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Agricultural and urban practices are correlated to changes in the resistome of riverine systems

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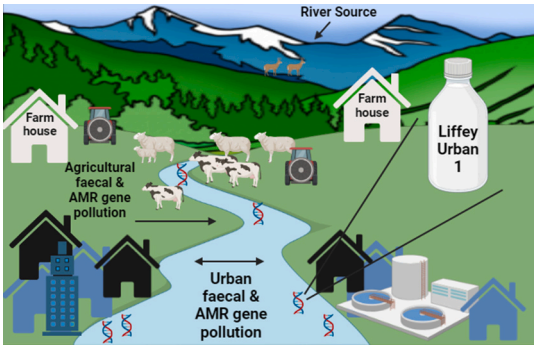
HIGHLIGHTS

- Riverine resistome composition is correlated to land use
- Riverine microbiome diversity is lower in agricultural and urban areas
- Riverine resistome diversity is higher in agricultural and urban areas
- Agricultural and human pollution affect microbiome and resistome composition
- A One Health approach important to address AMR pollution

GRAPHICAL ABSTRACT



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ABSTRACT

The objective of this study was to comprehensively characterise the resistome, the collective set of antimicrobial resistance genes in a given environment, of two rivers, from their source to discharge into the sea, as these flow through areas of different land use. Our findings reveal significant differences in the riverine resistome composition in areas of different land uses, with increased abundance and diversity of AMR in downstream agricultural and urban locations, with the resistome in urban areas more similar to the resistome in wastewater. The changes in resistome were accompanied by changes in microbial communities, with a reduction in microbial diversity in downstream agricultural and urban affected areas, driven mostly by increased relative abundance in the phyla, Bacteroidetes and Proteobacteria. These results provide insight into how pollution associated with agricultural and urban activities affects microbial communities and influences AMR in aquatic water bodies. These results add valuable insights to form effective strategies for mitigating and preserving aquatic ecosystems. Overall, our study highlights the critical role of the environment in the development and dissemination of AMR and underscores the importance of adopting a One Health approach to address this global public health threat.

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1. Introduction

Drug-resistant infections are one of the leading causes of death, and in 2019, global deaths attributable to bacterial antimicrobial resistance (AMR) surpassed HIV/AIDS and malaria (Collaborators, 2022). An estimated 3500 people die each day from AMR related health issues, and by 2050, modelling has estimated the AMR related global death toll will exceed that from cancer (O'Neill, 2016). AMR affects humans, animals, and their shared environment (Velazquez-Meza et al., 2022). To address the AMR public health threat, a One Health approach that acknowledges the shared roles played by different factors in both the cause and solution to the problem is now being commonly adopted and will help address this public health threat (Velazquez-Meza et al., 2022). Antimicrobials are widely used and found in the broader environment, creating selective pressures that promote the evolution and dissemination of antibiotic resistance genes (ARGs) (Koch et al., 2021; Polianciuc et al., 2020). This can occur through antimicrobials in agriculture and human healthcare and inadequate disposal of human and animal waste (Manyi-Loh et al., 2018; Singer et al., 2016). The critical role that the environment plays in the emergence and transmission of AMR is increasingly being recognised (Samreen et al., 2021) and in mitigating AMR, a collaborative and interdisciplinary effort is needed to manage the specific environmental factors contributing to its development.

Surveillance of the environment can provide valuable insights into regional resistance trends and areas of concern. Rivers and streams, for example, often serve as pathways for pollutants from agricultural and urban areas. Thus, any observed shifts in resistance or increased levels of clinically relevant resistance genes can be pinpointed to that region and complement traditional surveillance (Larsson and Flach, 2021). The use of antimicrobials in agriculture and animal husbandry, for example, can lead to the selection of resistant bacteria, which can then spread to water bodies. Similarly, untreated or poorly treated sewage in urban areas can release resistant bacteria and ARGs into nearby water bodies. The likelihood of the occurrence of these events is clearly related to specific land uses (agricultural and urban), and associations between different land uses and AMR profiles in river systems, with faecal pollution playing a key role, have previously been established (Nolan et al., 2023a, 2023b; Reynolds et al., 2020). Faecal pollution from agriculture and urban activities can significantly affect microbial communities in aquatic ecosystems, reducing overall diversity and altering community composition (Chen et al., 2018b; Labbate et al., 2016; Paruch et al., 2019).

A broad view that considers the contribution of various environments to resistance is therefore needed to better understand AMR. Traditional environmental AMR surveillance methods have their limitations. Analysis focusing on culturable bacteria or specific pathogens neglects the importance of AMR in a broader context by excluding non-pathogenic and unculturable environmental bacteria in the propagation of resistance (Bengtsson-Palme et al., 2018). Although targeted molecular methods such as PCR are valuable in surveillance, these have limitations regarding the prior selection of genes and the number of genes that can be analysed at any given time. In contrast, a metagenomic approach can allow for an examination of the resistome, the collective set of antimicrobial resistance genes in a given environment, in a non-targeted way, which allows for analysis without any bias in selecting targets (Franklin et al., 2021; Pillay et al., 2022). This approach allows for the analysis of the impact of land use practices on our water resources. Insights into how pollution associated with agricultural and urban activities affects microbial communities and drives AMR in aquatic water bodies can inform effective strategies for mitigating and preserving aquatic ecosystems.

Here we hypothesise that land use is correlated to changes in the resistome of two rivers, from source to discharge at sea. The correlation between AMR and faecal contamination of the two rivers studied here have been previously extensively analysed (Nolan et al., 2023a; Nolan et al., 2023b; Sala-Comorera et al., 2021; Reynolds et al., 2020). We here report the results of a metagenomic study expanding on our previous work. Our results highlight the effect of land use, with compositional changes in both resistome and microbial communities, and the potential impact of agricultural and anthropogenic practices on nearby waterbodies This study highlights the utility of a non-targeted metagenomic approach when studying environmental communities and AMR which may help mitigate and lower the potential risk these waters pose to recreational users.

2. Methods

2.1. Study area and sampling design

The land use designation was based on the Irish EPA Co-ORdinated INformation on the Environment (CORINE) land use maps. The River Liffey and Dodder are two rivers which converge in Dublin and discharge into Dublin Bay (Fig. 1). The River Liffey rises in the Wicklow Mountains and flows for 125 km, passing through peat and bog land, through agricultural lands in County Kildare and into the urban conurbation of Dublin before discharging into Dublin Bay. The catchment area of the River Liffey is approximately 1250 km^2. The River Dodder, with a catchment area of approximately 120 km^2, rises near the River Liffey in the Wicklow mountains, but with a stream length of 26 km, takes a more direct route through agricultural lands before entering Dublin Bay from the south of the city (Fig. 1).

Over two weeks in 2020, water samples (10 l) were collected ($n = 8$) from sites under the same dry weather conditions across the catchments of the Rivers Liffey and Dodder (Fig. 1; Supplementary Table 1). Using the EPA CORINE land use maps, four sampling locations were included on the River Liffey, two in agricultural and two in urban areas. In the River Dodder, one sample was taken at the source, located in peatland/bog, where there is the least impact from agricultural and urban activities. In addition to the source of the River Dodder, one sample was taken from agricultural and one from urban settings. One influent sample was taken from the Ringsend wastewater treatment plant serving the greater Dublin are (1.9 million p.e.).

2.2. Water collection and concentration

Water samples (10 l) were concentrated using ultrafiltration (Hill et al., 2005). Briefly, 10 l of river water samples were collected in 10 l carboys. Samples were transported to the laboratory and processed within 24 h of collection. Before filtration, sodium polyphosphate (10 mg/L) and Tween 80 (0.1 % v/v) were added to the water samples to aid in the recovery of microbes during ultrafiltration. Samples were concentrated using Tangential flow ultrafiltration using a hollow fibre membrane (Fresenius, Germany; pore size: 0.2 μm) and a Master Flex peristaltic pump (Avantor, USA). The ultrafiltration was performed until a final volume of approximately 100 ml of concentrate was obtained. Samples were further filtered with a 3 μm nitrocellulose filter membrane. The concentrate was then collected in sterile containers and stored at 4 °C until extraction.

2.3. Extraction of DNA and sequencing

Water samples of 100 ml volume were concentrated via 0.22 μm nitrocellulose filter membrane filtration. Filters were transferred into single-use polypropylene tubes containing ice-cold lysis buffer (5 M guanidine isothiocyanate, 100 mM EDTA [pH 8.0], 0.5 % [w/v] sodium lauroyl sarcosinate) and stored at −20 °C until extraction. DNA extraction was performed using the Qiagen PowerSoil Pro kit (Qiagen, Germany) (Gourmelon et al., 2007). Samples were quantified using a Qubit fluorometer (Thermo Fisher Scientific, USA), and the quality was checked using a nanodrop (Thermo Fisher Scientific, USA) per sequencing and quality control.

Extracted DNA sequencing was performed by NOVOGENE (Beijing, China) using the Illumina NovaSeq platform. Library preparation was

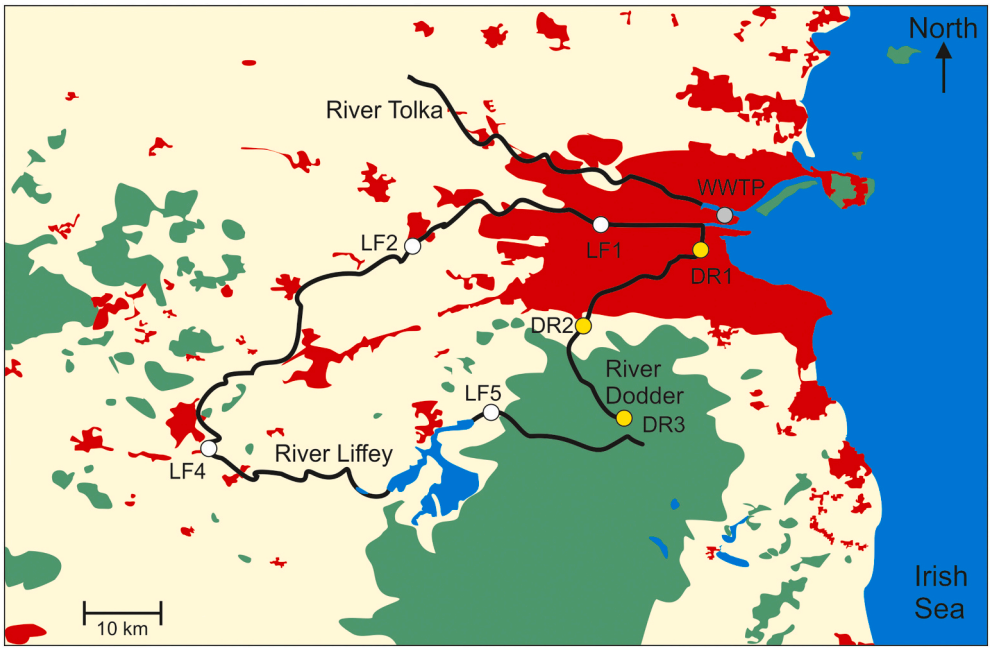

Fig. 1. Sampling points of the Rivers Liffey and Dodder, and an urban wastewater treatment plant. The source of the River Dodder located in peatland/bog (DR3), agricultural sampling point (DR2) and urban sampling point (DR1) were sequenced. Agricultural (LF5, LF4) and urban sampling (LF2, LF1) points of the River Liffey. WWTP: wastewater treatment plant. The colours match the defined land use as per the EPA CORINE characterisation. Red: urban, green: peatland/bog and yellow: agricultural land use.

carried out using a PCR-free protocol per the manufacturer's instructions. The sequencing depth was approximately 80 million reads per sample. Paired-end reads (2 × 250 bp) were generated, and the raw sequencing data were subjected to quality control and pre-processing as described below.

2.4. Bioinformatics analysis

2.4.1. Quality control

The raw sequence data obtained from the Illumina sequencing platform was first subjected to quality control using FastP software (v0.23.2) (Chen et al., 2018a). The software was used to remove adapter sequences, and low-quality reads and to trim the reads. The quality threshold used was Q15, corresponding to a base call accuracy of 99.9 %. The resulting high-quality paired-end reads were merged using PEAR software (v0.9.10) (Zhang et al., 2014).

2.4.2. Taxonomic classification

The high-quality paired-end reads were subjected to taxonomic classification using Kaiju software (v1.9.2) against the NCBI non-redundant protein database (RefSeq) (version 2021-09-12) (Menzel et al., 2016). In brief, Kaiju translates the metagenomic sequencing reads into the six possible reading frames and searches for maximum exact matches (MEMs) of amino acid sequences in the database of annotated proteins from microbial reference genomes. For a match to one or more database sequences for a read, Kaiju outputs the taxonomic identifier of the corresponding taxon or determines the lowest common ancestor (LCA). The database used for the search was downloaded on 01-10-2022. The Kaiju output was then imported into Phyloseq for further analysis.

2.4.3. Assembly and annotation

The high-quality paired-end reads were used for assembly using Metaspades software (v3.15.3) (Prjibelski et al., 2020). The assembly parameters included a minimum contig length of 500 bp. The resulting contigs were annotated and analysed using Abricate software (v1.0.1) against the CARD (Comprehensive Antibiotic Resistance Database) database (Jia et al., 2017; Torsten). A 90 % sequence identity and coverage cut-off point were annotated as ARGs. The quality of the assembly was assessed using the N50 statistic.

2.4.4. Microbial analysis

Analysis of taxonomic profiles was performed using R software (v4.1.2) with the Phyloseq and ggplot2 (v. 3.4.2) packages (McMurdie and Holmes, 2013; Wickham, 2016). Alpha diversity measures, including richness and evenness, were calculated using the Phyloseq package. Differential abundance analysis was performed using the DESeq2 package (Love et al., 2014).

2.5. Data analysis

Multivariate analyses were carried out on RStudio 4.2.2. Differences in land use were explored using a multivariate analysis of variance (PERMANOVA) using the EPA CORINE land use characterisation as fixed factors and ARGs annotated using Abricate software (v1.0.1) against the CARD as response variables. Subsequent univariate analysis of variance (Kruskal-Wallis) was used on significant differences between land uses. Descriptive multivariate analyses were carried out to highlight differences among land-impacted rivers. Hierarchical clustering was achieved using Wards (Ward.D2) linkage with Bray-Curtis distances. A Principal Component Analysis (PCA) was performed to detect land use clusters along the reaches of two rivers and and the influent of a wastewater treatment plant.

3. Results

3.1. Reduction in the diversity of microbial communities in downstream urban and agricultural sampling points

The observed Alpha diversity at the source of the River Dodder (DR3), together with agricultural and urban land use areas of the Liffey and Dodder catchments, showed the source exhibited the highest alpha diversity (observed: 7230, Chao1: 7260 ± 15, ACE: 7250 ± 15), followed by agricultural (LF5, LF4, DR2) and urban land uses (DR1 & LF1)

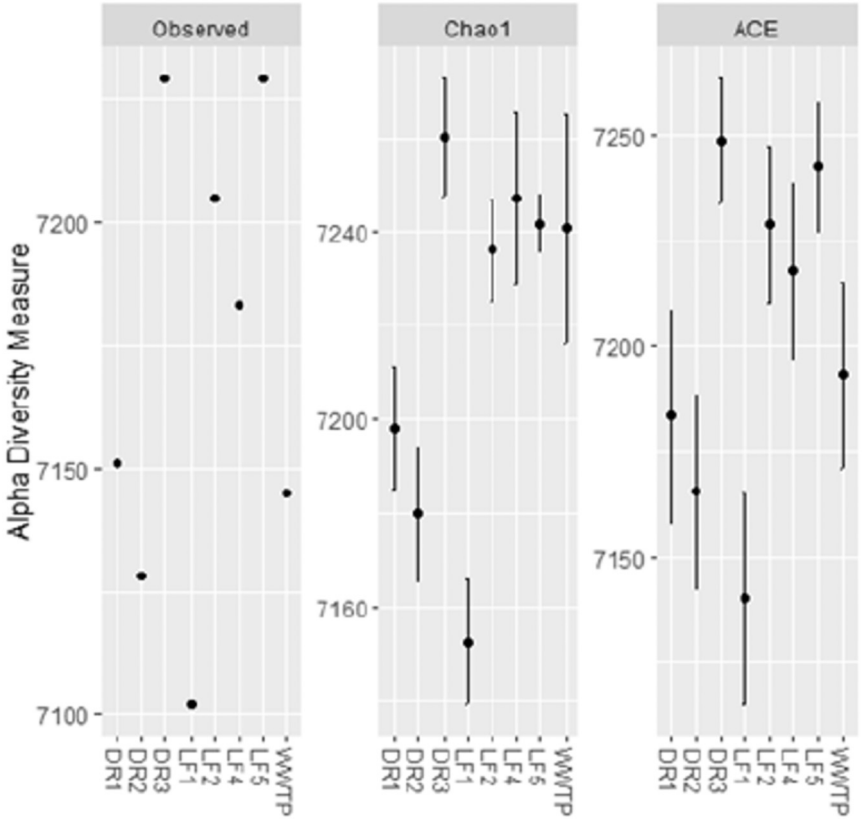

Fig. 2. Microbiome alpha diversity of sampling points on the Rivers. The Rivers Liffey (LF) and Dodder (DR) and an Urban wastewater (WWTP). The source of the River Dodder (DR3), agricultural sampling point (DR2) and urban sampling point (DR1) were sequenced. Agricultural (LF5, LF4) and urban sampling (LF2, LF1) points of the Rivers Liffey are shown. Observed, Chao1 and ACE indices are shown from left to right.

(Fig. 2). A reduction in observed Alpha diversity was seen in downstream agricultural sampling points in the Rivers Liffey and Dodder (LF5, LF4, DR2), with The lowest Alpha diversity observed in urban sampling points in these rivers.

Beta diversity of the microbial communities between the river samples revealed that most of the differences in microbial community composition were explained by PC1, which accounted for 48.2 % of the total diversity (Fig. 3). The distribution of samples along PC1 showed a clear separation between the source and agricultural/urban impacted areas, with the source having the highest alpha diversity metrics compared to the agricultural and urban impacted areas. Along PC2, which explained 23.2 % of the diversity, there was a separation between the urban and agricultural sampling points on one side, and the source sampling point and wastewater treatment plant sample on the other. These results indicate that the main differences in the microbial community composition of the river samples can be seen along PC1, with the separation between urban and agricultural sampling points, the source sampling point, and the wastewater treatment plant sample.

3.2. An increase in proteobacteria and bacteroidetes accompanied by a reduction in the diversity of microbial communities

To understand what differences land use may have on microbial communities and ecological health, a comparison of significant relative

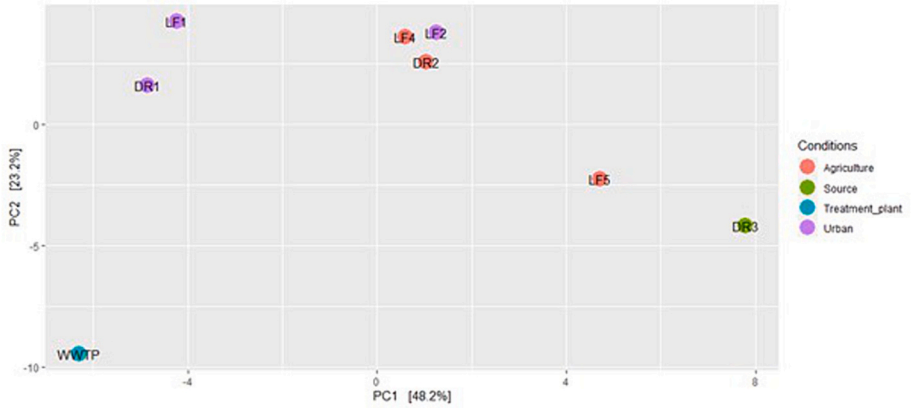

Fig. 3. Microbiome beta diversity (Bray-Curtis) at sampling points on the Rivers Liffey and Dodder. The Rivers Liffey (LF) and Dodder (DR) and an Urban wastewater (WWTP). The source of the River Dodder (DR3), agricultural sampling point (DR2) and urban sampling point (DR1) were sequenced. Agricultural (LF5, LF4) and urban sampling (LF2, LF1) points of the Rivers Liffey.

abundance of genera was investigated between land uses.

We analysed the relative abundance of bacterial phyla. We identified the most significant genera and their accompanying phyla as the river moves from its source through agricultural and urban land uses. We observed an increase in the relative abundance of Proteobacteria and Bacteroidetes in agricultural and urban sampling points, with the highest levels observed in urban sampling points (Fig. 4). The phyla, Proteobacteria and Bacteroidetes, showed the greatest differential abundance across the sampled sites. A greater diversity of phyla was observed at the source compared to agricultural and urban sampling points.

Compared to the source/bog land use, agricultural land use had an overall lower abundance of genera from the phyla Uroviricota, Proteobacteria, Preplamivirocota, and Actinobacteria, with a log2 fold change ranging from −2.5 to −12 (Fig. 5a). In contrast, urban land use had more genera from the phyla Proteobacteria, Bacteroidetes, Uroviricota, Actinobacteria, and Candidate Saccharibacteria compared to agricultural sites, with a log2 fold change ranging from 1.5 to 4.6. In addition, urban locations had a reduction in the abundance of genera from the phyla Plantomycetes, Uroviricota, Proteobacteria, and Gemmatimonadetes compared to agricultural sampling points, with a log2 fold change ranging from −1.18 to −3, was observed (Fig. 5b).

Furthermore, when comparing urban and wastewater samples, we found a higher abundance of genera from the phyla Proteobacteria, Phixviricota, Nucleocytoviricota, Firmicutes, Thaumarchaeota, Candidate Saccharibacteria, and Bacteroidetes, with a log2 fold change ranging from 2.7 to 9.65. In the wastewater sample, we observed a reduction in the abundance of genera from the phyla Proteobacteria, Uroviricota, Firmicutes, and Candidate Saccharibacteria, with a log2 fold change ranging from −2.6 to −7 (Fig. 5 d).

3.3. A change and clustering of the resistome of riverine systems based on their land use

The relative abundance of nine classes of antimicrobial resistance genes (ARG) was determined at sampling sites in the three different land use areas- source (bog), agriculture, and urban sites (Fig. 6). Nine classes of resistance genes that confer resistance to aminoglycoside, beta-lactam, diaminopyrimidine, disinfecting and antiseptics, lincosamide, macrolide, phenicol, sulfonamide and tetracyclines, were observed. The results showed that the relative abundance of ARGs varied among the different land use areas. A trend of increased abundance and diversity was seen upstream to downstream, as samples were taken from the source, through agricultural and urban sampling points. The highest relative abundance and diversity of ARGs was observed at the wastewater treatment plant, followed by urban, then agricultural and source sampling sites. Among the nine classes of ARGs, genes conferring resistance to the beta-lactams genes were the most prevalent in all four land uses, followed by disinfection and antiseptic agents and macrolide resistance genes. Our data identified a greater diversity of AMR classes in urban and wastewater samples compared to the source and agricultural sample sites. A greater relative abundance of macrolide resistance genes and genes conferring resistance to, or reduced susceptibility to, disinfecting agents and antiseptics was observed in the wastewater and urban samples.

Principal component analysis (PCA) was performed on the AMR gene data to further investigate the clustering of sampling sites based on their land use (Fig. 7). The PCA plot showed that the first two principal components explained 73 % of the total variation in the data. The source at the River Dodder (DR3) and Liffey agricultural samples (LF5, LF4) and the furthest upstream urban sample (LF2) were seen to cluster along the first principal component. Further separation along the second principal

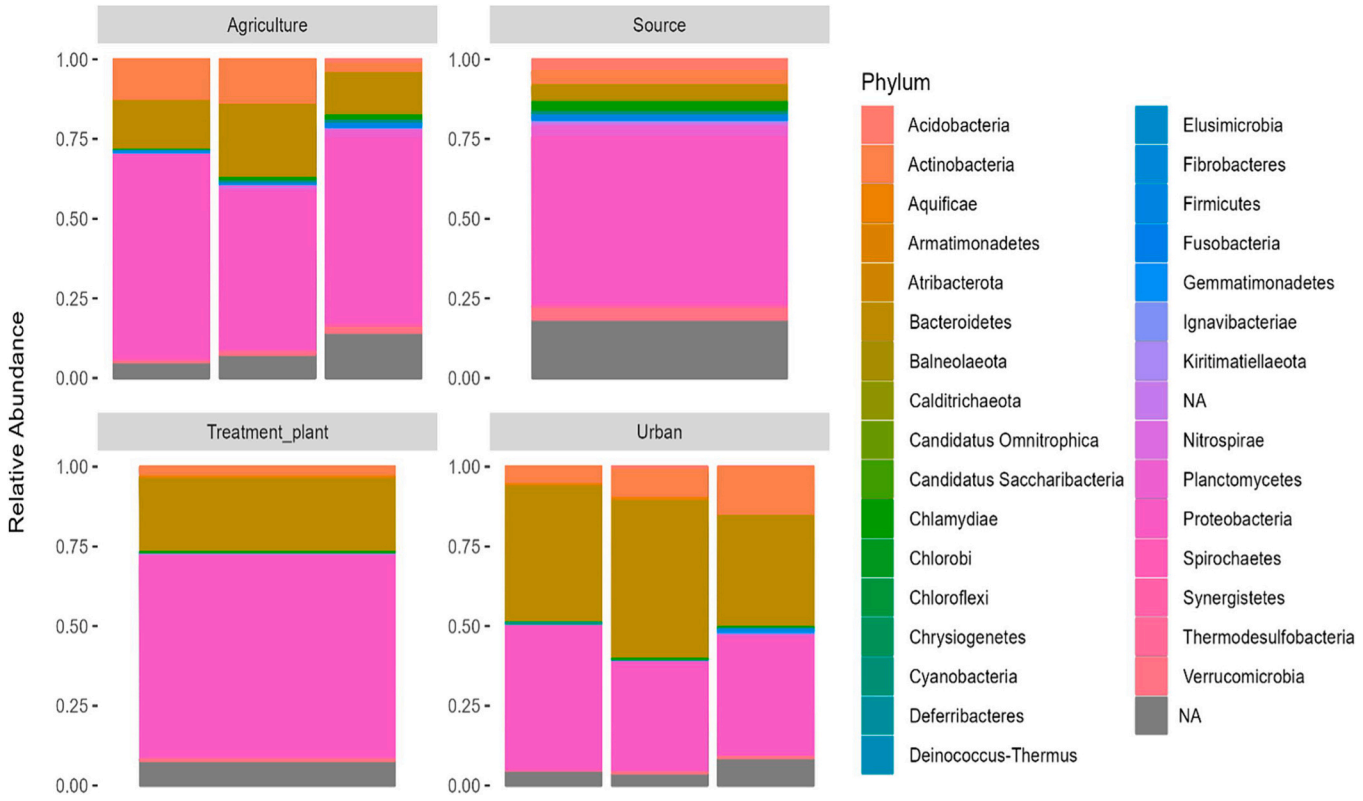

Fig. 4. The microbiome diversity at sampling points. The Rivers Liffey (LF) and Dodder (DR) and an Urban wastewater (WWTP) sample were characterised. Phylum (a) and Genus (b) with the most significant distribution are shown (more than 1 % abundance). DNA islolated from the source of the River Dodder (DR3), agricultural sampling point (DR2) and urban sampling point (DR1) were sequenced. In addition, DNA isolated at agricultural (LF5, LF4) and urban sampling (LF2, LF1) points of the Rivers Liffey were also characterised.

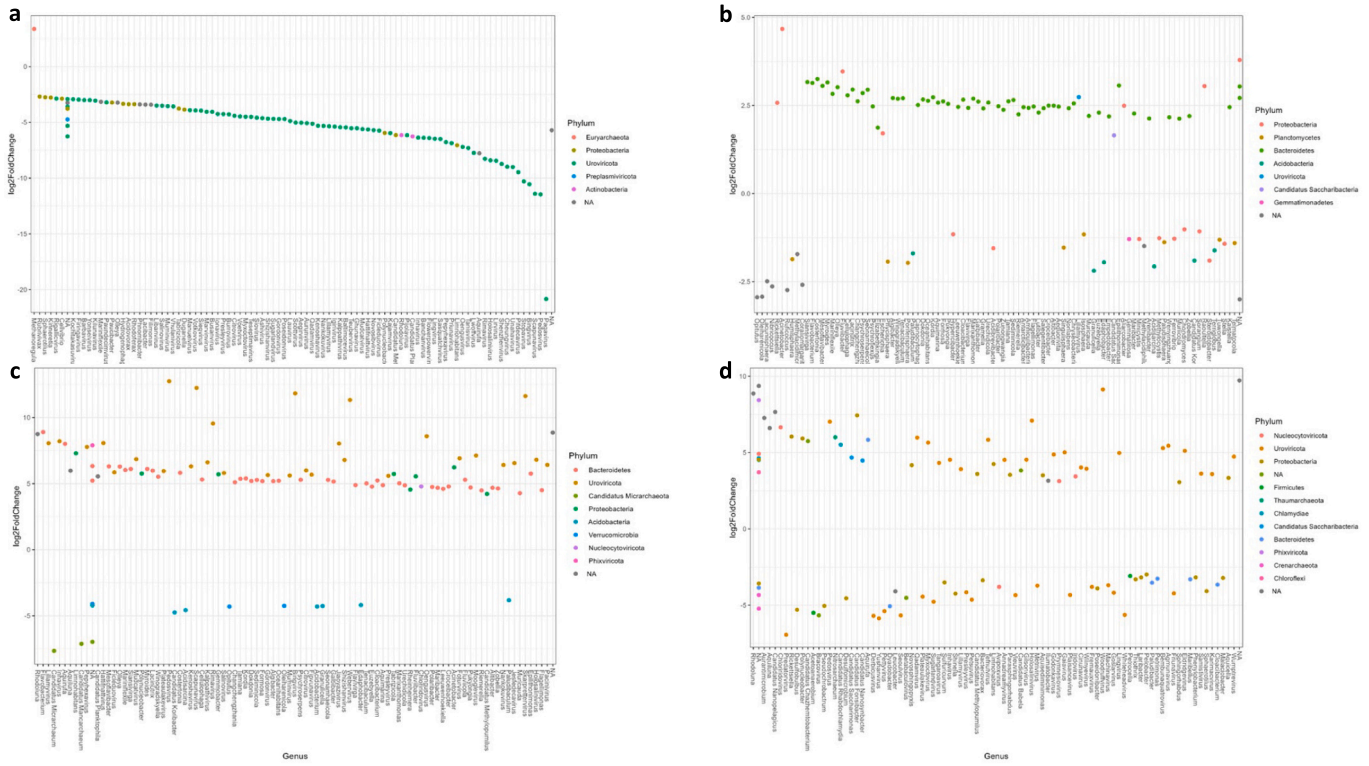

Fig. 5. Differential abundance analysis based on phylogeny using DeSeq2. The figure shows the significant differences in the abundance of the genus and its corresponding phylum between the two tested land uses. The source and agricultural sampling points (a), agricultural and urban sampling points (b), the source and urban sampling points (c) and urban and the WWTP sampling points (d). The relative Log Expression (RLE) method is based on scaling each sample by the median ratio of the sample counts over the geometric mean counts across samples. The results show a significant Log 2-fold change for the most significant 100 of 0.05 *p*-value threshold.

component was observed between the urban sampling points (LF1 and DR1). In contrast, the waste-water sample is separated from the urban and agricultural sites along the first principal component.

Hierarchical clustering of the ARGs data revealed two main clusters (Fig. 8). The first cluster contained the source sampling sites of the River Dodder (DR3), the agricultural points on the River Liffey (LF5, LF4) and the peri-urban site of the River Liffey (LF2). The second cluster contained the urban sites of the Rivers Dodder (DR1) and Liffey (LF1), alongside the wastewater sample (WWTP). These results depict that the ARG composition at the source and agricultural sites of the River Dodder and Liffey is distinct from that of the urban sites and that the ARG composition at the urban sites is more similar to the urban wastewater sample.

A significant effect of land use on the overall composition of ARGs across the two rivers was observed (PERMANOVA; Pillai's Trace on land use factor; F-ratio = 3.1998, a *p*-value of 0.012). Specifically, there were significant differences in the relative abundance of ARGs among different land use categories. However, the Kruskal-Wallis test showed no significant differences in the relative abundance of any individual ARGs between the land use groups. These results indicate that land use as a fixed factor is an important contributor to the composition of ARGs.

4. Discussion

In this study, our objective was to investigate the influence of land use on the resistome of riverine systems, with a specific emphasis on agricultural and urban activities. We hypothesised that these activities could impact both microbial and resistome diversity within these aquatic environments. By examining the resistome and evaluating variations in microbial composition, we aimed to gain insights into the potential implications for water quality and ecological health of these systems. Our study contributes to the expanding knowledge of the intricate relationship between land use, microbial communities, and AMR in rivers and streams.

We showed that the resistome in rivers at their source differed significantly from those at locations influenced by agricultural and urban land uses. ARGs were present at all sampling points, even in areas without agricultural or urban activities. This is perhaps not surprising given that AMR is naturally occurring, and clinically important ARGs are present even in the most remote locations (Hwengwere et al., 2022; Scott et al., 2020). While ARGs were detected at our source locations, our findings revealed a notable increase in the resistome diversity within agricultural and urban samples, with the highest diversity of ARGs identified in samples taken from urban settings. An interesting observation was the distinctive disparity between the urban and source/agricultural samples, primarily characterised by an increased relative abundance of ARGs associated with resistance to disinfecting agents, antiseptics, and macrolides.

The increase in the relative abundance of antiseptic, disinfectant, and macrolide resistance genes in urban areas provides crucial insights into how pollution affects rivers. These genes indicate that urban water bodies receive a complex mixture of contaminants, including residues from commonly used disinfectants and antiseptics in urban settings and microbes resistant to these chemicals (McLellan et al., 2015; Müller et al., 2020). Sources such as wastewater discharge and improper disposal of personal care products contribute to the contamination of urban streams (Khalid and Abdollahi, 2021; Reynolds et al., 2021; Wear et al., 2021). Macrolide resistance genes were particularly prevalent in wastewater and urban sampling points. The high prevalence of macrolides is consistent with a recent comprehensive study analysing sewage from 101 countries that revealed that genes conferring resistance to macrolides were among the most abundant in Europe and Central Asia

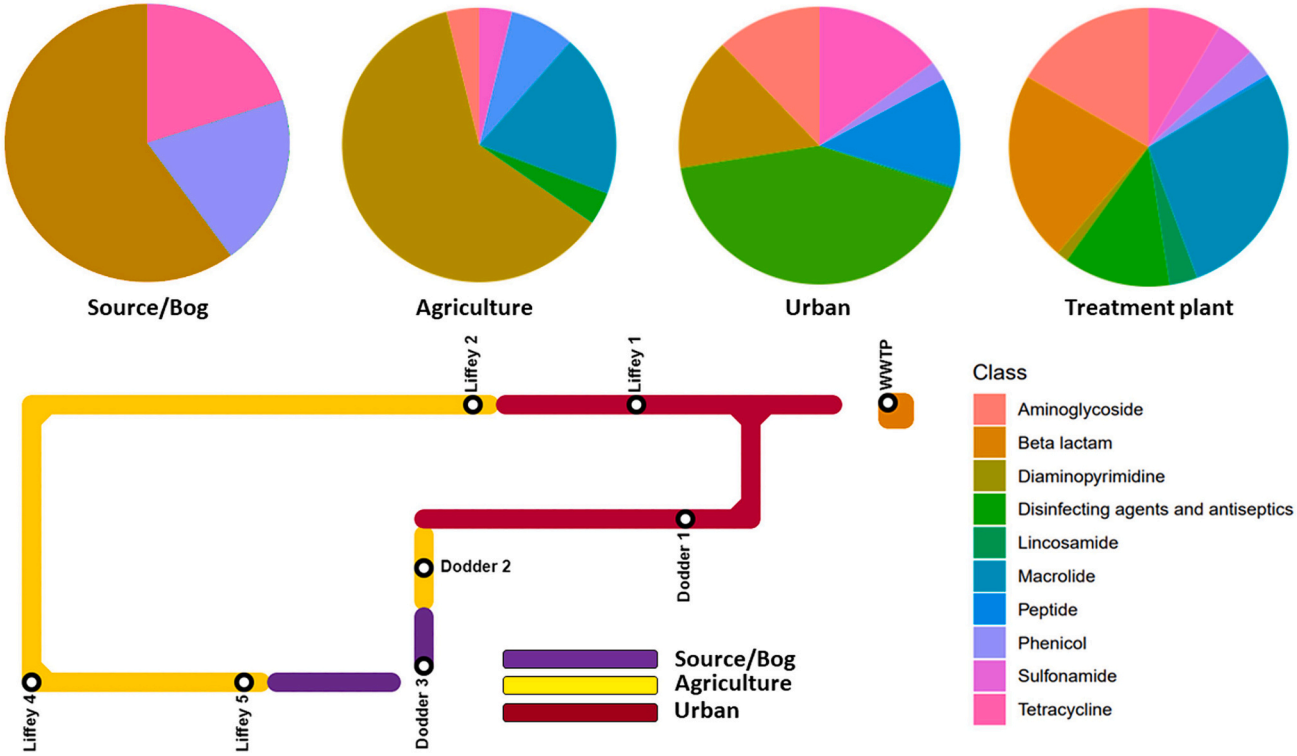

Fig. 6. Relative abundance of AMR class composition in relation to land use. The Rivers Liffey and Dodder and an urban wastewater sample resistome were characterised. Sites were grouped and annotated based on their assigned land use per the EPA CORINE characterisation. Agricultural (Liffey 5, Liffey 4, Dodder 2), urban (Liffey 2, Liffey 1, Dodder 1) and wastewater resistomes were characterised. The location of the sampling sites relative to land use are shown in a schematic overview of the rivers Dodder and Liffey.

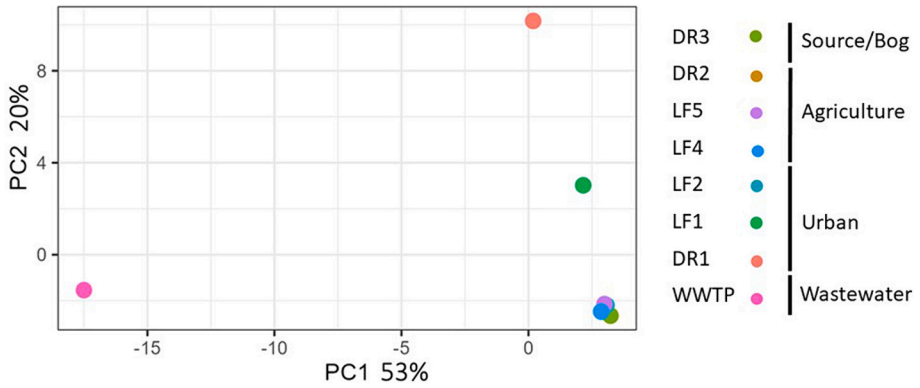

Fig. 7. Principle component analysis using classes of ARGs from rivers. Principal component analysis using the relative abundance of ARGs at the source of the River Dodder (DR3), agricultural sampling point (DR2) and urban sampling point (DR1). Agricultural (LF5, LF4) and urban sampling (LF2, LF1) points of the Rivers Liffey.

(Munk et al., 2022).

Moreover, the augmented diversity and relative abundance of disinfectants, antiseptics, and macrolides observed in urban areas coincide with distinct clustering patterns based on resistome profiles. Notably, the source and agricultural site samples exhibited similar resistome profiles. Though both areas exhibited less diversity in resistance genes than in urban areas, there was an increase in agricultural sampling points. Typical Urban sites exhibited closer clustering with the wastewater sample. This distinct clustering suggests that faecal pollution influences the resistome of urban environments. Waterbody contamination leading to the deterioration of water quality is attributed to various sources, underscoring the multifaceted nature of untreated wastewater pollution. In addition to the discharge of untreated and treated sewage from wastewater treatment plants, other significant contributors include combined sewer overflows during intense rainfall and misconnections in the pipe network of domestic sewer systems. The combined effect of these diverse sources significantly undermines the integrity of water bodies, posing substantial challenges to maintaining adequate water quality (Pazda et al., 2019; Reynolds et al., 2021; Waśko et al., 2022). In contrast to the urban sampling points and the wastewater sample, the source and agricultural site samples exhibited similar resistome profiles, but overall less diversity. Both the source and agriculture areas are likely to have similar pollution pressures, such as grazing adeer and sheep. However, an increase in agricultural practices is likely to lead to increased pollution levels and, thus, increase overall resistance. Our research highlighted this with increased resistance gene diversity in agricultural sampling points compared with the source. Agricultural sources of contamination include agricultural runoff, poorly maintained septic systems, and mismanagement of manure, which can contribute to the overall resistome and the pollution burden

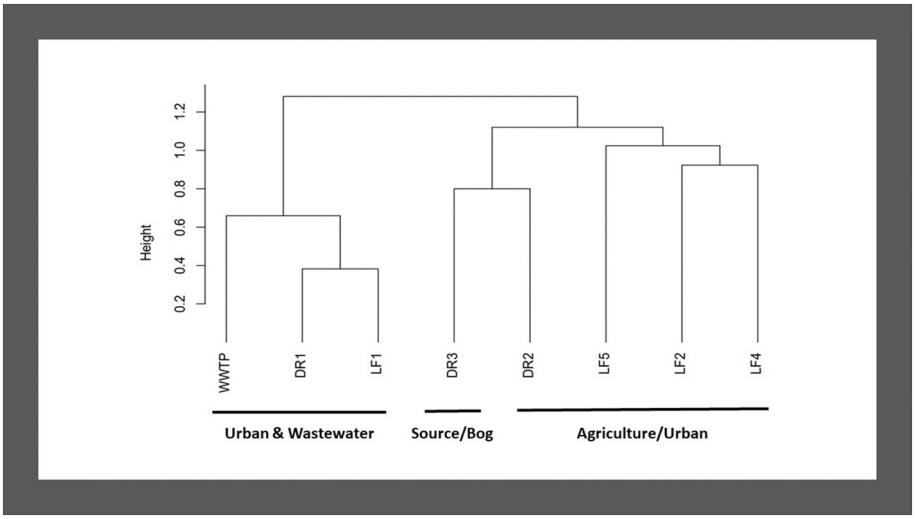

Fig. 8. Hierarchical clustering of sampling sites. Hierarchical clustering (Bray-Curtis- Ward.D2) of sampling sites using the relative abundance of ARGs for the source of the River Dodder (DR3), agricultural sampling point (DR2) and urban sampling point (DR1). Agricultural (LF5, LF4) and urban sampling (LF2, LF1) points of the Rivers Liffey.

in riverine systems (Lanyon et al., 2021).

Apart from the significant changes in the resistome profile, pollution can also have ecological ramifications, as highlighted in previous studies (Kraemer et al., 2019; Polianciuc et al., 2020). Our investigation revealed noteworthy shifts in the relative abundance of microbial phyla across diverse land uses, potentially leading to ecological consequences. Notably, we observed an overall decrease in diversity, including a significant reduction in environmental-associated microbes belonging to the phyla Verrucomicrobia, Planctomycetes, as well as the archaea Micrarchaeota, which are typically associated with acidic and peatland ecosystems (Freitas et al., 2012; Kaboré et al., 2020; Korzhenkov et al., 2019). The observed decrease in microbial diversity downstream in agricultural and urban areas is accompanied by an increased relative abundance of genera from certain bacterial phyla, particularly Proteobacteria and Bacteroidetes. Our findings, specifically those related to differential abundance, highlight significant variations in certain phyla associated with organic matter degradation and nutrient recycling (Maron et al., 2018; Philippot et al., 2013). These observed changes in the microbial community could provide insights into some of the external influences impacting the ecological health of these waters. Our results align with a recent Environmental Protection Agency (EPA) of Ireland report, which highlighted that the ecological health of the River Liffey catchment, one of Ireland's most closely monitored water bodies, ranks among the poorest due to elevated nutrient levels (EPA, 2022).

The observed changes in key phyla may influence organic matter degradation and nutrient recycling and release of nitrogen and phosphorus from organic sources. However, caution is advised in interpreting these findings. While prior studies have associated changes in bacterial phyla with nutrient release, directly attributing these alterations to harmful algal blooms may be an overinterpretation. The relationship between microbial shifts and harmful algal blooms is intricate and influenced by nutrient availability, environmental conditions, and microbial interactions (Anderson et al., 2002).

Most environmental AMR studies rely on approaches with a prior target selection and may consequently miss the important changes in resistome. Metagenomic sequencing can overcome this limitation by enabling the investigation of the total resistome. Our study demonstrates that agricultural and urban activities negatively impact microbial diversity and richness and suggests that changes in microbial communities across different land uses could have ecological implications. Furthermore, our results demonstrate that land use significantly impacts the resistome in riverine environments, and wastewater could be a possible culprit to the observed differences. These results highlight a need for surveillance and management of AMR in the environment to develop effective strategies for targeted reduction and disposal of AMR in urban and agricultural settings. Moreover, this approach can be an important resource to inform effective strategies to manage and conserve these valuable aquatic resources.

Further research could enhance the robustness of our findings by expanding the sampling scope to include a broader geographical representation encompassing rivers from diverse regions, which would contribute to a more comprehensive understanding of the interplay between land use and riverine resistome dynamics. Additionally, extending the study over a more extended time frame would provide insights into potential seasonal variations and long-term trends on the influence of land use on microbial communities and antimicrobial resistance.

5. Conclusion

In conclusion, this study further underscores the potential intricate relationship between riverine resistome and land use practices. The increase in the diversity and reduction in microbial communties points to pollution pressures associated within these landscapes. These pressures are likely to contribute to AMR and highlight the importance of a One Health approach to address the global public health threat of antimicrobial resistance. Metagenomic analysis provides a comprehensive and unbiased approach to studying environmental AMR. The results of this study can inform effective strategies for mitigating the impact of agricultural and urban pollution on aquatic ecosystems and lowering the potential risk these waters pose to human health.

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CRediT authorship contribution statement

Tristan M. Nolan: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Formal analysis, Data

curation, Conceptualization. **Niamh A. Martin:** Validation, Investigation. **Liam J. Reynolds:** Validation, Investigation. **Laura Sala-Comorera:** Validation. **Gregory M.P. O'Hare:** Writing – review & editing. **John J. O'Sullivan:** Writing – review & editing. **Wim G. Meijer:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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