Australian Government
Department of Industry, Science and Resources

Cooperative Research Centres Program

SAAFE CRC

Defining Meaningful Environmental Endpoints for AMR:

Moving Beyond MICs to Assess Minimal Selective Concentrations

Next Water Conference 2025

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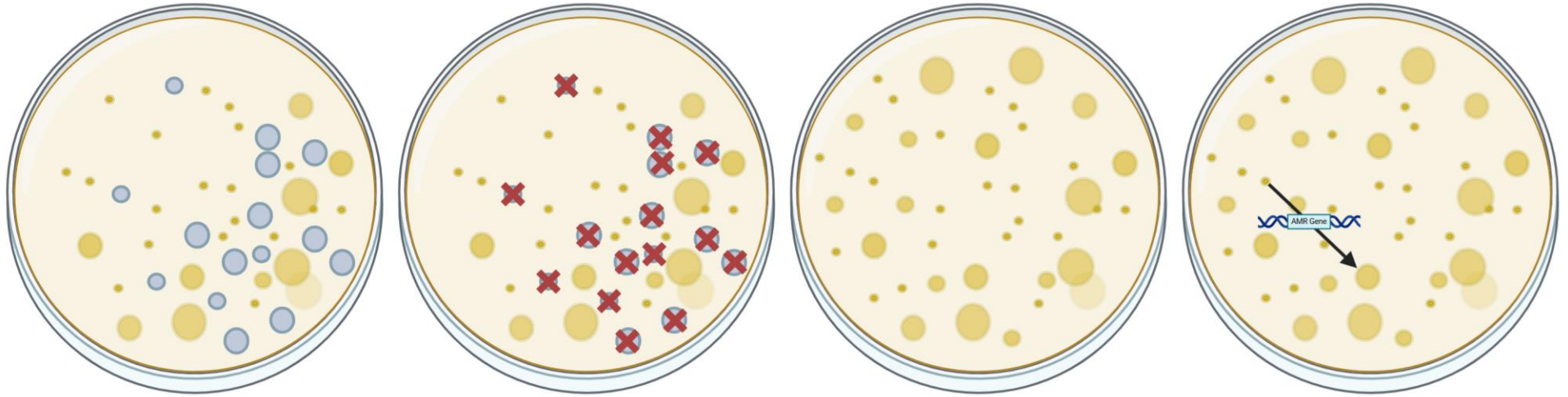
What is AMR?

- AMR = microorganisms evolve to survive antimicrobials
- Global health + ecological challenge
- Spread occurs in people, animals, cropping and the broader environment



1. Bacterial community, mix of sensitive and resistant.

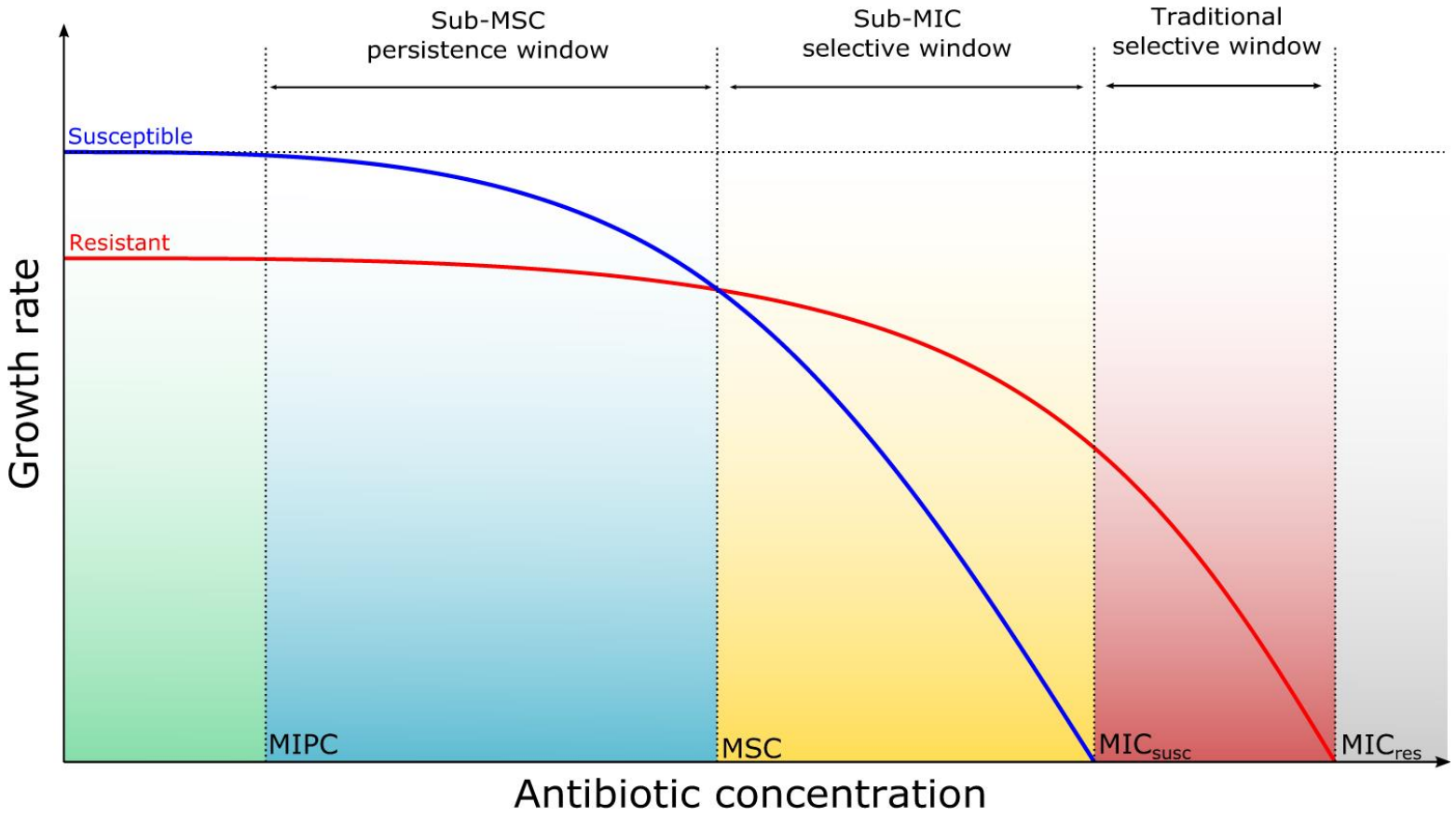
2. Antimicrobial exposure kills sensitive cells, but resistant strains remain

3. Resistant bacteria multiply

4. Antimicrobial resistance spreads

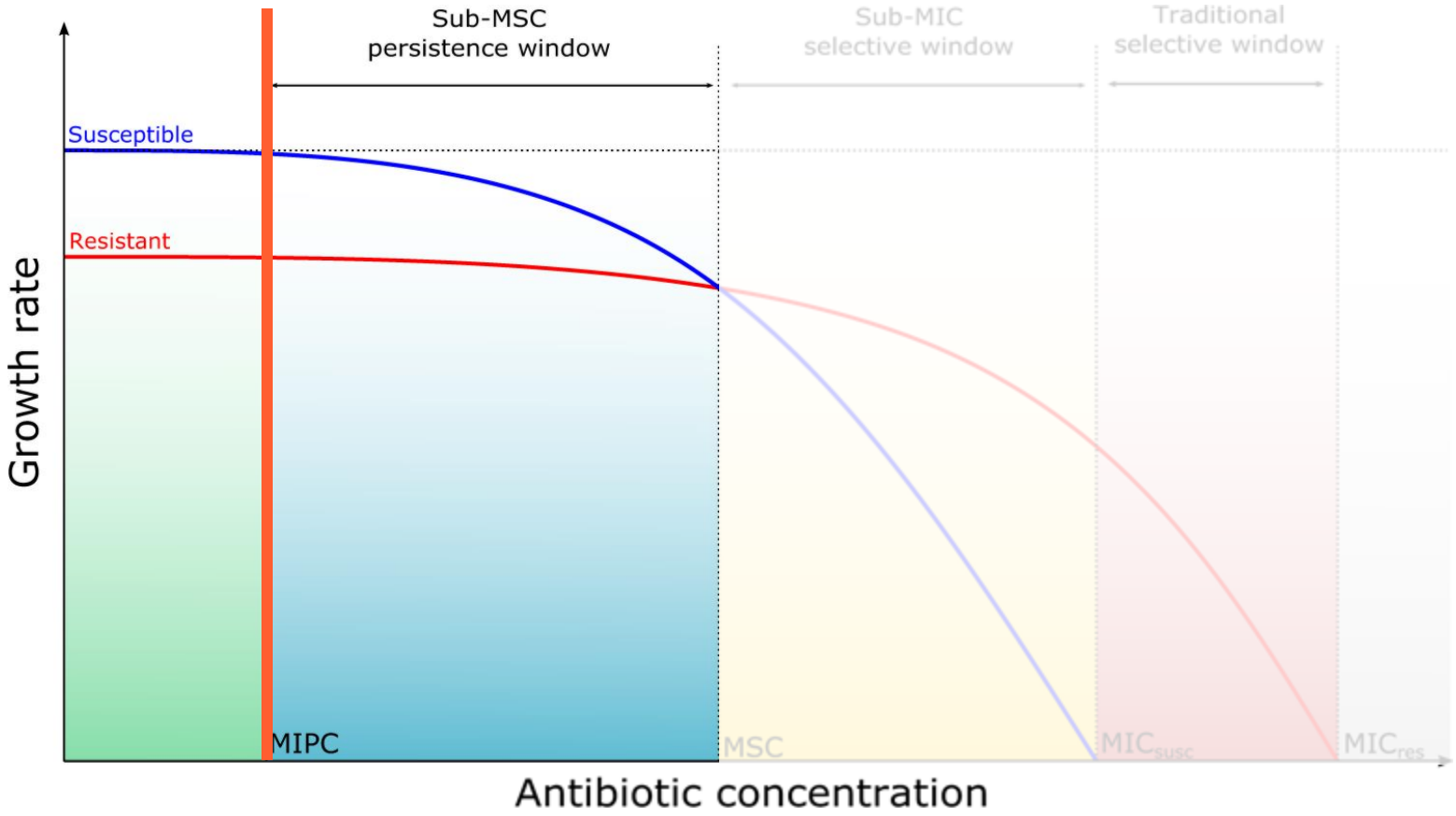
How do we measure resistance?

- “Resistance” defined clinically → when a drug no longer effective
- Based on dosing limits (can’t just keep increasing → toxicity, side effects)
- Clinical tool = MIC (Minimum Inhibitory Concentration)



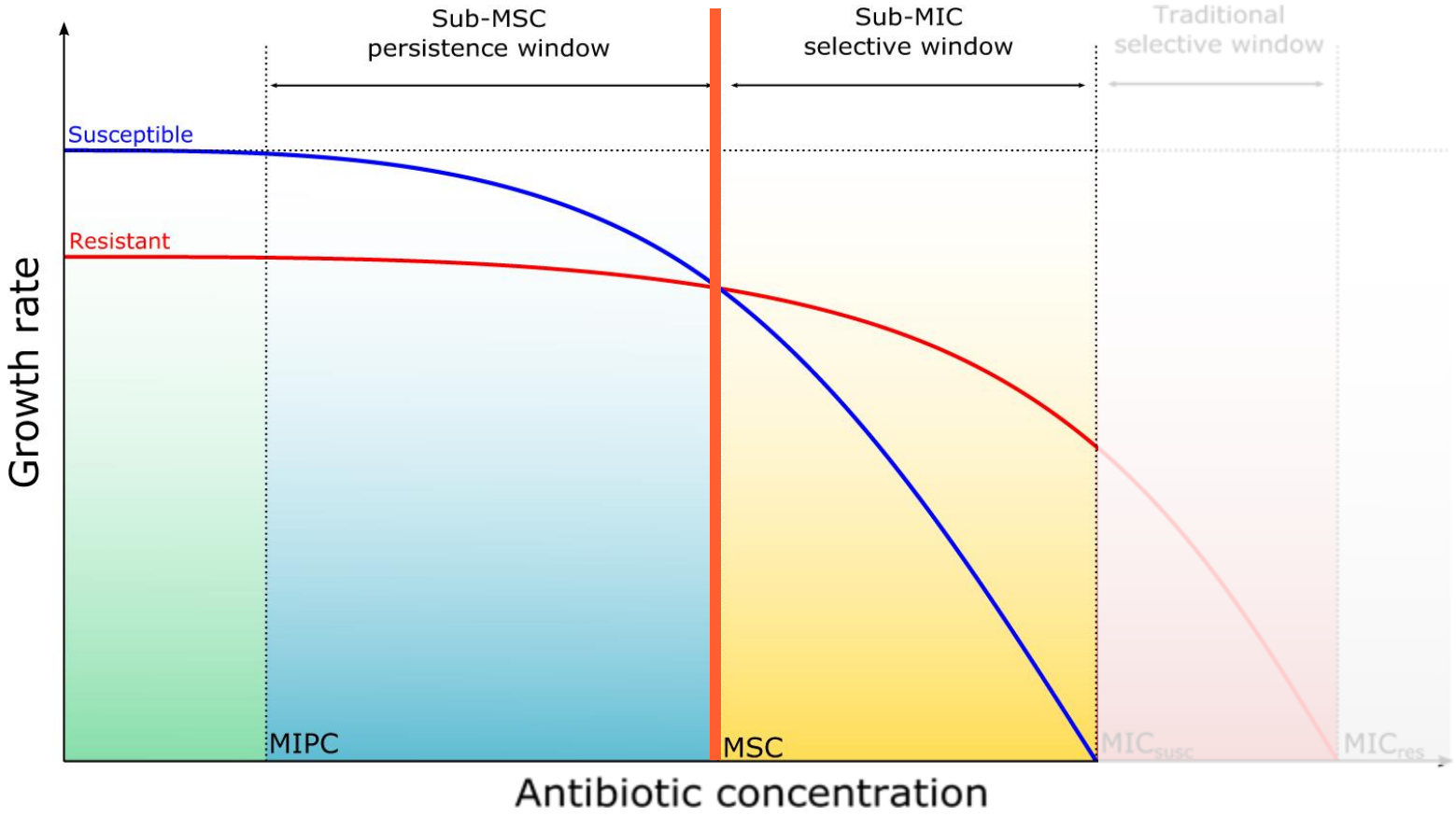
How do we measure resistance?

- MIPC = minimal increased persistence concentration



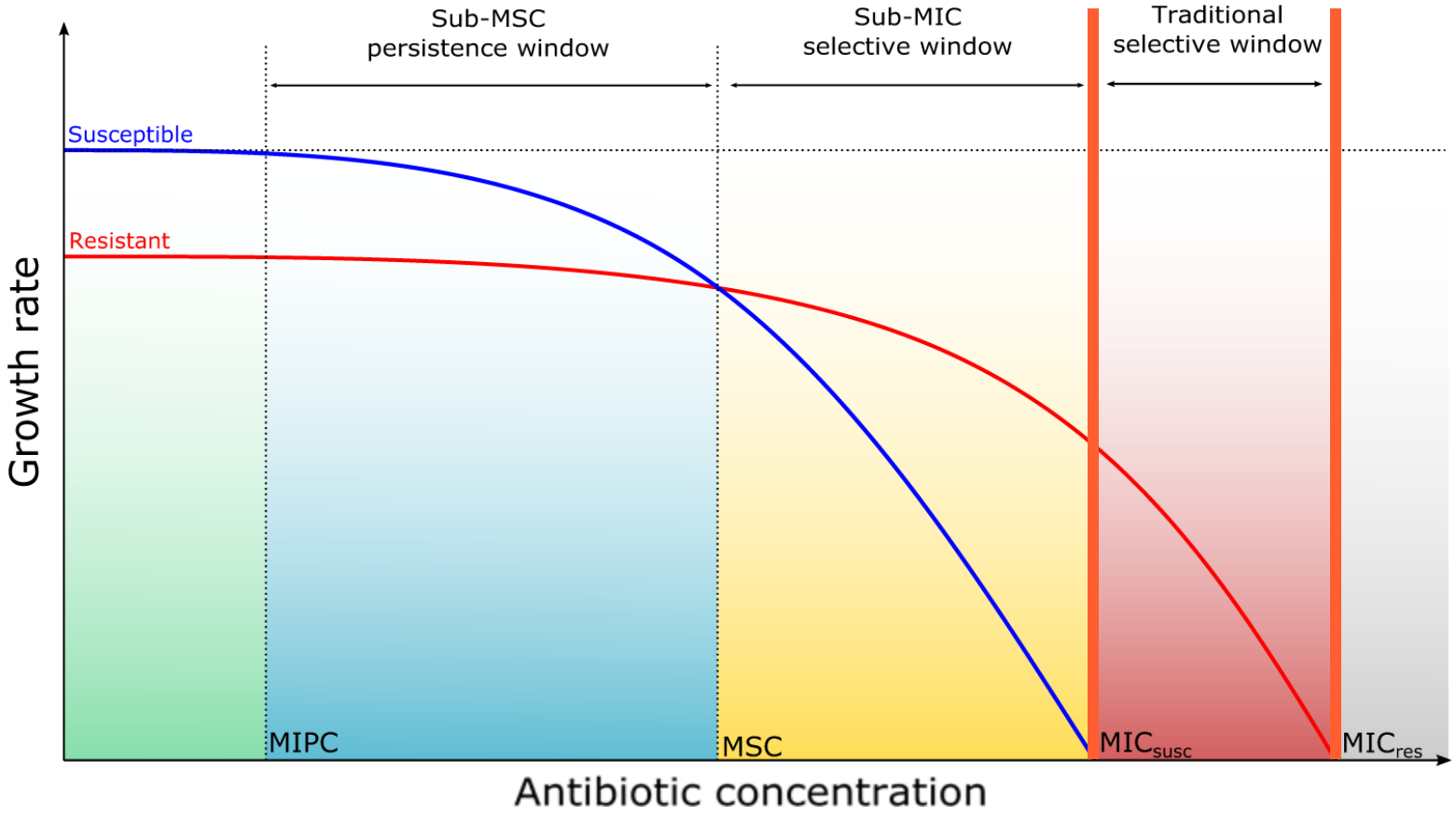
How do we measure resistance?

- MIPC = minimal increased persistence concentration
- MSC = minimal selective concentration

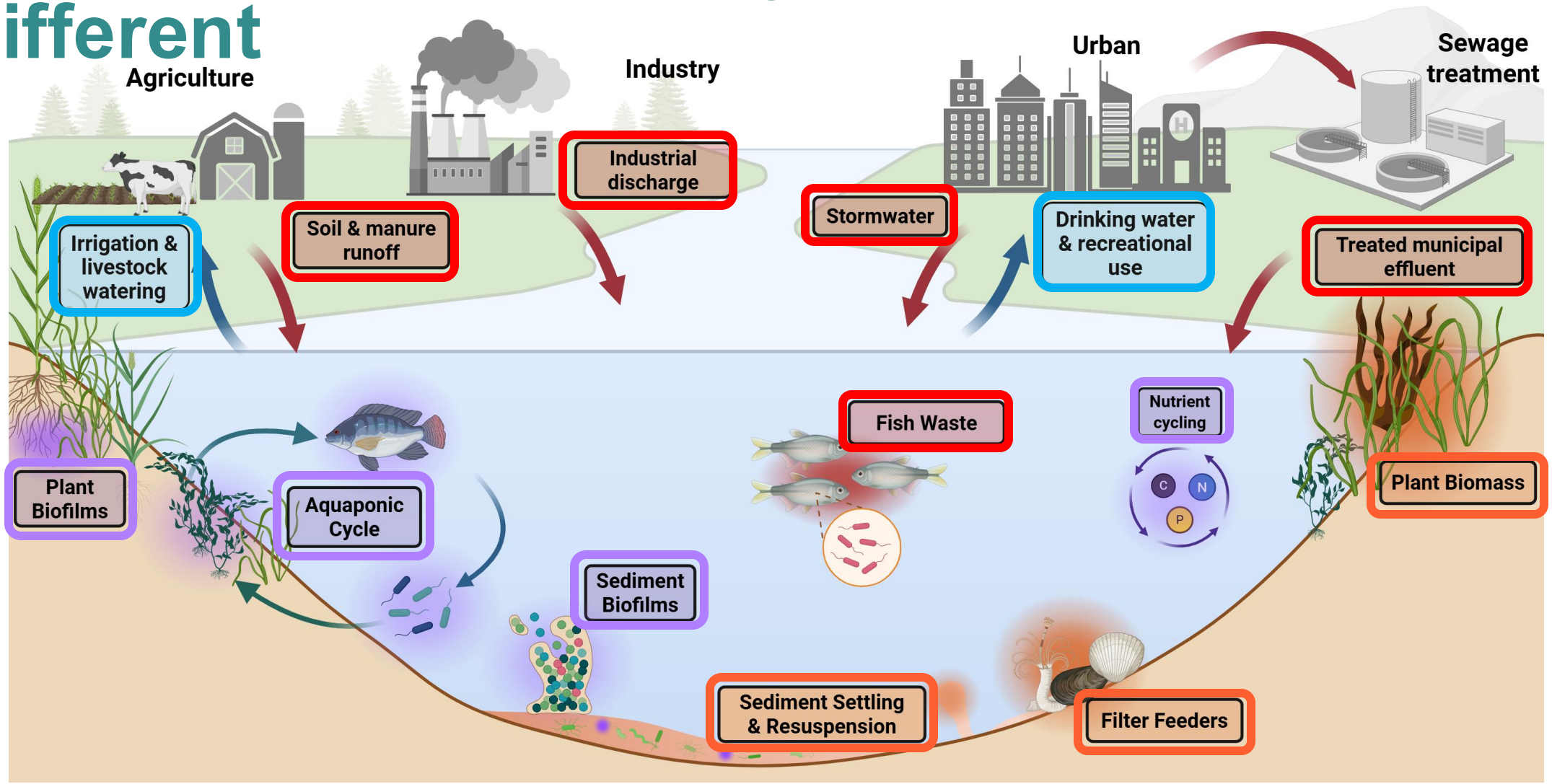


How do we measure resistance?

- MIPC = minimal increased persistence concentration
- MSC = minimum selective concentration
- MIC = minimum inhibitory concentration



From MIC to MSC: Why the environment is different



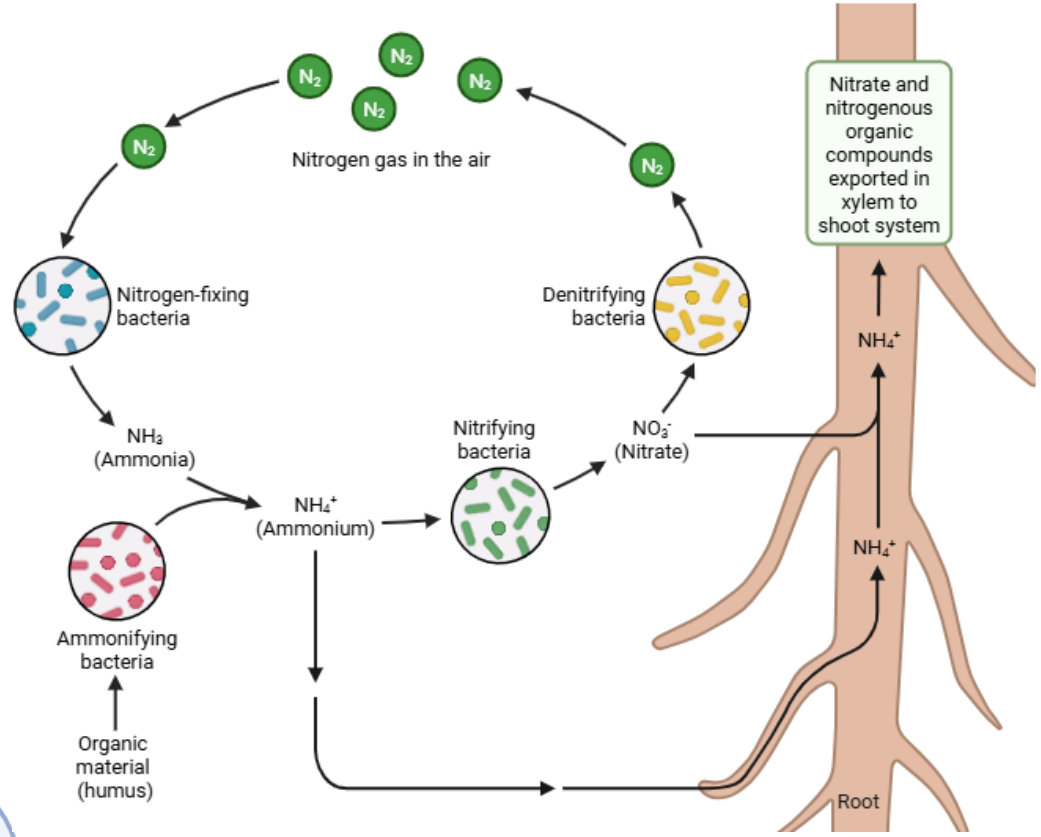
Why current guidelines fall short

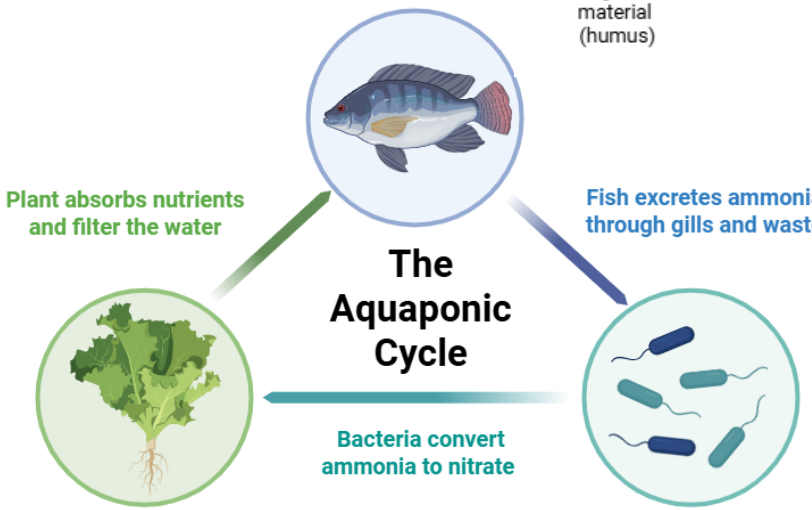
- Environmental guidelines rare or absent
- Attempts = crude (e.g., MIC ÷ 10)
  - Extrapolated from lab MIC data
- Resistance can evolve at much lower levels (MSCs) and due to shared genes/mutations in microbiomes
- Relevant in treated wastewater effluent, ag runoff, aquaculture, etc.

Ecologically meaningful endpoints

We need to look beyond growth inhibition:

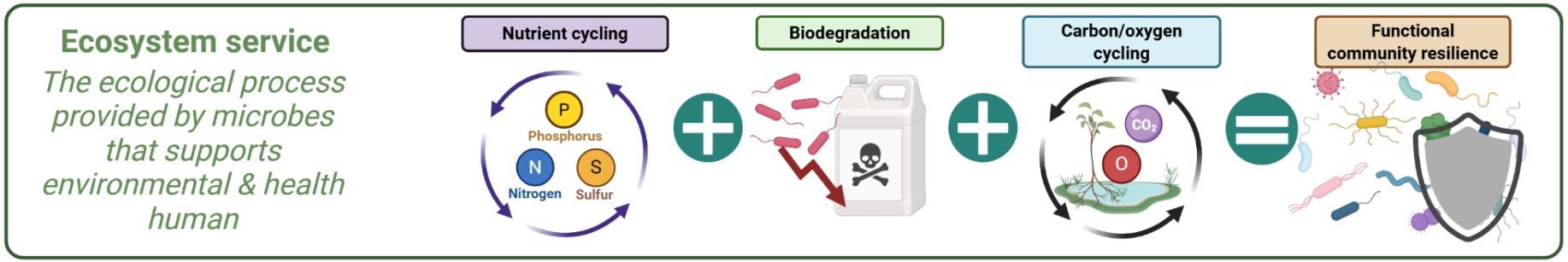
- Microbial community function (nutrient, carbon, oxygen cycling)
- Biofilm formation and stability in pipes, filters, and sediments
- Horizontal gene transfer potential
- Community structure shifts

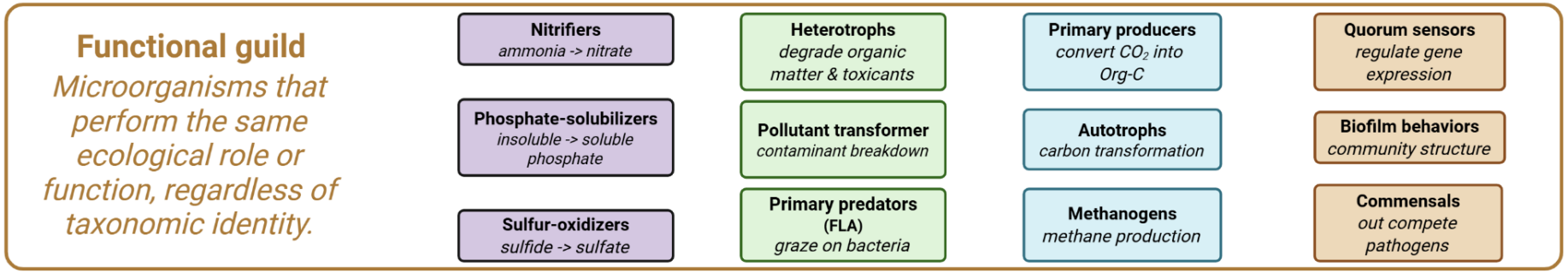




Why communities matter, not single species

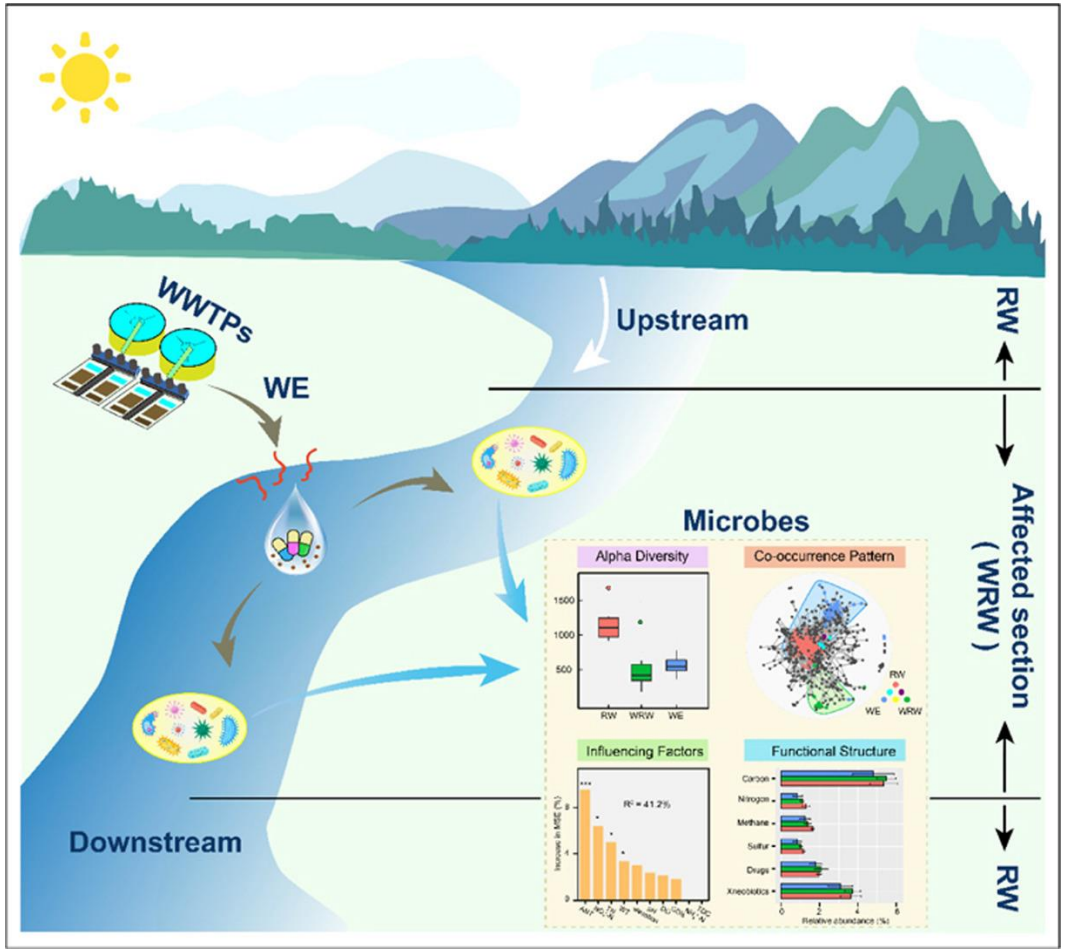
- Environments = multispecies biofilms & consortia
- Functional guilds (e.g., nitrifiers, denitrifiers, degraders) underpin treatment and water quality





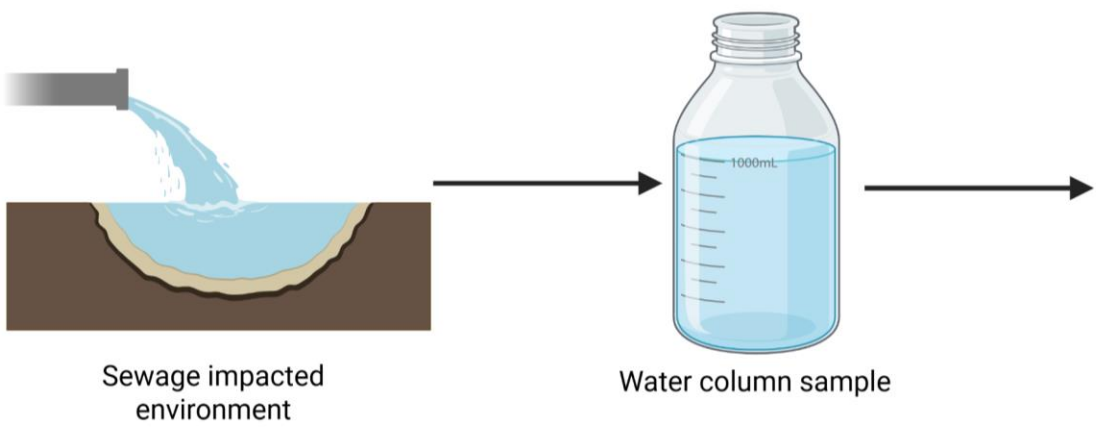
Case study: WWTP effluent → receiving waters

- Antibiotics detected in effluent:
  - CIP 10 ng/L, CLA 3 ng/L, CLI 3 ng/L, TRI 24 ng/L
- All well below clinical MICs
- Below current PNEC-res values
  - (e.g. CIP <64 ng/L, TRI <500 ng/L)
- Yet ARGs (*sul*1, *aad*A, *intI*1) increased downstream
- Suggests effluent loading + possible sub-MIC/MSC selection



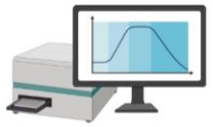
Bergland et al, 2015 doi: 10.1002/etc.2784

Tools we can use



Sewage impacted environment

Water column sample

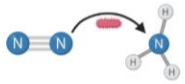


Bacterial growth inhibition assay
48 hr growth inhibition assay, spectrophotometric method

→ growth rate

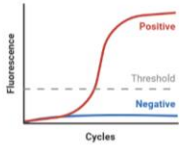
Chloroform fumigation-extraction
Measures C & N contained within microbial biomass

→ biomass



Acetylene reduction assay
Measures ethylene production from acetylene as a proxy for nitrogenase activity (N_2 fixation potential)

→ N-fixation

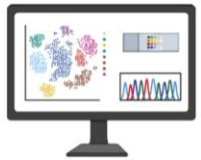


qPCR of functional genes
Measure abundance of functional and ARGs

→ change in gene copies

Metagenomics
Species richness and abundance profiling via amplicon sequencing.

→ shift in community or functional groups



Metatranscriptomics / Metaproteomics
Active functional genes / enzymes

→ change in gene expression

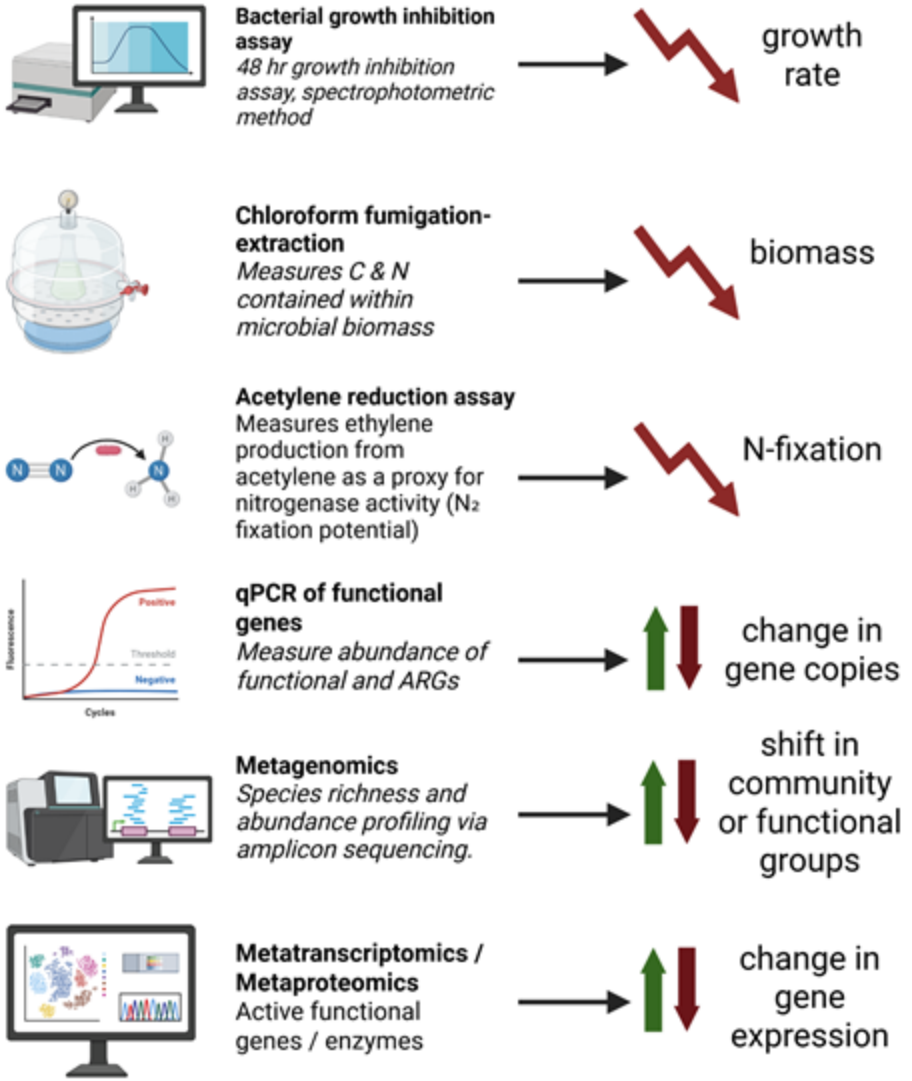
Tools we can use

Interpretation

Normal vs meaningful change?

Acute vs chronic?

Direction, magnitude & context

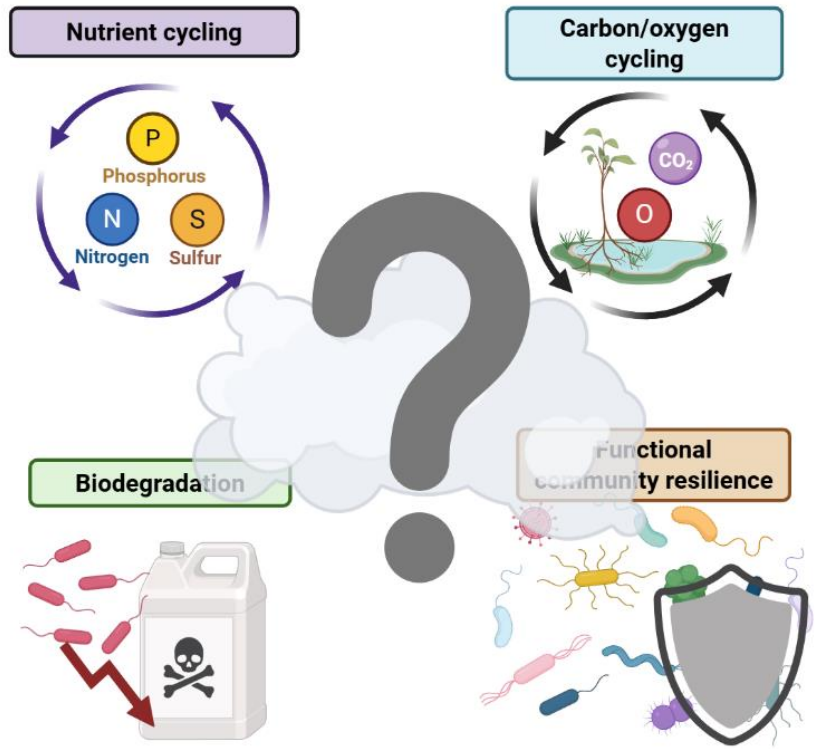


Where to from here?

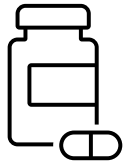
- Many unknowns remain:
  - Impacts on microbial community function & resilience
  - What is “normal” in these systems?
  - What counts as a meaningful change?

- We know what questions to ask of our tools and endpoints

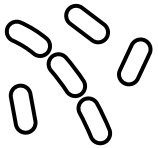
- MIC alone is not enough to assess environmental AMR risk



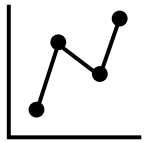
Take home messages



Antimicrobials can select resistance at levels far below MICs → MSCs are critical



Communities, not single species, drive ecosystem functions → we need endpoints that reflect this



Environmental guidelines must evolve → MIC-based thresholds underestimate risk

Asking the right questions of our tools and endpoints will help us protect microbial resilience and ecosystem health

Questions and Discussion

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