Underwater Dynamics:
How Eutrophication Shapes the River Resistome, Mobilome, and Virulome

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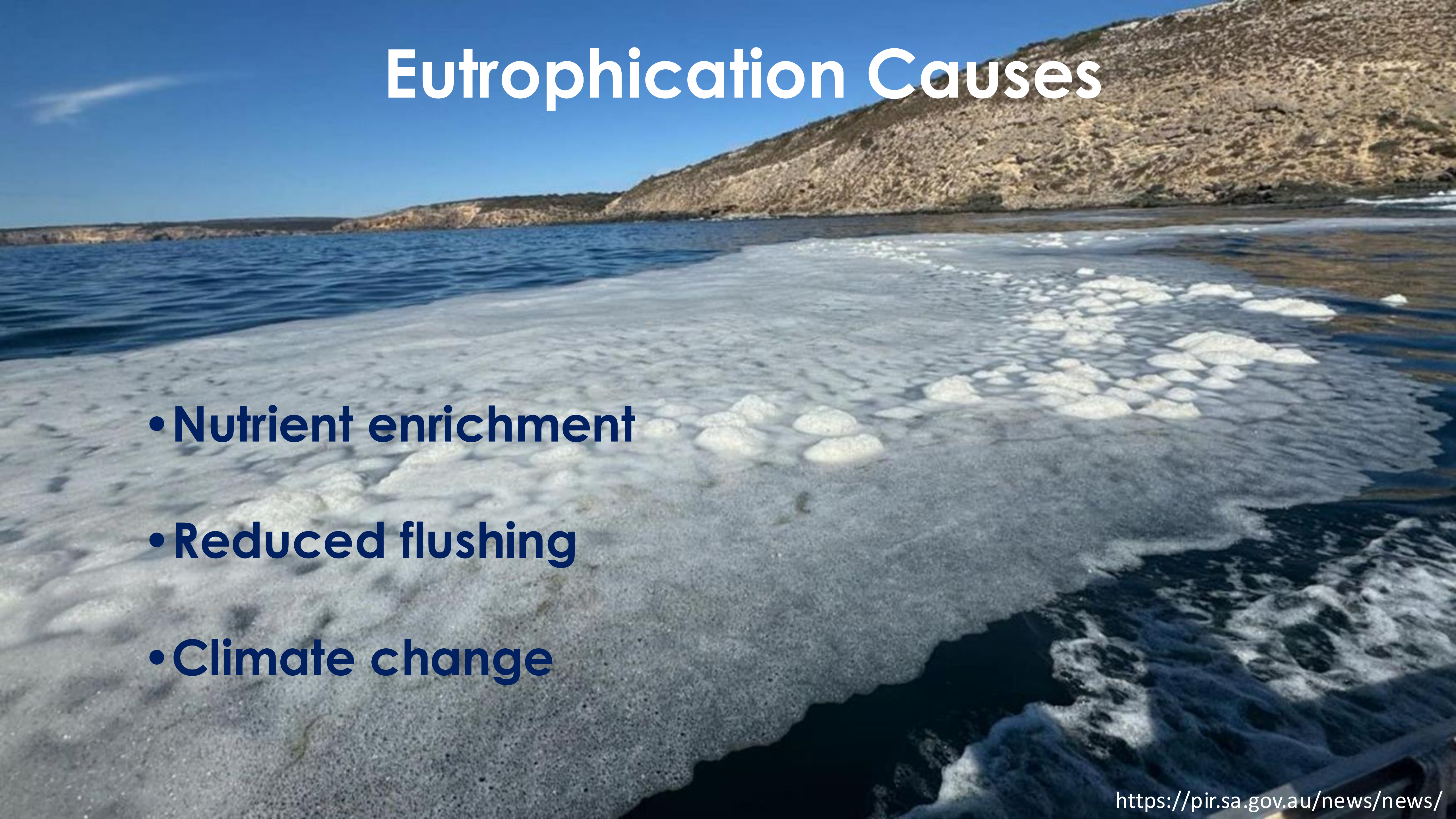
Eutrophication is a significant issue in South Australia

https://www.abc.net.au/news/2025-09-27/

Eutrophication Causes



- Nutrient enrichment
- Reduced flushing
- Climate change

https://pir.sa.gov.au/news/news/

Eutrophication Impacts

- Rivers, Lakes and Marine life
- Ecosystems
- Economy

https://edition.cnn.com/2025/07/22/

Eutrophication Management and Response

- **Monitoring:** river and marine ecosystems
- **Long-term solutions:** (e.g., improving nutrient management)

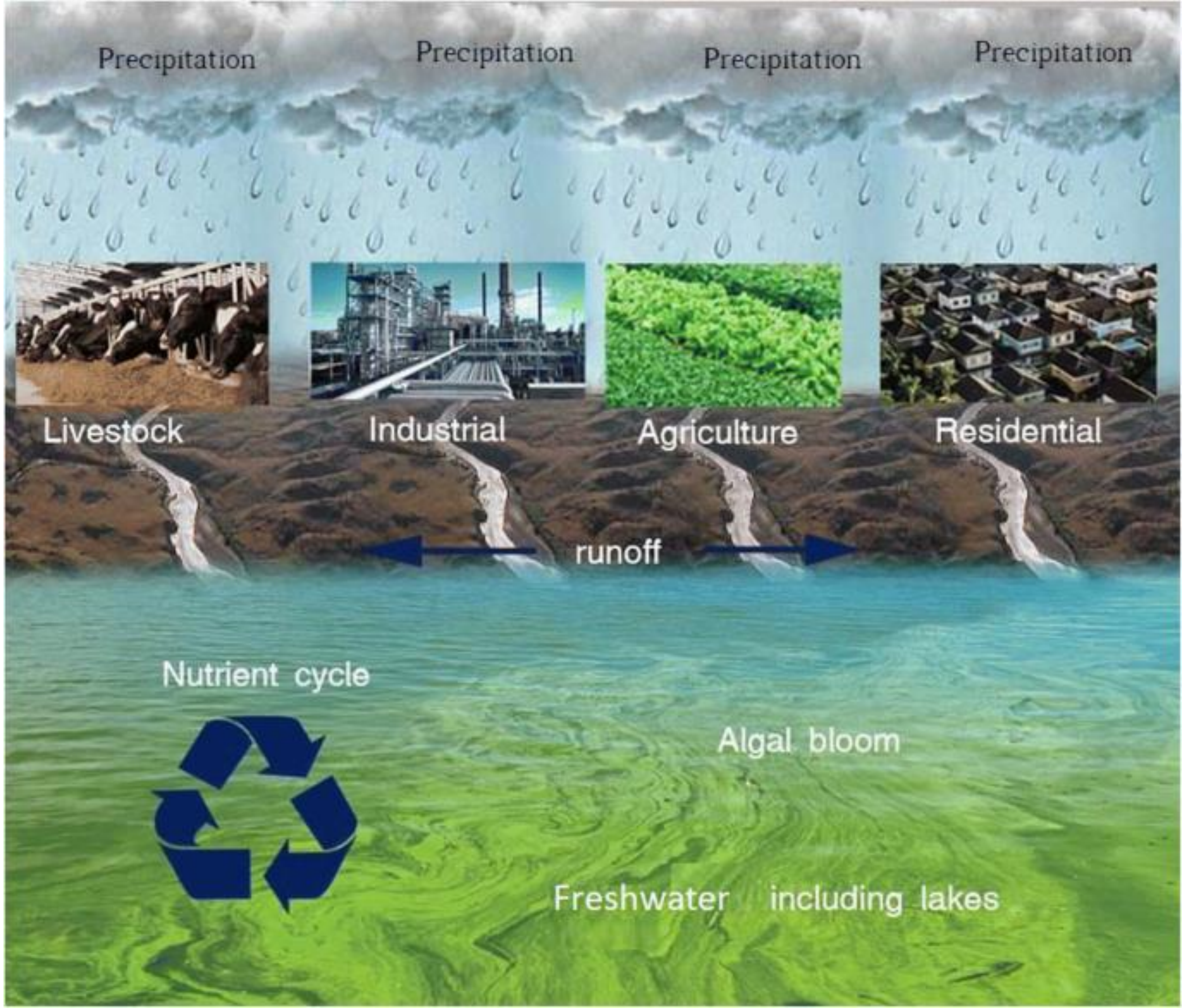
https://www.sbs.com.au/news/

Eutrophication is monitored by measuring:

- **nutrient levels** (nitrogen and phosphorus)
- **water quality indicators** (chlorophyll-a, dissolved oxygen, and transparency)
- **ecosystem health** (fish populations and the presence of specific algae types)

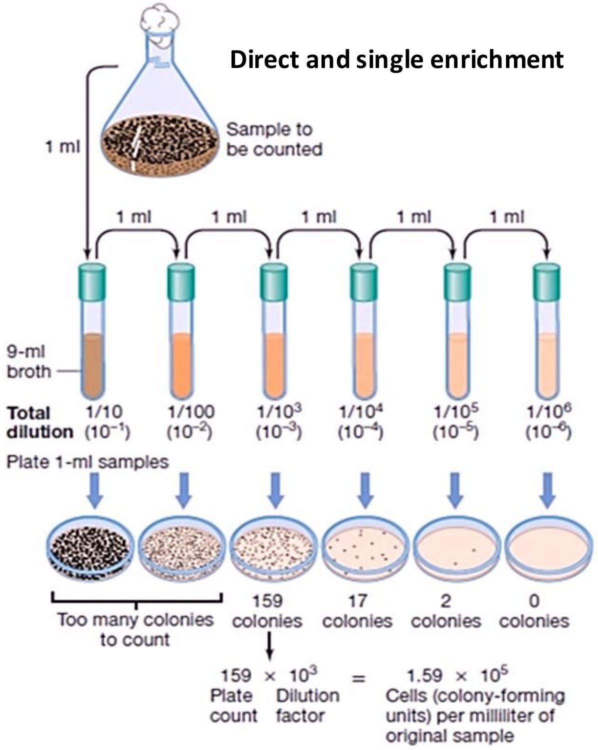
Objective:

Assess the impact of cultural or rapid eutrophication on the dynamics of antimicrobial resistance elements, virulence-associated genes, and bacterial community composition over time.



Land Use
Site A: Livestock grazing
Site B: Viticulture
Site C: Heavily Urbanized
Site D: Moderately Urbanized
Site E: Coastal

Culture-dependent methods



Direct and single enrichment

↓

MALDI-TOF

↓

Isolates DNA extractions

Gram negatives:

1. ***Klebsiella*** spp. : Brilliance™ ESBL Agar
2. ***Serratia*** spp.: Brilliance™ ESBL Agar
3. ***Citrobacter*** spp. : Brilliance™ ESBL Agar
4. ***Salmonella*** spp.: Brilliance™ Salmonella
5. ***Acinetobacter*** spp.: MacConkey agar
6. ***Pseudomonas*** spp.:
    - Pseudomonas Agar Plates
    - MacConkey agar
7. **Enterobacteriaceae**:
    - Colilert ™
    - MacConkey Agar
8. ***E.coli*** :
    - Brilliance™ ESBL Agar
    - Membrane faecal coliform (mFc) Agar +/- cefotaxime

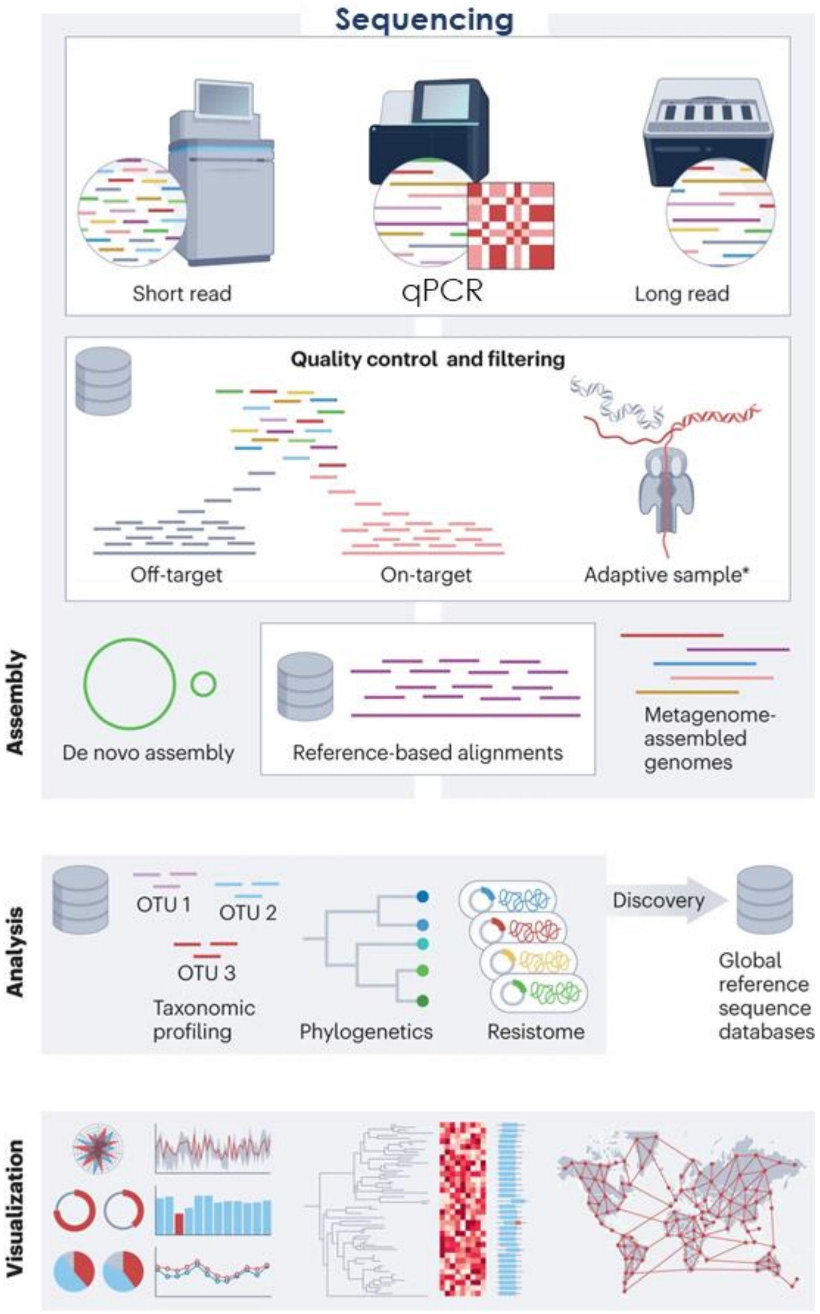
Gram positives:

9. ***E. faecium, E. faecalis, Enterococcus*** spp.: Slanetz-Bartley Agar
10. ***Staphylococcus*** spp.: Brilliance™ MRSA 2 Agar
11. ***Bacillus*** spp.: Brilliance™ MRSA 2 Agar
12. **MRSA**: Brilliance™ MRSA 2 Agar

Gram negatives and positives:

13. **CRE resistance microorganisms**:
    - CRE plates
    - Brilliance™ CRE Agar
14. **Colistin resistance microorganism**: colistin Agar

Genomics Toolbox



Modified from Djordjevic *et al.*,
Nature Reviews Genetics, **25**, 142-157 (**2024**)

Metagenomics

Biodiversity and community structure:

- Algae, Bacteria, Fungi, Viruses, Protist
- ARGs
- Biocides
- Mobile Genetic Elements (MGEs)
- Virulence Associated Genes (VAGs)

Whole Genome Sequencing

Phylogenetic trees

- Identify the distribution and relatedness of isolates
- Multiple ‘resolutions’ to refine the analyses in a step-wise fashion

Genotyping

- Presence/absence of key genes
  - ARGs
  - Virulence factors
  - Mobilisation (insertion sequences and plasmid replicons)

Multiplex qPCR assays:

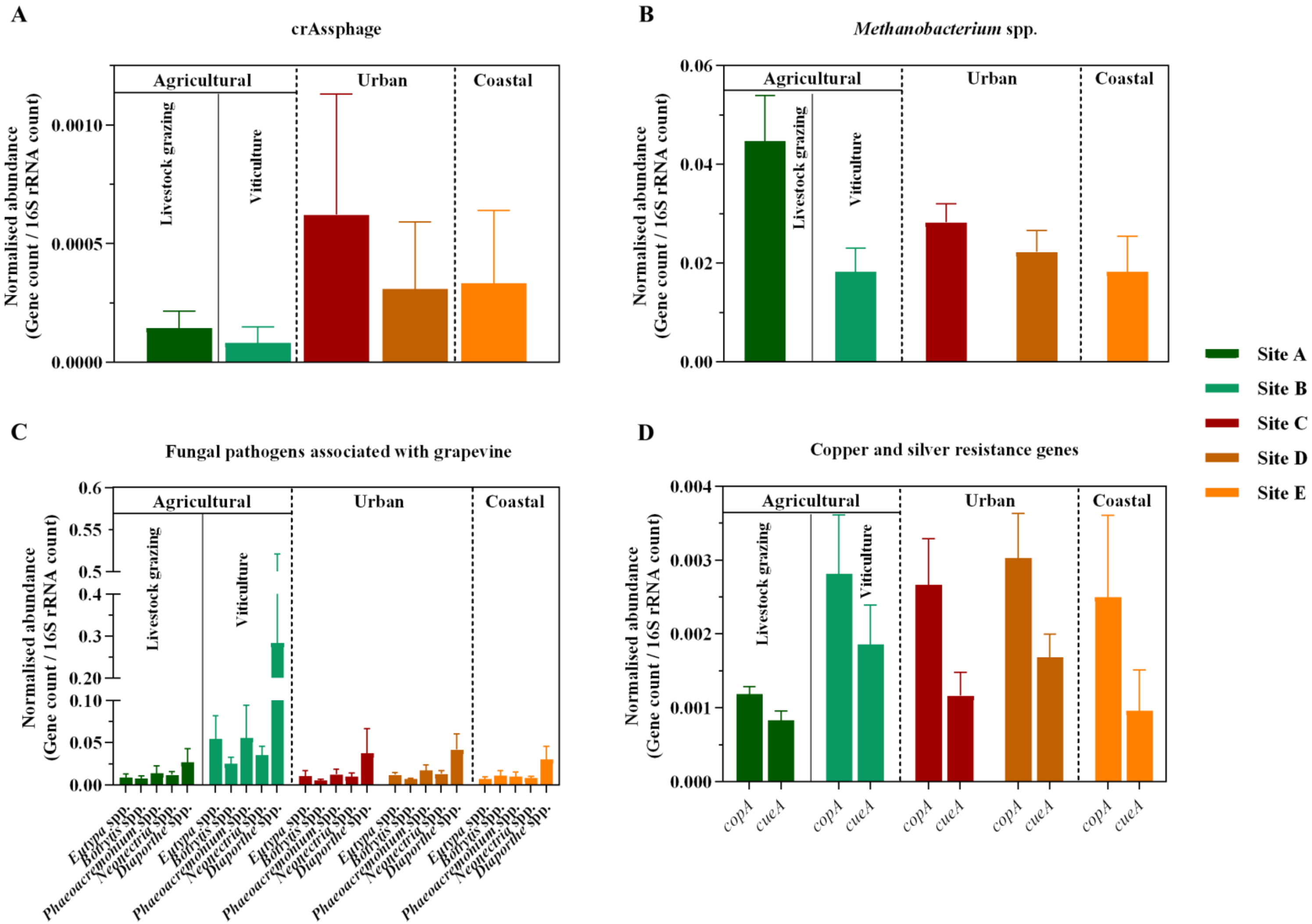
- Emerging and Present Human Opportunistic Pathogens and ARGs

Databases: e.g. Kaiju v1.7.3, ResFinder, MGE, DeepARG, CARD, Biocides, VFDB, VirulenceDB, MEGARes 2.0, Silva, PhyloSift, FastTree

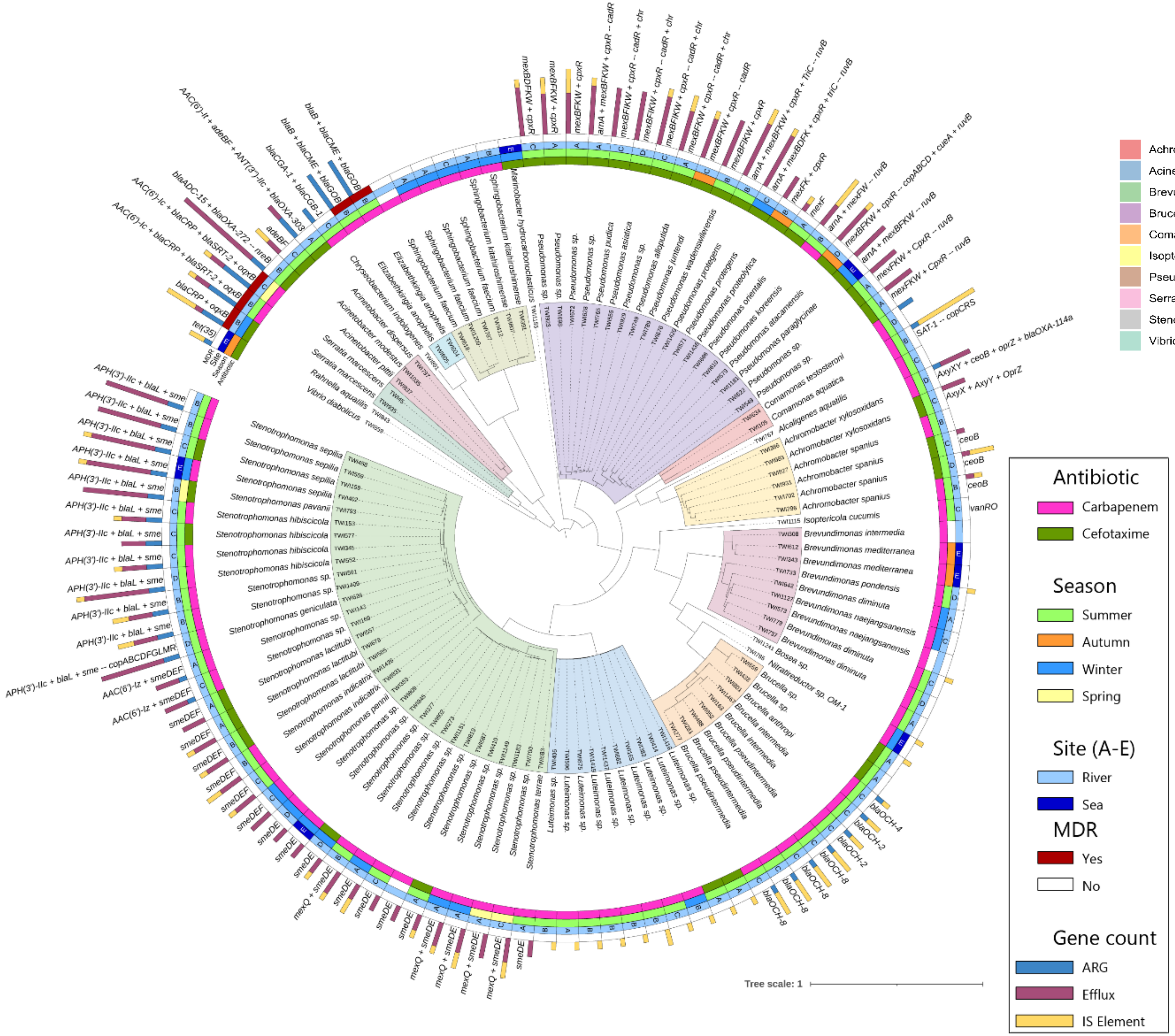
Variation in the Abundance of Urban and Agricultural Runoff Bioindicators

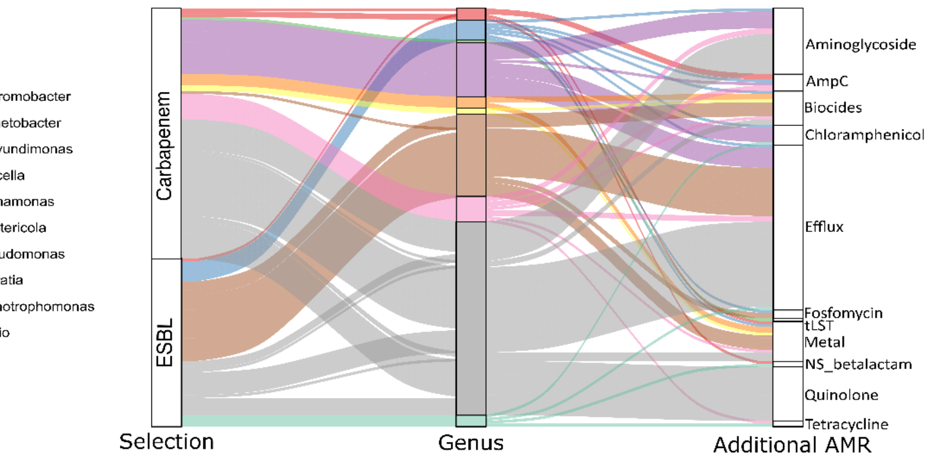
Catchment land use shapes microbial communities and the resistome.

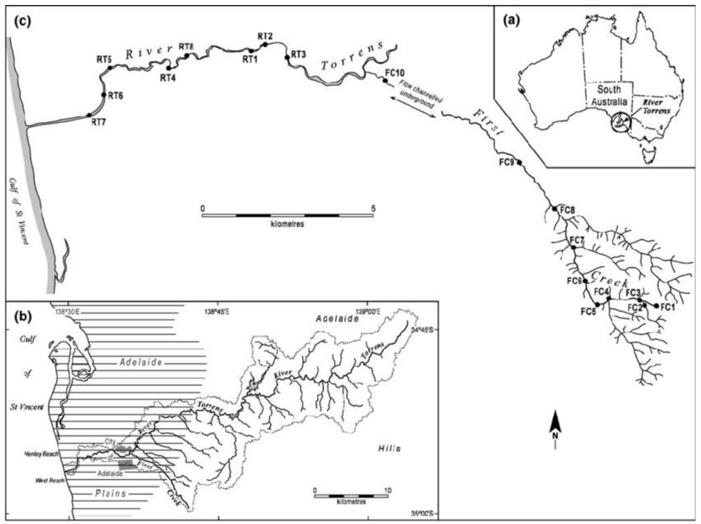
- ***Methanobacterium*** spp. and grapevine fungal pathogens show strong correlations with specific land-use types.
- Urban catchments exhibit elevated **crAssphage** levels, highlighting the impact of urbanization on river microbiomes, particularly during heavy rainfall events.



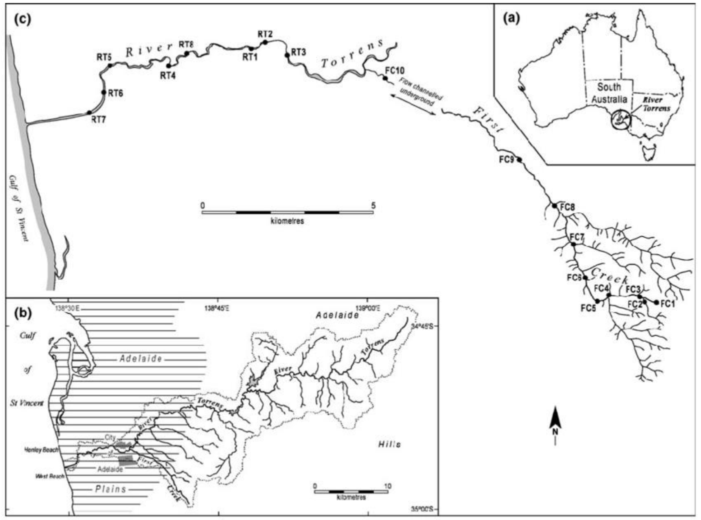
Seasonal Variation in Antimicrobial Determinants

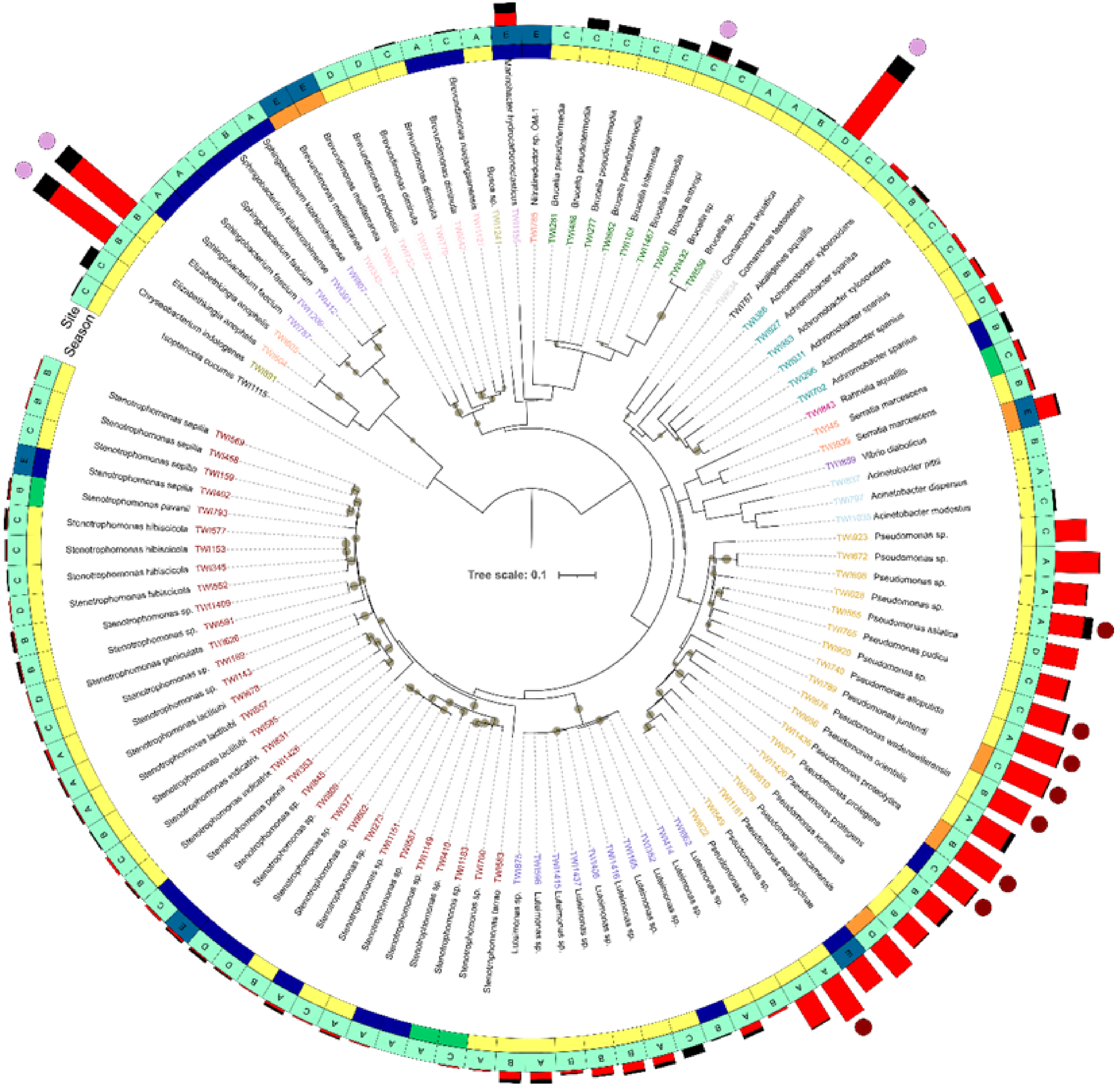






Seasonal Variation in Virulence Associated Genes



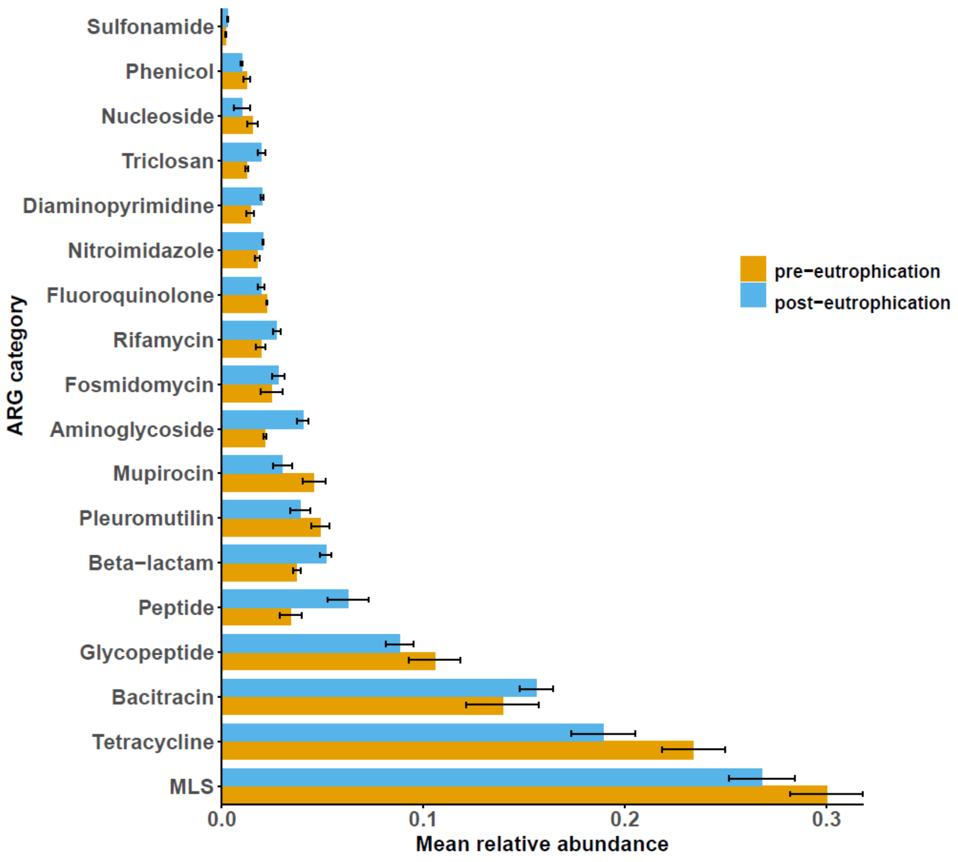


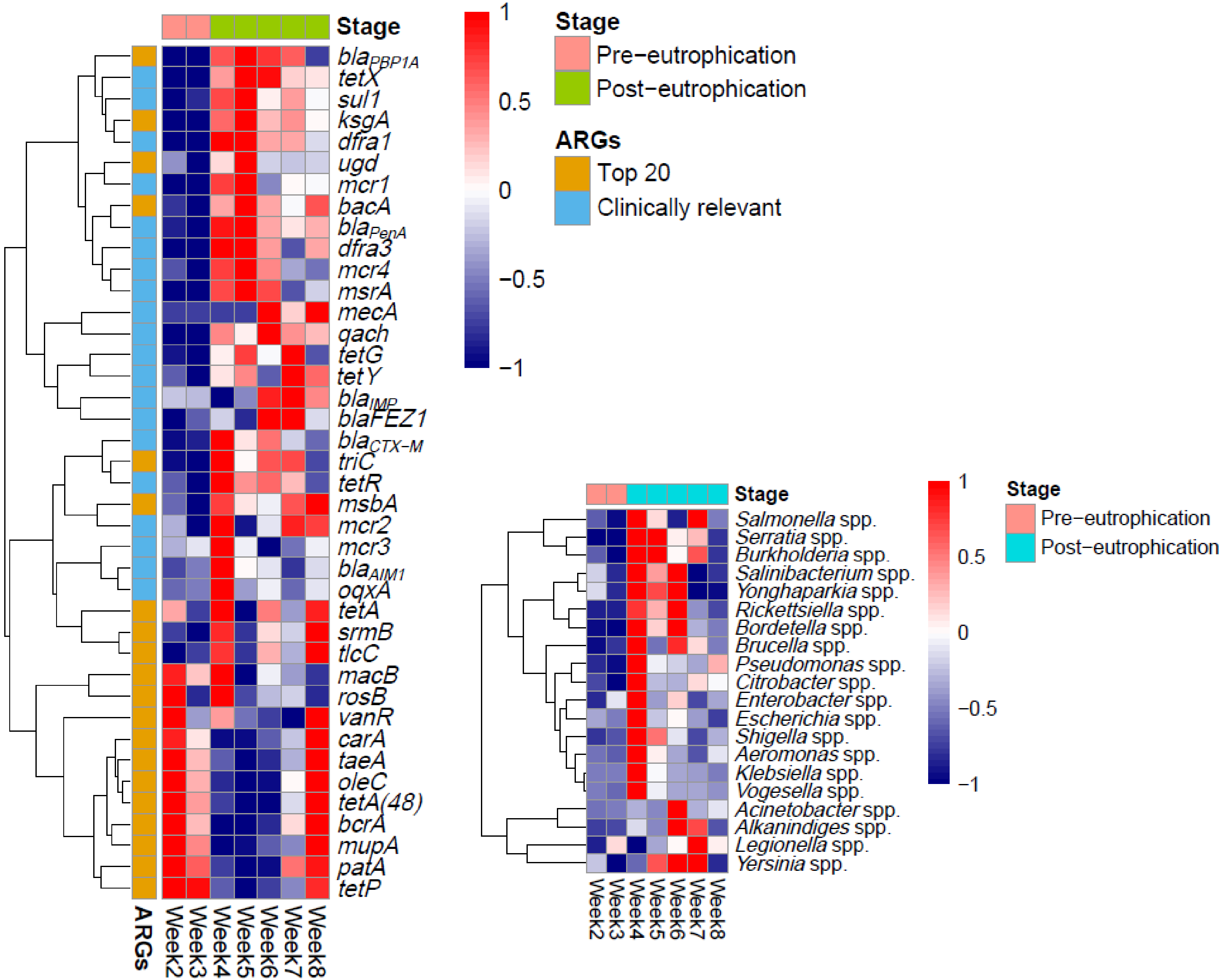
Season
- 🟩 Spring
- 🟨 Summer
- 🟦 Winter
- 🟧 Autumn

Site (A-E)
- 🟩 River
- 🟦 Sea

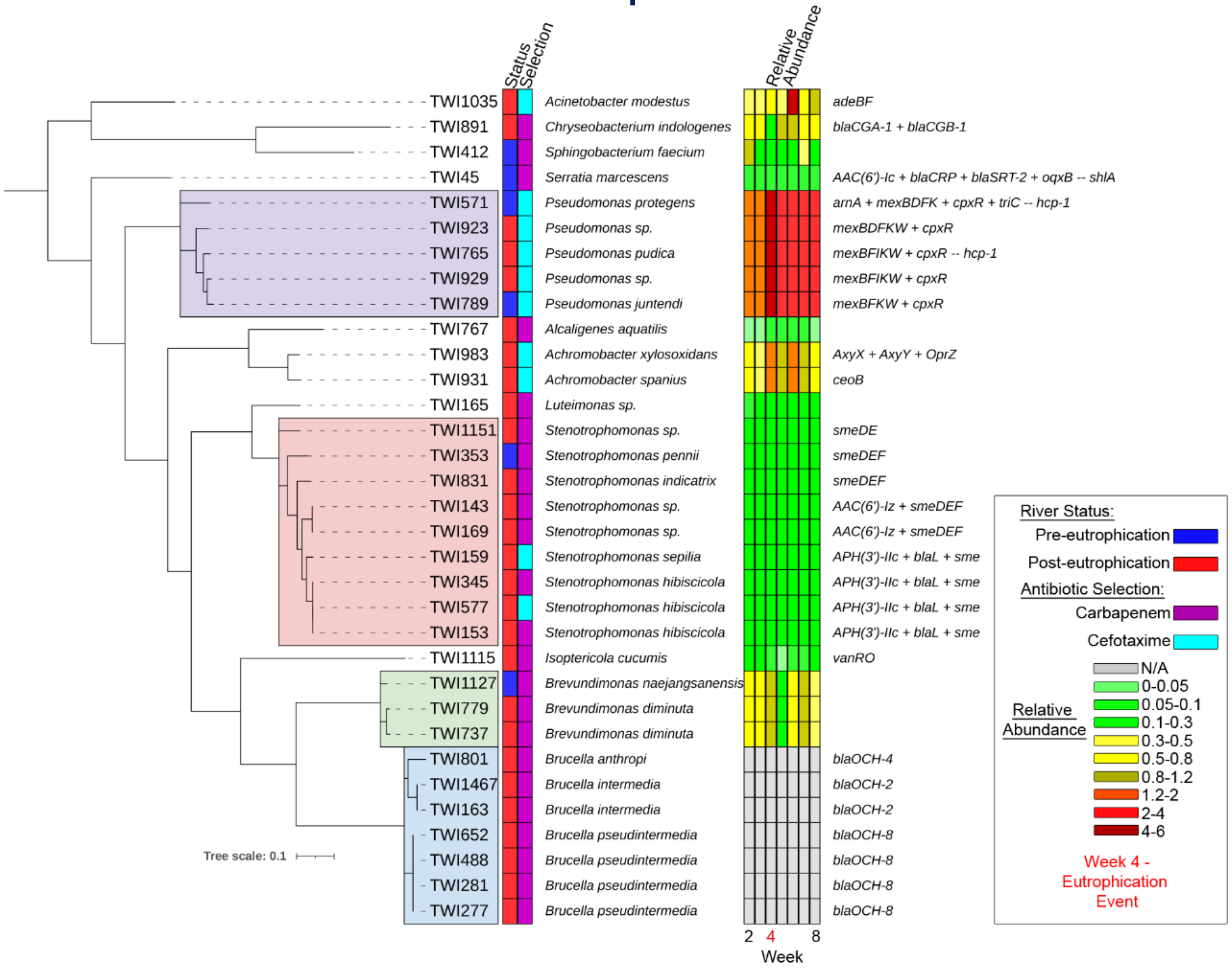
VAG
- ● astA
- ● hcp1 (T6SS)

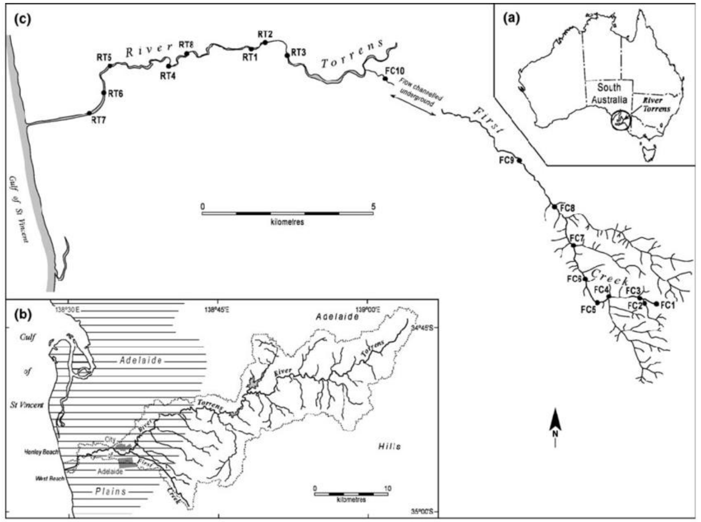
Eutrophication: variation in antibiotic resistance gene and bacterial pathogens





Post-eutrophication: presence of potential unknown and novel bacterial species





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