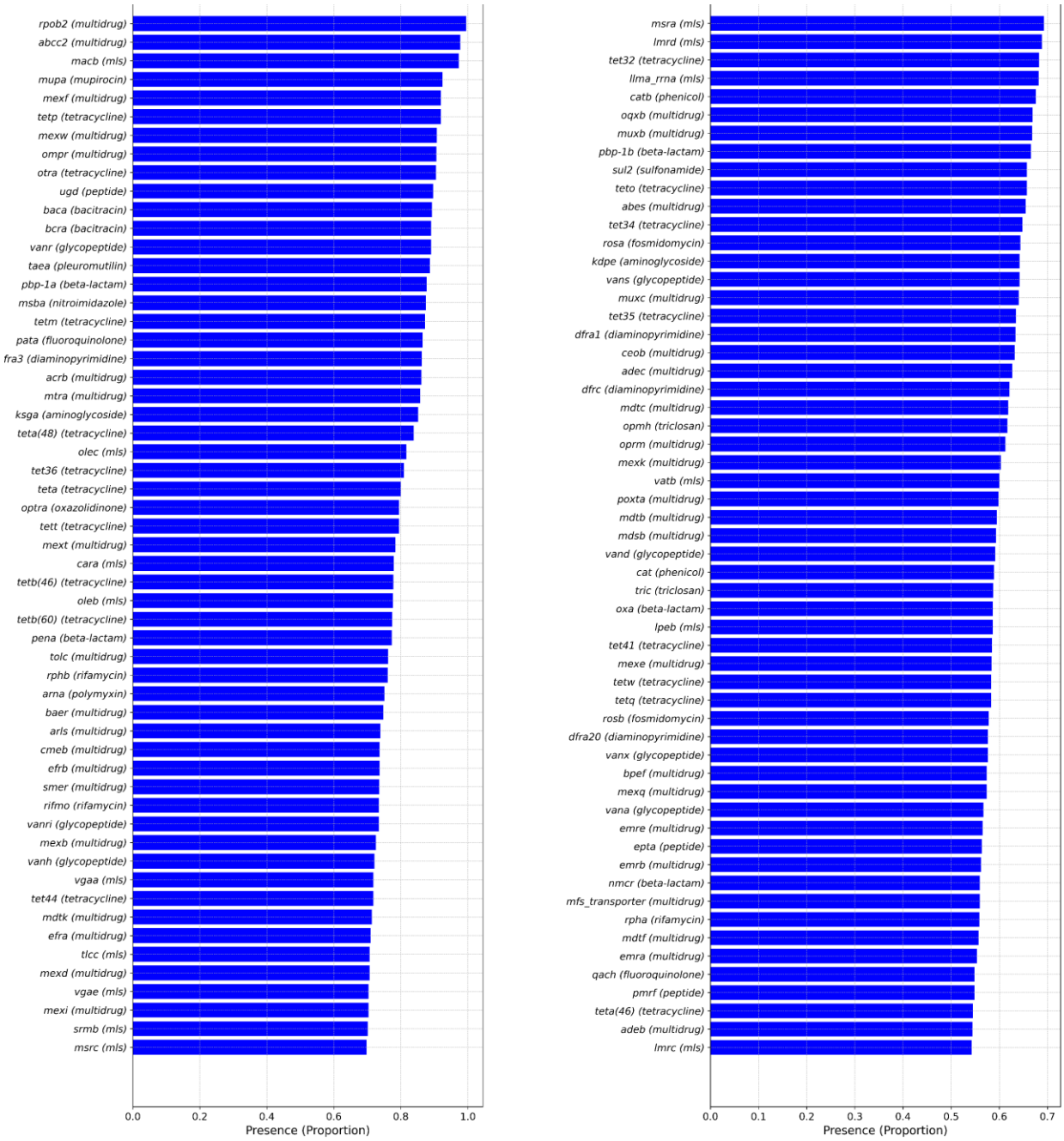


Figure 16. **The proportion of the core ARGs in the aquatic biome.** The proportion of aquatic core ARGs (n=113) is displayed across two bar plots, arranged in descending order of abundance from left to right. Core ARGs are defined based on a statistically significant presence across samples, identified through a binomial test with adjusted p-values below 0.05, rather than by arbitrary thresholds of occurrence.

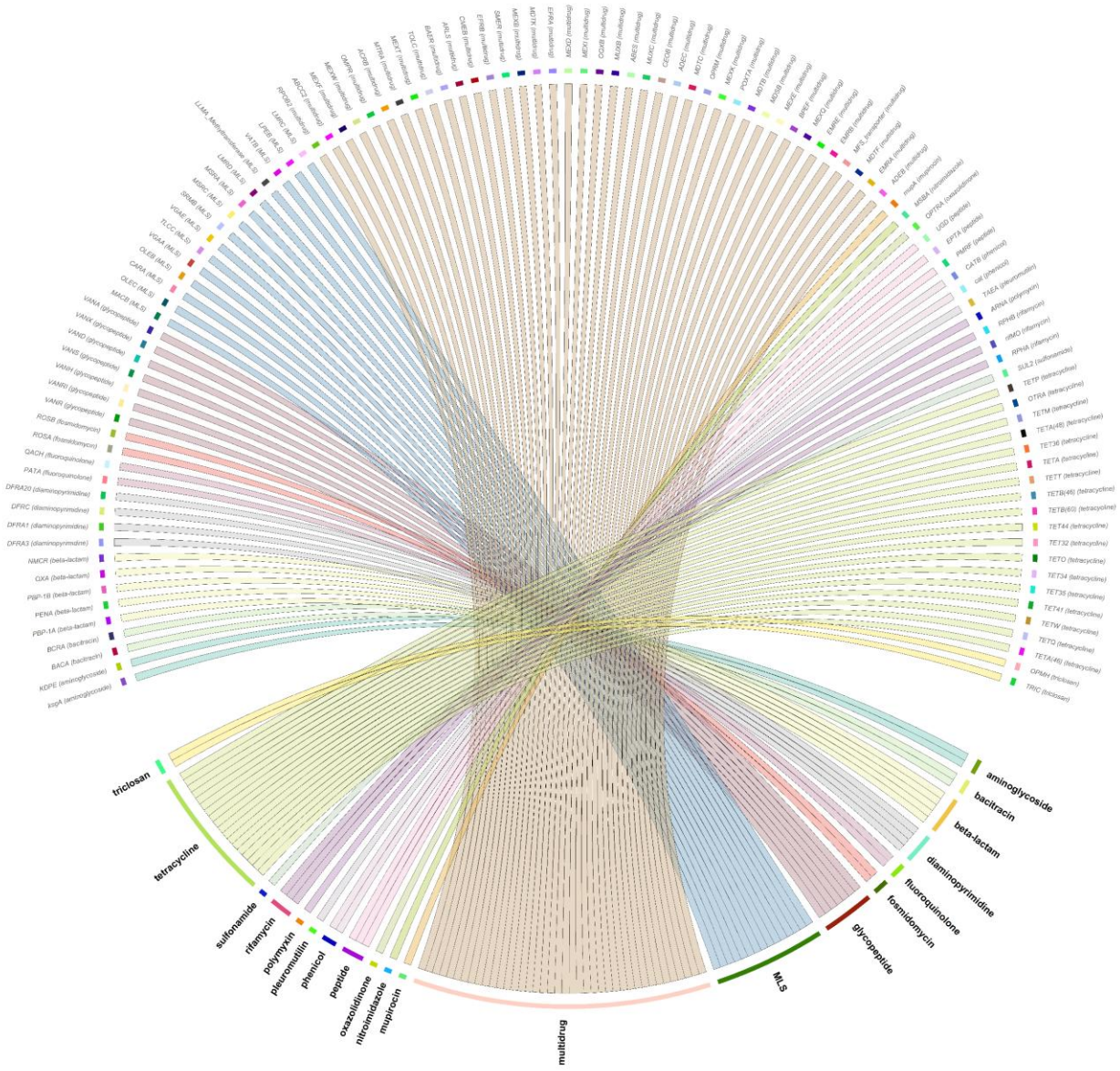


Figure 17. **The core resistome of aquatic biomes.** Chord diagram depicting the distribution of core aquatic ARGs (n=113) across 20 different resistance classes

In soil environments, the core resistome contained 179 ARGs (Figures 18, 19), with MDR genes being the most abundant (73 genes). The efflux pump genes *mexF* and *mexW* appeared in more than 97% of soil samples. The soil core resistome included thirteen beta-lactam resistance genes, with *pbp-1a* and *oxa* showing a mean presence of 71.6% across soil samples. Twenty-seven

tetracycline resistance genes were identified with a mean presence of 72.5%. The *vanR* gene showed high prevalence, while r*osA* and *bacA* appeared in 98.8% of soil samples.

Figure 18. **The proportion of the core ARGs in the soil biome.** The proportion of soil core ARGs (n=179) is displayed across two bar plots, arranged in descending order of abundance from left to right. Core ARGs are defined based on a statistically significant presence across samples, identified through a binomial test with adjusted p-values below 0.05, rather than by arbitrary thresholds of occurrence.

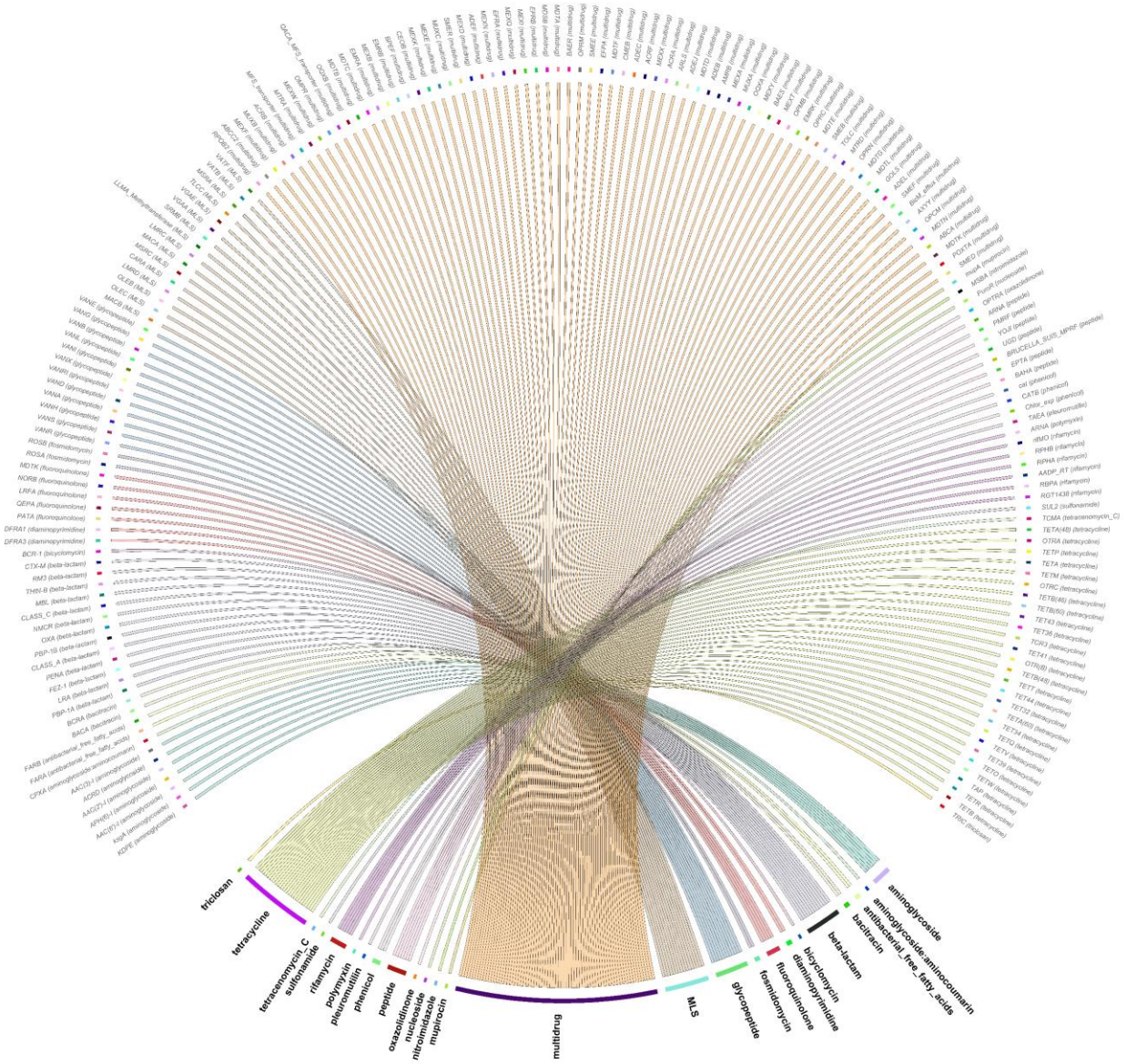


Figure 19. **The core resistome of the soil biome.** Chord diagram depicting the distribution of core ARGs (n=179) across 24 different resistance classes.

Comparative analysis revealed that the soil core resistome (179 ARGs) was 58.40% larger than the aquatic core resistome (113 ARGs) (Figure 20A). The environments shared 101 core ARGs, representing 52.88% of combined core resistomes. Soil environments contained 78 unique core

ARGs (40.83%), while aquatic environments had twelve unique core ARGs (6.28%). The differential abundance analysis (Figure 20B) mapped ARGs unique to each environment across resistance classes.

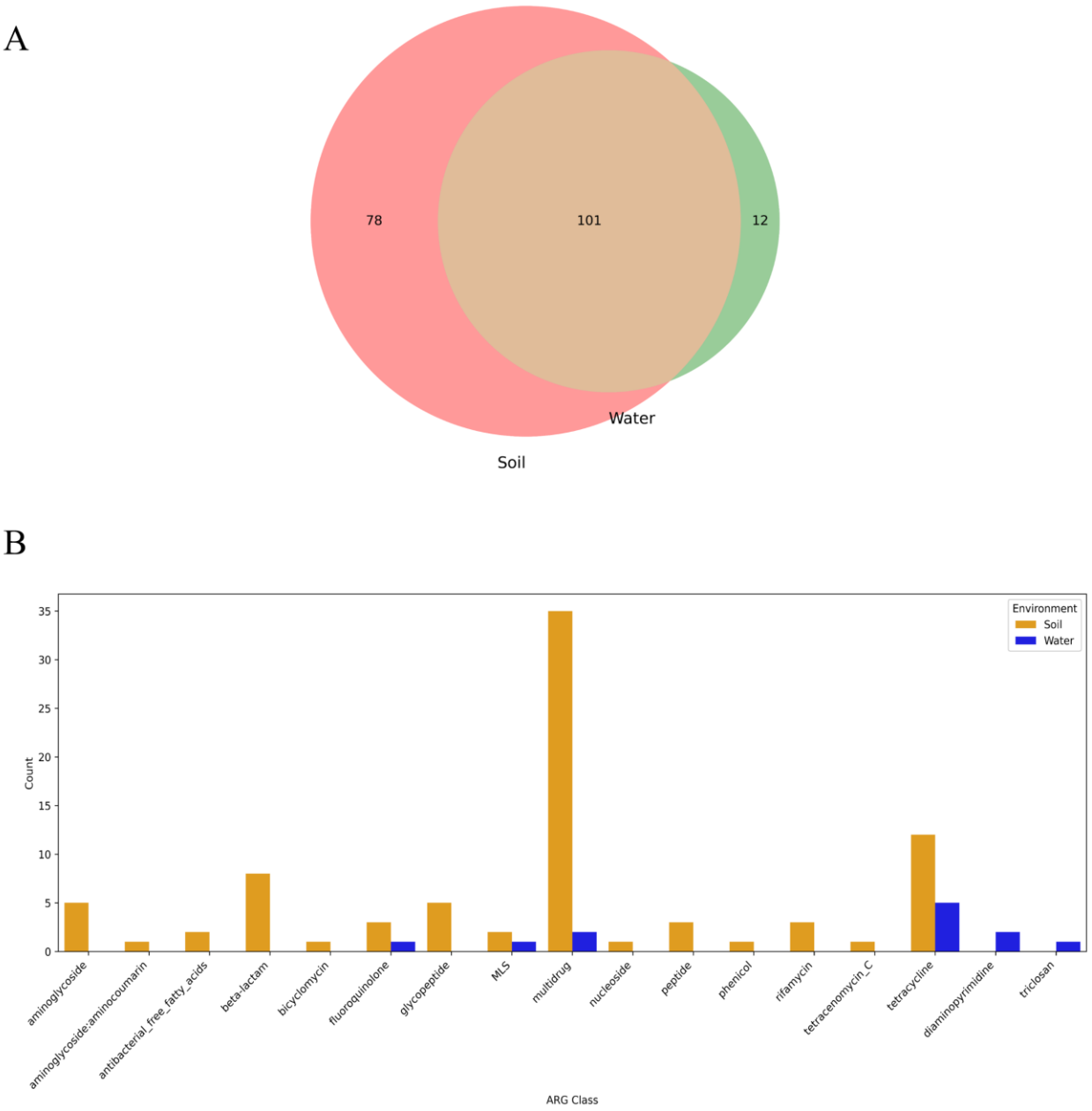


Figure 20. **Comparative analysis of core ARGs in soil and aquatic biomes.** (A) Venn diagram illustrating the number of core ARGs shared between and unique to soil and aquatic environments. Core ARGs are defined as those that are statistically pervasive across samples, determined by a binomial test with an adjusted p-value threshold of 0.05. These core genes represent baseline resistance present across a majority of samples in each environment. (B) Bar plot showing the distribution of ARGs that are unique to either soil or aquatic metagenomes. The presence of both orange and blue bars for certain classes, such as MDR, indicates that some genes in this class are unique to soil, while others are unique to aquatic environments.

4.1.11 The accessory resistome in soil and aquatic environments

The accessory resistome was identified by filtering out core ARGs from both soil and aquatic settings. The aquatic accessory resistome comprised 620 ARGs. Multidrug resistance genes showed the highest prevalence, present in 33.2% of aquatic metagenomes (Figure 21, 22). Beta-lactam resistance genes were the second most prevalent, appearing in 12.7% of aquatic metagenomes. Macrolide-lincosamide-streptogramin (MLS) resistance and tetracycline resistance genes appeared in 11.3% and 8.6% of samples respectively. Glycopeptide resistance and aminoglycoside resistance genes showed lower prevalence at 6.5% and 6.3%.

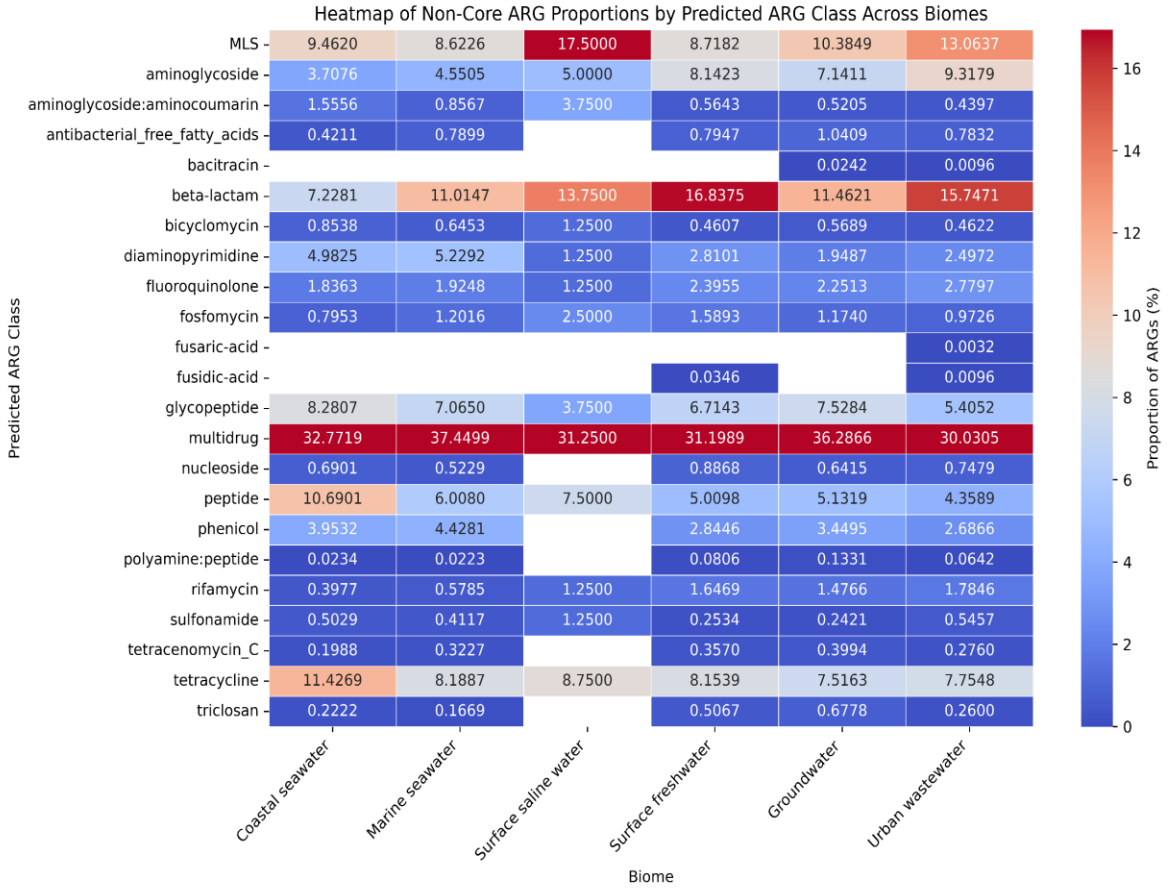


Figure 21. **Heatmap illustrating the normalised proportions of accessory ARG classes across aquatic biomes.** The heatmap highlights variations in the distribution of ARG classes within the accessory resistome across different environments.

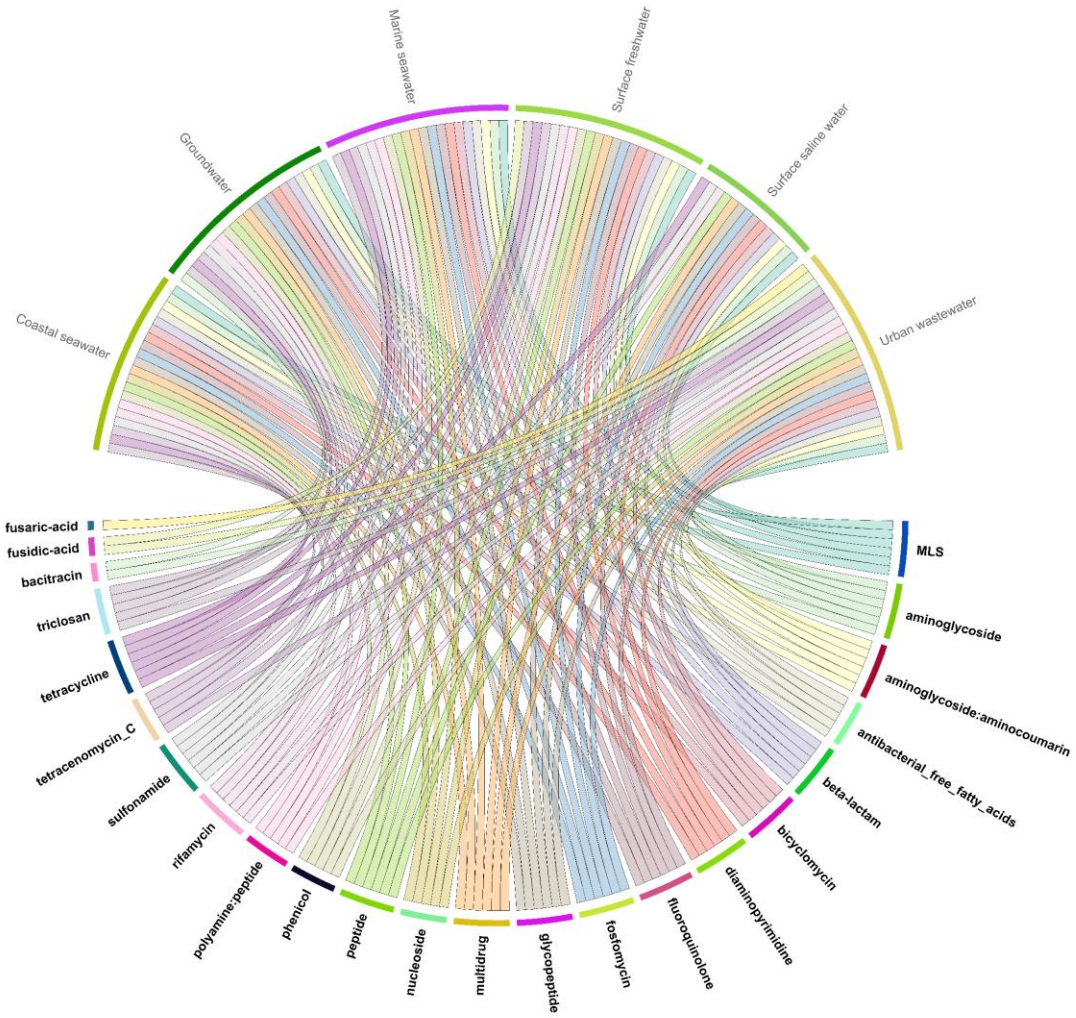


Figure 22. **Chord diagram demonstrating the distribution of the accessory resistome across aquatic biomes.** The diagram visually connects ARG classes to their respective biomes, emphasising the shared and distinct resistome patterns across environments.

The soil accessory resistome contained 542 genes. MDR genes appeared in 22.3% of soil metagenomes (Figure 23, 24), with the highest prevalence in anthropogenic soils at 28.95%. Beta-lactam resistance genes occurred in 16.9% of samples, while MLS resistance and aminoglycoside resistance genes appeared in 13.9% and 13.1% of samples respectively. Tetracycline resistance and fluoroquinolone resistance genes showed lower prevalence at 5.6% and 3.2%.

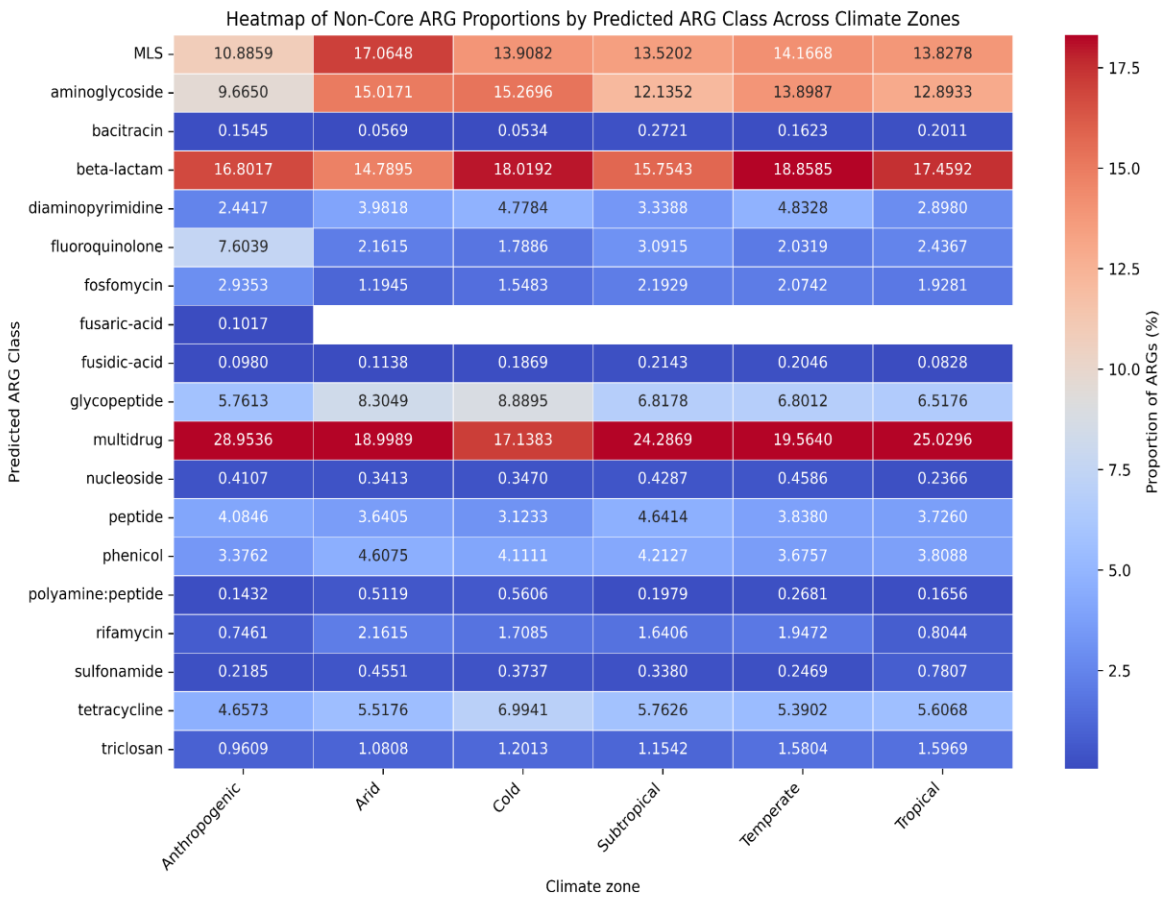


Figure 23. **Heatmap illustrating the normalised proportions of accessory ARG classes across soil biomes.** The heatmap highlights variations in the distribution of ARG classes within the accessory resistome across different environments.

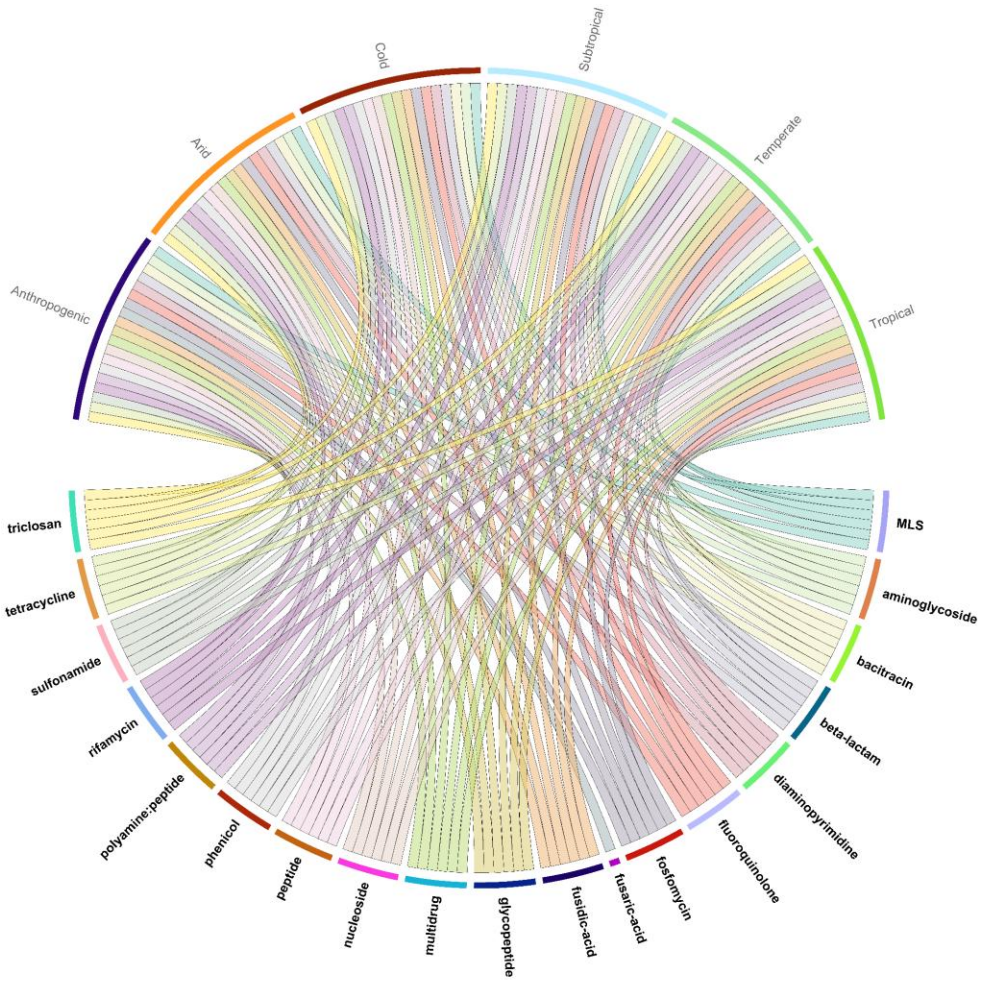


Figure 24. **Chord diagram demonstrating the distribution of the accessory resistome across soil biomes.** The diagram visually connects ARG classes to their respective biomes, emphasising the shared and distinct resistome patterns across environments.

Across both aquatic and soil environments, a consistent trend is observed: ARG classes abundant in the core resistome, such as multidrug resistance, beta-lactam resistance, MLS resistance and tetracycline resistance are similarly prevalent in the accessory resistome. This overlap suggests that the pressures driving core resistance also shape the accessory resistome, where the accessory resistome captures more localised or emerging resistance trends.

4.2 Discussion

4.2.1 Introduction to study findings

This study analysed 1,758 metagenomes to assess microbial species and ARG diversity across global soil and aquatic environments. Substantial differences were found between biomes, largely influenced by environmental factors and human activities. For instance, urban wastewater exhibited the highest diversity in aquatic settings (Figure 2A), with anthropogenic soils displaying elevated ARG diversity (Figure 10B). These findings underscore the complex dynamics of microbial and ARG diversity across different environments and, ultimately, the need for more judicious antimicrobial stewardship at a global level.

4.2.2 Diversity and drivers of microbial communities and antimicrobial resistance genes in aquatic environments

Urban wastewater showed the highest microbial species diversity across the aquatic metagenomes, characterised by a notably narrow interquartile range indicating consistent community structure. This can be attributed to the extensive influx of microorganisms from various human activities, particularly in wastewater treatment plants (WWTPs) (Pillay *et al.,* 2022). WWTPs receive a constant input of bacteria from domestic and industrial sources, including wastewater derived from hospitals, which increases the diversity of microbial species present (Khan *et al.,* 2021). The constant influx of nutrients and contaminants creates diverse niches that support this rich microbial community (Rizzo *et al.,* 2013). The selective pressures exerted by antibiotic residues and resistant bacteria within WWTPs contribute to the contamination of downstream environments, such as freshwater ecosystems and coastal areas (Rizzo *et al.,* 2013). The selective pressures from antibiotics and other contaminants have been shown to encourage the emergence and proliferation of resistant strains, leading to high ARG diversity (Qin *et al.,* 2020).

In addition to hosting high microbial diversity, urban wastewater is also a major reservoir of ARGs. This reflects the role of WWTPs as hotspots for ARG accumulation and potential dissemination (Pazda *et al.,* 2019; Qin *et al.,* 2020). These processes highlight the interconnectedness between urban wastewater, groundwater, and surface freshwater systems (Hemamalini *et al.,* 2022), where shared microbial community composition and ARG profiles pose risks to environmental and human health. This interconnection is particularly evident in the PCoA analysis (Figure 4A), where

these biomes cluster together, suggesting shared contamination sources and similar selective pressures. The major influence of WWTPs on downstream ecosystems highlights the need for improved wastewater management practices to mitigate the spread of antibiotic resistance (Rizzo *et al.,* 2013).

In contrast, coastal and marine environments, despite having high microbial diversity, show lower ARG diversity. This suggests that factors other than microbial diversity, such as salinity, temperature, and nutrient content, play important roles in shaping the resistome (Shu & Huang, 2022). Salinity, for instance, can act as a selective pressure, favouring microbial taxa adapted to saline conditions and concomitantly their inherent ARGs (Tan *et al.,* 2019). The distinct clustering of marine and coastal samples in the PCoA analysis (Figure 4A) reflects their unique ecological characteristics, shaped by oceanic conditions that create specialised niches for microbial communities (Zi *et al.,* 2024). Additionally, the lower human impact in these areas compared to urban wastewater settings might contribute to the reduced ARG diversity (Basili *et al.,* 2021).

Groundwater environments demonstrated considerable variability in microbial diversity, as evidenced by their broader interquartile range. This variability likely results from differences in groundwater depth, flow patterns, and nutrient availability, which collectively influence microbial community structure (Jiang *et al.,* 2021). The dynamic nature of these environments contributes to less stable but still diverse microbial communities, reflecting the complex interplay between physical and chemical factors in shaping microbial populations.

The correlation between microbial species abundance and ARG abundance ($r = 0.6936$, $p < 0.001$) observed in aquatic environments indicates that microbial community structure significantly influences ARG profiles (Figure 14). This coupling is supported by the PLSC model, which shows clear clusters corresponding to varying ARG levels across species. However, the decoupling observed in coastal seawater, where high microbial diversity does not correspond to high ARG diversity, suggests that environmental factors unique to marine settings might limit the spread and maintenance of ARGs (Shu & Huang, 2022).

The PCoA analyses further highlight the distinct patterns of microbial communities and ARG profiles across aquatic biomes. The clustering of freshwater biomes with urban wastewater suggests a major impact of human activities on these environments (Hutinel *et al.,* 2021), while the distinct clustering of marine and coastal seawater indicate unique resistome signatures shaped

by oceanic conditions (Shu & Huang, 2022). The higher percentage of variance captured in the ARG-based PCoA compared to the microbial community PCoA suggests that ARG profiles are shaped by more uniform selective pressures, whereas microbial communities are influenced by a wider range of environmental factors (Shu & Huang, 2022). This could be due to the widespread use of antibiotics and the resulting selection for resistance, leading to more consistent ARG profiles across different environments (Czekalski *et al.,* 2015). Furthermore, microbial interactions such as horizontal gene transfer, which plays a crucial role in the dissemination of ARGs, likely contribute to the distinct resistome compositions observed (Hutinel *et al.,* 2021).

4.2.3 Diversity and drivers of microbial communities and antimicrobial resistance genes in soil environments

Soil environments exhibit diverse microbial communities influenced by various factors such as temperature, moisture, organic matter, and human activities (Li *et al.,* 2018; Yang Y *et al.,* 2023). Markedly, the highest microbial diversity was observed in cold environments (mean Shannon index: 5.63) (Figure 2B), despite thermal conditions that typically limit biodiversity (Margesin & Collins, 2019). The uniformity in diversity across cold soil samples, evidenced by their compact interquartile range, suggests that microbial communities in these environments maintain stable populations despite harsh conditions. Cold environments typically present harsh conditions, including low temperatures and limited nutrient availability, which generally impose major stress on microorganisms (Margesin & Collins, 2019). To survive, microorganisms in these environments have evolved specialised adaptations, such as flexible enzyme structures and efficient nutrient uptake mechanisms, allowing them to persist under these constraints (D'Amico *et al.,* 2006; Margesin & Collins, 2019). Furthermore, the limited anthropogenic interference in these regions, such as reduced urbanisation and soil compaction, likely prevents disruptions that commonly reduce microbial diversity (Kumari *et al.,* 2024). These environments exhibited relatively low ARG diversity (mean Shannon index: 4.05), likely due to the reduced human activity and lower selective pressures, limiting the spread and proliferation of ARGs (Bachran *et al.,* 2019; Vásquez-Dean *et al.,* 2020).

Tropical and subtropical soils displayed high microbial diversity (mean Shannon indices: 5.55 and 5.54, respectively) (Figure 2B). These biomes provide complex and heterogeneous habitats due to their stable temperatures, high humidity and abundant resources, which create numerous

ecological niches (Zeng *et al.,* 2022; Yang N *et al.,* 2023). This environmental heterogeneity supports diverse microbial communities with various metabolic capabilities, allowing them to thrive (Zeng *et al.,* 2022; Yang N *et al.,* 2023). The stable environmental conditions in these regions contribute to the relatively narrow interquartile range observed in the Shannon diversity analysis, indicating consistent microbial community structures. Additionally, substantial plant diversity in these regions drives high microbial diversity (Xu *et al.,* 2023). Tropical and subtropical environments further exhibited high ARG diversity (mean Shannon indices: 4.84 and 5.12, respectively), possibly influenced by rich microbial diversity. Furthermore, agricultural activities in temperate, tropical and subtropical climates, may also introduce antibiotics and pollutants, creating selective pressures that drive ARG proliferation (Stiborova *et al.,* 2021; Cappelli *et al.,* 2022; Delgado-Baquerizo *et al.,* 2022).

Anthropogenic soils, such as those impacted by urbanisation and agriculture, exhibited the lowest microbial diversity, with a mean Shannon index of 4.92. This decrease is largely due to habitat disturbance, homogenisation, and the introduction of pollutants and chemical inputs, which alter natural soil structure and microbial habitats (Osburn *et al.,* 2023). Human activities often lead to soil compaction, reduced organic matter, and changes in pH. Collectively, these factors negatively impact the diversity of microbial communities (Osburn *et al.,* 2023; Kumari *et al.,* 2024). Frequent disturbances and reduced habitat heterogeneity further accelerate the decline in microbial diversity (Osburn *et al.,* 2023). These findings highlight the important impact of human activities on soil microbial communities and underscore the need for sustainable practices to preserve soil biodiversity (Dixit *et al.,* 2024). Despite the low microbial diversity, anthropogenic soils exhibited the highest ARG diversity (mean Shannon index: 5.71) (Figure 10B). This shift underscores the impact of human activities on ARG dissemination. Practices such as the application of manure and wastewater sludge as fertilisers coupled with heavy antibiotic use in agriculture introduce ideal conditions for the introduction and proliferation of ARGs in soil, enriching the resistome (Stiborova *et al.,* 2021). This finding highlights the critical role of anthropogenic activities in driving ARG proliferation in soil environments (Stiborova *et al.,* 2021).

Notably, arid soil environments showed the lowest ARG diversity (mean Shannon index: 3.15), despite relatively high microbial diversity. The narrow interquartile range in the Shannon diversity analysis suggests highly uniform microbial communities, likely adapted to the extreme

environmental stressors. This phenomenon can be attributed to limited water availability and lower organic matter content in arid soils, which likely restrict the spread and proliferation of ARGs (Bachran *et al.,* 2019; Vásquez-Dean *et al.,* 2020). Moisture is a critical factor in the distribution and abundance of ARGs (Kittredge *et al.,* 2022). In moist conditions, microbial cells are more likely to encounter each other and exchange genetic material, thereby increasing the spread of ARGs (Kittredge *et al.,* 2022; Suganya *et al.,* 2023). In dry, arid soils, the physical separation of microbial cells and the reduced mobility of genetic elements likely limit these interactions and consequently the spread of ARGs (Kittredge *et al.,* 2022; Suganya *et al.,* 2023). Furthermore, the scarcity of organic matter reduces microbial activity and the maintenance of ARGs within microbial communities (Delgado-Baquerizo *et al.,* 2022). These findings underscore the key roles that environmental conditions, such as moisture and nutrient availability, play in shaping ARG diversity in soils.

The positive linear correlation ($r = 0.6423$, $p < 0.001$) between microbial species abundance and ARG abundance suggests that soil microbial community structure plays a crucial role in shaping ARG profiles (Figure 15). This correlation is consistent with previous studies showing that diverse microbial communities can harbour a wide range of ARGs (Lu *et al.,* 2022). However, the high ARG diversity in anthropogenic soils despite low microbial diversity suggests that selective pressures from human activities, such as antibiotic use and pollution, play a more prominent role than microbial species richness in driving ARG dissemination in these environments (Stiborova *et al.,* 2021).

The PCoA analyses revealed distinct patterns of microbial communities and ARG profiles across soil biomes (Figure 4B). In the microbial species analysis, anthropogenic samples formed a well-defined and tightly clustered group, likely shaped by consistent human-driven selective pressures, whereas cold biomes displayed more dispersed and heterogeneous microbial community compositions. The considerable overlap between tropical and subtropical biomes suggests closer microbial community relationships, possibly due to similar environmental conditions (Zeng *et al.,* 2022; Yang N *et al.,* 2023). The tighter clustering observed in the ARG-based PCoA, compared to the microbial community PCoA, suggests that ARG profiles are shaped by more uniform and targeted selective pressures, specifically the presence of antibiotics. While microbial communities are shaped by a broad range of environmental factors (Osburn *et al.,* 2023), antibiotics selectively

exert pressure on specific resistance genes, leading to more consistent ARG profiles across environments. In contrast, microbial species diversity is influenced by multiple factors beyond antibiotic presence, resulting in greater variability in community composition across different settings (Czekalski *et al.,* 2015; Delgado-Baquerizo *et al.,* 2022). The higher percentage of variance captured in the ARG-based PCoA (52.39%) compared to the microbial community PCoA (25.27%) supports this, indicating that factors such as antibiotic use and horizontal gene transfer have a more pronounced effect on ARGs than on overall microbial diversity (Hutinel *et al.,* 2021; Zeng *et al.,* 2022).

4.2.4 Contrasting diversity and composition of microbial species and antimicrobial resistance genes in soil and aquatic biomes

The diversity and composition of microbial species and ARG profiles in soil and aquatic environments are shaped by distinct environmental conditions and anthropogenic pressures (Cycoń *et al.,* 2019; Checcucci *et al.,* 2020). Both biomes exhibit similarly high microbial species diversity, as indicated by the Shannon Diversity Index (soil: 5.409, water: 5.410) (Figure 3), despite the vastly different environmental conditions that characterise them.

In soils, microbial diversity is driven by factors such as organic matter content, moisture, and land-use practices such as agriculture and urbanisation (Delgado-Baquerizo *et al.,* 2022). The complex structure of soil creates numerous microhabitats, promoting high microbial diversity (Bach *et al.,* 2018). In contrast, aquatic environments experience dynamic conditions, including fluctuations in water chemistry, temperature, and hydrodynamic forces, which drive the formation of distinct microbial communities in freshwater and marine systems (Stanish *et al.,* 2016; Jiang *et al.,* 2021). Aquatic systems exhibit more variability in microbial composition, which is reflected in the broader clustering patterns observed in the PCoA analyses (Figure 5). These broader clusters indicate the influence of varied environmental conditions and contamination sources, especially in dynamic aquatic ecosystems (Pei *et al.,* 2006; Häder *et al.,* 2020).

In addition to the species-level diversity, the phylum-level analysis revealed distinct compositional patterns that further highlight the structural differences between soil and aquatic microbial communities (Figure 6). This differential distribution at the phylum level represents a fundamental distinction in how these environments maintain their similar species-level diversity. The broader phylum diversity in aquatic environments, coupled with more even distribution of abundant

populations across these phyla, suggests that aquatic ecosystems achieve their species diversity through multiple distinct evolutionary lineages. This strategy may enhance ecosystem resilience by maintaining diverse metabolic capabilities and adaptation potential across different taxonomic groups. In contrast, soil environments achieve comparable species diversity through greater specialisation within a more restricted set of phyla, as evidenced by the network analysis (Figure 7). This pattern suggests that soil environments may favour deeper evolutionary adaptation within successful phylogenetic groups, rather than maintaining diversity across broader taxonomic lines. These contrasting strategies for maintaining species diversity likely reflect the fundamental differences in environmental pressures and resource availability between soil and aquatic systems.

Although both biomes support similar levels of microbial species diversity, soil environments demonstrate higher ARG diversity (Figure 11). This is likely due to the persistent selective pressures in soils, driven by long-term contamination from agriculture, manure application, and antibiotic use, which promote horizontal gene transfer and ARG maintenance (Bach *et al.,* 2018; Checcucci *et al.,* 2020). The heterogeneous structure of soils further facilitates microbial interactions, promoting ARG dissemination (Bach *et al.,* 2018). In contrast, the dynamic nature of aquatic environments, including the dilution and dispersion of contaminants, leads to greater variability in ARG composition, reflected in the broader distribution of ARG diversity observed in aquatic systems (Pei *et al.,* 2006; Luo *et al.,* 2020).

Notably, anthropogenic soils exhibit the highest ARG diversity (Shannon index: 5.71), a pattern that underscores the substantial impact of human activities, particularly intensive agriculture and urban waste management, on soil resistomes (Stiborova *et al.,* 2021). While urban wastewater in aquatic environments also shows elevated ARG diversity (Shannon index: 4.28), the resistome in soil environments is far more diverse. This difference highlights the greater persistence of antibiotics and pollutants in soil, where ARGs can accumulate and persist due to prolonged exposure to contaminants (Checcucci *et al.,* 2020), compared to the more transient exposure seen in aquatic systems (Pei *et al.,* 2006).

4.2.5 Comparative dynamics of core and accessory resistomes in soil and aquatic environments

Comparative analysis of the core resistomes between soil and aquatic environments reveals substantial differences, reflecting the distinct ecological pressures and human impacts on these

biomes (Figure 20). The larger core resistome in soil (179 ARGs) compared to aquatic environments (113 ARGs) suggests a more extensive and stable presence of ARGs across various soil environments.

The broader presence of core ARGs in soil compared to aquatic environments can be attributed to several inherent characteristics of soil. The heterogeneous structure of soil creates diverse microhabitats that facilitate close microbial interactions (Bach *et al.,* 2018). This is supported by the fact that soil environments enable more direct contact between microorganisms, enhancing the potential for horizontal gene exchange of ARGs (Bach *et al.,* 2018; Cycoń *et al.,* 2019). Furthermore, the adsorption of antibiotics onto soil particles creates persistent hotspots of selective pressure, potentially maintaining and stabilising the resistome over time (Checcucci *et al.,* 2020).

In both environments, multidrug resistance (MDR) genes, particularly those involved in efflux systems such as *mexF*, *mexW*, and *acrB*, demonstrate high prevalence, being found in over 90% of samples (Figure 17, 19). These efflux pumps are essential for bacterial survival under diverse antibiotic pressures introduced by human activities (Alcalde-Rico *et al.,* 2016). The *mexF* and *mexW* genes, components of clinically relevant MDR efflux systems (Morita *et al.,* 2012), are particularly prevalent in soil environments, present in over 97% of samples. Additionally, the *ompR* gene, which forms part of a two-component regulatory system involved in antibiotic resistance regulation (Kenney & Anand, 2020), is present in over 90% of aquatic samples, indicating consistent exposure to antibiotic pressures (Barathe *et al.,* 2024).

Agricultural practices, such as the application of manure and wastewater sludge, introduce high loads of ARGs and antibiotics into soils, further reinforcing selective pressures and enhancing the stability of ARGs within soil environments (Stiborova *et al.,* 2021). This is particularly evident in the prevalence of genes conferring resistance to antibiotics commonly used in agriculture. For instance, tetracycline resistance genes *tetP* and *otrA* are highly prevalent in aquatic environments (91.9% and 90.5% respectively), reflecting the persistent use of tetracyclines in aquaculture and agriculture (Nelson & Levy, 2011; Amangelsin *et al.,* 2023). Similarly, in soil environments, beta-lactam resistance genes such as *pbp-1a* and *oxa* show high prevalence, likely due to the presence of beta-lactams in animal waste products used as fertilisers (Udikovic-Kolic *et al.,* 2014). Moreover, the soil core resistome shows distinctive patterns in glycopeptide resistance, particularly through *vanR*, which regulates vancomycin resistance (Hong *et al.,* 2008). This

prevalence reflects the widespread use of glycopeptide antibiotics in agricultural settings (Nilsson, 2012). Similarly, the nearly universal presence (98.8%) of *rosA* and *bacA* genes, mediating resistance to fosmidomycin and bacitracin respectively (Ghachi *et al.,* 2004), indicates the persistent impact of veterinary antibiotics and growth promoters in soil environments (Pérez *et al.,* 2014; Phillips, 1999).

In contrast, aquatic environments are more dynamic and experience greater dilution and dispersion of contaminants, leading to lower local concentrations of antibiotics and ARGs (Pei *et al.,* 2006). This reduces the selective pressure for resistance and the stability of the resistome in water (Pei *et al.,* 2006). However, certain resistance genes maintain high prevalence in aquatic environments. For example, the *macB* gene, conferring resistance to macrolides, lincosamides, and streptogramins (MLS), is found in 97.3% of aquatic samples. Additionally, *mupA*, conferring resistance to mupirocin and typically associated with MRSA treatment, is detected in 92.4% of aquatic samples, suggesting contamination from clinical settings where this antibiotic is routinely used (Antonov *et al.,* 2015).

Notably, 101 core ARGs are shared between soil and aquatic environments (Figure 20), highlighting the extent of overlap in resistance profiles. This overlap is likely due to the movement of ARGs between these environments, driven by wastewater runoff, irrigation, and the application of sludge, which transport ARGs from aquatic systems into soil (Rizzo *et al.,* 2013; Kodešová *et al.,* 2024). These shared ARGs suggest that the resistomes of both biomes are shaped, at least in part, by common anthropogenic factors and that the interconnectedness of these environments plays a role in the persistence and distribution of ARGs (Kodešová *et al.,* 2024).

However, each environment also harbours unique resistance genes, emphasising the distinct selective pressures that operate in each biome. Soil environments, in particular, exhibit a higher prevalence of MDR genes, with 35 core MDR genes that are unique to soil compared to aquatic settings, a difference likely driven by the extensive use of antibiotics in agriculture and the consequent selection for multidrug-resistant bacteria (Forsberg *et al.,* 2012; Alcalde-Rico *et al.,* 2016; Manyi-Loh *et al.,* 2018). These MDR genes play a critical role in sustaining high levels of resistance in soil environments (Forsberg *et al.,* 2012), highlighting the role of soil as a reservoir for ARGs that could be transferred to other environments.

The accessory resistome provides insights into more localised or emerging resistance patterns (Figures 21-24). In both soil and aquatic settings, anthropogenic activities contribute to a higher diversity of accessory ARGs, which include substantial proportions of genes conferring resistance to aminoglycosides, beta-lactams, tetracyclines, phenicol, and fluoroquinolones. For instance, in aquatic environments, MDR genes dominate the accessory resistome (33.2% of samples), particularly in surface freshwater and groundwater, indicating antibiotic pollution in these environments (Häder *et al.,* 2020). Similarly, beta-lactam resistance genes are prevalent (12.7%), especially in surface freshwater, possibly reflecting exposure to healthcare runoff and agricultural waste (Sta Ana *et al.,* 2021).

The presence of these genes in the accessory resistomes highlights the potential for emerging resistance driven by ongoing selective pressures (Rizzo *et al.,* 2013; Delgado-Baquerizo *et al.,* 2022). Despite the differences in the core and accessory resistomes, the trends observed in the accessory resistomes mirror those observed in the core resistomes. That is, ARGs conferring multidrug resistance or resistance to antibiotics such as MLS, glycopeptides, tetracyclines and beta-lactams remain highly prevalent within both the core and accessory resistomes. This suggests that similar selective pressures shape both the core and accessory resistomes.

4.2.6 Distribution and significance of ESKAPE pathogens in soil and aquatic biomes

The distribution of ESKAPE pathogens across soil and aquatic biomes reveals notable patterns influenced by both environmental conditions and anthropogenic activities (Figures 8, 9). These pathogens, known for their multidrug resistance, capacity to disseminate across diverse environmental and clinical settings and ability to cause serious nosocomial infections, pose a substantial threat to public health (Santajit & Indrawattana, 2016). The widespread presence of ESKAPE pathogens in both soil and aquatic environments underscores the critical need for effective management strategies to mitigate their spread and impact.

In soil environments, temperate biomes exhibit the highest proportion of ESKAPE pathogens (8.23%), predominantly driven by *Klebsiella pneumoniae* (5.17%) and *Staphylococcus aureus* (2.84%). This could be explained by the extensive agricultural and urban activities (such as construction and anthropogenic movement into these areas) in temperate regions, which facilitate the dissemination and proliferation of these pathogens (Barnes & Raymond, 2009; Manyi-Loh *et al.,* 2018; Denissen *et al.,* 2022). Contaminated fertilisers and wastewater used in agriculture

introduce substantial amounts of these pathogens into the soil, where they can persist and spread (Muurinen *et al.,* 2017). The substantial presence of *Enterobacter* species (2.65%) in anthropogenic soils highlights the impact of human activities as these species are commonly associated with urban environments and wastewater, underscoring the role of urban runoff and sewage sludge in pathogen dissemination (Moretto *et al.,* 2021; Abedinzadeh *et al.,* 2023).

Remote soil biomes show markedly lower ESKAPE pathogen levels, suggesting human activity as a key driver of pathogen abundance. For instance, cold soils contain 0.66% total ESKAPE proportion, with *Staphylococcus aureus* making up 0.47%, while tropical soils show just 0.26% total proportion, with *Klebsiella pneumoniae* at 0.13%. These lower proportions in less disturbed environments serve as a baseline for understanding human impact on pathogen distribution

In aquatic environments, urban wastewater (3.14%), groundwater (8.32%), and surface freshwater (4.81%) exhibit the highest proportions of ESKAPE pathogens, dominated by *Staphylococcus aureus* (5.40% in groundwater, 1.95% in urban wastewater, and 3.25% in surface freshwater) and *Klebsiella pneumoniae* (2.45% in groundwater, 0.73% in urban wastewater, and 1.28% in surface freshwater). WWTPs act as reservoirs and sources of ARGs and pathogens, receiving a mixture of domestic, industrial, and hospital wastewater containing high loads of antibiotics, resistant bacteria, and ARGs (Pazda *et al.,* 2019; Qin *et al.,* 2020). Despite treatment processes, substantial amounts of these contaminants can be discharged into the environment, contributing to the spread of resistance in downstream water bodies (Rizzo *et al.,* 2013).

The large proportions of ESKAPE pathogens in groundwater and surface freshwater are possibly due to contaminated runoff and effluent discharge from WWTPs. These findings are supported by the co-clustering of these biomes with urban wastewater in the aquatic microbial species PCoA analysis (Figure 4A). The presence of *Staphylococcus aureus* and *Klebsiella pneumoniae* in these environments reflects their adaptability to diverse conditions and their ability to persist (Denissen *et al.,* 2022; Venkateswaran *et al.,* 2023). Coastal and marine seawater show lower proportions of ESKAPE pathogens (1.14% and less), indicating different environmental pressures and possibly lower anthropogenic influence compared to freshwater and urban wastewater environments (Denissen *et al.,* 2022). Surface saline water exhibits the lowest proportions of ESKAPE species (0.09%), suggesting that extreme environmental pressures may limit the proliferation of these pathogens (Shu & Huang, 2022). However, the presence of these pathogens in marine

environments, albeit in low abundance, indicates their ability for long-range dispersal and successful establishment across various aquatic settings.

The consistent presence of ESKAPE pathogens in both soil and aquatic environments demonstrates their ability to persist and proliferate across diverse biomes (Denissen *et al.,* 2022). The substantially higher concentrations in urban wastewater, groundwater, surface freshwater, and anthropogenic soils point to human contamination as the primary driver of their distribution (Denissen *et al.,* 2022; Aguilar-Salazar *et al.,* 2023). This widespread distribution poses major risks to environmental and public health, as these pathogens can act as sources of AMR dissemination to other environments and ultimately to humans (Santajit & Indrawattana, 2016). The dominance of *Staphylococcus aureus* and *Klebsiella pneumoniae* in both settings aligns with their established prevalence in both clinical and non-clinical environments, highlighting their adaptability and potential for widespread dissemination (Aguilar-Salazar *et al.,* 2023; Venkateswaran *et al.,* 2023).

Chapter 5: Conclusions and recommendations

5.1 Limitations and future recommendations

While this study provides a comprehensive analysis of AMR across diverse global environments, several limitations must be acknowledged. First, the use of publicly available metagenomes, while beneficial for expanding the scope of the study, presents challenges in standardisation. The variability in sequencing strategies across different datasets introduces potential biases (Bengtsson-Palme *et al.,* 2017), even though data normalisation methods were applied. The normalisation methods may not fully account for differences in sequencing depth, library preparation, or the platforms used, which could influence the observed patterns in ARG and microbial taxa distributions (Bengtsson-Palme *et al.,* 2017). Furthermore, bioinformatic pipeline choices, particularly in genome assembly and ARG annotation, may impact the detection and classification of resistance genes, with potential limitations in identifying novel or divergent sequences (Hendriksen *et al.,* 2019).

Another notable limitation is the lack of time-controlled sampling. AMR is a dynamic phenomenon, with resistance profiles that can shift substantially over time (Javvadi & Mohan, 2024). The metagenomes used in this study were collected at different times and in different years. Without any temporal control, the findings represent a snapshot rather than a continuous or evolving picture of AMR. This temporal variability may obscure trends and lead to an underestimation or overestimation of resistance levels in certain environments. Additionally, the DNA-based metagenomic approach cannot capture the actual expression levels of resistance genes or their phenotypic manifestation in environmental communities (Waskito *et al.,* 2022).

Finally, while the study covers a broad range of biomes, it does not equally represent all possible environments where AMR may emerge or persist. This work encompasses a diverse range of soil and aquatic biomes, however, certain environments, particularly those less studied or harder to access, may harbour unique resistomes that are not reflected in this analysis. The uneven geographic distribution of samples, with some regions being underrepresented, could mask important regional patterns in AMR dissemination (Djordjevic *et al.,* 2024).

Future studies should address these limitations through time-controlled sampling approaches to capture temporal dynamics of resistance gene spread. The integration of metatranscriptomics with metagenomic data could provide insights into ARG expression patterns and functional resistance mechanisms (Waskito *et al.,* 2022). Systematic sampling across underrepresented regions and

biomes, particularly in developing nations where AMR poses a major threat, would enhance our understanding of global resistance patterns. Additionally, the development of standardised bioinformatic pipelines specifically optimised for environmental resistome analysis would improve comparability across studies (Hendriksen *et al.,* 2019).

5.2 Summary

This study provides a comprehensive analysis of ARGs across diverse soil and aquatic environments on a global scale. By examining 1,758 metagenomes, this work highlights the pervasive nature of AMR beyond clinical settings, emphasising the crucial roles that various environments and human activities play in shaping resistance profiles.

The findings reveal that soil environments, particularly those impacted by agriculture and urbanisation, harbour a more extensive core resistome compared to aquatic environments. This suggests that soil acts as a stable and abundant reservoir of resistance genes, likely due to persistent selective pressures from practices such as the application of manure, wastewater sludge, and antibiotics in agriculture. These activities enrich the soil resistome and promote the proliferation of ARGs, including multidrug resistance genes, which can persist and spread through environmental pathways.

In human-impacted soils, a dramatic reduction in microbial diversity was observed alongside an abundance of ESKAPE pathogens. This reduction in native microbial diversity may create ecological niches that allow harmful microorganisms to proliferate. The loss of beneficial microbial communities could disrupt natural competition enabling the proliferation of ESKAPE pathogens and leading to increased risks to environmental and public health.

Similar to soil environments, aquatic environments, especially urban wastewater, emerge as major hotspots for both microbial and ARG diversity. The high diversity observed is due to the influx of microorganisms and contaminants from various human sources, including domestic, industrial, and hospital effluents. Wastewater treatment plants, despite their role in mitigating pollution, can act as reservoirs and conduits for the dissemination of ARGs and ESKAPE pathogens into downstream ecosystems. The spill-over into groundwater and surface freshwater systems is particularly concerning, with these environments demonstrating the highest presence of ESKAPE pathogens. This linkage underscores the interconnectedness of water systems and highlights how contaminants from urban wastewater can affect broader aquatic environments.

The metagenomic insights provided in this study highlight the necessity of broadening AMR surveillance beyond clinical settings to include diverse environmental reservoirs. Focusing solely on healthcare environments overlooks sources of resistance genes and pathogens that contribute to the global AMR crisis. By adopting integrated approaches that consider the interconnectedness of different ecosystems, a more comprehensive understanding of AMR dissemination can be achieved. This facilitates the development of more effective strategies to combat its spread, taking into account the various pathways through which resistance genes and pathogens can move between environments.

Implementing sustainable practices is crucial in reducing anthropogenic impacts on environmental resistomes. Enhancing wastewater treatment technologies with additional filtration steps can help prevent the dissemination of ARGs and pathogens into water systems, particularly from hospital and industrial effluents where ESKAPE pathogens are abundant. In agriculture, promoting judicious antibiotic use through clear guidelines and monitoring systems, alongside proper treatment of organic waste before land application, can reduce the selective pressures driving resistance. These measures require coordinated policy frameworks that recognise the interconnected nature of soil and water systems in ARG spread. Regular environmental monitoring using standardised protocols is essential for tracking resistance patterns and evaluating the effectiveness of interventions. Such a systematic approach to environmental management, supported by evidence-based policies, would help mitigate the enrichment of resistomes in both soil and aquatic environments, helping to mitigate the AMR threat.

In conclusion, this study advances the understanding of the pervasive nature of AMR and the roles that environmental reservoirs and human activities play in its global dissemination. Recognising the importance of various environments—beyond clinical settings—in the evolution and spread of AMR is imperative. The findings emphasise the urgency of addressing AMR through coordinated efforts that encompass multiple sectors and disciplines. Future research should involve temporal monitoring to observe changes in resistome compositions and investigate the mechanisms underlying ARG dissemination across different environments. Such efforts will not only be instrumental in informing public health strategies but also in guiding the prudent use of antibiotics by identifying resistome profiles that predict antibiotic efficacy and resistance risks, ultimately contributing to a more effective response to this pressing global health challenge.

6. References

Abedinzadeh, A., Falahi Charkhabi, N., Aeini, M., & Amani, M. (2023). *Enterobacter* species, emerging plant-pathogenic bacteria, associated with decline and offshoot rot of date palm in Iran. European Journal of Plant Pathology. https://doi.org/10.1007/s10658-023-02666-3

Abraham, J. J. (1948). Some account of the history of the treatment of syphilis. British Journal of Venereal Diseases, 24(4), 153–160. https://doi.org/10.1136/sti.24.4.153

Aguilar-Salazar, A., Martínez-Vázquez, A. V., Aguilera-Arreola, G., de Jesus de Luna-Santillana, E., Cruz-Hernández, M. A., Escobedo-Bonilla, C. M., Lara-Ramírez, E., Sánchez-Sánchez, M., Guerrero, A., Rivera, G., & Bocanegra-Garcia, V. (2023). Prevalence of ESKAPE bacteria in surface water and wastewater sources: Multidrug resistance and molecular characterization, an updated review. Water, 15(18), Article 18. https://doi.org/10.3390/w15183200

Aminov, R. I. (2010). A brief history of the antibiotic era: Lessons learned and challenges for the future. Frontiers in Microbiology, 1, 134. https://doi.org/10.3389/fmicb.2010.00134

Anjum, M. F., Zankari, E., & Hasman, H. (2017). Molecular methods for detection of antimicrobial resistance. Microbiology Spectrum, 5(6), 10.1128/microbiolspec.arba-0011–2017. https://doi.org/10.1128/microbiolspec.arba-0011-2017

Arango-Argoty, G., Garner, E., Pruden, A., Heath, L. S., Vikesland, P., & Zhang, L. (2018). DeepARG: A deep learning approach for predicting antibiotic resistance genes from metagenomic data. Microbiome, 6(1), 23. https://doi.org/10.1186/s40168-018-0401-z

Arias, C. A., & Murray, B. E. (2012). The rise of the *Enterococcus*: Beyond vancomycin resistance. *Nature Reviews. Microbiology*, *10*(4), 266–278. https://doi.org/10.1038/nrmicro2761

Armstrong, G. L., Conn, L. A., & Pinner, R. W. (1999). Trends in infectious disease mortality in the United States during the 20th century. JAMA, 281(1), 61–66. https://doi.org/10.1001/jama.281.1.61

Bach, E. M., Williams, R. J., Hargreaves, S. K., Yang, F., & Hofmockel, K. S. (2018). Greatest soil microbial diversity found in micro-habitats. Soil Biology and Biochemistry, 118, 217–226. https://doi.org/10.1016/j.soilbio.2017.12.018

Bachran, M., Kluge, S., Lopez-Fernandez, M., & Cherkouk, A. (2019). Microbial diversity in an arid, naturally saline environment. Microbial Ecology, 78(2), 494–505. https://doi.org/10.1007/s00248-018-1301-2

Ballhausen, B., Kriegeskorte, A., Schleimer, N., Peters, G., & Becker, K. (2014). The *mecA* homolog *mecC* confers resistance against β-Lactams in *Staphylococcus aureus* irrespective of the genetic strain background. *Antimicrobial Agents and Chemotherapy*, *58*(7), 3791–3798. https://doi.org/10.1128/AAC.02731-13

Barber, M., & Rozwadowska-Dowzenko, M. (1948). Infection by penicillin-resistant staphylococci. The Lancet, 252(6530), 641–644. https://doi.org/10.1016/S0140-6736(48)92166-7

Barnes, R. T., & Raymond, P. A. (2009). The contribution of agricultural and urban activities to inorganic carbon fluxes within temperate watersheds. Chemical Geology, 266(3), 318–327. https://doi.org/10.1016/j.chemgeo.2009.06.018

Basili, M., Campanelli, A., Frapiccini, E., Luna, G. M., & Quero, G. M. (2021). Occurrence and distribution of microbial pollutants in coastal areas of the Adriatic Sea influenced by river discharge. Environmental Pollution, 285, 117672. https://doi.org/10.1016/j.envpol.2021.117672

Bassetti, M., Pecori, D., Sibani, M., Corcione, S., & De Rosa, F. G. (2015). Epidemiology and treatment of MDR Enterobacteriaceae. Current Treatment Options in Infectious Diseases, 7(4), 291–316. https://doi.org/10.1007/s40506-015-0065-1

Batchelor, M., Hopkins, K. L., Liebana, E., Slickers, P., Ehricht, R., Mafura, M., Aarestrup, F., Mevius, D., Clifton-Hadley, F. A., Woodward, M. J., Davies, R. H., Threlfall, E. J., & Anjum, M. F. (2008). Development of a miniaturised microarray-based assay for the rapid identification of antimicrobial resistance genes in Gram-negative bacteria. International Journal of Antimicrobial Agents, 31(5), 440–451. https://doi.org/10.1016/j.ijantimicag.2007.11.017

Bauer, A. W., Kirby, W. M., Sherris, J. C., & Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology, 45(4), 493–496. https://doi.org/10.1128/aac.1.6.451

Bengtsson-Palme, J., Larsson, D. G. J., & Kristiansson, E. (2017). Using metagenomics to investigate human and environmental resistomes. Journal of Antimicrobial Chemotherapy, 72(10), 2690–2703. https://doi.org/10.1093/jac/dkx199

Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: A practical and powerful approach to multiple testing. Journal of the Royal Statistical Society. Series B (Methodological), 57(1), 289–300. http://www.jstor.org/stable/2346101

Blaskovich, M. A. T., Hansford, K. A., Butler, M. S., Jia, Z., Mark, A. E., & Cooper, M. A. (2018). Developments in glycopeptide antibiotics. ACS Infectious Diseases, 4(5), 715–735. https://doi.org/10.1021/acsinfecdis.7b00258

Bray, J. R., & Curtis, J. T. (1957). An ordination of the upland forest communities of southern wisconsin. Ecological Monographs, 27(4), 325–349. https://doi.org/10.2307/1942268

Bueno, I., Beaudoin, A., Arnold, W. A., Kim, T., Frankson, L. E., LaPara, T. M., Kanankege, K., Wammer, K. H., & Singer, R. S. (2021). Quantifying and predicting antimicrobials and antimicrobial resistance genes in waterbodies through a holistic approach: A study in Minnesota, United States. Scientific Reports, 11(1), Article 1. https://doi.org/10.1038/s41598-021-98300-5

Cappelli, S. L., Domeignoz-Horta, L. A., Loaiza, V., & Laine, A.-L. (2022). Plant biodiversity promotes sustainable agriculture directly and via belowground effects. Trends in Plant Science, 27(7), 674–687. https://doi.org/10.1016/j.tplants.2022.02.003

Cason, C., D'Accolti, M., Soffritti, I., Mazzacane, S., Comar, M., & Caselli, E. (2022). Next-generation sequencing and PCR technologies in monitoring the hospital microbiome and its drug resistance. Frontiers in Microbiology, 13. https://doi.org/10.3389/fmicb.2022.969863

Checcucci, A., Trevisi, P., Luise, D., Modesto, M., Blasioli, S., Braschi, I., & Mattarelli, P. (2020). Exploring the animal waste resistome: The spread of antimicrobial resistance genes through the use of livestock manure. Frontiers in Microbiology, 11. https://doi.org/10.3389/fmicb.2020.01416

Chopra, I., Hesse, L., & O'Neill, A. (2002). Discovery and development of new anti-bacterial drugs. In H. van der Goot (Ed.), Pharmacochemistry Library (Vol. 32, pp. 213–225). Elsevier. https://doi.org/10.1016/S0165-7208(02)80022-8

Cuadrat, R. R. C., Sorokina, M., Andrade, B. G., Goris, T., & Dávila, A. M. R. (2020). Global ocean resistome revealed: Exploring antibiotic resistance gene abundance and distribution in TARA Oceans samples. GigaScience, 9(5), giaa046. https://doi.org/10.1093/gigascience/giaa046

Cui, L., & Su, X. (2009). Discovery, mechanisms of action and combination therapy of artemisinin. Expert Review of Anti-Infective Therapy, 7(8), 999–1013. https://doi.org/10.1586/eri.09.68

Cycoń, M., Mrozik, A., & Piotrowska-Seget, Z. (2019). Antibiotics in the soil environment—degradation and their impact on microbial activity and diversity. Frontiers in Microbiology, 10. https://doi.org/10.3389/fmicb.2019.00338

Czekalski, N., Sigdel, R., Birtel, J., Matthews, B., & Bürgmann, H. (2015). Does human activity impact the natural antibiotic resistance background? Abundance of antibiotic resistance genes in 21 Swiss lakes. Environment International, 81, 45–55. https://doi.org/10.1016/j.envint.2015.04.005

Dadgostar, P. (2019). Antimicrobial resistance: implications and costs. Infection and Drug Resistance, 12, 3903–3910. https://doi.org/10.2147/IDR.S234610

D'Amico, S., Collins, T., Marx, J., Feller, G., Gerday, C., & Gerday, C. (2006). Psychrophilic microorganisms: Challenges for life. EMBO Reports, 7(4), 385–389. https://doi.org/10.1038/sj.embor.7400662

Danko, D., Bezdan, D., Afshinnekoo, E., Ahsanuddin, S., Bhattacharya, C., Butler, D. J., Chng, K. R., Donnellan, D., Hecht, J., Jackson, K., Kuchin, K., Karasikov, M., Lyons, A., Mak, L., Meleshko, D., Mustafa, H., Mutai, B., Neches, R. Y., Ng, A., … Consortium, T. I. M. (2020). Global genetic cartography of urban metagenomes and anti-microbial resistance (p. 724526). bioRxiv. https://doi.org/10.1101/724526

Deekshit, V. K., & Srikumar, S. (2022). 'To be, or not to be'—The dilemma of 'silent' antimicrobial resistance genes in bacteria. Journal of Applied Microbiology, 133(5), 2902–2914. https://doi.org/10.1111/jam.15738

Delgado-Baquerizo, M., Hu, H.-W., Maestre, F. T., Guerra, C. A., Eisenhauer, N., Eldridge, D. J., Zhu, Y.-G., Chen, Q.-L., Trivedi, P., Du, S., Makhalanyane, T. P., Verma, J. P., Gozalo, B., Ochoa, V., Asensio, S., Wang, L., Zaady, E., Illán, J. G., Siebe, C., … He, J.-Z. (2022). The global

distribution and environmental drivers of the soil antibiotic resistome. Microbiome, 10(1), 219. https://doi.org/10.1186/s40168-022-01405-w

Denissen, J., Reyneke, B., Waso-Reyneke, M., Havenga, B., Barnard, T., Khan, S., & Khan, W. (2022). Prevalence of ESKAPE pathogens in the environment: Antibiotic resistance status, community-acquired infection and risk to human health. International Journal of Hygiene and Environmental Health, 244, 114006. https://doi.org/10.1016/j.ijheh.2022.114006

diCenzo, G. C., & Finan, T. M. (2017). The divided bacterial genome: Structure, function, and evolution. Microbiology and Molecular Biology Reviews : MMBR, 81(3), e00019-17. https://doi.org/10.1128/MMBR.00019-17

Dinos, G. P. (2017). The macrolide antibiotic renaissance. British Journal of Pharmacology, 174(18), 2967–2983. https://doi.org/10.1111/bph.13936

Dixit, M., Ghoshal, D., Lal Meena, A., Ghasal, P. C., Rai, A. K., Choudhary, J., & Dutta, D. (2024). Changes in soil microbial diversity under present land degradation scenario. Total Environment Advances, 10, 200104. https://doi.org/10.1016/j.teadva.2024.200104

Djordjevic, S. P., Jarocki, V. M., Seemann, T., Cummins, M. L., Watt, A. E., Drigo, B., Wyrsch, E. R., Reid, C. J., Donner, E., & Howden, B. P. (2024). Genomic surveillance for antimicrobial resistance—A One Health perspective. Nature Reviews Genetics, 25(2), Article 2. https://doi.org/10.1038/s41576-023-00649-y

Doi, Y., Wachino, J., & Arakawa, Y. (2016). Aminoglycoside resistance. Infectious Disease Clinics of North America, 30(2), 523–537. https://doi.org/10.1016/j.idc.2016.02.011

Falagas, M. E., & Karageorgopoulos, D. E. (2008). Pandrug resistance (PDR), extensive drug resistance (XDR), and multidrug resistance (MDR) among gram-negative bacilli: Need for international harmonization in terminology. Clinical Infectious Diseases, 46(7), 1121–1122. https://doi.org/10.1086/528867

Ferrer, M., Méndez-García, C., Bargiela, R., Chow, J., Alonso, S., García-Moyano, A., Bjerga, G. E. K., Steen, I. H., Schwabe, T., Blom, C., Vester, J., Weckbecker, A., Shahgaldian, P., de Carvalho, C. C. C. R., Meskys, R., Zanaroli, G., Glöckner, F. O., Fernández-Guerra, A., Thambisetty, S., … The INMARE Consortium. (2019). Decoding the ocean's microbiological secrets for marine

enzyme biodiscovery. *FEMS Microbiology Letters*, *366*(1), fny285. https://doi.org/10.1093/femsle/fny285

Fisher, R. A. (1915). Frequency distribution of the values of the correlation coefficient in samples from an indefinitely large population. Biometrika, 10(4), 507–521. https://doi.org/10.2307/2331838

Forbes, G. B. (1949). Infection with penicillin-resistant staphylococci In hospital and general practice. The British Medical Journal, 2(4627), 569–571. https://doi.org/10.1136/bmj.2.4627.569

Forsberg, K. J., Reyes, A., Wang, B., Selleck, E. M., Sommer, M. O. A., & Dantas, G. (2012). The shared antibiotic resistome of soil bacteria and human pathogens. Science (New York, N.Y.), 337(6098), 1107–1111. https://doi.org/10.1126/science.1220761

Fruchterman, T. M. J., & Reingold, E. M. (1991). Graph drawing by force-directed placement. Software: Practice and Experience, 21(11), 1129–1164. https://doi.org/10.1002/spe.4380211102

Frye, J. G., Lindsey, R. L., Rondeau, G., Porwollik, S., Long, F., McClelland, M., Jackson, C. R., Englen, M. D., Meinersmann, R. J., Berrang, M. E., Davis, J. A., Barrett, J. B., Turpin, J. B., Thitaram, S. N., & Fedorka-Cray, P. J. (2010). Development of a DNA microarray to detect antimicrobial resistance genes identified in the national center for biotechnology information database. Microbial Drug Resistance, 16(1), 9–19. https://doi.org/10.1089/mdr.2009.0082

Galhano, B. S. P., Ferrari, R. G., Panzenhagen, P., de Jesus, A. C. S., & Conte-Junior, C. A. (2021). Antimicrobial resistance gene detection methods for bacteria in animal-based goods: A brief review of highlights and advantages. Microorganisms, 9(5), 923. https://doi.org/10.3390/microorganisms9050923

Gavan, T. L., & Town, M. A. (1970). A microdilution method for antibiotic susceptibility testing: An evaluation. American Journal of Clinical Pathology, 53(6), 880–885. https://doi.org/10.1093/ajcp/53.6.880

Gaynes, R. (2017). The discovery of penicillin—new insights after more than 75 years of clinical use. Emerging Infectious Diseases, 23(5), 849–853. https://doi.org/10.3201/eid2305.161556

Gower, J. C. (1966). Some distance properties of latent root and vector methods used in multivariate analysis. Biometrika, 53(3/4), 325–338. https://doi.org/10.2307/2333639

Gruger, T., Nitiss, J. L., Maxwell, A., Zechiedrich, E. L., Heisig, P., Seeber, S., Pommier, Y., & Strumberg, D. (2004). A mutation in *Escherichia coli* DNA gyrase conferring quinolone resistance results in sensitivity to drugs targeting eukaryotic topoisomerase II. Antimicrobial Agents and Chemotherapy, 48(12), 4495–4504. https://doi.org/10.1128/AAC.48.12.4495-4504.2004

Häder, D.-P., Banaszak, A. T., Villafañe, V. E., Narvarte, M. A., González, R. A., & Helbling, E. W. (2020). Anthropogenic pollution of aquatic ecosystems: Emerging problems with global implications. Science of The Total Environment, 713, 136586. https://doi.org/10.1016/j.scitotenv.2020.136586

Harris, C. R., Millman, K. J., van der Walt, S. J., Gommers, R., Virtanen, P., Cournapeau, D., Wieser, E., Taylor, J., Berg, S., Smith, N. J., Kern, R., Picus, M., Hoyer, S., van Kerkwijk, M. H., Brett, M., Haldane, A., del Río, J. F., Wiebe, M., Peterson, P., … Oliphant, T. E. (2020). Array programming with NumPy. Nature, 585(7825), 357–362. https://doi.org/10.1038/s41586-020-2649-2

Havan, M., Kendirli, T., Parlar, Ö. T., Özcan, S., Yahşi, A., Erat, T., Öcal, D., Guriz, H., Özdemir, H., Karahan, Z. C., Çiftci, E., & İnce, E. (2023). Clinical management of a pandrug-resistant OXA-48 klebsiella pneumoniae infection in the pediatric intensive care unit. Microbial Drug Resistance, 29(6), 256–262. https://doi.org/10.1089/mdr.2022.0247

Hellewell, J., Horsfield, S. T., von Wachsmann, J., Gurbich, T. A., Finn, R. D., Iqbal, Z., Roberts, L. W., & Lees, J. A. (2024). CELEBRIMBOR: Core and accessory genes from metagenomes. *Bioinformatics*, *40*(9), btae542. https://doi.org/10.1093/bioinformatics/btae542

Hemamalini, N., Shanmugam, S. A., Kathirvelpandian, A., Deepak, A., Kaliyamurthi, V., & Suresh, E. (2022). A critical review on the antimicrobial resistance, antibiotic residue and metagenomics-assisted antimicrobial resistance gene detection in freshwater aquaculture environment. Aquaculture Research, 53(2), 344–366. https://doi.org/10.1111/are.15601

Hendriksen, R. S., Munk, P., Njage, P., van Bunnik, B., McNally, L., Lukjancenko, O., Röder, T., Nieuwenhuijse, D., Pedersen, S. K., Kjeldgaard, J., Kaas, R. S., Clausen, P. T. L. C., Vogt, J. K., Leekitcharoenphon, P., van de Schans, M. G. M., Zuidema, T., de Roda Husman, A. M., Rasmussen, S., Petersen, B., … Aarestrup, F. M. (2019). Global monitoring of antimicrobial

resistance based on metagenomics analyses of urban sewage. Nature Communications, 10(1), Article 1. https://doi.org/10.1038/s41467-019-08853-3

Hendriksen, R. S., Bortolaia, V., Tate, H., Tyson, G. H., Aarestrup, F. M., & McDermott, P. F. (2019). Using genomics to track global antimicrobial resistance. *Frontiers in Public Health*, *7*. https://doi.org/10.3389/fpubh.2019.00242

Hewitt, W. L. (1973). The cephalosporins: 1973. The Journal of Infectious Diseases, 128, S312–S319. https://doi.org/10.1093/infdis/128.supplement_2.s312

Hover, B. M., Kim, S.-H., Katz, M., Charlop-Powers, Z., Owen, J. G., Ternei, M. A., Maniko, J., Estrela, A. B., Molina, H., Park, S., Perlin, D. S., & Brady, S. F. (2018). Culture-independent discovery of the malacidins as calcium-dependent antibiotics with activity against multidrug-resistant Gram-positive pathogens. Nature Microbiology, 3(4), 415–422. https://doi.org/10.1038/s41564-018-0110-1

Hunduma, D., Amenu, K., Desta, H., Grace, D., Agga, G. E., & Kerro Dego, O. (2023). Prevalence and antimicrobial resistance of *Escherichia coli* O157:H7 and *Salmonella*, and the prevalence of *Staphylococcus aureus* in dairy cattle and camels under pastoral production system. Antibiotics, 13(1), 26. https://doi.org/10.3390/antibiotics13010026

Hunter, J. D. (2007). Matplotlib: A 2D graphics environment. Computing in Science & Engineering, 9(3), 90–95. Computing in Science & Engineering. https://doi.org/10.1109/MCSE.2007.55

Hutinel, M., Fick, J., Larsson, D. G. J., & Flach, C.-F. (2021). Investigating the effects of municipal and hospital wastewaters on horizontal gene transfer. Environmental Pollution, 276, 116733. https://doi.org/10.1016/j.envpol.2021.116733

Iseman, M. D. (2002). Tuberculosis therapy: Past, present and future. European Respiratory Journal, 20(36 suppl), 87S – 94s. https://doi.org/10.1183/09031936.02.00309102

Javvadi, Y., & Mohan, S. V. (2024). Temporal dynamics and persistence of resistance genes to broad spectrum antibiotics in an urban community. Npj Clean Water, 7(1), 1–12. https://doi.org/10.1038/s41545-024-00349-y

Jia, B., Raphenya, A. R., Alcock, B., Waglechner, N., Guo, P., Tsang, K. K., Lago, B. A., Dave, B. M., Pereira, S., Sharma, A. N., Doshi, S., Courtot, M., Lo, R., Williams, L. E., Frye, J. G., Elsayegh, T., Sardar, D., Westman, E. L., Pawlowski, A. C., … McArthur, A. G. (2017). CARD 2017: Expansion and model-centric curation of the comprehensive antibiotic resistance database. Nucleic Acids Research, 45(D1), D566–D573. https://doi.org/10.1093/nar/gkw1004

Jiang, T., Sun, S., Chen, Y., Qian, Y., Guo, J., Dai, R., & An, D. (2021). Microbial diversity characteristics and the influence of environmental factors in a large drinking-water source. Science of The Total Environment, 769, 144698. https://doi.org/10.1016/j.scitotenv.2020.144698

Jin, M., Osman, M., Green, B. A., Yang, Y., Ahuja, A., Lu, Z., & Cazer, C. L. (2023). Evidence for the transmission of antimicrobial resistant bacteria between humans and companion animals: A scoping review. One Health, 17, 100593. https://doi.org/10.1016/j.onehlt.2023.100593

K, S., Vasanthrao, R., & Chattopadhyay, I. (2023). Impact of environment on transmission of antibiotic-resistant superbugs in humans and strategies to lower dissemination of antibiotic resistance. Folia Microbiologica, 68(5), 657–675. https://doi.org/10.1007/s12223-023-01083-7

Karam, G., Chastre, J., Wilcox, M. H., & Vincent, J.-L. (2016). Antibiotic strategies in the era of multidrug resistance. Critical Care, 20(1), 136. https://doi.org/10.1186/s13054-016-1320-7

Kerantzas, C. A., & Jacobs, W. R. (2017). Origins of combination therapy for tuberculosis: Lessons for future antimicrobial development and application. mBio, 8(2), e01586-16. https://doi.org/10.1128/mBio.01586-16

Kers, J. G., & Saccenti, E. (2022). The power of microbiome studies: Some considerations on which alpha and beta metrics to use and how to report results. Frontiers in Microbiology, 12. https://doi.org/10.3389/fmicb.2021.796025

Khan, M. T., Shah, I. A., Ihsanullah, I., Naushad, Mu., Ali, S., Shah, S. H. A., & Mohammad, A. W. (2021). Hospital wastewater as a source of environmental contamination: An overview of management practices, environmental risks, and treatment processes. Journal of Water Process Engineering, 41, 101990. https://doi.org/10.1016/j.jwpe.2021.101990

Khezri, A., Avershina, E., & Ahmad, R. (2020). Plasmid identification and plasmid-mediated antimicrobial gene detection in Norwegian isolates. Microorganisms, 9(1), 52. https://doi.org/10.3390/microorganisms9010052

Kim, H.-S. L., Rodriguez, R. D., Morris, S. K., Zhao, S., & Donato, J. J. (2020). Identification of a novel plasmid-borne gentamicin resistance gene in nontyphoidal *Salmonella* isolated from retail turkey. Antimicrobial Agents and Chemotherapy, 64(11), 10.1128/aac.00867-20. https://doi.org/10.1128/aac.00867-20

Kittredge, H. A., Dougherty, K. M., & Evans, S. E. (2022). Dead but not forgotten: how extracellular DNA, moisture, and space modulate the horizontal transfer of extracellular antibiotic resistance genes in soil. Applied and Environmental Microbiology, 88(7). https://doi.org/10.1128/aem.02280-21

Ko, K. K. K., Chng, K. R., & Nagarajan, N. (2022). Metagenomics-enabled microbial surveillance. Nature Microbiology, 7(4), Article 4. https://doi.org/10.1038/s41564-022-01089-w

Kodešová, R., Švecová, H., Klement, A., Fér, M., Nikodem, A., Fedorova, G., Rieznyk, O., Kočárek, M., Sadchenko, A., Chroňáková, A., & Grabic, R. (2024). Contamination of water, soil, and plants by micropollutants from reclaimed wastewater and sludge from a wastewater treatment plant. Science of The Total Environment, 907, 167965. https://doi.org/10.1016/j.scitotenv.2023.167965

Kodzius, R., & Gojobori, T. (2015). Marine metagenomics as a source for bioprospecting. *Marine Genomics*, *24*, 21–30. https://doi.org/10.1016/j.margen.2015.07.001

Kolde, R. (2019). pheatmap: pretty heatmaps (Version 1.0.12) [Computer software]. https://cran.r-project.org/web/packages/pheatmap/index.html

Köser, C. U., Ellington, M. J., & Peacock, S. J. (2014). Whole-genome sequencing to control antimicrobial resistance. Trends in Genetics, 30(9), 401–407. https://doi.org/10.1016/j.tig.2014.07.003

Kotwani, A., Joshi, J., & Kaloni, D. (2021). Pharmaceutical effluent: A critical link in the interconnected ecosystem promoting antimicrobial resistance. Environmental Science and

Pollution Research International, 28(25), 32111–32124. https://doi.org/10.1007/s11356-021-14178-w

Ku, Y.-S., Wang, Z., Duan, S., & Lam, H.-M. (2021). Rhizospheric Communication through mobile genetic element transfers for the regulation of microbe–plant interactions. Biology, 10(6), 477. https://doi.org/10.3390/biology10060477

Kumar, K., C. Gupta, S., Chander, Y., & Singh, A. K. (2005). Antibiotic use in agriculture and its impact on the terrestrial environment. in advances in agronomy (Vol. 87, pp. 1–54). Academic Press. https://doi.org/10.1016/S0065-2113(05)87001-4

Kumari, S., Devi, M., Thakur, K., Minhas, B., Bhatt, A. K., & Kaushik, N. (2024). Impact of anthropogenic activities on microbial diversity and soil health. In R. K. Bhatia & A. Walia (Eds.), Advancements in Microbial Biotechnology for Soil Health (pp. 227–248). Springer Nature. https://doi.org/10.1007/978-981-99-9482-3_11

Landecker, H. (2019). Antimicrobials before antibiotics: War, peace, and disinfectants. Palgrave Communications, 5(1), Article 1. https://doi.org/10.1057/s41599-019-0251-8

Levy, S. B., & Marshall, B. (2004). Antibacterial resistance worldwide: Causes, challenges and responses. Nature Medicine, 10(12), Article 12. https://doi.org/10.1038/nm1145

Li, L., Xu, M., Eyakub Ali, M., Zhang, W., Duan, Y., & Li, D. (2018). Factors affecting soil microbial biomass and functional diversity with the application of organic amendments in three contrasting cropland soils during a field experiment. PLoS ONE, 13(9), e0203812. https://doi.org/10.1371/journal.pone.0203812

Liu, B., & Pop, M. (2009). ARDB--antibiotic resistance genes database. Nucleic Acids Research, 37(Database issue), D443-447. https://doi.org/10.1093/nar/gkn656

Lobanovska, M., & Pilla, G. (2017). Penicillin's discovery and antibiotic resistance: Lessons for the future? The Yale Journal of Biology and Medicine, 90(1), 135–145. https://pmc.ncbi.nlm.nih.gov/articles/PMC5369031/

Low, C. X., Tan, L. T.-H., Ab Mutalib, N.-S., Pusparajah, P., Goh, B.-H., Chan, K.-G., Letchumanan, V., & Lee, L.-H. (2021). Unveiling the impact of antibiotics and alternative methods

for animal husbandry: A Review. Antibiotics, 10(5), 578. https://doi.org/10.3390/antibiotics10050578

Lu, J., Yuan, Q., Wang, X., Gong, L., An, X., & Liu, J. (2022). Antibiotics and microbial community-induced antibiotic-resistant genes distribution in soil and sediment in the eastern coastline of China. Environmental Monitoring and Assessment, 194(9), 607. https://doi.org/10.1007/s10661-022-10295-2

Lynch, S. V., & Pedersen, O. (2016). The human intestinal microbiome in health and disease. *The New England Journal of Medicine*, *375*(24), 2369–2379. https://doi.org/10.1056/NEJMra1600266

Magalhães, C., Lima, M., Trieu-Cuot, P., & Ferreira, P. (2021). To give or not to give antibiotics is not the only question. The Lancet Infectious Diseases, 21(7), e191–e201. https://doi.org/10.1016/S1473-3099(20)30602-2

Magiorakos, A.-P., Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M. E., Giske, C. G., Harbarth, S., Hindler, J. F., Kahlmeter, G., Olsson-Liljequist, B., Paterson, D. L., Rice, L. B., Stelling, J., Struelens, M. J., Vatopoulos, A., Weber, J. T., & Monnet, D. L. (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. Clinical Microbiology and Infection, 18(3), 268–281. https://doi.org/10.1111/j.1469-0691.2011.03570.x

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 : A Journal of Synthetic Chemistry and Natural Product Chemistry, 23(4), 795. https://doi.org/10.3390/molecules23040795

Mapbox. (n.d.). Mapbox APIs. from https://www.mapbox.com

Mardis, E. R. (2008). Next-generation DNA sequencing methods. *Annual Review of Genomics and Human Genetics*, *9*, 387–402. https://doi.org/10.1146/annurev.genom.9.081307.164359

Margesin, R., & Collins, T. (2019). Microbial ecology of the cryosphere (glacial and permafrost habitats): Current knowledge. Applied Microbiology and Biotechnology, 103(6), 2537–2549. https://doi.org/10.1007/s00253-019-09631-3

Marshall, B. M., & Levy, S. B. (2011). Food animals and antimicrobials: Impacts on human health. Clinical Microbiology Reviews, 24(4), 718–733. https://doi.org/10.1128/cmr.00002-11

Mason, C., Afshinnekoo, E., Ahsannudin, S., Ghedin, E., Read, T., Fraser, C., Dudley, J., Hernandez, M., Bowler, C., Stolovitzky, G., Chernonetz, A., Gray, A., Darling, A., Burke, C., Łabaj, P. P., Graf, A., Noushmehr, H., Moraes, s., Dias-Neto, E., … The MetaSUB International Consortium. (2016). The metagenomics and metadesign of the subways and urban biomes (MetaSUB) international consortium inaugural meeting report. Microbiome, 4(1), 24. https://doi.org/10.1186/s40168-016-0168-z

Matuschek, E., Brown, D. F. J., & Kahlmeter, G. (2014). Development of the EUCAST disk diffusion antimicrobial susceptibility testing method and its implementation in routine microbiology laboratories. Clinical Microbiology and Infection, 20(4), O255–O266. https://doi.org/10.1111/1469-0691.12373

Mckinney, W. (2011). pandas: A foundational python library for data analysis and statistics. Python High Performance Science Computer. https://docslib.org/doc/4231522/a-foundational-python-library-for-data-analysis-and-statistics

Meyer, F., Paarmann, D., D'Souza, M., Olson, R., Glass, E., Kubal, M., Paczian, T., Rodriguez, A., Stevens, R., Wilke, A., Wilkening, J., & Edwards, R. (2008). The metagenomics RAST server – a public resource for the automatic phylogenetic and functional analysis of metagenomes. BMC Bioinformatics, 9(1), 386. https://doi.org/10.1186/1471-2105-9-386

Mikheenko, A., Saveliev, V., & Gurevich, A. (2016). MetaQUAST: Evaluation of metagenome assemblies. Bioinformatics, 32(7), 1088–1090. https://doi.org/10.1093/bioinformatics/btv697

Mirzaei, B., Bazgir, Z. N., Goli, H. R., Iranpour, F., Mohammadi, F., & Babaei, R. (2020). Prevalence of multi-drug resistant (MDR) and extensively drug-resistant (XDR) phenotypes of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolated in clinical samples from Northeast of Iran. BMC Research Notes, 13(1), 380. https://doi.org/10.1186/s13104-020-05224-w

Moretto, V. T., Cordeiro, S. M., Bartley, P. S., Silva, L. K., Ponce-Terashima, R., Reis, M. G., Blanton, R. E., & Barbosa, L. M. (2021). Antimicrobial-resistant enterobacteria in surface waters

with fecal contamination from urban and rural communities. Revista Da Sociedade Brasileira de Medicina Tropical, 54, e0724. https://doi.org/10.1590/0037-8682-0724-2020

Mulchandani, R., Wang, Y., Gilbert, M., & Boeckel, T. P. V. (2023). Global trends in antimicrobial use in food-producing animals: 2020 to 2030. PLOS Global Public Health, 3(2), e0001305. https://doi.org/10.1371/journal.pgph.0001305

Munk, P., Brinch, C., Møller, F. D., Petersen, T. N., Hendriksen, R. S., Seyfarth, A. M., Kjeldgaard, J. S., Svendsen, C. A., van Bunnik, B., Berglund, F., Larsson, D. G. J., Koopmans, M., Woolhouse, M., & Aarestrup, F. M. (2022). Genomic analysis of sewage from 101 countries reveals global landscape of antimicrobial resistance. Nature Communications, 13(1), 7251. https://doi.org/10.1038/s41467-022-34312-7

Murray, C. J. L., Ikuta, K. S., Sharara, F., Swetschinski, L., Aguilar, G. R., Gray, A., Han, C., Bisignano, C., Rao, P., Wool, E., Johnson, S. C., Browne, A. J., Chipeta, M. G., Fell, F., Hackett, S., Haines-Woodhouse, G., Hamadani, B. H. K., Kumaran, E. A. P., McManigal, B., … Naghavi, M. (2022). Global burden of bacterial antimicrobial resistance in 2019: A systematic analysis. The Lancet, 399(10325), 629–655. https://doi.org/10.1016/S0140-6736(21)02724-0

Muurinen, J., Stedtfeld, R., Karkman, A., Pärnänen, K., Tiedje, J., & Virta, M. (2017). Influence of manure application on the environmental resistome under Finnish agricultural practice with restricted antibiotic use. Environmental Science & Technology, 51(11), 5989–5999. https://doi.org/10.1021/acs.est.7b00551

Nelson, M. L., & Levy, S. B. (2011). The history of the tetracyclines. Annals of the New York Academy of Sciences, 1241, 17–32. https://doi.org/10.1111/j.1749-6632.2011.06354.x

Nicolaou, K. C., & Rigol, S. (2018). A brief history of antibiotics and select advances in their synthesis. The Journal of Antibiotics, 71(2), Article 2. https://doi.org/10.1038/ja.2017.62

Oniciuc, E. A., Likotrafiti, E., Alvarez-Molina, A., Prieto, M., Santos, J. A., & Alvarez-Ordóñez, A. (2018). The present and future of whole genome sequencing (WGS) and whole metagenome sequencing (WMS) for surveillance of antimicrobial resistant microorganisms and antimicrobial resistance genes across the food chain. Genes, 9(5), 268. https://doi.org/10.3390/genes9050268

Ordine, J. V. W., de Souza, G. M., Tamasco, G., Virgilio, S., Fernandes, A. F. T., Silva-Rocha, R., & Guazzaroni, M.-E. (2023). Metagenomic insights for antimicrobial resistance surveillance in soils with different land uses in Brazil. Antibiotics, 12(2), 334. https://doi.org/10.3390/antibiotics12020334

Osburn, E. D., Yang, G., Rillig, M. C., & Strickland, M. S. (2023). Evaluating the role of bacterial diversity in supporting soil ecosystem functions under anthropogenic stress. ISME Communications, 3(1), 66. https://doi.org/10.1038/s43705-023-00273-1

Ovung, A., & Bhattacharyya, J. (2021). Sulfonamide drugs: Structure, antibacterial property, toxicity, and biophysical interactions. Biophysical Reviews, 13(2), 259–272. https://doi.org/10.1007/s12551-021-00795-9

Pazda, M., Kumirska, J., Stepnowski, P., & Mulkiewicz, E. (2019). Antibiotic resistance genes identified in wastewater treatment plant systems – A review. Science of The Total Environment, 697, 134023. https://doi.org/10.1016/j.scitotenv.2019.134023

Pedregosa, F., Varoquaux, G., Gramfort, A., Michel, V., Thirion, B., Grisel, O., Blondel, M., Prettenhofer, P., Weiss, R., Dubourg, V., Vanderplas, J., Passos, A., Cournapeau, D., Brucher, M., Perrot, M., & Duchesnay, É. (2011). Scikit-learn: Machine learning in python. J. Mach. Learn. Res., 12(null), 2825–2830. https://dl.acm.org/doi/10.5555/1953048.2078195

Pei, R., Kim, S.-C., Carlson, K. H., & Pruden, A. (2006). Effect of river landscape on the sediment concentrations of antibiotics and corresponding antibiotic resistance genes (ARG). Water Research, 40(12), 2427–2435. https://doi.org/10.1016/j.watres.2006.04.017

Pham, T. D. M., Ziora, Z. M., & Blaskovich, M. A. T. (2019). Quinolone antibiotics. MedChemComm, 10(10), 1719–1739. https://doi.org/10.1039/c9md00120d

Pillay, S., Calderón-Franco, D., Urhan, A., & Abeel, T. (2022). Metagenomic-based surveillance systems for antibiotic resistance in non-clinical settings. Frontiers in Microbiology, 13. https://www.frontiersin.org/journals/microbiology/articles/10.3389/fmicb.2022.1066995

Pruitt, K. D., Tatusova, T., & Maglott, D. R. (2007). NCBI reference sequences (RefSeq): A curated non-redundant sequence database of genomes, transcripts and proteins. Nucleic Acids Research, 35(Database issue), D61–D65. https://doi.org/10.1093/nar/gkl842

Qin, K., Wei, L., Li, J., Lai, B., Zhu, F., Yu, H., Zhao, Q., & Wang, K. (2020). A review of ARGs in WWTPs: Sources, stressors and elimination. Chinese Chemical Letters, 31(10), 2603–2613. https://doi.org/10.1016/j.cclet.2020.04.057

Quince, C., Walker, A. W., Simpson, J. T., Loman, N. J., & Segata, N. (2017). Shotgun metagenomics, from sampling to analysis. Nature Biotechnology, 35(9), Article 9. https://doi.org/10.1038/nbt.3935

Raza, S., Jo, H., Kim, J., Shin, H., Hur, H.-G., & Unno, T. (2021). Metagenomic exploration of antibiotic resistome in treated wastewater effluents and their receiving water. Science of The Total Environment, 765, 142755. https://doi.org/10.1016/j.scitotenv.2020.142755

Reardon, S. (2023). Antibiotic use in farming set to soar despite drug-resistance fears. Nature, 614(7948), 397–397. https://doi.org/10.1038/d41586-023-00284-x

Reddy, S., Kaur, K., Barathe, P., Shriram, V., Govarthanan, M., & Kumar, V. (2022). Antimicrobial resistance in urban river ecosystems. Microbiological Research, 263, 127135. https://doi.org/10.1016/j.micres.2022.127135

Rizzo, L., Manaia, C., Merlin, C., Schwartz, T., Dagot, C., Ploy, M. C., Michael, I., & Fatta-Kassinos, D. (2013). Urban wastewater treatment plants as hotspots for antibiotic resistant bacteria and genes spread into the environment: A review. Science of The Total Environment, 447, 345–360. https://doi.org/10.1016/j.scitotenv.2013.01.032

Roberts, L. W., Forde, B. M., Hurst, T., Ling, W., Nimmo, G. R., Bergh, H., George, N., Hajkowicz, K., McNamara, J. F., Lipman, J., Permana, B., Schembri, M. A., Paterson, D., Beatson, S. A., & Harris, P. N. A. (2021). Genomic surveillance, characterization and intervention of a polymicrobial multidrug-resistant outbreak in critical care. Microbial Genomics, 7(3), mgen000530. https://doi.org/10.1099/mgen.0.000530

Rodríguez-Melcón, C., Alonso-Calleja, C., García-Fernández, C., Carballo, J., & Capita, R. (2021). Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) for twelve antimicrobials (Biocides and Antibiotics) in eight strains of *Listeria monocytogenes*. Biology, 11(1), 46. https://doi.org/10.3390/biology11010046

Santajit, S., & Indrawattana, N. (2016). Mechanisms of antimicrobial resistance in ESKAPE pathogens. BioMed Research International, 2016, 2475067. https://doi.org/10.1155/2016/2475067

Sarstedt, M., Ringle, C. M., & Hair, J. F. (2017). Partial least squares structural equation modeling. in C. Homburg, M. Klarmann, & A. Vomberg (Eds.), Handbook of Market Research (pp. 1–40). Springer International Publishing. https://doi.org/10.1007/978-3-319-05542-8_15-1

Seabold, S., & Perktold, J. (2010). Statsmodels: Econometric and statistical modeling with python. Proceedings of the 9th Python in Science Conference, 2010. https://doi.org/10.25080/Majora-92bf1922-011

Seber, G. A. F., & Lee, A. J. (2012). Linear regression analysis. John Wiley & Sons. http://dx.doi.org/10.1002/9780471722199

Segerman, B. (2012). The genetic integrity of bacterial species: The core genome and the accessory genome, two different stories. *Frontiers in Cellular and Infection Microbiology*, *2*. https://www.frontiersin.org/articles/10.3389/fcimb.2012.00116

Shannon, C. E. (1948). A mathematical theory of communication. Bell System Technical Journal, 27(3), 379–423. https://doi.org/10.1002/j.1538-7305.1948.tb01338.x

Shu, W.-S., & Huang, L.-N. (2022). Microbial diversity in extreme environments. Nature Reviews Microbiology, 20(4), 219–235. https://doi.org/10.1038/s41579-021-00648-y

Sirijatuphat, R., Sripanidkulchai, K., Boonyasiri, A., Rattanaumpawan, P., Supapueng, O., Kiratisin, P., & Thamlikitkul, V. (2018). Implementation of global antimicrobial resistance surveillance system (GLASS) in patients with bacteremia. PLoS ONE, 13(1), e0190132. https://doi.org/10.1371/journal.pone.0190132

Skender, B. (2024). The demise of the antibiotic pipeline: The Bayer case. Humanities and Social Sciences Communications, 11(1), 1–10. https://doi.org/10.1057/s41599-024-03584-3

Solden, L., Lloyd, K., & Wrighton, K. (2016). The bright side of microbial dark matter: Lessons learned from the uncultivated majority. Current Opinion in Microbiology, 31, 217–226. https://doi.org/10.1016/j.mib.2016.04.020

Stanish, L. F., Hull, N. M., Robertson, C. E., Harris, J. K., Stevens, M. J., Spear, J. R., & Pace, N. R. (2016). Factors influencing bacterial diversity and community composition in municipal drinking waters in the Ohio river basin, USA. PLoS ONE, 11(6), e0157966. https://doi.org/10.1371/journal.pone.0157966

Stiborova, H., Kracmarova, M., Vesela, T., Biesiekierska, M., Cerny, J., Balik, J., & Demnerova, K. (2021). Impact of long-term manure and sewage sludge application to soil as organic fertilizer on the incidence of pathogenic microorganisms and antibiotic resistance genes. Agronomy, 11(7), Article 7. https://doi.org/10.3390/agronomy11071423

Stokes, J. M., Yang, K., Swanson, K., Jin, W., Cubillos-Ruiz, A., Donghia, N. M., MacNair, C. R., French, S., Carfrae, L. A., Bloom-Ackermann, Z., Tran, V. M., Chiappino-Pepe, A., Badran, A. H., Andrews, I. W., Chory, E. J., Church, G. M., Brown, E. D., Jaakkola, T. S., Barzilay, R., & Collins, J. J. (2020). A deep learning approach to antibiotic discovery. Cell, 180(4), 688-702.e13. https://doi.org/10.1016/j.cell.2020.01.021

Su, M., Satola, S. W., & Read, T. D. (2019). Genome-based prediction of bacterial antibiotic resistance. Journal of Clinical Microbiology, 57(3), 10.1128/jcm.01405-18. https://doi.org/10.1128/jcm.01405-18

Sunagawa, S., Acinas, S. G., Bork, P., Bowler, C., Eveillard, D., Gorsky, G., Guidi, L., Iudicone, D., Karsenti, E., Lombard, F., Ogata, H., Pesant, S., Sullivan, M. B., Wincker, P., & de Vargas, C. (2020). Tara oceans: Towards global ocean ecosystems biology. Nature Reviews Microbiology, 18(8), Article 8. https://doi.org/10.1038/s41579-020-0364-5

Talat, A., Blake, K. S., Dantas, G., & Khan, A. U. (2023). Metagenomic insight into microbiome and antibiotic resistance genes of high clinical concern in urban and rural hospital wastewater of northern India origin: A major reservoir of antimicrobial resistance. Microbiology Spectrum, 11(2), e04102-22. https://doi.org/10.1128/spectrum.04102-22

Tan, L., Wang, F., Liang, M., Wang, X., Das, R., Mao, D., & Luo, Y. (2019). Antibiotic resistance genes attenuated with salt accumulation in saline soil. Journal of Hazardous Materials, 374, 35–42. https://doi.org/10.1016/j.jhazmat.2019.04.020

The Universal Protein Resource (UniProt). (2008). Nucleic Acids Research, 36(Database issue), D190–D195. https://doi.org/10.1093/nar/gkm895

Tiwari, H. K., Sapkota, D., & Sen, M. R. (2008). High prevalence of multidrug-resistant MRSA in a tertiary care hospital of northern India. Infection and Drug Resistance, 1, 57–61. https://doi.org/10.2147/idr.s4105

Turnbaugh, P. J., Ley, R. E., Hamady, M., Fraser-Liggett, C. M., Knight, R., & Gordon, J. I. (2007). The human microbiome project. *Nature*, *449*(7164), 804–810. https://doi.org/10.1038/nature06244

Uddin, T. M., Chakraborty, A. J., Khusro, A., Zidan, B. R. M., Mitra, S., Emran, T. B., Dhama, K., Ripon, Md. K. H., Gajdács, M., Sahibzada, M. U. K., Hossain, Md. J., & Koirala, N. (2021). Antibiotic resistance in microbes: History, mechanisms, therapeutic strategies and future prospects. Journal of Infection and Public Health, 14(12), 1750–1766. https://doi.org/10.1016/j.jiph.2021.10.020

Van Boeckel, T. P., Brower, C., Gilbert, M., Grenfell, B. T., Levin, S. A., Robinson, T. P., Teillant, A., & Laxminarayan, R. (2015). Global trends in antimicrobial use in food animals. Proceedings of the National Academy of Sciences of the United States of America, 112(18), 5649–5654. https://doi.org/10.1073/pnas.1503141112

Vásquez-Dean, J., Maza, F., Morel, I., Pulgar, R., & González, M. (2020). Microbial communities from arid environments on a global scale. A systematic review. Biological Research, 53(1), 29. https://doi.org/10.1186/s40659-020-00296-1

Venkateswaran, P., Vasudevan, S., David, H., Shaktivel, A., Shanmugam, K., Neelakantan, P., & Solomon, A. P. (2023). Revisiting ESKAPE pathogens: Virulence, resistance, and combating strategies focusing on quorum sensing. Frontiers in Cellular and Infection Microbiology, 13. https://doi.org/10.3389/fcimb.2023.1159798

Ventola, C. L. (2015). The antibiotic resistance crisis. Pharmacy and Therapeutics, 40(4), 277–283. https://pmc.ncbi.nlm.nih.gov/articles/PMC4378521/

Vincent, J. G., Vincent, H. W., & Morton, J. (1944). Filter paper disc modification of the Oxford cup penicillin determination. Proceedings of the Society for Experimental Biology and Medicine, 55(3), 162–164. https://doi.org/10.3181/00379727-55-14502

Virtanen, P., Gommers, R., Oliphant, T. E., Haberland, M., Reddy, T., Cournapeau, D., Burovski, E., Peterson, P., Weckesser, W., Bright, J., van der Walt, S. J., Brett, M., Wilson, J., Millman, K. J., Mayorov, N., Nelson, A. R. J., Jones, E., Kern, R., Larson, E., … van Mulbregt, P. (2020). SciPy 1.0: Fundamental algorithms for scientific computing in Python. Nature Methods, 17(3), 261–272. https://doi.org/10.1038/s41592-019-0686-2

Waddington, C., Carey, M. E., Boinett, C. J., Higginson, E., Veeraraghavan, B., & Baker, S. (2022). Exploiting genomics to mitigate the public health impact of antimicrobial resistance. Genome Medicine, 14(1), 15. https://doi.org/10.1186/s13073-022-01020-2

Wang, M., Wei, H., Zhao, Y., Shang, L., Di, L., Lyu, C., & Liu, J. (2019). Analysis of multidrug-resistant bacteria in 3223 patients with hospital-acquired infections (HAI) from a tertiary general hospital in China. Bosnian Journal of Basic Medical Sciences, 19(1), 86–93. https://doi.org/10.17305/bjbms.2018.3826

Waskito, L. A., Rezkitha, Y. A. A., Vilaichone, R., Wibawa, I. D. N., Mustika, S., Sugihartono, T., & Miftahussurur, M. (2022). Antimicrobial resistance profile by metagenomic and metatranscriptomic approach in clinical practice: Opportunity and challenge. *Antibiotics*, *11*(5), 654. https://doi.org/10.3390/antibiotics11050654

Waskom, M. L. (2021). seaborn: Statistical data visualization. Journal of Open Source Software, 6(60), 3021. https://doi.org/10.21105/joss.03021

Willmann, M., Bezdan, D., Zapata, L., Susak, H., Vogel, W., Schröppel, K., Liese, J., Weidenmaier, C., Autenrieth, I. B., Ossowski, S., & Peter, S. (2015). Analysis of a long-term outbreak of XDR *Pseudomonas aeruginosa*: A molecular epidemiological study. Journal of Antimicrobial Chemotherapy, 70(5), 1322–1330. https://doi.org/10.1093/jac/dku546

Wood, D. E., Lu, J., & Langmead, B. (2019). Improved metagenomic analysis with Kraken 2. Genome Biology, 20(1), 257. https://doi.org/10.1186/s13059-019-1891-0

Xiao, T., & Zhou, W. (2020). The third generation sequencing: The advanced approach to genetic diseases. *Translational Pediatrics*, *9*(2), 163–173. https://doi.org/10.21037/tp.2020.03.06

Xu, S., Yuan, Y., Song, P., Cui, M., Zhao, R., Song, X., Cao, M., Zhang, Y., & Yang, J. (2023). The spatial patterns of diversity and their relationships with environments in rhizosphere microorganisms and host plants differ along elevational gradients. Frontiers in Microbiology, 14. https://doi.org/10.3389/fmicb.2023.1079113

Yang, N., Wang, Y., Liu, B., Zhang, J., Hua, J., Liu, D., Bhople, P., Zhang, Y., Zhang, H., Zhang, C., Ruan, H., & Wang, W. (2023). Exploration of soil microbial diversity and community structure along mid-subtropical elevation gradients in southeast China. forests, 14(4), Article 4. https://doi.org/10.3390/f14040769

Yang, Y., Qiu, K., Xie, Y., Li, X., Zhang, S., Liu, W., Huang, Y., Cui, L., Wang, S., & Bao, P. (2023). Geographical, climatic, and soil factors control the altitudinal pattern of rhizosphere microbial diversity and its driving effect on root zone soil multifunctionality in mountain ecosystems. Science of The Total Environment, 904, 166932. https://doi.org/10.1016/j.scitotenv.2023.166932

Yooseph, S., Sutton, G., Rusch, D. B., Halpern, A. L., Williamson, S. J., Remington, K., Eisen, J. A., Heidelberg, K. B., Manning, G., Li, W., Jaroszewski, L., Cieplak, P., Miller, C. S., Li, H., Mashiyama, S. T., Joachimiak, M. P., Belle, C. van, Chandonia, J.-M., Soergel, D. A., … Venter, J. C. (2007). The sorcerer II global ocean sampling expedition: Expanding the universe of protein families. *PLOS Biology*, *5*(3), e16. https://doi.org/10.1371/journal.pbio.0050016

Zeng, Q., Lebreton, A., Man, X., Jia, L., Wang, G., Gong, S., Buée, M., Wu, G., Dai, Y., Yang, Z., & Martin, F. M. (2022). Ecological drivers of the soil microbial diversity and composition in primary old-growth forest and secondary woodland in a subtropical evergreen broad-leaved Forest biome in the Ailao mountains, China. Frontiers in Microbiology, 13. https://doi.org/10.3389/fmicb.2022.908257

Zi, F., Wang, B., Yang, L., Huo, Q., Wang, Z., Ren, D., Huo, B., Song, Y., & Chen, S. (2024). Ecology of saline watersheds: An investigation of the functional communities and drivers of benthic fauna in typical water bodies of the Irtysh River Basin. Biology, 13(1), 27. https://doi.org/10.3390/biology13010027

Zheng, D., Yin, G., Liu, M., Hou, L., Yang, Y., Van Boeckel, T. P., Zheng, Y., & Li, Y. (2022). Global biogeography and projection of soil antibiotic resistance genes. Science Advances, 8(46), eabq8015. https://doi.org/10.1126/sciadv.abq8015

7. Supplementary Data

7.1 Supplementary Table 1

MG RAST ID	Latitude	Longitude	Type	Biome
mgm4465942	42.582778	-85.366667	Soil	Anthropogenic
mgm4465943	42.599444	-85.366667	Soil	Anthropogenic
mgm4465947	42.599444	-85.366667	Soil	Anthropogenic
mgm4510860	43.15	2.55	Soil	Anthropogenic
mgm4510861	43.15	2.55	Soil	Anthropogenic
mgm4510862	43.15	2.55	Soil	Anthropogenic
mgm4510863	43.15	2.55	Soil	Anthropogenic
mgm4510864	43.15	2.55	Soil	Anthropogenic
mgm4510865	43.15	2.55	Soil	Anthropogenic
mgm4510867	43.15	2.55	Soil	Anthropogenic
mgm4510868	43.15	2.55	Soil	Anthropogenic
mgm4527652	55.6346944	13.4061667	Soil	Anthropogenic
mgm4528934	55.6346944	13.4061667	Soil	Anthropogenic
mgm4529373	55.6346944	13.4061667	Soil	Anthropogenic
mgm4529786	55.6346944	13.4061667	Soil	Anthropogenic
mgm4537190	51.481481	0.222231	Soil	Anthropogenic
mgm4537191	51.481481	0.222231	Soil	Anthropogenic
mgm4623913	19.2	73.8	Soil	Anthropogenic
mgm4626750	21.08839	105.727278	Soil	Anthropogenic
mgm4626751	21.088395	105.727361	Soil	Anthropogenic
mgm4631721	32.778609	35.023668	Soil	Anthropogenic
mgm4631724	32.778609	35.023668	Soil	Anthropogenic
mgm4635904	22.11	112.77	Soil	Anthropogenic
mgm4635905	22.11	112.77	Soil	Anthropogenic
mgm4636837	42.4439614	-76.501881	Soil	Anthropogenic
mgm4637195	42.4439614	-76.501881	Soil	Anthropogenic
mgm4637196	42.4439614	-76.501881	Soil	Anthropogenic
mgm4637197	42.4439614	-76.501881	Soil	Anthropogenic
mgm4667212	23.579592	47.586947	Soil	Anthropogenic
mgm4667213	23.579592	47.586947	Soil	Anthropogenic
mgm4667214	23.579592	47.586947	Soil	Anthropogenic
mgm4667215	23.579592	47.586947	Soil	Anthropogenic
mgm4815682	3.52	11.32	Soil	Anthropogenic
mgm4815683	7.19	13.34	Soil	Anthropogenic
mgm4815684	12.23	1.3	Soil	Anthropogenic
mgm4815685	7.19	13.35	Soil	Anthropogenic

mgm4815686	3.52	11.31	Soil	Anthropogenic
mgm4815687	12.23	1.29	Soil	Anthropogenic
mgm4819059	60.9852833	25.6513472	Soil	Anthropogenic
mgm4819060	-26.702306	27.0996667	Soil	Anthropogenic
mgm4819061	60.1896333	24.9761444	Soil	Anthropogenic
mgm4819062	47.52686	19.01319	Soil	Anthropogenic
mgm4819063	47.51509	18.98505	Soil	Anthropogenic
mgm4819064	47.5358	19.01722	Soil	Anthropogenic
mgm4819065	61.0136833	25.6816194	Soil	Anthropogenic
mgm4819066	60.9707472	25.7037444	Soil	Anthropogenic
mgm4819067	60.2209306	24.9232361	Soil	Anthropogenic
mgm4819068	60.9629944	25.6528889	Soil	Anthropogenic
mgm4819069	39.3306833	-76.624933	Soil	Anthropogenic
mgm4819070	60.9933972	25.707175	Soil	Anthropogenic
mgm4819071	-26.701472	27.0899722	Soil	Anthropogenic
mgm4819072	60.2087111	24.8769444	Soil	Anthropogenic
mgm4819073	61.0136833	25.6816194	Soil	Anthropogenic
mgm4819074	-26.858944	27.0498611	Soil	Anthropogenic
mgm4819075	-26.679361	27.1141944	Soil	Anthropogenic
mgm4819076	39.4875667	-76.689417	Soil	Anthropogenic
mgm4819077	47.58763	19.0298	Soil	Anthropogenic
mgm4819078	-26.716778	27.1011111	Soil	Anthropogenic
mgm4819079	60.9629944	25.6528889	Soil	Anthropogenic
mgm4819080	47.53897	19.01736	Soil	Anthropogenic
mgm4819081	61.0797194	25.6993111	Soil	Anthropogenic
mgm4819082	47.51509	18.98505	Soil	Anthropogenic
mgm4819083	60.9698639	25.6529056	Soil	Anthropogenic
mgm4819084	60.2233833	25.0227222	Soil	Anthropogenic
mgm4819085	47.53556	19.01747	Soil	Anthropogenic
mgm4819086	39.3295833	-76.62445	Soil	Anthropogenic
mgm4819087	60.9698639	25.6529056	Soil	Anthropogenic
mgm4819088	61.0136833	25.6816194	Soil	Anthropogenic
mgm4819089	47.52702	19.01423	Soil	Anthropogenic
mgm4819090	60.2379861	24.9038278	Soil	Anthropogenic
mgm4819091	60.2274833	25.0117389	Soil	Anthropogenic
mgm4819092	39.3447833	-76.625817	Soil	Anthropogenic
mgm4819093	-26.708333	27.1101944	Soil	Anthropogenic
mgm4819094	-26.677556	27.09225	Soil	Anthropogenic
mgm4819095	39.3420667	-76.60105	Soil	Anthropogenic

mgm4819096	47.53556	19.01747	Soil	Anthropogenic
mgm4819098	60.9924306	25.7018944	Soil	Anthropogenic
mgm4819099	47.52686	19.01319	Soil	Anthropogenic
mgm4819100	61.0056306	25.6559083	Soil	Anthropogenic
mgm4819101	60.1737861	24.9329278	Soil	Anthropogenic
mgm4819102	60.2259583	24.9788528	Soil	Anthropogenic
mgm4819103	-26.70125	27.0793056	Soil	Anthropogenic
mgm4819104	39.3475167	-76.620433	Soil	Anthropogenic
mgm4819105	47.58763	19.0298	Soil	Anthropogenic
mgm4819106	39.3426667	-76.6237	Soil	Anthropogenic
mgm4819107	60.9707472	25.7037444	Soil	Anthropogenic
mgm4819109	47.52648	19.01307	Soil	Anthropogenic
mgm4819110	60.1737861	24.9329278	Soil	Anthropogenic
mgm4819111	-26.713389	27.0824444	Soil	Anthropogenic
mgm4819112	60.1983833	24.964875	Soil	Anthropogenic
mgm4819113	60.1657667	24.9389806	Soil	Anthropogenic
mgm4819114	39.3447833	-76.625817	Soil	Anthropogenic
mgm4819115	47.51509	18.98505	Soil	Anthropogenic
mgm4819116	60.2087111	24.8769444	Soil	Anthropogenic
mgm4819117	61.0616167	25.6600167	Soil	Anthropogenic
mgm4819118	39.3447833	-76.625817	Soil	Anthropogenic
mgm4819119	60.9854306	25.6450389	Soil	Anthropogenic
mgm4819120	61.0627861	25.5402611	Soil	Anthropogenic
mgm4819121	39.3526167	-76.610283	Soil	Anthropogenic
mgm4819122	39.3440667	-76.601317	Soil	Anthropogenic
mgm4819123	47.5287	18.89726	Soil	Anthropogenic
mgm4819124	60.2574722	24.9214667	Soil	Anthropogenic
mgm4819125	61.0136833	25.6816194	Soil	Anthropogenic
mgm4819126	39.33	-76.618883	Soil	Anthropogenic
mgm4819127	60.9854306	25.6450389	Soil	Anthropogenic
mgm4819128	60.2209306	24.9232361	Soil	Anthropogenic
mgm4819129	60.9921667	25.7140333	Soil	Anthropogenic
mgm4819131	-26.858944	27.0498611	Soil	Anthropogenic
mgm4819132	39.4825	-76.682767	Soil	Anthropogenic
mgm4819133	47.51509	18.98505	Soil	Anthropogenic
mgm4819134	60.9629944	25.6528889	Soil	Anthropogenic
mgm4819135	39.3346333	-76.60935	Soil	Anthropogenic
mgm4819136	60.3077028	24.5324028	Soil	Anthropogenic
mgm4819137	60.9855306	25.6506361	Soil	Anthropogenic

mgm4819138	60.9698639	25.6529056	Soil	Anthropogenic
mgm4819139	-26.637306	27.1972222	Soil	Anthropogenic
mgm4819140	61.0921111	25.7387778	Soil	Anthropogenic
mgm4819141	39.3440667	-76.601317	Soil	Anthropogenic
mgm4819142	47.53924	19.01614	Soil	Anthropogenic
mgm4819143	60.2460972	25.1708056	Soil	Anthropogenic
mgm4819144	60.9933972	25.707175	Soil	Anthropogenic
mgm4819145	60.2672417	25.0124806	Soil	Anthropogenic
mgm4819146	39.4811833	-76.6888	Soil	Anthropogenic
mgm4819147	-26.701472	27.0899722	Soil	Anthropogenic
mgm4819148	60.2015861	24.8586861	Soil	Anthropogenic
mgm4819149	60.2259583	24.9788528	Soil	Anthropogenic
mgm4819150	47.52686	19.01319	Soil	Anthropogenic
mgm4819151	47.5163	18.98259	Soil	Anthropogenic
mgm4819152	61.0056306	25.6559083	Soil	Anthropogenic
mgm4819153	60.2379861	24.9038278	Soil	Anthropogenic
mgm4819154	61.0921111	25.7387778	Soil	Anthropogenic
mgm4819155	39.3262333	-76.622017	Soil	Anthropogenic
mgm4819156	60.9653417	25.6996667	Soil	Anthropogenic
mgm4819157	47.53924	19.01614	Soil	Anthropogenic
mgm4819158	39.3447833	-76.625817	Soil	Anthropogenic
mgm4819159	60.9158556	25.5550306	Soil	Anthropogenic
mgm4819160	47.53556	19.01747	Soil	Anthropogenic
mgm4819161	47.54482	19.00874	Soil	Anthropogenic
mgm4819162	47.52723	18.90282	Soil	Anthropogenic
mgm4819163	61.0098056	25.6774639	Soil	Anthropogenic
mgm4819165	-26.729	27.09625	Soil	Anthropogenic
mgm4819166	60.9653417	25.6996667	Soil	Anthropogenic
mgm4819167	47.53924	19.01614	Soil	Anthropogenic
mgm4819168	60.9933972	25.707175	Soil	Anthropogenic
mgm4819169	60.9924306	25.7018944	Soil	Anthropogenic
mgm4819170	39.3550333	-76.609033	Soil	Anthropogenic
mgm4819171	47.5163	18.98259	Soil	Anthropogenic
mgm4819172	-26.729	27.09625	Soil	Anthropogenic
mgm4819173	60.2919083	25.14645	Soil	Anthropogenic
mgm4819174	-26.694583	27.0936667	Soil	Anthropogenic
mgm4819175	39.3550333	-76.609033	Soil	Anthropogenic
mgm4819176	60.2233833	25.0227222	Soil	Anthropogenic
mgm4819177	47.53924	19.01614	Soil	Anthropogenic

mgm4819178	61.0056306	25.6559083	Soil	Anthropogenic
mgm4819179	60.287625	24.7954806	Soil	Anthropogenic
mgm4819180	60.9924306	25.7018944	Soil	Anthropogenic
mgm4819181	47.51557	18.97242	Soil	Anthropogenic
mgm4819182	60.1984667	24.9698194	Soil	Anthropogenic
mgm4819183	60.193975	24.9774778	Soil	Anthropogenic
mgm4819184	61.0616167	25.6600167	Soil	Anthropogenic
mgm4819185	47.51557	18.97242	Soil	Anthropogenic
mgm4819187	39.3420667	-76.60105	Soil	Anthropogenic
mgm4819188	60.2209306	24.9232361	Soil	Anthropogenic
mgm4819189	60.2274833	25.0117389	Soil	Anthropogenic
mgm4819190	-26.677556	27.09225	Soil	Anthropogenic
mgm4819191	-26.694583	27.0936667	Soil	Anthropogenic
mgm4819192	47.53594	19.0176	Soil	Anthropogenic
mgm4819193	60.1983833	24.964875	Soil	Anthropogenic
mgm4819194	60.9854306	25.6450389	Soil	Anthropogenic
mgm4819195	-26.743056	27.0977778	Soil	Anthropogenic
mgm4819196	39.4825	-76.682767	Soil	Anthropogenic
mgm4819197	60.2574722	24.9214667	Soil	Anthropogenic
mgm4819198	60.9629944	25.6528889	Soil	Anthropogenic
mgm4819199	-26.679361	27.1141944	Soil	Anthropogenic
mgm4819200	39.4908667	-76.690433	Soil	Anthropogenic
mgm4819201	61.0062472	25.65335	Soil	Anthropogenic
mgm4819202	61.0797194	25.6993111	Soil	Anthropogenic
mgm4819203	47.5163	18.98259	Soil	Anthropogenic
mgm4819204	47.52723	18.90282	Soil	Anthropogenic
mgm4819205	47.52702	19.01423	Soil	Anthropogenic
mgm4819206	-26.717111	27.1175	Soil	Anthropogenic
mgm4819207	39.3526167	-76.610283	Soil	Anthropogenic
mgm4819208	60.1983833	24.964875	Soil	Anthropogenic
mgm4819209	61.0921111	25.7387778	Soil	Anthropogenic
mgm4819210	39.3426667	-76.6237	Soil	Anthropogenic
mgm4819211	60.2274833	25.0117389	Soil	Anthropogenic
mgm4819212	-26.696361	27.0797222	Soil	Anthropogenic
mgm4819213	39.3262333	-76.622017	Soil	Anthropogenic
mgm4819214	39.4968467	-76.688917	Soil	Anthropogenic
mgm4819215	60.1983833	24.964875	Soil	Anthropogenic
mgm4819216	60.9854306	25.6450389	Soil	Anthropogenic
mgm4819217	60.9852833	25.6513472	Soil	Anthropogenic

mgm4819218	60.1657667	24.9389806	Soil	Anthropogenic
mgm4819220	-26.679361	27.1141944	Soil	Anthropogenic
mgm4819221	47.52723	18.90282	Soil	Anthropogenic
mgm4819222	60.3077028	24.5324028	Soil	Anthropogenic
mgm4819223	-26.694583	27.0936667	Soil	Anthropogenic
mgm4819224	61.0153361	25.6653667	Soil	Anthropogenic
mgm4819225	60.193975	24.9774778	Soil	Anthropogenic
mgm4819226	60.1737861	24.9329278	Soil	Anthropogenic
mgm4819227	60.2919083	25.14645	Soil	Anthropogenic
mgm4819228	-26.690444	27.0722222	Soil	Anthropogenic
mgm4819229	-26.70125	27.0793056	Soil	Anthropogenic
mgm4819230	47.52702	19.01423	Soil	Anthropogenic
mgm4819231	60.2233833	25.0227222	Soil	Anthropogenic
mgm4819232	39.3427167	-76.5994	Soil	Anthropogenic
mgm4819233	60.287625	24.7954806	Soil	Anthropogenic
mgm4819234	47.53556	19.01747	Soil	Anthropogenic
mgm4819235	-26.702306	27.0996667	Soil	Anthropogenic
mgm4819236	39.3427167	-76.5994	Soil	Anthropogenic
mgm4819237	60.2460972	25.1708056	Soil	Anthropogenic
mgm4819238	39.3295833	-76.62445	Soil	Anthropogenic
mgm4819239	47.58746	19.03558	Soil	Anthropogenic
mgm4819240	47.53923	19.01723	Soil	Anthropogenic
mgm4819241	61.0153361	25.6653667	Soil	Anthropogenic
mgm4819242	60.2379861	24.9038278	Soil	Anthropogenic
mgm4819243	-26.680444	27.0818611	Soil	Anthropogenic
mgm4819244	47.52782	18.8985	Soil	Anthropogenic
mgm4819245	47.52782	18.8985	Soil	Anthropogenic
mgm4819246	47.58746	19.03558	Soil	Anthropogenic
mgm4819247	60.1914972	24.926775	Soil	Anthropogenic
mgm4819248	47.53923	19.01723	Soil	Anthropogenic
mgm4819249	60.9653417	25.6996667	Soil	Anthropogenic
mgm4819250	39.3428167	-76.600417	Soil	Anthropogenic
mgm4819251	39.33	-76.618883	Soil	Anthropogenic
mgm4819252	-26.717111	27.1175	Soil	Anthropogenic
mgm4819253	39.3428167	-76.600417	Soil	Anthropogenic
mgm4819254	-26.637306	27.1972222	Soil	Anthropogenic
mgm4819255	47.52723	18.90282	Soil	Anthropogenic
mgm4819256	-26.717111	27.1175	Soil	Anthropogenic
mgm4819257	-26.743056	27.0977778	Soil	Anthropogenic

mgm4819258	47.52686	19.01319	Soil	Anthropogenic
mgm4819259	60.9855306	25.6506361	Soil	Anthropogenic
mgm4819260	-26.713389	27.0824444	Soil	Anthropogenic
mgm4819261	47.5287	18.89726	Soil	Anthropogenic
mgm4819262	-26.701472	27.0899722	Soil	Anthropogenic
mgm4819263	60.9852833	25.6513472	Soil	Anthropogenic
mgm4819264	60.1914972	24.926775	Soil	Anthropogenic
mgm4819265	-26.696361	27.0797222	Soil	Anthropogenic
mgm4819266	-26.680444	27.0818611	Soil	Anthropogenic
mgm4819267	47.53923	19.01723	Soil	Anthropogenic
mgm4819268	61.0056306	25.6559083	Soil	Anthropogenic
mgm4819269	60.2919083	25.14645	Soil	Anthropogenic
mgm4819270	60.9653417	25.6996667	Soil	Anthropogenic
mgm4819271	47.5287	18.89726	Soil	Anthropogenic
mgm4819272	60.9924306	25.7018944	Soil	Anthropogenic
mgm4819274	61.0098056	25.6774639	Soil	Anthropogenic
mgm4819275	60.2233833	25.0227222	Soil	Anthropogenic
mgm4819276	47.5163	18.98259	Soil	Anthropogenic
mgm4819277	60.9707472	25.7037444	Soil	Anthropogenic
mgm4819278	-26.708333	27.1101944	Soil	Anthropogenic
mgm4819279	47.52702	19.01423	Soil	Anthropogenic
mgm4819280	39.3295833	-76.62445	Soil	Anthropogenic
mgm4819281	60.9698639	25.6529056	Soil	Anthropogenic
mgm4819282	-26.713389	27.0824444	Soil	Anthropogenic
mgm4819283	60.9158556	25.5550306	Soil	Anthropogenic
mgm4819284	-26.690444	27.0722222	Soil	Anthropogenic
mgm4819285	47.53897	19.01736	Soil	Anthropogenic
mgm4819286	60.3077028	24.5324028	Soil	Anthropogenic
mgm4819287	39.3445	-76.600667	Soil	Anthropogenic
mgm4819288	61.0797194	25.6993111	Soil	Anthropogenic
mgm4819289	-26.716778	27.1011111	Soil	Anthropogenic
mgm4819290	47.5287	18.89726	Soil	Anthropogenic
mgm4819291	60.9158556	25.5550306	Soil	Anthropogenic
mgm4819292	60.1914972	24.926775	Soil	Anthropogenic
mgm4819293	47.51524	18.98487	Soil	Anthropogenic
mgm4819294	60.287625	24.7954806	Soil	Anthropogenic
mgm4819295	60.287625	24.7954806	Soil	Anthropogenic
mgm4819296	-26.696361	27.0797222	Soil	Anthropogenic
mgm4819297	60.1914972	24.926775	Soil	Anthropogenic

mgm4819298	60.1896333	24.9761444	Soil	Anthropogenic
mgm4819299	39.4908667	-76.690433	Soil	Anthropogenic
mgm4819300	-26.637306	27.1972222	Soil	Anthropogenic
mgm4819301	39.4811833	-76.6888	Soil	Anthropogenic
mgm4819302	-26.679361	27.1141944	Soil	Anthropogenic
mgm4819303	61.0797194	25.6993111	Soil	Anthropogenic
mgm4819304	60.2672417	25.0124806	Soil	Anthropogenic
mgm4819305	60.9852833	25.6513472	Soil	Anthropogenic
mgm4819306	60.9921667	25.7140333	Soil	Anthropogenic
mgm4819307	61.0616167	25.6600167	Soil	Anthropogenic
mgm4819308	60.9855306	25.6506361	Soil	Anthropogenic
mgm4819309	-26.677556	27.09225	Soil	Anthropogenic
mgm4819310	61.0098056	25.6774639	Soil	Anthropogenic
mgm4819312	47.54482	19.00874	Soil	Anthropogenic
mgm4819313	-26.694583	27.0936667	Soil	Anthropogenic
mgm4819314	-26.738694	27.0832222	Soil	Anthropogenic
mgm4819315	61.0627861	25.5402611	Soil	Anthropogenic
mgm4819316	60.3077028	24.5324028	Soil	Anthropogenic
mgm4819317	60.2259583	24.9788528	Soil	Anthropogenic
mgm4819318	-26.690444	27.0722222	Soil	Anthropogenic
mgm4819319	47.51557	18.97242	Soil	Anthropogenic
mgm4819321	47.51524	18.98487	Soil	Anthropogenic
mgm4819322	47.59222	19.02959	Soil	Anthropogenic
mgm4819323	60.2209306	24.9232361	Soil	Anthropogenic
mgm4819326	39.4968467	-76.688917	Soil	Anthropogenic
mgm4819328	60.9933972	25.707175	Soil	Anthropogenic
mgm4819330	47.52782	18.8985	Soil	Anthropogenic
mgm4819332	60.1737861	24.9329278	Soil	Anthropogenic
mgm4819334	60.1657667	24.9389806	Soil	Anthropogenic
mgm4819336	-26.677556	27.09225	Soil	Anthropogenic
mgm4819338	47.5358	19.01722	Soil	Anthropogenic
mgm4819340	-26.729	27.09625	Soil	Anthropogenic
mgm4819342	47.58763	19.0298	Soil	Anthropogenic
mgm4819344	-26.696361	27.0797222	Soil	Anthropogenic
mgm4819346	-26.637306	27.1972222	Soil	Anthropogenic
mgm4819348	47.58763	19.0298	Soil	Anthropogenic
mgm4819349	47.53594	19.0176	Soil	Anthropogenic
mgm4819350	60.1984667	24.9698194	Soil	Anthropogenic
mgm4819351	60.2574722	24.9214667	Soil	Anthropogenic

mgm4819352	61.0616167	25.6600167	Soil	Anthropogenic
mgm4819353	61.0153361	25.6653667	Soil	Anthropogenic
mgm4819354	61.0098056	25.6774639	Soil	Anthropogenic
mgm4819355	47.59222	19.02959	Soil	Anthropogenic
mgm4819356	60.2015861	24.8586861	Soil	Anthropogenic
mgm4819357	60.2919083	25.14645	Soil	Anthropogenic
mgm4819358	47.52648	19.01307	Soil	Anthropogenic
mgm4654026	31.033333	34.9	Soil	Arid
mgm4654027	31.033333	34.9	Soil	Arid
mgm4654028	31.033333	34.9	Soil	Arid
mgm4654029	30.866667	34.766667	Soil	Arid
mgm4654030	30.866667	34.766667	Soil	Arid
mgm4654031	30.866667	34.766667	Soil	Arid
mgm4664124	-29.164658	116.786696	Soil	Arid
mgm4664125	-29.164658	116.786696	Soil	Arid
mgm4664126	-29.164658	116.786696	Soil	Arid
mgm4664127	-29.164658	116.786696	Soil	Arid
mgm4664128	-29.164658	116.786696	Soil	Arid
mgm4664129	-29.164658	116.786696	Soil	Arid
mgm4664130	-29.164658	116.786696	Soil	Arid
mgm4664131	-29.164658	116.786696	Soil	Arid
mgm4664132	-29.164658	116.786696	Soil	Arid
mgm4664133	-29.164658	116.786696	Soil	Arid
mgm4664134	-29.164658	116.786696	Soil	Arid
mgm4664135	-29.164658	116.786696	Soil	Arid
mgm4664136	-29.164658	116.786696	Soil	Arid
mgm4664137	-29.164658	116.786696	Soil	Arid
mgm4664138	-29.164658	116.786696	Soil	Arid
mgm4664147	-29.164658	116.786696	Soil	Arid
mgm4670116	48.3	-110.66056	Soil	Arid
mgm4670122	48.3	-110.66056	Soil	Arid
mgm4440306	49.705	-124.35167	Aquatic	Coastal seawater
mgm4441143	41.091	-71.602	Aquatic	Coastal seawater
mgm4441144	38.94	-74.685	Aquatic	Coastal seawater
mgm4441152	44.1372	-63.644	Aquatic	Coastal seawater
mgm4441153	43.632	-66.8472	Aquatic	Coastal seawater
mgm4441582	44.112	-64.9467	Aquatic	Coastal seawater
mgm4441585	36.00389	-75.3947	Aquatic	Coastal seawater
mgm4441617	-6.3167	39.55	Aquatic	Coastal seawater

mgm4441618	-6.1167	39.1167	Aquatic	Coastal seawater
mgm4441658	39.4178	-75.5142	Aquatic	Coastal seawater
mgm4442708	44.1372	-63.644	Aquatic	Coastal seawater
mgm4442709	43.632	-66.8472	Aquatic	Coastal seawater
mgm4447483	-17.478417	-39.028083	Aquatic	Coastal seawater
mgm4447551	-17.478417	-39.028083	Aquatic	Coastal seawater
mgm4447862	-17.478417	-39.028083	Aquatic	Coastal seawater
mgm4449259	16.7758333	-88.075555	Aquatic	Coastal seawater
mgm4450891	-12.37444	-77	Aquatic	Coastal seawater
mgm4450892	-12.37444	-77	Aquatic	Coastal seawater
mgm4452038	-12.37444	-77	Aquatic	Coastal seawater
mgm4452039	-12.37444	-77	Aquatic	Coastal seawater
mgm4452042	-12.37444	-77	Aquatic	Coastal seawater
mgm4452043	-12.37444	-77	Aquatic	Coastal seawater
mgm4453304	-17.478417	-39.028083	Aquatic	Coastal seawater
mgm4453371	-17.478417	-39.028083	Aquatic	Coastal seawater
mgm4460178	48.016667	-122.30056	Aquatic	Coastal seawater
mgm4460179	48.368056	-122.71667	Aquatic	Coastal seawater
mgm4460180	47.700556	-122.45056	Aquatic	Coastal seawater
mgm4460182	47.333333	-122.43472	Aquatic	Coastal seawater
mgm4460188	47.883333	-122.36667	Aquatic	Coastal seawater
mgm4460189	47.683889	-122.40694	Aquatic	Coastal seawater
mgm4460190	47.661667	-122.43333	Aquatic	Coastal seawater
mgm4460676	-12.37444	-77	Aquatic	Coastal seawater
mgm4460677	-12.37444	-77	Aquatic	Coastal seawater
mgm4460734	-12.37444	-77	Aquatic	Coastal seawater
mgm4460735	-12.37444	-77	Aquatic	Coastal seawater
mgm4460736	-12.37444	-77	Aquatic	Coastal seawater
mgm4461588	-12.37444	-77	Aquatic	Coastal seawater
mgm4461589	-12.37444	-77	Aquatic	Coastal seawater
mgm4559920	1.338083	-49.37105	Aquatic	Coastal seawater
mgm4559921	0.949317	-48.67375	Aquatic	Coastal seawater
mgm4559922	0.267833	-47.161683	Aquatic	Coastal seawater
mgm4559926	3.505517	-50.500033	Aquatic	Coastal seawater
mgm4562962	-17.9	-39	Aquatic	Coastal seawater
mgm4562963	-17.9	-39	Aquatic	Coastal seawater
mgm4629609	37.069333	-8.331139	Aquatic	Coastal seawater
mgm4629610	37.069333	-8.331139	Aquatic	Coastal seawater
mgm4629611	37.069333	-8.331139	Aquatic	Coastal seawater

mgm4629612	37.069333	-8.331139	Aquatic	Coastal seawater
mgm4629613	37.069333	-8.331139	Aquatic	Coastal seawater
mgm4629614	37.069333	-8.331139	Aquatic	Coastal seawater
mgm4629615	37.069333	-8.331139	Aquatic	Coastal seawater
mgm4629616	37.069333	-8.331139	Aquatic	Coastal seawater
mgm4629617	37.069333	-8.331139	Aquatic	Coastal seawater
mgm4629618	37.069333	-8.331139	Aquatic	Coastal seawater
mgm4629619	37.069333	-8.331139	Aquatic	Coastal seawater
mgm4629620	37.069333	-8.331139	Aquatic	Coastal seawater
mgm4629621	37.069333	-8.331139	Aquatic	Coastal seawater
mgm4629622	37.069333	-8.331139	Aquatic	Coastal seawater
mgm4629623	37.069333	-8.331139	Aquatic	Coastal seawater
mgm4629624	37.069333	-8.331139	Aquatic	Coastal seawater
mgm4629625	37.069333	-8.331139	Aquatic	Coastal seawater
mgm4629626	37.069333	-8.331139	Aquatic	Coastal seawater
mgm4629627	37.069333	-8.331139	Aquatic	Coastal seawater
mgm4629628	37.069333	-8.331139	Aquatic	Coastal seawater
mgm4662524	-45.7971	171.02087	Aquatic	Coastal seawater
mgm4662527	-45.7971	171.02087	Aquatic	Coastal seawater
mgm4668298	24.83414	121.96191	Aquatic	Coastal seawater
mgm4668300	24.83499	121.96207	Aquatic	Coastal seawater
mgm4668301	24.83499	121.96207	Aquatic	Coastal seawater
mgm4668302	24.83499	121.96207	Aquatic	Coastal seawater
mgm4668303	24.83414	121.96191	Aquatic	Coastal seawater
mgm4668304	24.83414	121.96191	Aquatic	Coastal seawater
mgm4668305	24.83442	121.96201	Aquatic	Coastal seawater
mgm4679649	9.844167	104.296667	Aquatic	Coastal seawater
mgm4679741	9.844167	104.296667	Aquatic	Coastal seawater
mgm4679742	9.8472	104.2975	Aquatic	Coastal seawater
mgm4679743	9.8472	104.2975	Aquatic	Coastal seawater
mgm4698363	41.226579	-8.71988	Aquatic	Coastal seawater
mgm4698364	41.226579	-8.71988	Aquatic	Coastal seawater
mgm4699428	42.48853	3.14293	Aquatic	Coastal seawater
mgm4699429	42.48853	3.14293	Aquatic	Coastal seawater
mgm4699430	42.48853	3.14293	Aquatic	Coastal seawater
mgm4699432	42.48853	3.14293	Aquatic	Coastal seawater
mgm4699433	42.48853	3.14293	Aquatic	Coastal seawater
mgm4699434	42.48853	3.14293	Aquatic	Coastal seawater
mgm4699435	42.48853	3.14293	Aquatic	Coastal seawater

mgm4699436	42.48853	3.14293	Aquatic	Coastal seawater
mgm4699437	42.48853	3.14293	Aquatic	Coastal seawater
mgm4699438	42.48853	3.14293	Aquatic	Coastal seawater
mgm4699439	42.48853	3.14293	Aquatic	Coastal seawater
mgm4699440	42.48853	3.14293	Aquatic	Coastal seawater
mgm4699441	42.48853	3.14293	Aquatic	Coastal seawater
mgm4699442	42.48853	3.14293	Aquatic	Coastal seawater
mgm4699443	42.48853	3.14293	Aquatic	Coastal seawater
mgm4699444	42.48853	3.14293	Aquatic	Coastal seawater
mgm4699445	42.48853	3.14293	Aquatic	Coastal seawater
mgm4699446	42.48853	3.14293	Aquatic	Coastal seawater
mgm4699447	42.48853	3.14293	Aquatic	Coastal seawater
mgm4699448	42.48853	3.14293	Aquatic	Coastal seawater
mgm4699449	42.48853	3.14293	Aquatic	Coastal seawater
mgm4699450	42.48853	3.14293	Aquatic	Coastal seawater
mgm4699451	42.48853	3.14293	Aquatic	Coastal seawater
mgm4699452	42.48853	3.14293	Aquatic	Coastal seawater
mgm4699453	42.48853	3.14293	Aquatic	Coastal seawater
mgm4699454	42.48853	3.14293	Aquatic	Coastal seawater
mgm4699455	42.48853	3.14293	Aquatic	Coastal seawater
mgm4699456	42.48853	3.14293	Aquatic	Coastal seawater
mgm4699457	42.48853	3.14293	Aquatic	Coastal seawater
mgm4699458	42.48853	3.14293	Aquatic	Coastal seawater
mgm4699459	42.48853	3.14293	Aquatic	Coastal seawater
mgm4699460	42.48853	3.14293	Aquatic	Coastal seawater
mgm4699461	42.48853	3.14293	Aquatic	Coastal seawater
mgm4699462	42.48853	3.14293	Aquatic	Coastal seawater
mgm4699463	42.48853	3.14293	Aquatic	Coastal seawater
mgm4699464	42.48853	3.14293	Aquatic	Coastal seawater
mgm4699465	42.48853	3.14293	Aquatic	Coastal seawater
mgm4699466	42.48853	3.14293	Aquatic	Coastal seawater
mgm4699467	42.48853	3.14293	Aquatic	Coastal seawater
mgm4709843	34.2501	-119.9063	Aquatic	Coastal seawater
mgm4709844	34.2501	-119.9063	Aquatic	Coastal seawater
mgm4709845	34.2501	-119.9063	Aquatic	Coastal seawater
mgm4709846	34.2501	-119.9063	Aquatic	Coastal seawater
mgm4709847	34.2501	-119.9063	Aquatic	Coastal seawater
mgm4709848	34.2501	-119.9063	Aquatic	Coastal seawater
mgm4709849	34.2501	-119.9063	Aquatic	Coastal seawater

mgm4709850	34.2501	-119.9063	Aquatic	Coastal seawater
mgm4709851	34.2501	-119.9063	Aquatic	Coastal seawater
mgm4709852	34.2501	-119.9063	Aquatic	Coastal seawater
mgm4709853	34.2501	-119.9063	Aquatic	Coastal seawater
mgm4709854	34.2501	-119.9063	Aquatic	Coastal seawater
mgm4709855	34.2501	-119.9063	Aquatic	Coastal seawater
mgm4709856	34.2501	-119.9063	Aquatic	Coastal seawater
mgm4709857	34.2501	-119.9063	Aquatic	Coastal seawater
mgm4709858	34.2501	-119.9063	Aquatic	Coastal seawater
mgm4709859	34.2501	-119.9063	Aquatic	Coastal seawater
mgm4709860	34.2501	-119.9063	Aquatic	Coastal seawater
mgm4709861	34.2501	-119.9063	Aquatic	Coastal seawater
mgm4709862	34.2501	-119.9063	Aquatic	Coastal seawater
mgm4713199	34.2501	-119.9063	Aquatic	Coastal seawater
mgm4713204	34.2501	-119.9063	Aquatic	Coastal seawater
mgm4713208	34.2501	-119.9063	Aquatic	Coastal seawater
mgm4713210	34.2501	-119.9063	Aquatic	Coastal seawater
mgm4713211	34.2501	-119.9063	Aquatic	Coastal seawater
mgm4713213	34.2501	-119.9063	Aquatic	Coastal seawater
mgm4713216	34.2501	-119.9063	Aquatic	Coastal seawater
mgm4713218	34.2501	-119.9063	Aquatic	Coastal seawater
mgm4713220	34.2501	-119.9063	Aquatic	Coastal seawater
mgm4713221	34.2501	-119.9063	Aquatic	Coastal seawater
mgm4713226	34.2501	-119.9063	Aquatic	Coastal seawater
mgm4713228	34.2501	-119.9063	Aquatic	Coastal seawater
mgm4713230	34.2501	-119.9063	Aquatic	Coastal seawater
mgm4713232	34.2501	-119.9063	Aquatic	Coastal seawater
mgm4719936	31.21667	29.96667	Aquatic	Coastal seawater
mgm4719938	41.6666	2.8	Aquatic	Coastal seawater
mgm4719939	35.086353	-2.214658	Aquatic	Coastal seawater
mgm4719940	53.225417	-4.159028	Aquatic	Coastal seawater
mgm4719941	48.359	-4.552	Aquatic	Coastal seawater
mgm4719942	65.9449	-22.4192	Aquatic	Coastal seawater
mgm4719943	27.041533	33.9082	Aquatic	Coastal seawater
mgm4719944	31.383607	-80.866685	Aquatic	Coastal seawater
mgm4719945	43.333333	-1.925	Aquatic	Coastal seawater
mgm4719946	43.22639	5.74583	Aquatic	Coastal seawater
mgm4719947	65.48868	-18.0608	Aquatic	Coastal seawater
mgm4719948	39.134347	-9.384778	Aquatic	Coastal seawater

mgm4719950	43.57	13.595	Aquatic	Coastal seawater
mgm4719951	66.00659	-18.19753	Aquatic	Coastal seawater
mgm4719952	51.458433	2.350467	Aquatic	Coastal seawater
mgm4719953	59.8822	23.2538	Aquatic	Coastal seawater
mgm4719954	45.08	13.61	Aquatic	Coastal seawater
mgm4719955	45.31435	12.508317	Aquatic	Coastal seawater
mgm4719956	37.167	-7.504	Aquatic	Coastal seawater
mgm4719957	32.86698	-117.25725	Aquatic	Coastal seawater
mgm4719958	57.8498	-5.6495	Aquatic	Coastal seawater
mgm4719959	56.9631	-2.1031	Aquatic	Coastal seawater
mgm4719960	51.361369	3.118856	Aquatic	Coastal seawater
mgm4719961	38.885507	-76.5416	Aquatic	Coastal seawater
mgm4719962	39.3322	-75.4699	Aquatic	Coastal seawater
mgm4719963	35.086353	-2.214658	Aquatic	Coastal seawater
mgm4719964	38.48435	21.31689	Aquatic	Coastal seawater
mgm4719965	51.334817	2.50215	Aquatic	Coastal seawater
mgm4719966	50.151	-4.13	Aquatic	Coastal seawater
mgm4719967	43.8444	-69.6409	Aquatic	Coastal seawater
mgm4719968	32.7524	-79.89954	Aquatic	Coastal seawater
mgm4719969	64.208333	-22.015	Aquatic	Coastal seawater
mgm4719972	51.481583	2.451483	Aquatic	Coastal seawater
mgm4719974	31.98282	-81.01667	Aquatic	Coastal seawater
mgm4719975	59.622	10.6282	Aquatic	Coastal seawater
mgm4719976	54.8333	10	Aquatic	Coastal seawater
mgm4719977	-34.6759	-54.2752	Aquatic	Coastal seawater
mgm4719978	29.4667	34.9291	Aquatic	Coastal seawater
mgm4719979	35.1927	-2.88005	Aquatic	Coastal seawater
mgm4719980	51.74835	2.698	Aquatic	Coastal seawater
mgm4719981	45.325568	13.33189	Aquatic	Coastal seawater
mgm4719983	38.41333	27.03421	Aquatic	Coastal seawater
mgm4719984	51.307333	2.849333	Aquatic	Coastal seawater
mgm4719985	33.55	-118.4	Aquatic	Coastal seawater
mgm4719986	43.175843	27.908643	Aquatic	Coastal seawater
mgm4719987	-36.292794	174.818567	Aquatic	Coastal seawater
mgm4719988	41.6666666	18.5833333	Aquatic	Coastal seawater
mgm4719989	44.66666	-1.16666	Aquatic	Coastal seawater
mgm4719991	45.4142	12.4378	Aquatic	Coastal seawater
mgm4719992	27.4694	-80.283366	Aquatic	Coastal seawater
mgm4719993	51.7423	-8.3112	Aquatic	Coastal seawater

mgm4719995	36.997655	-7.973119	Aquatic	Coastal seawater
mgm4719996	27.61578	-82.72587	Aquatic	Coastal seawater
mgm4719997	35.35	25.29	Aquatic	Coastal seawater
mgm4719998	55.03306	-1.43278	Aquatic	Coastal seawater
mgm4719999	51.277933	2.613667	Aquatic	Coastal seawater
mgm4720000	40.808	14.25	Aquatic	Coastal seawater
mgm4720001	53.580926	8.148636	Aquatic	Coastal seawater
mgm4720002	32.74675	-9.036667	Aquatic	Coastal seawater
mgm4720003	64.208333	-22.015	Aquatic	Coastal seawater
mgm4720004	33.259611	-8.499222	Aquatic	Coastal seawater
mgm4720005	1.2726	103.9206	Aquatic	Coastal seawater
mgm4720007	74.31	-20.3043	Aquatic	Coastal seawater
mgm4720008	44.6936	-63.6403	Aquatic	Coastal seawater
mgm4720009	24.7449	-80.78375	Aquatic	Coastal seawater
mgm4720010	48.7778	-3.9375	Aquatic	Coastal seawater
mgm4720011	44.6936	-63.6403	Aquatic	Coastal seawater
mgm4720012	41.6835	-8.8341	Aquatic	Coastal seawater
mgm4720014	60.16121	5.11504	Aquatic	Coastal seawater
mgm4720015	42.252939	27.415647	Aquatic	Coastal seawater
mgm4720016	35	33	Aquatic	Coastal seawater
mgm4720017	-45.7442	170.7706	Aquatic	Coastal seawater
mgm4720018	35.661	24.99	Aquatic	Coastal seawater
mgm4720019	45.502	12.4176	Aquatic	Coastal seawater
mgm4720020	44	13	Aquatic	Coastal seawater
mgm4720021	65.4877	-18.06032	Aquatic	Coastal seawater
mgm4720022	39.415067	-9.218828	Aquatic	Coastal seawater
mgm4720023	74.31	-20.3043	Aquatic	Coastal seawater
mgm4720024	38.757283	-8.966333	Aquatic	Coastal seawater
mgm4720025	54.18194	7.9	Aquatic	Coastal seawater
mgm4720026	45.4125	12.5265	Aquatic	Coastal seawater
mgm4720028	46.44155	30.77595	Aquatic	Coastal seawater
mgm4720029	39.14039	-9.38011	Aquatic	Coastal seawater
mgm4720030	43.8604	-69.5781	Aquatic	Coastal seawater
mgm4720032	41.524467	-70.672174	Aquatic	Coastal seawater
mgm4720033	51.37485	3.218333	Aquatic	Coastal seawater
mgm4720034	59.89961	10.71999	Aquatic	Coastal seawater
mgm4720035	43.9475	12.935	Aquatic	Coastal seawater
mgm4720036	51.2695	2.90465	Aquatic	Coastal seawater
mgm4720037	66.00696	-18.19041	Aquatic	Coastal seawater

mgm4720039	34.32444	135.12083	Aquatic	Coastal seawater
mgm4720040	51.441017	3.13995	Aquatic	Coastal seawater
mgm4720041	45.4568	12.2605	Aquatic	Coastal seawater
mgm4720042	-34.37	-54.16	Aquatic	Coastal seawater
mgm4720044	59.81618	10.59863	Aquatic	Coastal seawater
mgm4720045	35.362826	33.287118	Aquatic	Coastal seawater
mgm4720046	41.1416	-8.6668	Aquatic	Coastal seawater
mgm4720047	38.6667	-9.4367	Aquatic	Coastal seawater
mgm4720048	34.7181	-76.6707	Aquatic	Coastal seawater
mgm4720050	32.0694	34.843	Aquatic	Coastal seawater
mgm4720051	43.43287	2.90056	Aquatic	Coastal seawater
mgm4720052	51.796139	1.012958	Aquatic	Coastal seawater
mgm4720053	45.70092	13.71003	Aquatic	Coastal seawater
mgm4720054	-33.93572	18.47147	Aquatic	Coastal seawater
mgm4720055	65.7064	-18.1181	Aquatic	Coastal seawater
mgm4720056	37.005053	-7.973119	Aquatic	Coastal seawater
mgm4720057	38.26861	15.63708	Aquatic	Coastal seawater
mgm4720058	8.5216	81.0521	Aquatic	Coastal seawater
mgm4720060	51.580317	2.7897	Aquatic	Coastal seawater
mgm4720061	51.185917	2.702133	Aquatic	Coastal seawater
mgm4720062	35.82	-5.75	Aquatic	Coastal seawater
mgm4720063	26.10293	-80.09315	Aquatic	Coastal seawater
mgm4720065	38.676942	-9.012392	Aquatic	Coastal seawater
mgm4720066	38.6792	-76.1742	Aquatic	Coastal seawater
mgm4720067	51.471583	3.0592	Aquatic	Coastal seawater
mgm4720069	58.957	-2.9726	Aquatic	Coastal seawater
mgm4720070	51.434733	2.810867	Aquatic	Coastal seawater
mgm4720071	41.6666666	18.5833333	Aquatic	Coastal seawater
mgm4720072	42.49	3.14	Aquatic	Coastal seawater
mgm4720073	40.145122	-8.869328	Aquatic	Coastal seawater
mgm4720074	69.023323	-105.34339	Aquatic	Coastal seawater
mgm4720075	-34.42	-54.16	Aquatic	Coastal seawater
mgm4720076	51.682717	2.414967	Aquatic	Coastal seawater
mgm4720077	30.2484	-88.74825	Aquatic	Coastal seawater
mgm4720078	66.1316	-18.7902	Aquatic	Coastal seawater
mgm4720079	35.661	24.99	Aquatic	Coastal seawater
mgm4720080	33.583917	-7.700639	Aquatic	Coastal seawater
mgm4720081	65.9449	-22.4192	Aquatic	Coastal seawater
mgm4722298	36.5533	-6.5669	Aquatic	Coastal seawater

mgm4722308	-32.2401	17.7103	Aquatic	Coastal seawater
mgm4770402	35.6	2.9	Aquatic	Coastal seawater
mgm4770403	32.4	9.02	Aquatic	Coastal seawater
mgm4781023	28.9433333	-88.97	Aquatic	Coastal seawater
mgm4842562	-20.55278	-70.73178	Aquatic	Coastal seawater
mgm4842563	-20.55278	-70.73178	Aquatic	Coastal seawater
mgm4842564	-20.55278	-70.73178	Aquatic	Coastal seawater
mgm4842565	-20.55278	-70.73178	Aquatic	Coastal seawater
mgm4443231	80.00048	-85.83945	Soil	Cold
mgm4443232	80.00048	-85.83945	Soil	Cold
mgm4449125	81.517	-62.283	Soil	Cold
mgm4449126	81.517	-62.283	Soil	Cold
mgm4450123	78.925833	11.943333	Soil	Cold
mgm4450124	78.925833	11.943333	Soil	Cold
mgm4450125	78.942222	11.8157	Soil	Cold
mgm4450126	78.942222	11.8157	Soil	Cold
mgm4450127	78.942222	11.8157	Soil	Cold
mgm4450729	81.517	-62.283	Soil	Cold
mgm4450731	81.517	-62.283	Soil	Cold
mgm4469340	64.677812	-157.83325	Soil	Cold
mgm4469538	64.677812	-157.83325	Soil	Cold
mgm4469540	64.677812	-157.83325	Soil	Cold
mgm4470009	64.677812	-157.83325	Soil	Cold
mgm4470010	64.677812	-157.83325	Soil	Cold
mgm4470253	64.677812	-157.83325	Soil	Cold
mgm4470254	64.677812	-157.83325	Soil	Cold
mgm4470255	64.677812	-157.83325	Soil	Cold
mgm4470315	64.677812	-157.83325	Soil	Cold
mgm4470378	64.677812	-157.83325	Soil	Cold
mgm4470381	64.677812	-157.83325	Soil	Cold
mgm4470382	64.677812	-157.83325	Soil	Cold
mgm4491237	64.677812	-157.83325	Soil	Cold
mgm4491382	64.677812	-157.83325	Soil	Cold
mgm4530050	79.415	-90.758333	Soil	Cold
mgm4667018	-76.160767	162.01583	Soil	Cold
mgm4667019	-77.0247	161.759167	Soil	Cold
mgm4667020	-77.01915	161.782183	Soil	Cold
mgm4667021	-77.0262	161.674833	Soil	Cold
mgm4667023	-77.018183	161.741783	Soil	Cold

mgm4667025	-76.822633	162.107033	Soil	Cold
mgm4667026	-77.046033	161.359317	Soil	Cold
mgm4667027	-76.869067	161.752467	Soil	Cold
mgm4667028	-77.018367	161.709783	Soil	Cold
mgm4667029	-77.023067	161.713233	Soil	Cold
mgm4667030	-76.952217	161.404383	Soil	Cold
mgm4667031	-76.11045	162.013983	Soil	Cold
mgm4667032	-76.72885	161.01165	Soil	Cold
mgm4667033	-77.0109	161.767217	Soil	Cold
mgm4667034	-76.654617	161.09295	Soil	Cold
mgm4667036	-76.967117	161.162167	Soil	Cold
mgm4670117	43.5144444	-110.71167	Soil	Cold
mgm4670118	43.2958333	-110.6575	Soil	Cold
mgm4670119	43.5144444	-110.71167	Soil	Cold
mgm4670120	43.5694444	-110.31111	Soil	Cold
mgm4670121	43.2958333	-110.6575	Soil	Cold
mgm4670123	43.5694444	-110.31111	Soil	Cold
mgm4689228	78.54	11.44	Soil	Cold
mgm4689230	78.54	11.44	Soil	Cold
mgm4689231	78.54	11.44	Soil	Cold
mgm4689232	78.54	11.44	Soil	Cold
mgm4689233	78.54	11.44	Soil	Cold
mgm4689234	78.54	11.44	Soil	Cold
mgm4689235	78.54	11.44	Soil	Cold
mgm4689236	78.54	11.44	Soil	Cold
mgm4689237	78.54	11.44	Soil	Cold
mgm4689238	78.54	11.44	Soil	Cold
mgm4689239	78.54	11.44	Soil	Cold
mgm4689240	78.54	11.44	Soil	Cold
mgm4737777	69.07	-105.03	Soil	Cold
mgm4737779	74.28	-20.32	Soil	Cold
mgm4737781	69.07	-105.03	Soil	Cold
mgm4737788	69.07	-105.03	Soil	Cold
mgm4737791	74.28	-20.32	Soil	Cold
mgm4451759	62.717	29.062	Aquatic	Groundwater
mgm4451761	62.717	29.062	Aquatic	Groundwater
mgm4453297	62.717	29.062	Aquatic	Groundwater
mgm4470906	10.239333	-61.604991	Aquatic	Groundwater
mgm4470907	57.02	-111.55	Aquatic	Groundwater

mgm4470909	57.02	-111.55	Aquatic	Groundwater
mgm4470910	57.02	-111.55	Aquatic	Groundwater
mgm4492624	57.02	-111.55	Aquatic	Groundwater
mgm4492774	57.02	-111.55	Aquatic	Groundwater
mgm4492775	57.02	-111.55	Aquatic	Groundwater
mgm4492776	57.02	-111.55	Aquatic	Groundwater
mgm4507669	57.02	-111.55	Aquatic	Groundwater
mgm4527898	57.02	-111.55	Aquatic	Groundwater
mgm4559934	55.07	-110.53	Aquatic	Groundwater
mgm4564417	56.97	-111.39	Aquatic	Groundwater
mgm4606815	46.047772	-77.409993	Aquatic	Groundwater
mgm4606816	46.047772	-77.409993	Aquatic	Groundwater
mgm4606817	46.047772	-77.409993	Aquatic	Groundwater
mgm4606818	46.047772	-77.409993	Aquatic	Groundwater
mgm4606856	46.047772	-77.409993	Aquatic	Groundwater
mgm4606871	46.047772	-77.409993	Aquatic	Groundwater
mgm4606957	46.047772	-77.409993	Aquatic	Groundwater
mgm4606964	46.047772	-77.409993	Aquatic	Groundwater
mgm4607040	46.047772	-77.409993	Aquatic	Groundwater
mgm4607056	46.047772	-77.409993	Aquatic	Groundwater
mgm4608555	46.047772	-77.409993	Aquatic	Groundwater
mgm4609351	46.047772	-77.409993	Aquatic	Groundwater
mgm4609352	46.047772	-77.409993	Aquatic	Groundwater
mgm4610088	46.047772	-77.409993	Aquatic	Groundwater
mgm4795328	29.424	-98.493	Aquatic	Groundwater
mgm4795329	29.424	-98.493	Aquatic	Groundwater
mgm4795332	29.424	-98.493	Aquatic	Groundwater
mgm4795333	36.154	-98.992	Aquatic	Groundwater
mgm4795334	36.154	-95.992	Aquatic	Groundwater
mgm4795335	38.523	-77.29	Aquatic	Groundwater
mgm4795339	38.523	-77.29	Aquatic	Groundwater
mgm4795340	36.154	-98.992	Aquatic	Groundwater
mgm4795341	36.154	-98.992	Aquatic	Groundwater
mgm4795342	36.154	-98.992	Aquatic	Groundwater
mgm4795673	36.154	-98.992	Aquatic	Groundwater
mgm4795675	38.523	-77.29	Aquatic	Groundwater
mgm4795676	40.518	-74.412	Aquatic	Groundwater
mgm4795677	40.518	-74.412	Aquatic	Groundwater
mgm4795678	38.523	-77.29	Aquatic	Groundwater

mgm4795679	38.523	-77.29	Aquatic	Groundwater
mgm4795845	38.6	-77.162	Aquatic	Groundwater
mgm4795846	38.6	-77.162	Aquatic	Groundwater
mgm4795847	38.6	-77.162	Aquatic	Groundwater
mgm4795922	38.6	-77.162	Aquatic	Groundwater
mgm4795924	38.6	-77.162	Aquatic	Groundwater
mgm4795927	38.6	-77.162	Aquatic	Groundwater
mgm4966255	-37.202777	174.873884	Aquatic	Groundwater
mgm4966256	-37.165378	174.870224	Aquatic	Groundwater
mgm4966257	-37.192702	174.900955	Aquatic	Groundwater
mgm4966258	-36.105879	174.516346	Aquatic	Groundwater
mgm4966259	-37.130213	174.755722	Aquatic	Groundwater
mgm4966399	-38.615545	175.836022	Aquatic	Groundwater
mgm4441133	-12.0925	96.88167	Aquatic	Marine seawater
mgm4441134	-10.4461	88.3027	Aquatic	Marine seawater
mgm4441135	-26.035	50.123	Aquatic	Marine seawater
mgm4441145	-1.97389	-95.01472	Aquatic	Marine seawater
mgm4441146	-10.1314	-135.4494	Aquatic	Marine seawater
mgm4441147	-8.505	80.3756	Aquatic	Marine seawater
mgm4441148	-4.6136	55.5086	Aquatic	Marine seawater
mgm4441149	-4.635	56.8361	Aquatic	Marine seawater
mgm4441150	-4.6625	60.5231	Aquatic	Marine seawater
mgm4441151	-23.2161	52.3061	Aquatic	Marine seawater
mgm4441155	-10.94361	92.05889	Aquatic	Marine seawater
mgm4441156	-9.59694	84.1957	Aquatic	Marine seawater
mgm4441568	-23.2161	52.3061	Aquatic	Marine seawater
mgm4441570	31.175	-64.324	Aquatic	Marine seawater
mgm4441571	31.175	-64.324	Aquatic	Marine seawater
mgm4441573	31.175	-64.324	Aquatic	Marine seawater
mgm4441574	32.1748	-64.01017	Aquatic	Marine seawater
mgm4441589	10.7164	-80.2544	Aquatic	Marine seawater
mgm4441591	8.1292	-79.6911	Aquatic	Marine seawater
mgm4441593	5.55278	-87.0878	Aquatic	Marine seawater
mgm4441594	1.2642	-90.295	Aquatic	Marine seawater
mgm4441595	1.2161	-90.42278	Aquatic	Marine seawater
mgm4441597	0.30111	-91.65167	Aquatic	Marine seawater
mgm4441598	-0.59389	-91.06944	Aquatic	Marine seawater
mgm4441599	-1.2283	-90.4292	Aquatic	Marine seawater
mgm4441600	-0.3831	-90.2797	Aquatic	Marine seawater

mgm4441601	1.3891367	-91.81694	Aquatic	Marine seawater
mgm4441602	-0.02083	-91.1978	Aquatic	Marine seawater
mgm4441603	-17.47583	-149.8122	Aquatic	Marine seawater
mgm4441604	-15.1436	-147.435	Aquatic	Marine seawater
mgm4441606	12.0925	96.88167	Aquatic	Marine seawater
mgm4441607	-10.4461	88.3027	Aquatic	Marine seawater
mgm4441608	-10.4461	88.30278	Aquatic	Marine seawater
mgm4441609	-8.505	80.3756	Aquatic	Marine seawater
mgm4441610	-7.0075	76.3314	Aquatic	Marine seawater
mgm4441611	-4.99028	64.9767	Aquatic	Marine seawater
mgm4441613	-4.6136	55.5086	Aquatic	Marine seawater
mgm4441614	-29.3489	43.2156	Aquatic	Marine seawater
mgm4441615	-30.8983	40.4203	Aquatic	Marine seawater
mgm4441616	-32.39917	36.5919	Aquatic	Marine seawater
mgm4441621	70.6933	-136.4233	Aquatic	Marine seawater
mgm4441622	73.16167	159.44667	Aquatic	Marine seawater
mgm4441661	5.64	-86.565	Aquatic	Marine seawater
mgm4441662	0.2722	-91.633	Aquatic	Marine seawater
mgm4442626	-0.30111	-91.65167	Aquatic	Marine seawater
mgm4443733	12.2836	-56.118	Aquatic	Marine seawater
mgm4443734	15.03833	-31.9567	Aquatic	Marine seawater
mgm4443735	11.7522	-46.1675	Aquatic	Marine seawater
mgm4443736	12.2836	56.118	Aquatic	Marine seawater
mgm4443737	11.7522	-46.1675	Aquatic	Marine seawater
mgm4443738	11.7633	-46.17167	Aquatic	Marine seawater
mgm4443740	12.001	-40.1022	Aquatic	Marine seawater
mgm4443741	-15.055	-155.01833	Aquatic	Marine seawater
mgm4443742	-15	-178.75	Aquatic	Marine seawater
mgm4443743	-15.055	-155.01833	Aquatic	Marine seawater
mgm4443744	15	-178.75	Aquatic	Marine seawater
mgm4443763	12.0067	-40.1133	Aquatic	Marine seawater
mgm4466309	5.86646	-162.11346	Aquatic	Marine seawater
mgm4466592	-9.91672	-150.21072	Aquatic	Marine seawater
mgm4466597	-11.43911	-151.81964	Aquatic	Marine seawater
mgm4466599	-11.44423	-151.81709	Aquatic	Marine seawater
mgm4466736	-0.38188	-159.998	Aquatic	Marine seawater
mgm4466737	-0.36537	-160.006	Aquatic	Marine seawater
mgm4466738	-0.36902	-160.00819	Aquatic	Marine seawater
mgm4466739	-0.369017	-160.00819	Aquatic	Marine seawater

mgm4466740	6.3856	-162.3486	Aquatic	Marine seawater
mgm4466741	6.387	-162.386	Aquatic	Marine seawater
mgm4466745	1.99095	-157.48251	Aquatic	Marine seawater
mgm4466809	2.0085833	-157.48945	Aquatic	Marine seawater
mgm4466810	-4.03326	-154.95094	Aquatic	Marine seawater
mgm4466812	-3.99531	-154.94452	Aquatic	Marine seawater
mgm4466813	5.8692	-162.0755	Aquatic	Marine seawater
mgm4466820	-5.6222	-155.88002	Aquatic	Marine seawater
mgm4466825	3.82595	-159.34957	Aquatic	Marine seawater
mgm4466835	3.84085	-159.36047	Aquatic	Marine seawater
mgm4466841	3.8122	-159.3386	Aquatic	Marine seawater
mgm4466844	-10.05835	-152.30954	Aquatic	Marine seawater
mgm4466845	4.70242	-160.39212	Aquatic	Marine seawater
mgm4466846	4.6867167	-160.42023	Aquatic	Marine seawater
mgm4487647	-20.53	-29.33	Aquatic	Marine seawater
mgm4559924	8.02225	-50.982	Aquatic	Marine seawater
mgm4601897	-65.27	118.98	Aquatic	Marine seawater
mgm4601899	-65.27	118.98	Aquatic	Marine seawater
mgm4616090	-65.27	118.98	Aquatic	Marine seawater
mgm4616091	-65.27	118.98	Aquatic	Marine seawater
mgm4661579	-17.96505	-38.704766	Aquatic	Marine seawater
mgm4661580	-17.96505	-38.704766	Aquatic	Marine seawater
mgm4662525	-45.8361	171.5481	Aquatic	Marine seawater
mgm4662526	-45.84373	171.53933	Aquatic	Marine seawater
mgm4662528	-45.8361	171.5481	Aquatic	Marine seawater
mgm4662529	-45.84373	171.53933	Aquatic	Marine seawater
mgm4713197	17.538	-149.8289	Aquatic	Marine seawater
mgm4713198	17.538	-149.8289	Aquatic	Marine seawater
mgm4713200	17.538	-149.8289	Aquatic	Marine seawater
mgm4713202	17.538	-149.8289	Aquatic	Marine seawater
mgm4713203	17.538	-149.8289	Aquatic	Marine seawater
mgm4713205	17.538	-149.8289	Aquatic	Marine seawater
mgm4713206	17.538	-149.8289	Aquatic	Marine seawater
mgm4713207	17.538	-149.8289	Aquatic	Marine seawater
mgm4713209	17.538	-149.8289	Aquatic	Marine seawater
mgm4713212	17.538	-149.8289	Aquatic	Marine seawater
mgm4713214	17.538	-149.8289	Aquatic	Marine seawater
mgm4713215	17.538	-149.8289	Aquatic	Marine seawater
mgm4713217	17.538	-149.8289	Aquatic	Marine seawater

mgm4713222	17.538	-149.8289	Aquatic	Marine seawater
mgm4713223	17.538	-149.8289	Aquatic	Marine seawater
mgm4713224	17.538	-149.8289	Aquatic	Marine seawater
mgm4713225	17.538	-149.8289	Aquatic	Marine seawater
mgm4713227	17.538	-149.8289	Aquatic	Marine seawater
mgm4713229	17.538	-149.8289	Aquatic	Marine seawater
mgm4713231	17.538	-149.8289	Aquatic	Marine seawater
mgm4719937	32.7747	-16.828664	Aquatic	Marine seawater
mgm4719949	21.28656	-157.84351	Aquatic	Marine seawater
mgm4719970	-8.703761	40.659875	Aquatic	Marine seawater
mgm4719971	21.2888	-156.86362	Aquatic	Marine seawater
mgm4719973	32.64605	-16.910158	Aquatic	Marine seawater
mgm4719982	21.26882	-157.72231	Aquatic	Marine seawater
mgm4719990	-41.839834	-83.18995	Aquatic	Marine seawater
mgm4719994	16.802575	88.08165	Aquatic	Marine seawater
mgm4720006	38.64	-28.13	Aquatic	Marine seawater
mgm4720013	32.741808	-16.711281	Aquatic	Marine seawater
mgm4720027	37.4257	-25.3156	Aquatic	Marine seawater
mgm4720031	34.41	-9.51	Aquatic	Marine seawater
mgm4720038	-17.2894	-149.53985	Aquatic	Marine seawater
mgm4720043	78.453333	-2.829667	Aquatic	Marine seawater
mgm4720049	37.4328	-25.19	Aquatic	Marine seawater
mgm4720059	38.5297	-28.601778	Aquatic	Marine seawater
mgm4720068	32.822	32.954	Aquatic	Marine seawater
mgm4722276	-20.4091	-3.1759	Aquatic	Marine seawater
mgm4722277	-20.9354	-35.1803	Aquatic	Marine seawater
mgm4722278	-31.0266	4.665	Aquatic	Marine seawater
mgm4722279	34.6712	-71.3093	Aquatic	Marine seawater
mgm4722280	35.3671	-127.7422	Aquatic	Marine seawater
mgm4722281	42.2038	17.715	Aquatic	Marine seawater
mgm4722282	6.0001	73.8955	Aquatic	Marine seawater
mgm4722283	27.16	34.835	Aquatic	Marine seawater
mgm4722284	36.5533	-6.5669	Aquatic	Marine seawater
mgm4722285	25.5264	-88.394	Aquatic	Marine seawater
mgm4722286	34.1132	-49.9181	Aquatic	Marine seawater
mgm4722287	-8.9111	-142.5571	Aquatic	Marine seawater
mgm4722288	-30.1367	-43.2899	Aquatic	Marine seawater
mgm4722289	39.2305	-70.0377	Aquatic	Marine seawater
mgm4722290	-60.2287	-60.6476	Aquatic	Marine seawater

mgm4722291	20.8183	63.5047	Aquatic	Marine seawater
mgm4722292	6.3332	-102.9432	Aquatic	Marine seawater
mgm4722293	21.9467	38.2517	Aquatic	Marine seawater
mgm4722294	23.36	37.2183	Aquatic	Marine seawater
mgm4722295	-21.146	-104.787	Aquatic	Marine seawater
mgm4722296	39.3888	19.3905	Aquatic	Marine seawater
mgm4722297	18.3967	39.875	Aquatic	Marine seawater
mgm4722299	-22.3368	40.3412	Aquatic	Marine seawater
mgm4722300	35.759	14.2574	Aquatic	Marine seawater
mgm4722301	0.0003	-153.6759	Aquatic	Marine seawater
mgm4722302	-5.2529	-85.1545	Aquatic	Marine seawater
mgm4722303	0.0033	71.6428	Aquatic	Marine seawater
mgm4722304	18.3967	39.875	Aquatic	Marine seawater
mgm4722305	20.8183	63.5047	Aquatic	Marine seawater
mgm4722306	-29.7238	-101.1604	Aquatic	Marine seawater
mgm4722307	-47.1863	-58.2902	Aquatic	Marine seawater
mgm4722309	-30.1367	-43.2899	Aquatic	Marine seawater
mgm4722310	21.9467	38.2517	Aquatic	Marine seawater
mgm4722311	-9.1504	-140.5216	Aquatic	Marine seawater
mgm4722312	39.3888	19.3905	Aquatic	Marine seawater
mgm4722313	-8.7789	-17.9099	Aquatic	Marine seawater
mgm4722314	-47.1863	-58.2902	Aquatic	Marine seawater
mgm4722315	31.5213	-158.9958	Aquatic	Marine seawater
mgm4781021	27.3741667	-90.569722	Aquatic	Marine seawater
mgm4781022	27.4244444	-91.968056	Aquatic	Marine seawater
mgm4781024	28.7027778	-88.366111	Aquatic	Marine seawater
mgm4781025	28.7027778	-88.366111	Aquatic	Marine seawater
mgm4781026	27.7838889	-91.516944	Aquatic	Marine seawater
mgm4857030	45.325568	13.33189	Aquatic	Marine seawater
mgm4857031	45.4125	12.5265	Aquatic	Marine seawater
mgm4857032	45.31435	12.508317	Aquatic	Marine seawater
mgm4857033	45.08	13.61	Aquatic	Marine seawater
mgm4857034	45.502	12.4176	Aquatic	Marine seawater
mgm4445990	36.816667	115.916667	Soil	Subtropical
mgm4445993	36.816667	115.916667	Soil	Subtropical
mgm4445994	36.816667	115.916667	Soil	Subtropical
mgm4445996	36.816667	115.916667	Soil	Subtropical
mgm4451033	-23.89694	-46.20778	Soil	Subtropical
mgm4451034	-23.90167	-45.25083	Soil	Subtropical

mgm4451035	-25.08389	-45.25083	Soil	Subtropical
mgm4451036	-25.08389	-47.95056	Soil	Subtropical
mgm4582104	-22.41	-47.38	Soil	Subtropical
mgm4582105	-22.41	-47.38	Soil	Subtropical
mgm4582106	-22.41	-47.38	Soil	Subtropical
mgm4582107	-22.41	-47.38	Soil	Subtropical
mgm4582108	-22.41	-47.38	Soil	Subtropical
mgm4582109	-22.41	-47.38	Soil	Subtropical
mgm4582110	-22.41	-47.38	Soil	Subtropical
mgm4582111	-22.41	-47.38	Soil	Subtropical
mgm4582112	-22.41	-47.38	Soil	Subtropical
mgm4582113	-22.41	-47.38	Soil	Subtropical
mgm4582114	-22.41	-47.38	Soil	Subtropical
mgm4582115	-22.41	-47.38	Soil	Subtropical
mgm4582116	-22.41	-47.38	Soil	Subtropical
mgm4582117	-22.41	-47.38	Soil	Subtropical
mgm4582118	-22.41	-47.38	Soil	Subtropical
mgm4582119	-22.41	-47.38	Soil	Subtropical
mgm4582120	-22.41	-47.38	Soil	Subtropical
mgm4582121	-22.41	-47.38	Soil	Subtropical
mgm4582122	-22.41	-47.38	Soil	Subtropical
mgm4582123	-22.41	-47.38	Soil	Subtropical
mgm4582124	-22.41	-47.38	Soil	Subtropical
mgm4582125	-22.41	-47.38	Soil	Subtropical
mgm4582126	-22.41	-47.38	Soil	Subtropical
mgm4582127	-22.41	-47.38	Soil	Subtropical
mgm4582128	-22.41	-47.38	Soil	Subtropical
mgm4582129	-22.41	-47.38	Soil	Subtropical
mgm4582130	-22.41	-47.38	Soil	Subtropical
mgm4582131	-22.41	-47.38	Soil	Subtropical
mgm4582132	-22.41	-47.38	Soil	Subtropical
mgm4582133	-22.41	-47.38	Soil	Subtropical
mgm4582134	-22.41	-47.38	Soil	Subtropical
mgm4582135	-22.41	-47.38	Soil	Subtropical
mgm4582136	-22.41	-47.38	Soil	Subtropical
mgm4582137	-22.41	-47.38	Soil	Subtropical
mgm4582138	-22.41	-47.38	Soil	Subtropical
mgm4582139	-22.41	-47.38	Soil	Subtropical
mgm4582140	-22.41	-47.38	Soil	Subtropical

mgm4582141	-22.41	-47.38	Soil	Subtropical
mgm4582142	-22.41	-47.38	Soil	Subtropical
mgm4582143	-22.41	-47.38	Soil	Subtropical
mgm4582144	-22.41	-47.38	Soil	Subtropical
mgm4582145	-22.41	-47.38	Soil	Subtropical
mgm4582146	-22.41	-47.38	Soil	Subtropical
mgm4582147	-22.41	-47.38	Soil	Subtropical
mgm4582148	-22.41	-47.38	Soil	Subtropical
mgm4582149	-22.41	-47.38	Soil	Subtropical
mgm4582150	-22.41	-47.38	Soil	Subtropical
mgm4582151	-22.41	-47.38	Soil	Subtropical
mgm4582152	-22.41	-47.38	Soil	Subtropical
mgm4582153	-22.41	-47.38	Soil	Subtropical
mgm4582154	-22.41	-47.38	Soil	Subtropical
mgm4582155	-22.41	-47.38	Soil	Subtropical
mgm4582156	-22.41	-47.38	Soil	Subtropical
mgm4582157	-22.41	-47.38	Soil	Subtropical
mgm4582158	-22.41	-47.38	Soil	Subtropical
mgm4582159	-22.41	-47.38	Soil	Subtropical
mgm4582160	-22.41	-47.38	Soil	Subtropical
mgm4600198	19.402222	-98.048333	Soil	Subtropical
mgm4600199	19.402222	-98.048333	Soil	Subtropical
mgm4600200	19.402222	-98.048333	Soil	Subtropical
mgm4615217	-22.666667	-48.166667	Soil	Subtropical
mgm4615218	-22.666667	-48.166667	Soil	Subtropical
mgm4615220	-22.666667	-48.166667	Soil	Subtropical
mgm4615241	-22.666667	-48.166667	Soil	Subtropical
mgm4615243	-22.666667	-48.166667	Soil	Subtropical
mgm4615270	-22.666667	-48.166667	Soil	Subtropical
mgm4615272	-22.666667	-48.166667	Soil	Subtropical
mgm4615273	-22.666667	-48.166667	Soil	Subtropical
mgm4615274	-22.666667	-48.166667	Soil	Subtropical
mgm4615275	-22.666667	-48.166667	Soil	Subtropical
mgm4615276	-22.666667	-48.166667	Soil	Subtropical
mgm4615277	-22.666667	-48.166667	Soil	Subtropical
mgm4615280	-22.666667	-48.166667	Soil	Subtropical
mgm4615281	-22.666667	-48.166667	Soil	Subtropical
mgm4615282	-22.666667	-48.166667	Soil	Subtropical
mgm4620456	-22.666667	-48.166667	Soil	Subtropical

mgm4622702	-23.579722	-47.586667	Soil	Subtropical
mgm4622703	-23.579722	-47.586667	Soil	Subtropical
mgm4622704	-23.579722	-47.586667	Soil	Subtropical
mgm4626287	22.11	112.77	Soil	Subtropical
mgm4626288	22.11	112.77	Soil	Subtropical
mgm4626289	22.11	112.77	Soil	Subtropical
mgm4626290	22.11	112.77	Soil	Subtropical
mgm4626291	22.11	112.77	Soil	Subtropical
mgm4626292	22.11	112.77	Soil	Subtropical
mgm4626293	22.11	112.77	Soil	Subtropical
mgm4626294	22.11	112.77	Soil	Subtropical
mgm4626295	22.11	112.77	Soil	Subtropical
mgm4626743	21.08839	105.727278	Soil	Subtropical
mgm4626744	21.08839	105.727278	Soil	Subtropical
mgm4626745	21.08839	105.727278	Soil	Subtropical
mgm4626746	21.08839	105.727278	Soil	Subtropical
mgm4626747	21.08839	105.727278	Soil	Subtropical
mgm4626748	21.08839	105.727278	Soil	Subtropical
mgm4626753	21.08839	105.727278	Soil	Subtropical
mgm4626754	21.08839	105.727278	Soil	Subtropical
mgm4626755	21.08839	105.727278	Soil	Subtropical
mgm4626756	21.08839	105.727278	Soil	Subtropical
mgm4628823	22.11	112.77	Soil	Subtropical
mgm4628824	22.11	112.77	Soil	Subtropical
mgm4628825	22.11	112.77	Soil	Subtropical
mgm4628826	22.11	112.77	Soil	Subtropical
mgm4628827	22.11	112.77	Soil	Subtropical
mgm4628828	22.11	112.77	Soil	Subtropical
mgm4628829	22.11	112.77	Soil	Subtropical
mgm4628830	22.11	112.77	Soil	Subtropical
mgm4628831	22.11	112.77	Soil	Subtropical
mgm4658538	19.402222	-98.048333	Soil	Subtropical
mgm4658539	19.402222	-98.048333	Soil	Subtropical
mgm4658540	19.402222	-98.048333	Soil	Subtropical
mgm4702966	37.4599	126.9519	Soil	Subtropical
mgm4702967	37.4599	126.9519	Soil	Subtropical
mgm4702968	37.4599	126.9519	Soil	Subtropical
mgm4702969	37.4599	126.9519	Soil	Subtropical
mgm4702970	37.4599	126.9519	Soil	Subtropical

mgm4702971	37.4599	126.9519	Soil	Subtropical
mgm4702972	37.4599	126.9519	Soil	Subtropical
mgm4702973	37.4599	126.9519	Soil	Subtropical
mgm4702974	37.4599	126.9519	Soil	Subtropical
mgm4702975	37.4599	126.9519	Soil	Subtropical
mgm4702976	37.4599	126.9519	Soil	Subtropical
mgm4702977	37.4599	126.9519	Soil	Subtropical
mgm4702978	37.4599	126.9519	Soil	Subtropical
mgm4702979	37.4599	126.9519	Soil	Subtropical
mgm4702980	37.4599	126.9519	Soil	Subtropical
mgm4702981	37.4599	126.9519	Soil	Subtropical
mgm4702982	37.4599	126.9519	Soil	Subtropical
mgm4702983	37.4599	126.9519	Soil	Subtropical
mgm4702984	37.4599	126.9519	Soil	Subtropical
mgm4702985	37.4599	126.9519	Soil	Subtropical
mgm4702986	37.4599	126.9519	Soil	Subtropical
mgm4702987	37.4599	126.9519	Soil	Subtropical
mgm4702988	37.4599	126.9519	Soil	Subtropical
mgm4702989	37.4599	126.9519	Soil	Subtropical
mgm4702990	37.4599	126.9519	Soil	Subtropical
mgm4702991	37.4599	126.9519	Soil	Subtropical
mgm4702992	37.4599	126.9519	Soil	Subtropical
mgm4702993	37.4599	126.9519	Soil	Subtropical
mgm4702994	37.4599	126.9519	Soil	Subtropical
mgm4702995	37.4599	126.9519	Soil	Subtropical
mgm4707558	37.4599	126.9519	Soil	Subtropical
mgm4707559	37.4599	126.9519	Soil	Subtropical
mgm4707560	37.4599	126.9519	Soil	Subtropical
mgm4707561	37.4599	126.9519	Soil	Subtropical
mgm4707562	37.4599	126.9519	Soil	Subtropical
mgm4707563	37.4599	126.9519	Soil	Subtropical
mgm4707564	37.4599	126.9519	Soil	Subtropical
mgm4707565	37.4599	126.9519	Soil	Subtropical
mgm4707566	37.4599	126.9519	Soil	Subtropical
mgm4707567	37.4599	126.9519	Soil	Subtropical
mgm4707568	37.4599	126.9519	Soil	Subtropical
mgm4707569	37.4599	126.9519	Soil	Subtropical
mgm4707570	37.4599	126.9519	Soil	Subtropical
mgm4707571	37.4599	126.9519	Soil	Subtropical

mgm4707572	37.4599	126.9519	Soil	Subtropical
mgm4707573	37.4599	126.9519	Soil	Subtropical
mgm4707574	37.4599	126.9519	Soil	Subtropical
mgm4707575	37.4599	126.9519	Soil	Subtropical
mgm4707576	37.4599	126.9519	Soil	Subtropical
mgm4707577	37.4599	126.9519	Soil	Subtropical
mgm4707578	37.4599	126.9519	Soil	Subtropical
mgm4707579	37.4599	126.9519	Soil	Subtropical
mgm4707580	37.4599	126.9519	Soil	Subtropical
mgm4707581	37.4599	126.9519	Soil	Subtropical
mgm4707582	37.4599	126.9519	Soil	Subtropical
mgm4707583	37.4599	126.9519	Soil	Subtropical
mgm4707584	37.4599	126.9519	Soil	Subtropical
mgm4707585	37.4599	126.9519	Soil	Subtropical
mgm4707586	37.4599	126.9519	Soil	Subtropical
mgm4707587	37.4599	126.9519	Soil	Subtropical
mgm4707588	37.4599	126.9519	Soil	Subtropical
mgm4707589	37.4599	126.9519	Soil	Subtropical
mgm4441590	9.1644	-79.83611	Aquatic	Surface freshwater
mgm4467029	42.1469444	-8.0144444	Aquatic	Surface freshwater
mgm4467058	31.26	120.12	Aquatic	Surface freshwater
mgm4467059	40.531	-84.499	Aquatic	Surface freshwater
mgm4470937	39.109484	-84.545377	Aquatic	Surface freshwater
mgm4470954	39.109484	-84.545377	Aquatic	Surface freshwater
mgm4511246	43	-91	Aquatic	Surface freshwater
mgm4511247	44	-91	Aquatic	Surface freshwater
mgm4511248	47	-95	Aquatic	Surface freshwater
mgm4511249	45	-94	Aquatic	Surface freshwater
mgm4511250	45	-94	Aquatic	Surface freshwater
mgm4511251	44	-93	Aquatic	Surface freshwater
mgm4511252	44	-93	Aquatic	Surface freshwater
mgm4511253	43	-93	Aquatic	Surface freshwater
mgm4511254	44	-93	Aquatic	Surface freshwater
mgm4511255	44	-92	Aquatic	Surface freshwater
mgm4511256	44	-92	Aquatic	Surface freshwater
mgm4511257	44	-92	Aquatic	Surface freshwater
mgm4516288	39.3037632	-0.3226403	Aquatic	Surface freshwater
mgm4516289	39.3037632	-0.3226403	Aquatic	Surface freshwater
mgm4516290	39.3037632	-0.3226403	Aquatic	Surface freshwater

mgm4517540	47.561024	-122.35012	Aquatic	Surface freshwater
mgm4517541	47.403256	-123.14116	Aquatic	Surface freshwater
mgm4517542	47.584796	-122.36873	Aquatic	Surface freshwater
mgm4517543	47.700556	-122.45056	Aquatic	Surface freshwater
mgm4517544	47.333333	-122.43472	Aquatic	Surface freshwater
mgm4517545	47.686743	-122.40378	Aquatic	Surface freshwater
mgm4517546	47.661667	-122.43333	Aquatic	Surface freshwater
mgm4622705	-23.5794	-47.5882	Aquatic	Surface freshwater
mgm4622706	-23.5794	-47.5882	Aquatic	Surface freshwater
mgm4622707	-23.5794	-47.5882	Aquatic	Surface freshwater
mgm4690154	42.2	-81.2	Aquatic	Surface freshwater
mgm4690155	42.2	-81.2	Aquatic	Surface freshwater
mgm4690156	42.2	-81.2	Aquatic	Surface freshwater
mgm4690157	42.2	-81.2	Aquatic	Surface freshwater
mgm4690158	42.2	-81.2	Aquatic	Surface freshwater
mgm4690159	42.2	-81.2	Aquatic	Surface freshwater
mgm4690160	42.2	-81.2	Aquatic	Surface freshwater
mgm4690161	42.2	-81.2	Aquatic	Surface freshwater
mgm4690162	42.2	-81.2	Aquatic	Surface freshwater
mgm4690163	42.2	-81.2	Aquatic	Surface freshwater
mgm4690164	42.2	-81.2	Aquatic	Surface freshwater
mgm4690165	42.2	-81.2	Aquatic	Surface freshwater
mgm4690166	42.2	-81.2	Aquatic	Surface freshwater
mgm4690168	42.2	-81.2	Aquatic	Surface freshwater
mgm4690169	42.2	-81.2	Aquatic	Surface freshwater
mgm4690209	15.098333	-23.623333	Aquatic	Surface freshwater
mgm4692683	42.2	-81.2	Aquatic	Surface freshwater
mgm4692684	42.2	-81.2	Aquatic	Surface freshwater
mgm4706087	42.2	-81.2	Aquatic	Surface freshwater
mgm4712785	31.4137069	121.709913	Aquatic	Surface freshwater
mgm4712786	31.4415378	121.691631	Aquatic	Surface freshwater
mgm4712787	31.4137069	121.709913	Aquatic	Surface freshwater
mgm4712788	31.4415378	121.691631	Aquatic	Surface freshwater
mgm4739190	5.58368333	101.3451	Aquatic	Surface freshwater
mgm4739192	5.46115	101.323283	Aquatic	Surface freshwater
mgm4739347	5.46115	101.323283	Aquatic	Surface freshwater
mgm4739348	5.58368333	101.3451	Aquatic	Surface freshwater
mgm4742954	40.80612	-96.686063	Aquatic	Surface freshwater
mgm4753763	40.784236	-96.643326	Aquatic	Surface freshwater

mgm4754113	40.80612	-96.686063	Aquatic	Surface freshwater
mgm4754114	40.80612	-96.686063	Aquatic	Surface freshwater
mgm4754463	40.80612	-96.686063	Aquatic	Surface freshwater
mgm4754464	40.80612	-96.686063	Aquatic	Surface freshwater
mgm4754519	40.801736	-96.679853	Aquatic	Surface freshwater
mgm4754900	40.801736	-96.679853	Aquatic	Surface freshwater
mgm4754901	40.795436	-96.670673	Aquatic	Surface freshwater
mgm4754902	40.784236	-96.643326	Aquatic	Surface freshwater
mgm4754903	40.795436	-96.670673	Aquatic	Surface freshwater
mgm4755173	40.80612	-96.686063	Aquatic	Surface freshwater
mgm4755174	40.80612	-96.686063	Aquatic	Surface freshwater
mgm4755175	40.80612	-96.686063	Aquatic	Surface freshwater
mgm4755527	40.80612	-96.686063	Aquatic	Surface freshwater
mgm4755528	40.80612	-96.686063	Aquatic	Surface freshwater
mgm4755664	40.80612	-96.686063	Aquatic	Surface freshwater
mgm4755667	40.80612	-96.686063	Aquatic	Surface freshwater
mgm4755755	40.80612	-96.686063	Aquatic	Surface freshwater
mgm4755756	40.80612	-96.686063	Aquatic	Surface freshwater
mgm4755757	40.80612	-96.686063	Aquatic	Surface freshwater
mgm4755759	40.80612	-96.686063	Aquatic	Surface freshwater
mgm4759283	41.535207	-70.641308	Aquatic	Surface freshwater
mgm4759285	41.535207	-70.641308	Aquatic	Surface freshwater
mgm4885516	54.1992889	78.1680083	Aquatic	Surface freshwater
mgm4885517	54.1992889	78.1680083	Aquatic	Surface freshwater
mgm4885518	54.1992889	78.1680083	Aquatic	Surface freshwater
mgm4885519	54.1992889	78.1680083	Aquatic	Surface freshwater
mgm4885520	54.1992889	78.1680083	Aquatic	Surface freshwater
mgm4440438	32.6	-117.1072	Aquatic	Surface saline water
mgm4516291	37.72955	-0.7741	Aquatic	Surface saline water
mgm4516292	37.72955	-0.7741	Aquatic	Surface saline water
mgm4516293	37.72955	-0.7741	Aquatic	Surface saline water
mgm4683415	34.1865028	49.8443056	Aquatic	Surface saline water
mgm4683416	34.1956389	49.8343611	Aquatic	Surface saline water
mgm4683417	34.1888972	49.8397	Aquatic	Surface saline water
mgm4449249	45.39926	-93.26529	Soil	Temperate
mgm4449252	45.39926	-93.26529	Soil	Temperate
mgm4449255	45.39926	-93.26529	Soil	Temperate
mgm4449256	45.39926	-93.26529	Soil	Temperate
mgm4449258	45.39926	-93.26529	Soil	Temperate

mgm4449284	45.39926	-93.26529	Soil	Temperate
mgm4449356	42.44158	-85.37609	Soil	Temperate
mgm4449357	45.39926	-93.26529	Soil	Temperate
mgm4449358	42.44158	-85.37609	Soil	Temperate
mgm4449359	42.44158	-85.37609	Soil	Temperate
mgm4449360	42.44158	-85.37609	Soil	Temperate
mgm4449362	42.44158	-85.37609	Soil	Temperate
mgm4449363	42.44158	-85.37609	Soil	Temperate
mgm4449364	42.44158	-85.37609	Soil	Temperate
mgm4449365	42.44158	-85.37609	Soil	Temperate
mgm4449877	45.39926	-93.26529	Soil	Temperate
mgm4450130	48.99	14.16	Soil	Temperate
mgm4450131	48.99	14.16	Soil	Temperate
mgm4450171	48.99	14.16	Soil	Temperate
mgm4450172	48.99	14.16	Soil	Temperate
mgm4450628	48.99	14.16	Soil	Temperate
mgm4450629	48.99	14.16	Soil	Temperate
mgm4450630	48.99	14.16	Soil	Temperate
mgm4450631	48.99	14.16	Soil	Temperate
mgm4453246	51.481481	0.222231	Soil	Temperate
mgm4453247	51.481481	0.222231	Soil	Temperate
mgm4453254	51.481481	0.222231	Soil	Temperate
mgm4453256	51.481481	0.222231	Soil	Temperate
mgm4453257	51.481481	0.222231	Soil	Temperate
mgm4453261	51.481481	0.222231	Soil	Temperate
mgm4453274	51.481481	0.222231	Soil	Temperate
mgm4453406	51.481481	0.222231	Soil	Temperate
mgm4453407	51.481481	0.222231	Soil	Temperate
mgm4453433	51.481481	0.222231	Soil	Temperate
mgm4453434	51.481481	0.222231	Soil	Temperate
mgm4453435	51.481481	0.222231	Soil	Temperate
mgm4453436	51.481481	0.222231	Soil	Temperate
mgm4465556	36.96971	-3.46027	Soil	Temperate
mgm4465558	36.95744	-3.46336	Soil	Temperate
mgm4485389	51.97	5.64	Soil	Temperate
mgm4485390	51.97	5.64	Soil	Temperate
mgm4485391	51.97	5.64	Soil	Temperate
mgm4485392	51.97	5.64	Soil	Temperate
mgm4485393	51.97	5.64	Soil	Temperate

mgm4485394	51.97	5.64	Soil	Temperate
mgm4485395	51.97	5.64	Soil	Temperate
mgm4485396	51.97	5.64	Soil	Temperate
mgm4485397	51.97	5.64	Soil	Temperate
mgm4485398	51.97	5.64	Soil	Temperate
mgm4485399	51.97	5.64	Soil	Temperate
mgm4485400	51.97	5.64	Soil	Temperate
mgm4485401	51.97	5.64	Soil	Temperate
mgm4485402	51.97	5.64	Soil	Temperate
mgm4485403	51.97	5.64	Soil	Temperate
mgm4539063	45.4026492	-93.202066	Soil	Temperate
mgm4539064	45.4026492	-93.202066	Soil	Temperate
mgm4541641	45.4026492	-93.202066	Soil	Temperate
mgm4541642	45.4026492	-93.202066	Soil	Temperate
mgm4541644	45.4026492	-93.202066	Soil	Temperate
mgm4541645	45.4026492	-93.202066	Soil	Temperate
mgm4541646	45.4026492	-93.202066	Soil	Temperate
mgm4541647	45.4026492	-93.202066	Soil	Temperate
mgm4541648	45.4026492	-93.202066	Soil	Temperate
mgm4541649	45.4026492	-93.202066	Soil	Temperate
mgm4541650	45.4026492	-93.202066	Soil	Temperate
mgm4541651	45.4026492	-93.202066	Soil	Temperate
mgm4543770	42.394828	-85.373855	Soil	Temperate
mgm4543771	42.394828	-85.373855	Soil	Temperate
mgm4543772	42.394828	-85.373855	Soil	Temperate
mgm4543773	42.394828	-85.373855	Soil	Temperate
mgm4543774	42.394828	-85.373855	Soil	Temperate
mgm4543775	42.394828	-85.373855	Soil	Temperate
mgm4543776	42.394828	-85.373855	Soil	Temperate
mgm4543777	42.394828	-85.373855	Soil	Temperate
mgm4543778	42.394828	-85.373855	Soil	Temperate
mgm4543779	42.394828	-85.373855	Soil	Temperate
mgm4543780	42.394828	-85.373855	Soil	Temperate
mgm4543781	42.394828	-85.373855	Soil	Temperate
mgm4543782	42.394828	-85.373855	Soil	Temperate
mgm4543783	42.394828	-85.373855	Soil	Temperate
mgm4543784	42.394828	-85.373855	Soil	Temperate
mgm4543785	42.394828	-85.373855	Soil	Temperate
mgm4543786	42.394828	-85.373855	Soil	Temperate

mgm4543787	42.394828	-85.373855	Soil	Temperate
mgm4543788	42.394828	-85.373855	Soil	Temperate
mgm4543789	42.394828	-85.373855	Soil	Temperate
mgm4543790	42.394828	-85.373855	Soil	Temperate
mgm4565458	-34.6	-71.12	Soil	Temperate
mgm4565459	-33.58	-71.37	Soil	Temperate
mgm4565460	-33.58	-71.37	Soil	Temperate
mgm4565461	-34.6	-71.12	Soil	Temperate
mgm4565462	-32.87	-71.12	Soil	Temperate
mgm4565463	-32.87	-71.12	Soil	Temperate
mgm4569530	37.5034138	126.766031	Soil	Temperate
mgm4569531	37.5034138	126.766031	Soil	Temperate
mgm4569533	37.5034138	126.766031	Soil	Temperate
mgm4569534	37.5034138	126.766031	Soil	Temperate
mgm4583655	37.22619	-80.42829	Soil	Temperate
mgm4615008	43.348961	-84.028456	Soil	Temperate
mgm4615009	43.349067	-84.02845	Soil	Temperate
mgm4615010	43.34905	-84.028389	Soil	Temperate
mgm4615011	43.3575	-84.009972	Soil	Temperate
mgm4615012	43.358528	-84.008667	Soil	Temperate
mgm4615013	43.357694	-84.009722	Soil	Temperate
mgm4615014	43.355383	-84.040067	Soil	Temperate
mgm4615015	43.355567	-84.040083	Soil	Temperate
mgm4615016	43.3557	-84.039883	Soil	Temperate
mgm4623639	46.682	-114.027	Soil	Temperate
mgm4623640	46.682	-114.027	Soil	Temperate
mgm4623641	46.682	-114.027	Soil	Temperate
mgm4623642	46.682	-114.027	Soil	Temperate
mgm4623643	46.682	-114.027	Soil	Temperate
mgm4623644	46.682	-114.027	Soil	Temperate
mgm4623645	46.682	-114.027	Soil	Temperate
mgm4623646	46.682	-114.027	Soil	Temperate
mgm4623647	46.682	-114.027	Soil	Temperate
mgm4623648	46.682	-114.027	Soil	Temperate
mgm4623649	46.682	-114.027	Soil	Temperate
mgm4623650	46.682	-114.027	Soil	Temperate
mgm4623651	46.682	-114.027	Soil	Temperate
mgm4623652	46.682	-114.027	Soil	Temperate
mgm4623653	46.682	-114.027	Soil	Temperate

mgm4623654	46.682	-114.027	Soil	Temperate
mgm4623655	46.682	-114.027	Soil	Temperate
mgm4623656	46.682	-114.027	Soil	Temperate
mgm4623657	46.682	-114.027	Soil	Temperate
mgm4623658	46.682	-114.027	Soil	Temperate
mgm4623659	46.682	-114.027	Soil	Temperate
mgm4623660	46.682	-114.027	Soil	Temperate
mgm4623661	46.682	-114.027	Soil	Temperate
mgm4623662	46.682	-114.027	Soil	Temperate
mgm4623663	46.682	-114.027	Soil	Temperate
mgm4623664	46.682	-114.027	Soil	Temperate
mgm4623665	46.682	-114.027	Soil	Temperate
mgm4623666	46.682	-114.027	Soil	Temperate
mgm4623667	46.682	-114.027	Soil	Temperate
mgm4623668	46.682	-114.027	Soil	Temperate
mgm4623669	46.682	-114.027	Soil	Temperate
mgm4623670	46.682	-114.027	Soil	Temperate
mgm4623671	46.682	-114.027	Soil	Temperate
mgm4623672	46.682	-114.027	Soil	Temperate
mgm4623673	46.682	-114.027	Soil	Temperate
mgm4623674	46.682	-114.027	Soil	Temperate
mgm4623675	46.682	-114.027	Soil	Temperate
mgm4623676	46.682	-114.027	Soil	Temperate
mgm4623677	46.682	-114.027	Soil	Temperate
mgm4623678	46.682	-114.027	Soil	Temperate
mgm4623679	46.682	-114.027	Soil	Temperate
mgm4623680	46.682	-114.027	Soil	Temperate
mgm4623681	46.682	-114.027	Soil	Temperate
mgm4623682	46.682	-114.027	Soil	Temperate
mgm4623683	46.682	-114.027	Soil	Temperate
mgm4623684	46.682	-114.027	Soil	Temperate
mgm4623685	46.682	-114.027	Soil	Temperate
mgm4623686	46.682	-114.027	Soil	Temperate
mgm4623687	46.682	-114.027	Soil	Temperate
mgm4623688	46.682	-114.027	Soil	Temperate
mgm4623689	46.682	-114.027	Soil	Temperate
mgm4623690	46.682	-114.027	Soil	Temperate
mgm4623691	46.682	-114.027	Soil	Temperate
mgm4623692	46.682	-114.027	Soil	Temperate

mgm4623693	46.682	-114.027	Soil	Temperate
mgm4623694	46.682	-114.027	Soil	Temperate
mgm4623695	46.682	-114.027	Soil	Temperate
mgm4623697	46.682	-114.027	Soil	Temperate
mgm4623698	46.682	-114.027	Soil	Temperate
mgm4623699	46.682	-114.027	Soil	Temperate
mgm4623700	46.682	-114.027	Soil	Temperate
mgm4623701	46.682	-114.027	Soil	Temperate
mgm4623702	46.682	-114.027	Soil	Temperate
mgm4625855	45.6810341	-73.437497	Soil	Temperate
mgm4625856	45.6810341	-73.437497	Soil	Temperate
mgm4626132	45.6810341	-73.437497	Soil	Temperate
mgm4626133	45.6810341	-73.437497	Soil	Temperate
mgm4626134	45.6810341	-73.437497	Soil	Temperate
mgm4626135	45.6810341	-73.437497	Soil	Temperate
mgm4626136	45.6810341	-73.437497	Soil	Temperate
mgm4626137	45.6810341	-73.437497	Soil	Temperate
mgm4626138	45.6810341	-73.437497	Soil	Temperate
mgm4626139	45.6810341	-73.437497	Soil	Temperate
mgm4626140	45.6810341	-73.437497	Soil	Temperate
mgm4626321	45.6810341	-73.437497	Soil	Temperate
mgm4626322	45.6810341	-73.437497	Soil	Temperate
mgm4626323	45.6810341	-73.437497	Soil	Temperate
mgm4626324	45.6810341	-73.437497	Soil	Temperate
mgm4626325	45.6810341	-73.437497	Soil	Temperate
mgm4626327	45.6810341	-73.437497	Soil	Temperate
mgm4626328	45.6810341	-73.437497	Soil	Temperate
mgm4627252	49.0437647	13.6171381	Soil	Temperate
mgm4633085	41.920556	-93.749722	Soil	Temperate
mgm4633087	41.920556	-93.749722	Soil	Temperate
mgm4633089	41.920556	-93.749722	Soil	Temperate
mgm4633090	41.920556	-93.749722	Soil	Temperate
mgm4633092	41.920556	-93.749722	Soil	Temperate
mgm4633093	41.920556	-93.749722	Soil	Temperate
mgm4633098	41.920556	-93.749722	Soil	Temperate
mgm4633120	41.920556	-93.749722	Soil	Temperate
mgm4633122	41.920556	-93.749722	Soil	Temperate
mgm4633188	41.920556	-93.749722	Soil	Temperate
mgm4635362	41.920556	-93.749722	Soil	Temperate

mgm4654021	33	35.233333	Soil	Temperate
mgm4654022	33	35.233333	Soil	Temperate
mgm4654023	31.7	35.05	Soil	Temperate
mgm4654024	31.7	35.05	Soil	Temperate
mgm4654025	31.7	35.05	Soil	Temperate
mgm4654032	33	35.233333	Soil	Temperate
mgm4446153	18.3	-65.833	Soil	Tropical
mgm4450243	-23.903944	-46.42975	Soil	Tropical
mgm4477749	-11.68325	-55.835766	Soil	Tropical
mgm4477751	-11.68325	-55.835766	Soil	Tropical
mgm4477755	-11.68325	-55.835766	Soil	Tropical
mgm4477757	-11.68325	-55.835766	Soil	Tropical
mgm4477789	-11.68325	-55.835766	Soil	Tropical
mgm4477790	-11.68325	-55.835766	Soil	Tropical
mgm4478030	-11.68325	-55.835766	Soil	Tropical
mgm4478037	-11.741266	-56.253339	Soil	Tropical
mgm4478038	-11.741266	-56.253339	Soil	Tropical
mgm4478222	-11.741266	-56.253339	Soil	Tropical
mgm4478283	-11.741266	-56.253339	Soil	Tropical
mgm4478290	-11.68325	-55.835766	Soil	Tropical
mgm4478291	-11.68325	-55.835766	Soil	Tropical
mgm4478292	-11.68325	-55.835766	Soil	Tropical
mgm4478294	-11.68325	-55.835766	Soil	Tropical
mgm4478934	-11.741266	-56.253339	Soil	Tropical
mgm4478936	-11.68325	-55.835766	Soil	Tropical
mgm4478937	-11.68325	-55.835766	Soil	Tropical
mgm4478938	-11.68325	-55.835766	Soil	Tropical
mgm4478939	-11.741266	-56.253339	Soil	Tropical
mgm4478940	-11.741266	-56.253339	Soil	Tropical
mgm4478941	-11.741266	-56.253339	Soil	Tropical
mgm4478943	-11.741266	-56.253339	Soil	Tropical
mgm4479311	-11.741266	-56.253339	Soil	Tropical
mgm4577671	-15.6094	-47.7433	Soil	Tropical
mgm4578924	-15.6094	-47.7433	Soil	Tropical
mgm4578925	-15.6094	-47.7433	Soil	Tropical
mgm4578926	-15.6094	-47.7433	Soil	Tropical
mgm4578927	-15.6094	-47.7433	Soil	Tropical
mgm4579275	-8.37677	-63.631439	Soil	Tropical
mgm4579276	-8.37677	-63.631439	Soil	Tropical

mgm4579277	-8.37677	-63.631439	Soil	Tropical
mgm4582230	4.96495396	117.821125	Soil	Tropical
mgm4582231	4.96495396	117.821125	Soil	Tropical
mgm4582232	4.94980342	117.807104	Soil	Tropical
mgm4582233	4.94980342	117.807104	Soil	Tropical
mgm4582234	4.95908973	117.803962	Soil	Tropical
mgm4582235	4.95908973	117.803962	Soil	Tropical
mgm4582236	4.97135547	117.78609	Soil	Tropical
mgm4582237	4.97135547	117.78609	Soil	Tropical
mgm4582238	4.97051904	117.815888	Soil	Tropical
mgm4582243	5.09315978	117.636106	Soil	Tropical
mgm4582244	5.09574501	117.629826	Soil	Tropical
mgm4582245	5.09574501	117.629826	Soil	Tropical
mgm4582246	5.09517437	117.645543	Soil	Tropical
mgm4582247	5.09517437	117.645543	Soil	Tropical
mgm4582248	5.02092395	117.752247	Soil	Tropical
mgm4582249	5.02092395	117.752247	Soil	Tropical
mgm4582250	4.98554293	117.860351	Soil	Tropical
mgm4582251	4.98554293	117.860351	Soil	Tropical
mgm4582252	4.93340406	117.981119	Soil	Tropical
mgm4582253	4.93340406	117.981119	Soil	Tropical
mgm4582254	5.02155938	117.790187	Soil	Tropical
mgm4582261	5.07986499	117.706104	Soil	Tropical
mgm4582262	5.08483613	117.708229	Soil	Tropical
mgm4582263	5.08483613	117.708229	Soil	Tropical
mgm4582267	5.07999223	117.783439	Soil	Tropical
mgm4582268	4.49215142	117.773377	Soil	Tropical
mgm4582270	4.49783569	117.780593	Soil	Tropical
mgm4582272	4.49018318	117.723698	Soil	Tropical
mgm4582273	4.49018318	117.723698	Soil	Tropical
mgm4582274	4.49793728	117.727391	Soil	Tropical
mgm4582275	4.49793728	117.727391	Soil	Tropical
mgm4582276	4.93717088	117.946666	Soil	Tropical
mgm4582277	4.93717088	117.946666	Soil	Tropical
mgm4582785	4.96495396	117.821125	Soil	Tropical
mgm4582786	4.94980342	117.807104	Soil	Tropical
mgm4582787	4.95908973	117.803962	Soil	Tropical
mgm4582788	4.97135547	117.78609	Soil	Tropical
mgm4582789	4.97051904	117.815888	Soil	Tropical

mgm4582790	4.92604374	117.949045	Soil	Tropical
mgm4582791	5.09315978	117.636106	Soil	Tropical
mgm4582792	5.09574501	117.629826	Soil	Tropical
mgm4582793	5.09517437	117.645543	Soil	Tropical
mgm4582794	5.02092395	117.752247	Soil	Tropical
mgm4582795	4.98554293	117.860351	Soil	Tropical
mgm4582796	4.93340406	117.981119	Soil	Tropical
mgm4582797	5.02155938	117.790187	Soil	Tropical
mgm4582798	5.02811881	117.787334	Soil	Tropical
mgm4582799	5.08978615	117.680529	Soil	Tropical
mgm4582800	5.07986499	117.706104	Soil	Tropical
mgm4582801	5.08483613	117.708229	Soil	Tropical
mgm4582802	5.08372326	117.77605	Soil	Tropical
mgm4582803	5.07999223	117.783439	Soil	Tropical
mgm4582804	4.49215142	117.773377	Soil	Tropical
mgm4582805	4.49783569	117.780593	Soil	Tropical
mgm4582806	4.49018318	117.723698	Soil	Tropical
mgm4582807	4.49793728	117.727391	Soil	Tropical
mgm4582808	4.93717088	117.946665	Soil	Tropical
mgm4597434	-4.5585849	105.406808	Soil	Tropical
mgm4597435	-4.5585849	105.406808	Soil	Tropical
mgm4597436	-4.5585849	105.406808	Soil	Tropical
mgm4597437	-4.5585849	105.406808	Soil	Tropical
mgm4597438	-4.5585849	105.406808	Soil	Tropical
mgm4597439	-4.5585849	105.406808	Soil	Tropical
mgm4597440	-4.5585849	105.406808	Soil	Tropical
mgm4597441	-4.5585849	105.406808	Soil	Tropical
mgm4597442	-4.5585849	105.406808	Soil	Tropical
mgm4597443	-4.5585849	105.406808	Soil	Tropical
mgm4597444	-4.5585849	105.406808	Soil	Tropical
mgm4597445	-4.5585849	105.406808	Soil	Tropical
mgm4597446	-4.5585849	105.406808	Soil	Tropical
mgm4597447	-4.5585849	105.406808	Soil	Tropical
mgm4597448	-4.5585849	105.406808	Soil	Tropical
mgm4597449	-4.5585849	105.406808	Soil	Tropical
mgm4612674	-3.2666667	-60.2	Soil	Tropical
mgm4612675	-3.2666667	-60.2	Soil	Tropical
mgm4612676	-3.2666667	-60.2	Soil	Tropical
mgm4612677	-3.2666667	-60.2	Soil	Tropical

mgm4612679	-3.2666667	-60.2	Soil	Tropical
mgm4612898	-3.2666667	-60.2	Soil	Tropical
mgm4612899	-3.2666667	-60.2	Soil	Tropical
mgm4612900	-3.2666667	-60.2	Soil	Tropical
mgm4612901	-3.2666667	-60.2	Soil	Tropical
mgm4612902	-3.2666667	-60.2	Soil	Tropical
mgm4612985	-3.2666667	-60.2	Soil	Tropical
mgm4612986	-3.2666667	-60.2	Soil	Tropical
mgm4612989	-3.2666667	-60.2	Soil	Tropical
mgm4612990	-3.2666667	-60.2	Soil	Tropical
mgm4612991	-3.2666667	-60.2	Soil	Tropical
mgm4704026	4.6537	114.51952	Soil	Tropical
mgm4704028	4.6537	114.51952	Soil	Tropical
mgm4704031	4.6537	114.51952	Soil	Tropical
mgm4704033	4.6537	114.51952	Soil	Tropical
mgm4704036	4.6537	114.51952	Soil	Tropical
mgm4704040	4.6537	114.51952	Soil	Tropical
mgm4737782	2.51	104.09	Soil	Tropical
mgm4737783	2.03	103.82	Soil	Tropical
mgm4737784	1.91	103.18	Soil	Tropical
mgm4737785	2.51	104.09	Soil	Tropical
mgm4737786	3.14	101.38	Soil	Tropical
mgm4737787	2.03	103.82	Soil	Tropical
mgm4737789	2.03	103.82	Soil	Tropical
mgm4737790	1.91	103.18	Soil	Tropical
mgm4737792	2.51	104.09	Soil	Tropical
mgm4737793	1.91	103.18	Soil	Tropical
mgm4527699	2	101	Aquatic	Urban wastewater
mgm4537910	22.28	114.14	Aquatic	Urban wastewater
mgm4537912	22.28	114.14	Aquatic	Urban wastewater
mgm4550606	49.513414	6.017925	Aquatic	Urban wastewater
mgm4611649	57.04951	9.864788	Aquatic	Urban wastewater
mgm4622641	37.222	-80.426	Aquatic	Urban wastewater
mgm4622642	37.222	-80.426	Aquatic	Urban wastewater
mgm4622643	37.222	-80.426	Aquatic	Urban wastewater
mgm4622644	37.222	-80.426	Aquatic	Urban wastewater
mgm4622645	37.222	-80.426	Aquatic	Urban wastewater
mgm4622651	37.222	-80.426	Aquatic	Urban wastewater
mgm4622652	37.222	-80.426	Aquatic	Urban wastewater

mgm4622653	37.222	-80.426	Aquatic	Urban wastewater
mgm4622654	37.222	-80.426	Aquatic	Urban wastewater
mgm4622655	37.222	-80.426	Aquatic	Urban wastewater
mgm4628867	37.222	-80.426	Aquatic	Urban wastewater
mgm4628870	37.222	-80.426	Aquatic	Urban wastewater
mgm4628871	37.222	-80.426	Aquatic	Urban wastewater
mgm4628872	37.222	-80.426	Aquatic	Urban wastewater
mgm4628873	37.222	-80.426	Aquatic	Urban wastewater
mgm4628874	37.222	-80.426	Aquatic	Urban wastewater
mgm4628875	37.222	-80.426	Aquatic	Urban wastewater
mgm4633656	55.883293	12.497252	Aquatic	Urban wastewater
mgm4633657	55.883293	12.497252	Aquatic	Urban wastewater
mgm4633836	55.883293	12.497252	Aquatic	Urban wastewater
mgm4633837	55.883293	12.497252	Aquatic	Urban wastewater
mgm4682197	37.222	-80.426	Aquatic	Urban wastewater
mgm4682198	37.222	-80.426	Aquatic	Urban wastewater
mgm4682199	37.222	-80.426	Aquatic	Urban wastewater
mgm4682200	37.222	-80.426	Aquatic	Urban wastewater
mgm4682201	37.222	-80.426	Aquatic	Urban wastewater
mgm4682202	37.222	-80.426	Aquatic	Urban wastewater
mgm4682203	37.222	-80.426	Aquatic	Urban wastewater
mgm4682204	37.222	-80.426	Aquatic	Urban wastewater
mgm4682205	37.222	-80.426	Aquatic	Urban wastewater
mgm4682206	37.222	-80.426	Aquatic	Urban wastewater
mgm4682207	37.222	-80.426	Aquatic	Urban wastewater
mgm4682208	37.222	-80.426	Aquatic	Urban wastewater
mgm4682209	37.222	-80.426	Aquatic	Urban wastewater
mgm4682210	37.222	-80.426	Aquatic	Urban wastewater
mgm4682211	37.222	-80.426	Aquatic	Urban wastewater
mgm4682212	37.222	-80.426	Aquatic	Urban wastewater
mgm4682213	37.222	-80.426	Aquatic	Urban wastewater
mgm4682214	37.222	-80.426	Aquatic	Urban wastewater
mgm4682215	37.222	-80.426	Aquatic	Urban wastewater
mgm4682216	37.222	-80.426	Aquatic	Urban wastewater
mgm4682217	37.222	-80.426	Aquatic	Urban wastewater
mgm4682218	37.222	-80.426	Aquatic	Urban wastewater
mgm4691131	37.222	-80.426	Aquatic	Urban wastewater
mgm4691133	37.222	-80.426	Aquatic	Urban wastewater
mgm4691134	37.222	-80.426	Aquatic	Urban wastewater

mgm4691135	37.222	-80.426	Aquatic	Urban wastewater
mgm4691136	37.222	-80.426	Aquatic	Urban wastewater
mgm4691137	37.222	-80.426	Aquatic	Urban wastewater
mgm4691138	37.222	-80.426	Aquatic	Urban wastewater
mgm4691140	37.222	-80.426	Aquatic	Urban wastewater
mgm4691141	37.222	-80.426	Aquatic	Urban wastewater
mgm4691142	37.222	-80.426	Aquatic	Urban wastewater
mgm4691143	37.222	-80.426	Aquatic	Urban wastewater
mgm4691144	37.222	-80.426	Aquatic	Urban wastewater
mgm4691145	37.222	-80.426	Aquatic	Urban wastewater
mgm4691146	37.222	-80.426	Aquatic	Urban wastewater
mgm4691147	37.222	-80.426	Aquatic	Urban wastewater
mgm4691149	37.222	-80.426	Aquatic	Urban wastewater
mgm4691150	37.222	-80.426	Aquatic	Urban wastewater
mgm4691151	37.222	-80.426	Aquatic	Urban wastewater
mgm4691152	37.222	-80.426	Aquatic	Urban wastewater
mgm4691153	37.222	-80.426	Aquatic	Urban wastewater
mgm4691154	37.222	-80.426	Aquatic	Urban wastewater
mgm4691155	37.222	-80.426	Aquatic	Urban wastewater
mgm4691156	37.222	-80.426	Aquatic	Urban wastewater
mgm4691157	37.222	-80.426	Aquatic	Urban wastewater
mgm4691158	37.222	-80.426	Aquatic	Urban wastewater
mgm4691159	37.222	-80.426	Aquatic	Urban wastewater
mgm4691160	37.222	-80.426	Aquatic	Urban wastewater
mgm4711609	39.9075	116.3972	Aquatic	Urban wastewater
mgm4711610	39.9075	116.3972	Aquatic	Urban wastewater
mgm4711611	39.9075	116.3972	Aquatic	Urban wastewater
mgm4713021	39.5	116.2	Aquatic	Urban wastewater
mgm4713028	39.5	116.2	Aquatic	Urban wastewater
mgm4713031	39.5	116.2	Aquatic	Urban wastewater
mgm4713033	39.5	116.2	Aquatic	Urban wastewater
mgm4713037	39.5	116.2	Aquatic	Urban wastewater
mgm4715759	46.8182	8.2275	Aquatic	Urban wastewater
mgm4715760	46.8182	8.2275	Aquatic	Urban wastewater
mgm4715763	46.8182	8.2275	Aquatic	Urban wastewater
mgm4715765	46.8182	8.2275	Aquatic	Urban wastewater
mgm4715768	46.8182	8.2275	Aquatic	Urban wastewater
mgm4715770	46.8182	8.2275	Aquatic	Urban wastewater
mgm4715773	46.8182	8.2275	Aquatic	Urban wastewater

mgm4715777	46.8182	8.2275	Aquatic	Urban wastewater
mgm4715778	46.8182	8.2275	Aquatic	Urban wastewater
mgm4715779	46.8182	8.2275	Aquatic	Urban wastewater
mgm4715780	46.8182	8.2275	Aquatic	Urban wastewater
mgm4715782	46.8182	8.2275	Aquatic	Urban wastewater
mgm4715843	46.8182	8.2275	Aquatic	Urban wastewater
mgm4715849	46.8182	8.2275	Aquatic	Urban wastewater
mgm4715856	46.8182	8.2275	Aquatic	Urban wastewater
mgm4715858	46.8182	8.2275	Aquatic	Urban wastewater
mgm4715963	46.8182	8.2275	Aquatic	Urban wastewater
mgm4715964	46.8182	8.2275	Aquatic	Urban wastewater
mgm4715967	46.8182	8.2275	Aquatic	Urban wastewater
mgm4715968	46.8182	8.2275	Aquatic	Urban wastewater
mgm4715970	46.8182	8.2275	Aquatic	Urban wastewater
mgm4715971	46.8182	8.2275	Aquatic	Urban wastewater
mgm4715972	46.8182	8.2275	Aquatic	Urban wastewater
mgm4715973	46.8182	8.2275	Aquatic	Urban wastewater
mgm4715975	46.8182	8.2275	Aquatic	Urban wastewater
mgm4715977	46.8182	8.2275	Aquatic	Urban wastewater
mgm4715979	46.8182	8.2275	Aquatic	Urban wastewater
mgm4716115	46.8182	8.2275	Aquatic	Urban wastewater
mgm4716707	18.918611	-99.234167	Aquatic	Urban wastewater
mgm4717011	18.918611	-99.234167	Aquatic	Urban wastewater
mgm4717032	18.918611	-99.234167	Aquatic	Urban wastewater
mgm4717073	46.8182	8.2275	Aquatic	Urban wastewater
mgm4717074	46.8182	8.2275	Aquatic	Urban wastewater
mgm4717076	46.8182	8.2275	Aquatic	Urban wastewater
mgm4717083	46.8182	8.2275	Aquatic	Urban wastewater
mgm4717089	46.8182	8.2275	Aquatic	Urban wastewater
mgm4717090	46.8182	8.2275	Aquatic	Urban wastewater
mgm4717093	46.8182	8.2275	Aquatic	Urban wastewater
mgm4717096	46.8182	8.2275	Aquatic	Urban wastewater
mgm4717101	46.8182	8.2275	Aquatic	Urban wastewater
mgm4717157	46.8182	8.2275	Aquatic	Urban wastewater
mgm4717158	46.8182	8.2275	Aquatic	Urban wastewater
mgm4717159	46.8182	8.2275	Aquatic	Urban wastewater
mgm4717161	46.8182	8.2275	Aquatic	Urban wastewater
mgm4717162	46.8182	8.2275	Aquatic	Urban wastewater
mgm4717164	46.8182	8.2275	Aquatic	Urban wastewater

mgm4717167	46.8182	8.2275	Aquatic	Urban wastewater
mgm4717168	46.8182	8.2275	Aquatic	Urban wastewater
mgm4717169	46.8182	8.2275	Aquatic	Urban wastewater
mgm4717171	46.8182	8.2275	Aquatic	Urban wastewater
mgm4722850	33.650555	131.076903	Aquatic	Urban wastewater
mgm4723073	33.650555	131.076903	Aquatic	Urban wastewater
mgm4723074	33.650555	131.076903	Aquatic	Urban wastewater
mgm4723078	33.650555	131.076903	Aquatic	Urban wastewater
mgm4725194	33.650555	131.076903	Aquatic	Urban wastewater
mgm4726716	33.650555	131.076903	Aquatic	Urban wastewater
mgm4726717	33.650555	131.076903	Aquatic	Urban wastewater
mgm4726718	33.650555	131.076903	Aquatic	Urban wastewater
mgm4726719	33.650555	131.076903	Aquatic	Urban wastewater
mgm4728683	32	118	Aquatic	Urban wastewater
mgm4728684	32	118	Aquatic	Urban wastewater
mgm4738433	36	118	Aquatic	Urban wastewater
mgm4738618	31.6	118.5	Aquatic	Urban wastewater
mgm4739196	31.6	118.5	Aquatic	Urban wastewater
mgm4739515	31.6	118.5	Aquatic	Urban wastewater
mgm4739516	31.6	118.5	Aquatic	Urban wastewater
mgm4739517	31.6	118.5	Aquatic	Urban wastewater
mgm4739982	36	118	Aquatic	Urban wastewater
mgm4739983	36	118	Aquatic	Urban wastewater
mgm4754248	40.838482	-96.687679	Aquatic	Urban wastewater
mgm4754406	40.838482	-96.687679	Aquatic	Urban wastewater
mgm4773098	36.0104	-84.2696	Aquatic	Urban wastewater
mgm4773099	36.0104	-84.2696	Aquatic	Urban wastewater
mgm4773100	36.0104	-84.2696	Aquatic	Urban wastewater
mgm4775281	15.476	38.828	Aquatic	Urban wastewater
mgm4775320	0.21347	37.61867	Aquatic	Urban wastewater
mgm4775461	0.21347	37.61867	Aquatic	Urban wastewater
mgm4775550	8.18	39.476	Aquatic	Urban wastewater
mgm4775675	45.97	40.52	Aquatic	Urban wastewater
mgm4775851	1.997	35.353	Aquatic	Urban wastewater
mgm4775867	4.046	38.458	Aquatic	Urban wastewater
mgm4775884	0.02946	38.458	Aquatic	Urban wastewater
mgm4775917	33.269	37.10077	Aquatic	Urban wastewater
mgm4775941	27.824	28.247	Aquatic	Urban wastewater
mgm4776218	17.57	62.438	Aquatic	Urban wastewater

mgm4776224	0.48637	37.44057	Aquatic	Urban wastewater
mgm4783684	36.229494	139.783547	Aquatic	Urban wastewater
mgm4783685	36.229494	139.783547	Aquatic	Urban wastewater
mgm4783686	36.229494	139.783547	Aquatic	Urban wastewater
mgm4783845	36.229455	139.783536	Aquatic	Urban wastewater
mgm4783846	36.229455	139.783536	Aquatic	Urban wastewater
mgm4785061	37.55	127.06	Aquatic	Urban wastewater
mgm4791579	37.55	127.06	Aquatic	Urban wastewater
mgm4798502	38.872848	141.579113	Aquatic	Urban wastewater
mgm4798503	38.872848	141.579113	Aquatic	Urban wastewater
mgm4798504	38.872848	141.579113	Aquatic	Urban wastewater
mgm4799984	38.872848	141.579113	Aquatic	Urban wastewater
mgm4799988	38.872848	141.579113	Aquatic	Urban wastewater
mgm4799991	38.872848	141.579113	Aquatic	Urban wastewater
mgm4801003	38.872848	141.579113	Aquatic	Urban wastewater
mgm4801004	38.872848	141.579113	Aquatic	Urban wastewater
mgm4801800	38.872848	141.579113	Aquatic	Urban wastewater
mgm4804501	1.3521	103.8198	Aquatic	Urban wastewater
mgm4804502	1.3521	103.8198	Aquatic	Urban wastewater
mgm4804503	1.3521	103.8198	Aquatic	Urban wastewater
mgm4806959	38.872848	141.579113	Aquatic	Urban wastewater
mgm4806960	38.872848	141.579113	Aquatic	Urban wastewater
mgm4806961	38.872848	141.579113	Aquatic	Urban wastewater
mgm4827496	38.872848	141.579113	Aquatic	Urban wastewater
mgm4827497	38.872848	141.579113	Aquatic	Urban wastewater
mgm4827498	38.872848	141.579113	Aquatic	Urban wastewater
mgm4828662	38.872848	141.579113	Aquatic	Urban wastewater
mgm4828663	38.872848	141.579113	Aquatic	Urban wastewater
mgm4830571	36.0104	-84.2696	Aquatic	Urban wastewater
mgm4830867	36.0104	-84.2696	Aquatic	Urban wastewater
mgm4840855	38.872848	141.579113	Aquatic	Urban wastewater
mgm4840856	38.872848	141.579113	Aquatic	Urban wastewater
mgm4840857	38.872848	141.579113	Aquatic	Urban wastewater
mgm4852302	38.872848	141.579113	Aquatic	Urban wastewater
mgm4852303	38.872848	141.579113	Aquatic	Urban wastewater
mgm4852304	38.872848	141.579113	Aquatic	Urban wastewater
mgm4852331	38.872848	141.579113	Aquatic	Urban wastewater
mgm4852332	38.872848	141.579113	Aquatic	Urban wastewater
mgm4852338	38.872848	141.579113	Aquatic	Urban wastewater

mgm4852339	38.872848	141.579113	Aquatic	Urban wastewater
mgm4852340	38.872848	141.579113	Aquatic	Urban wastewater
mgm4855228	38.872848	141.579113	Aquatic	Urban wastewater
mgm4861219	38.872848	141.579113	Aquatic	Urban wastewater
mgm4861220	38.872848	141.579113	Aquatic	Urban wastewater
mgm4861221	38.872848	141.579113	Aquatic	Urban wastewater

Supplementary Table 1. The list of all metagenomes analysed in this study, their MG-RAST ID, co-ordinates and biome classification.