



A panoramic view of the virosphere in three wastewater treatment plants by integrating viral-like particle-concentrated and traditional non-concentrated metagenomic approaches

Jiayu Zhang^{1,2}, Aixi Tang¹, Tao Jin³, Deshou Sun^{1,5}, Fangliang Guo¹, Huaxin Lei¹,
Lin Lin¹, Wensheng Shu^{3,4}, Pingfeng Yu⁶, Xiaoyan Li¹, Bing Li^{1*}

¹ Tsinghua Shenzhen International Graduate School, Tsinghua University

² Research Center for Eco-environmental Engineering, Dongguan University of Technology

³ Guangdong Magigene Biotechnology Co., Ltd.

⁴ School of Life Sciences, South China Normal University

⁵ Shenzhen Tongchen Biotechnology Co., Limited, Shenzhen, 457001, China.

⁶ College of Environmental and Resource Sciences, Zhejiang University



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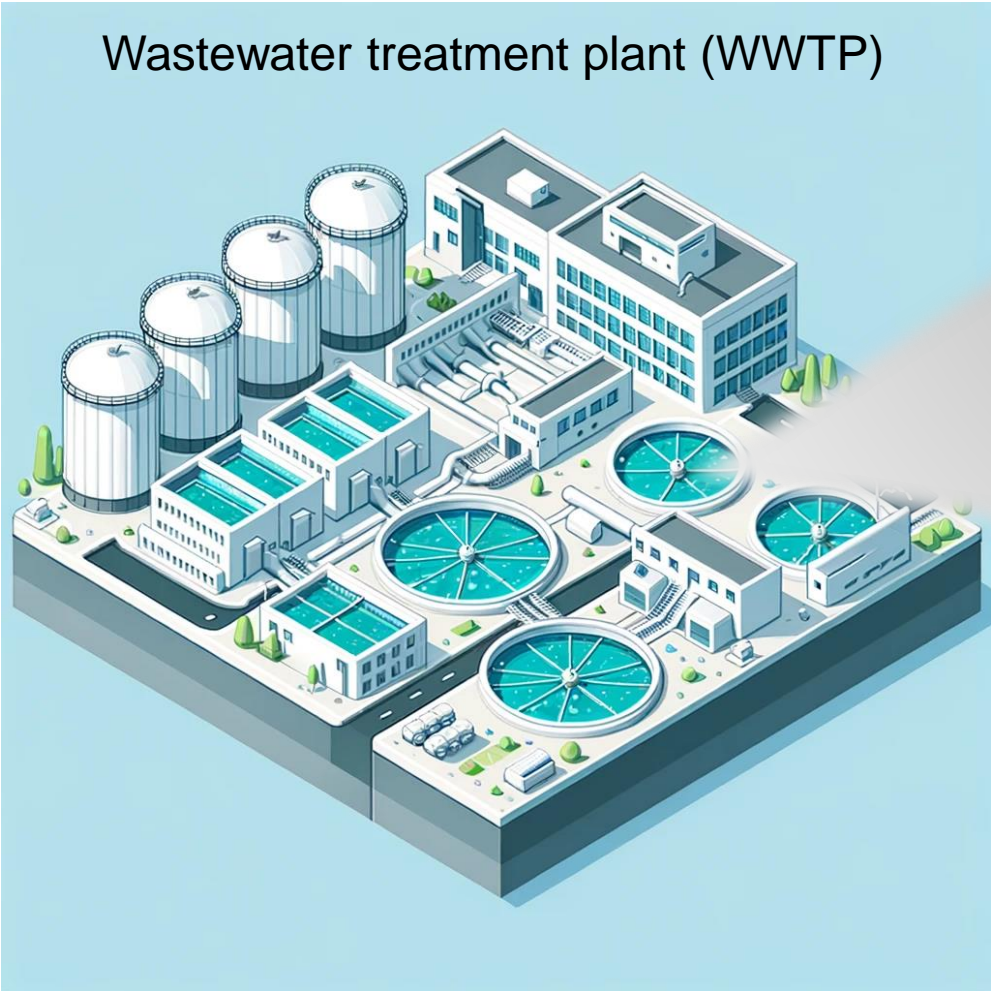
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Introduction

Viruses are the most abundant and diverse biological entities in wastewater treatment plants (WWTPs). However, our understanding of viruses in WWTPs remains limited.

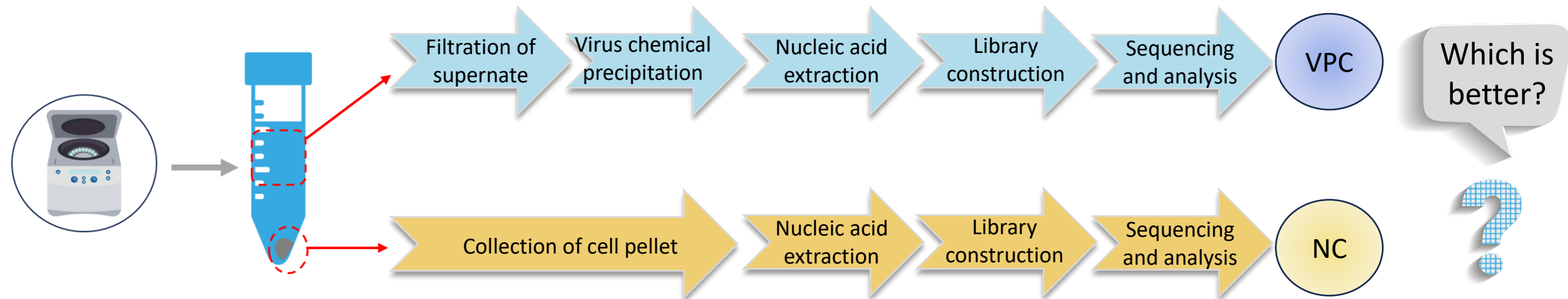
Wastewater treatment plant (WWTP)





Introduction

- When investigating viromes via metagenomics, researchers typically employ either viral-like particle-concentrated (VPC, targeting free viral-like particle fraction) or non-concentrated (NC, targeting cellular fraction) sample preparation methods.
- Gaps remain in our understanding of the virome acquired through different sample preparation methods.
- This study aimed to unveil the distinct advantages of two methods in capturing the complexity of viral communities and their functions in WWTPs.

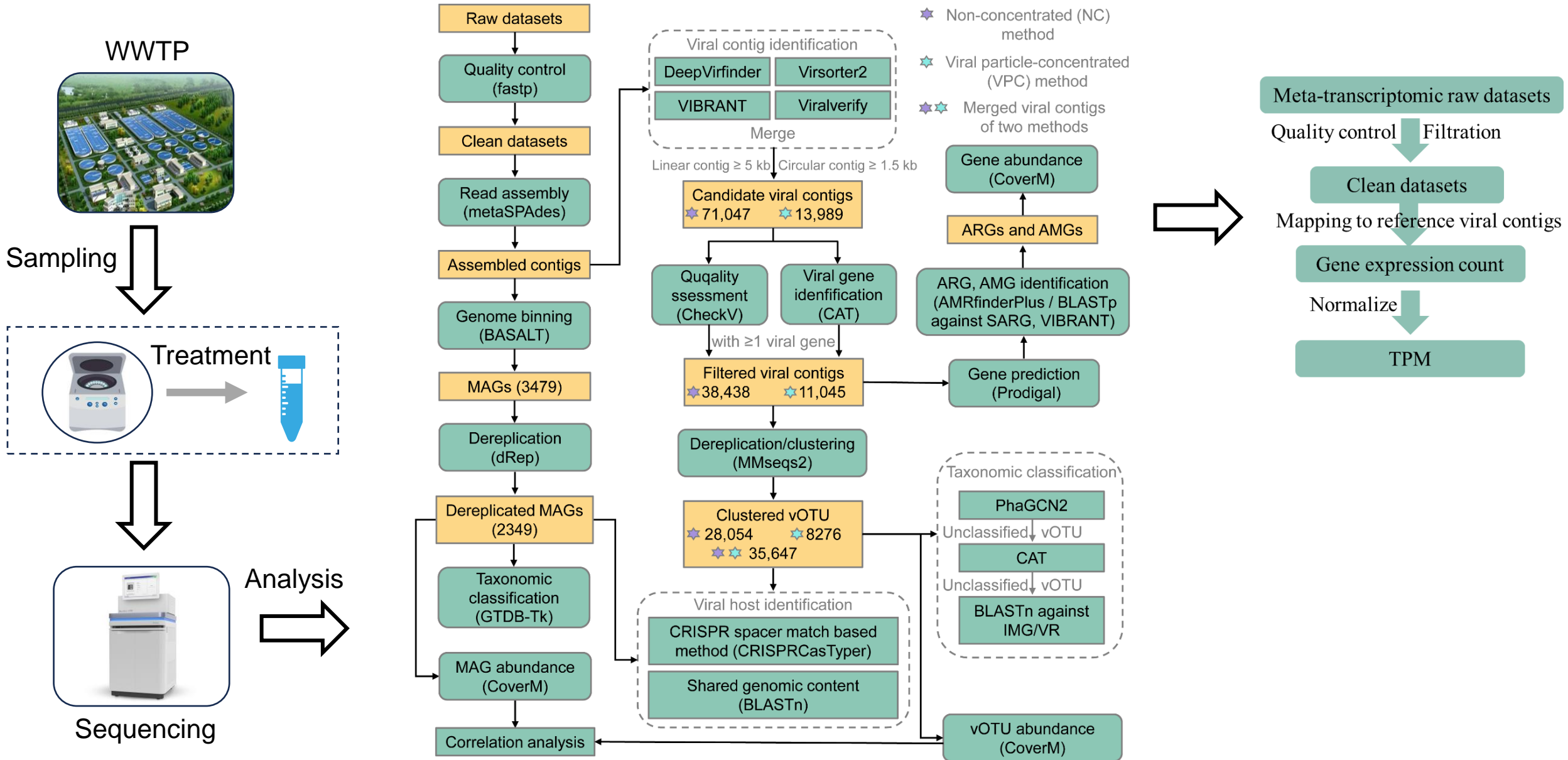


Pipelines for VPC and NC approaches



Methods

Pipeline for viromic analysis and meta-transcriptomic analysis





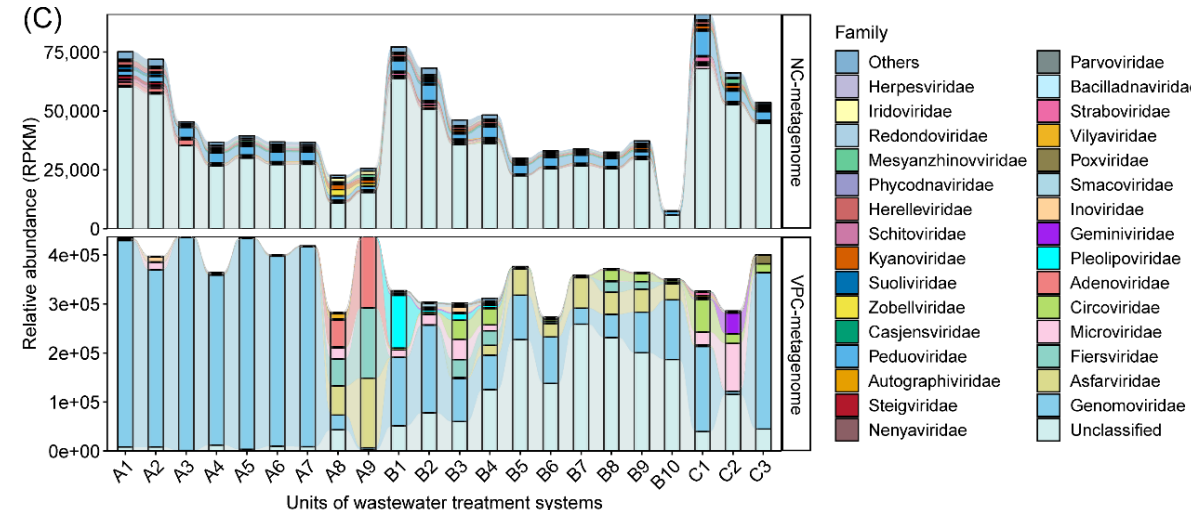
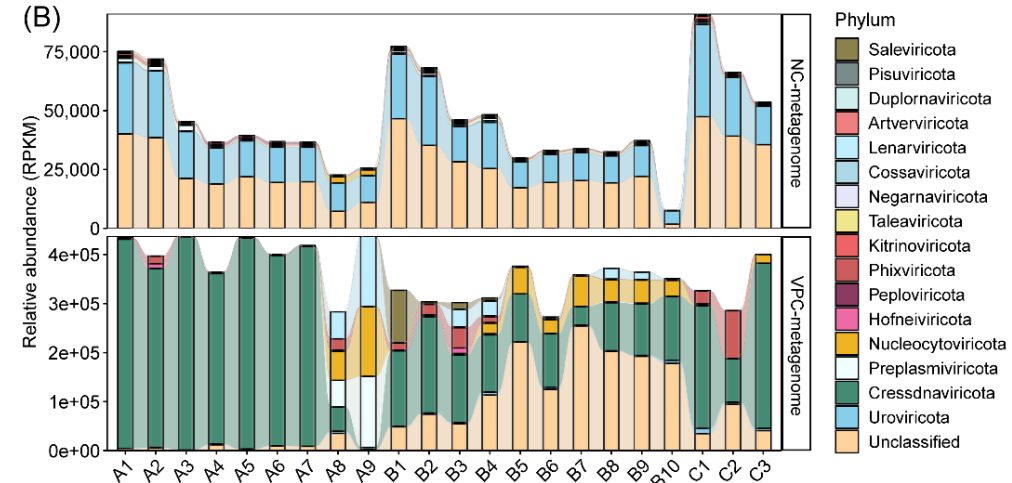
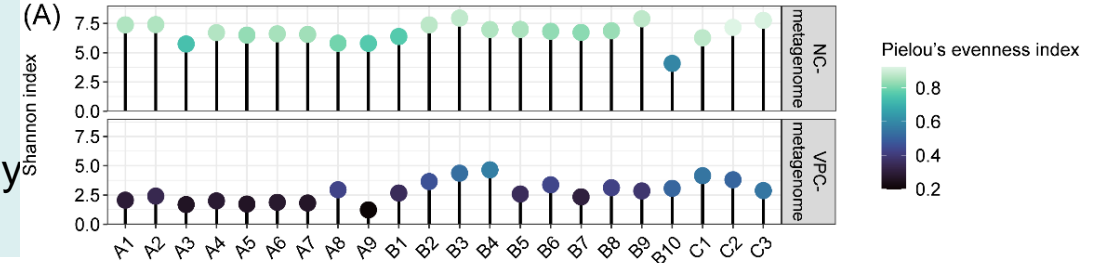
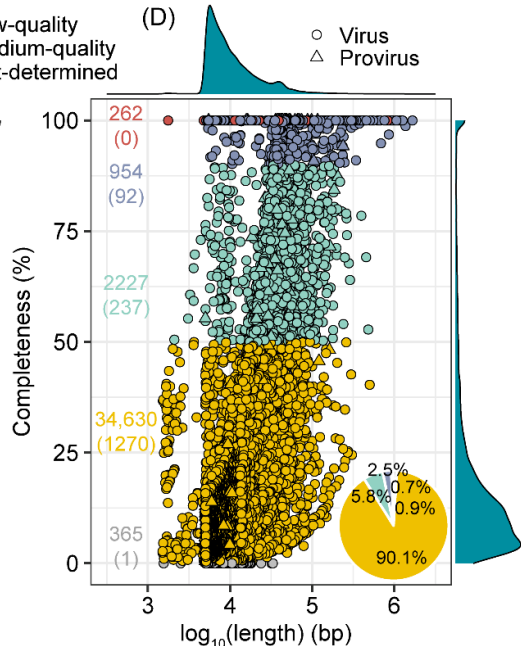
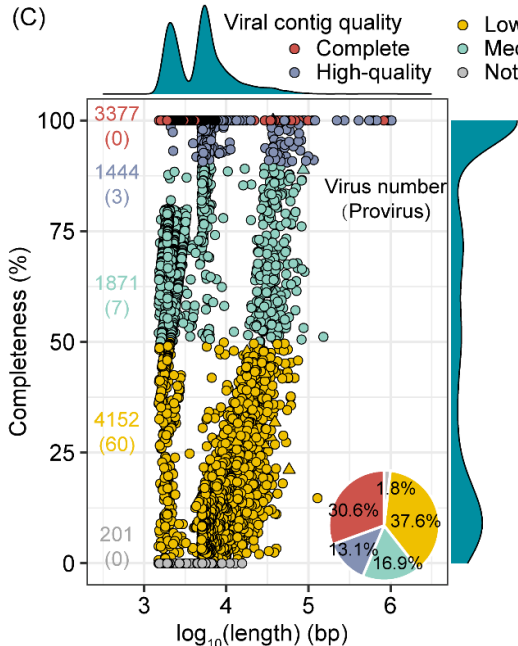
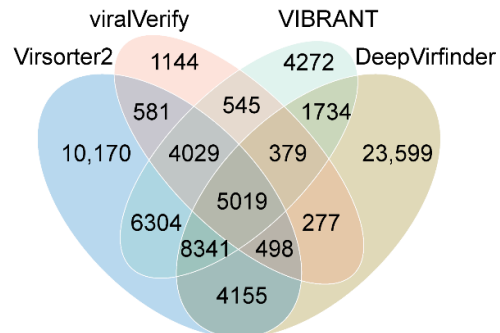
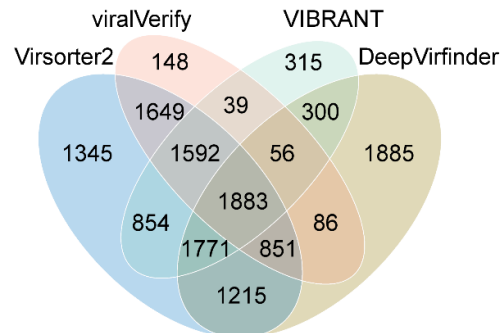
Results and discussion

Comparison of NC vs. VPC viromes

- VPC: Viral contigs with high quality. Dominated by Cressdnaviricota
- NC: Numerous viral contigs with high diversity. Dominated by Uroviricota

(A) VPC metagenome : 13,989 viral contigs

(B) NC metagenome : 71,047 viral contigs





Results and discussion

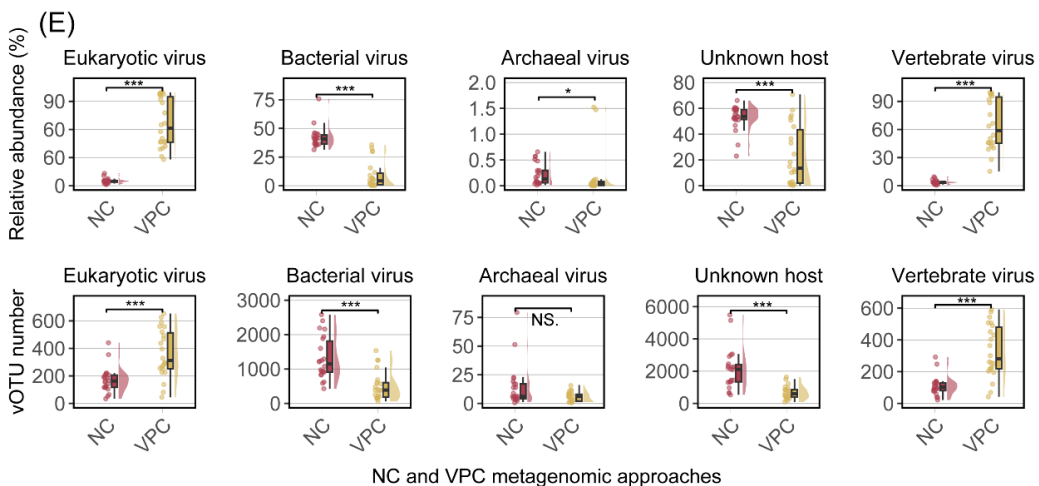
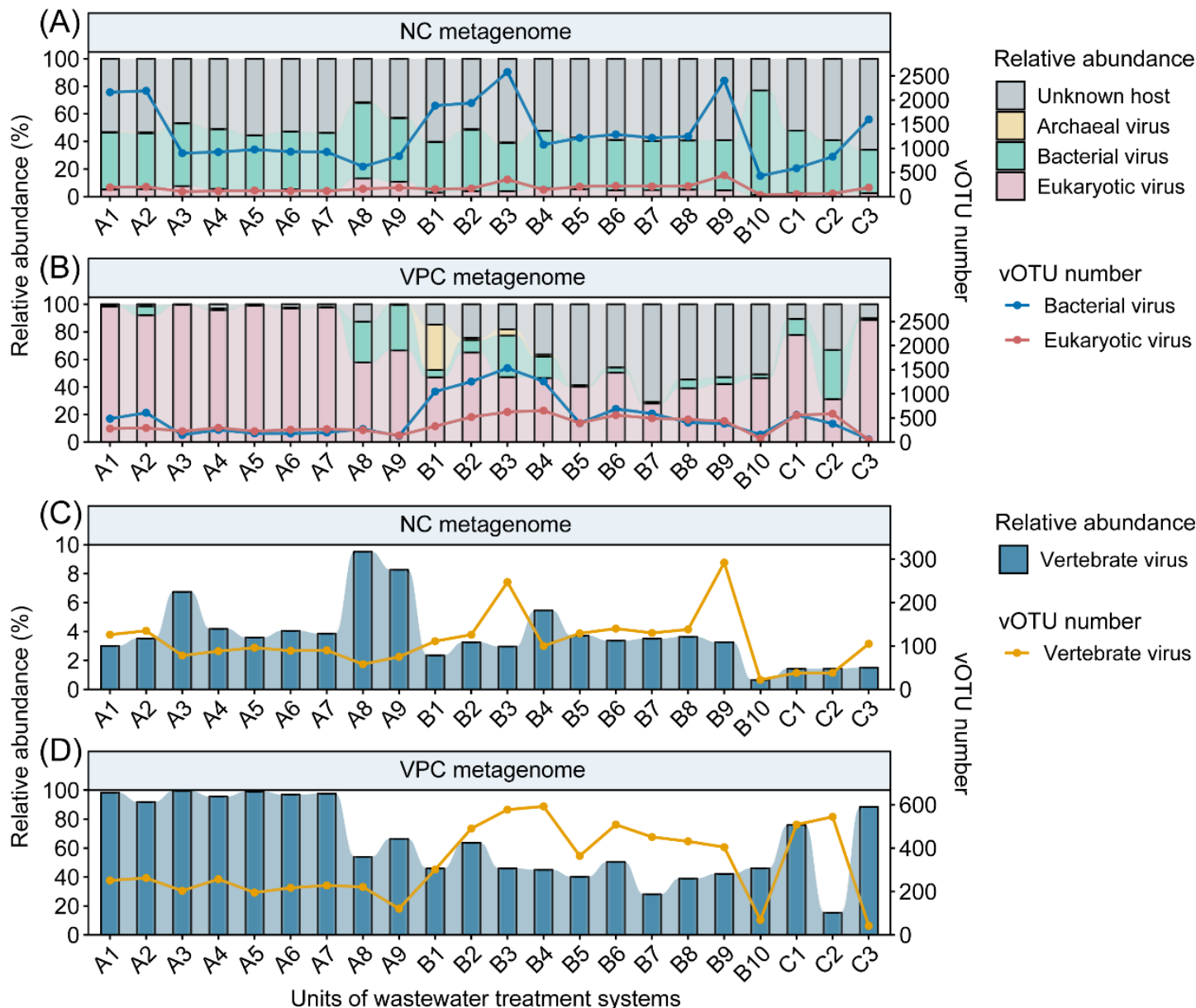
Dynamics of viruses infecting diverse hosts across wastewater treatment processes

NC: Dominated by bacteriophage viruses.

VPC: Dominated by eukaryotic viruses.

The NC method, mainly targeting cellular components such as bacteria, is more suitable for studying bacteriophages in wastewater.

The VPC method targets free virus particles and is more efficient for investigating free-living eukaryotic viruses, especially those vertebrate viruses that infect humans or animals.

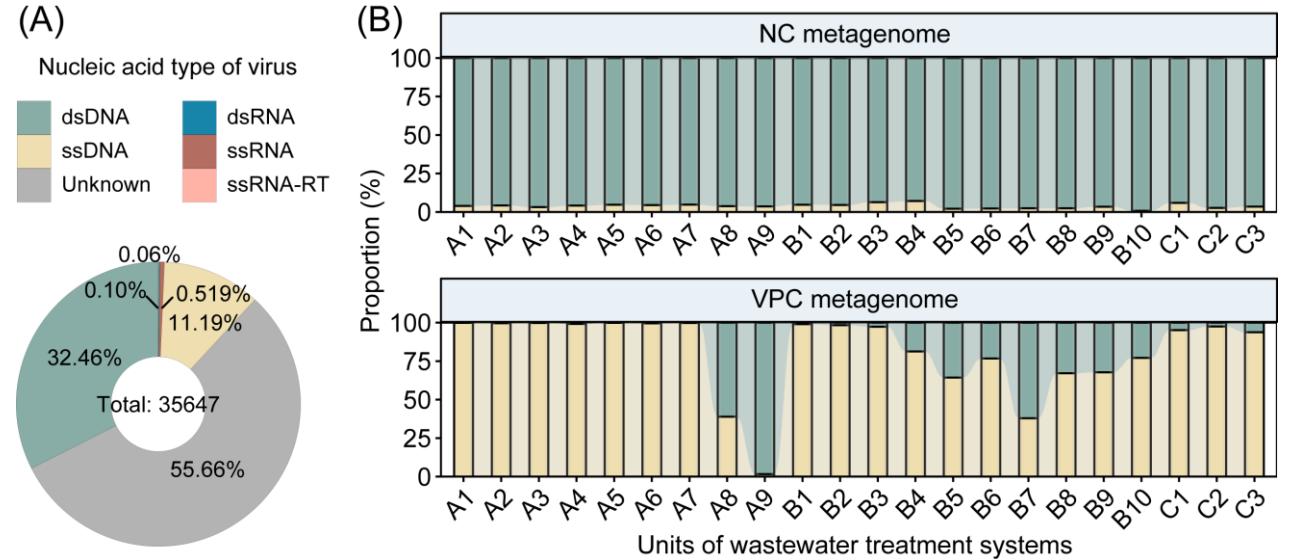




Results and discussion

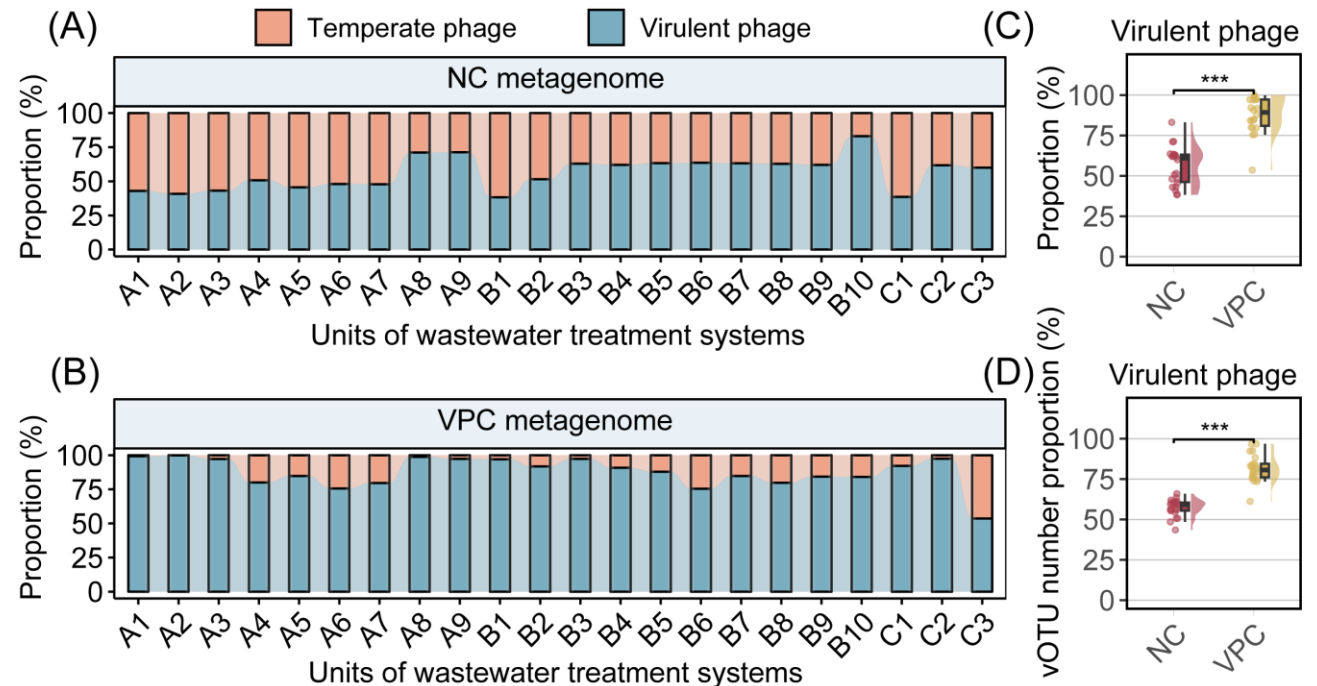
Viral nucleic acid types

- **NC method:** Most were dsDNA viruses
- **VPC method:** Most were ssDNA viruses



Bacteriophage life cycle types

- **NC method:** 56.1% of bacteriophages were lytic
- **VPC method:** 87.7% of bacteriophages were lytic

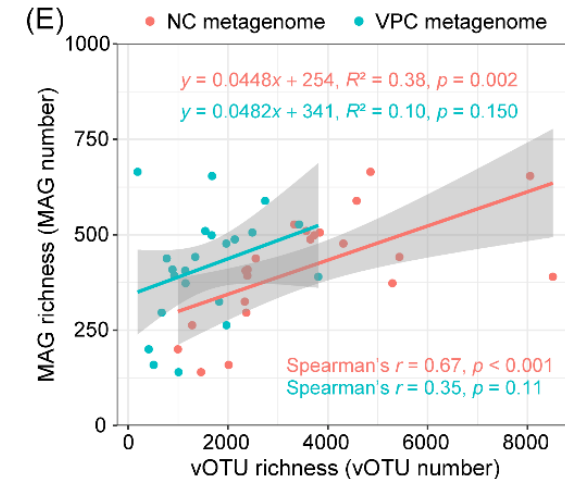
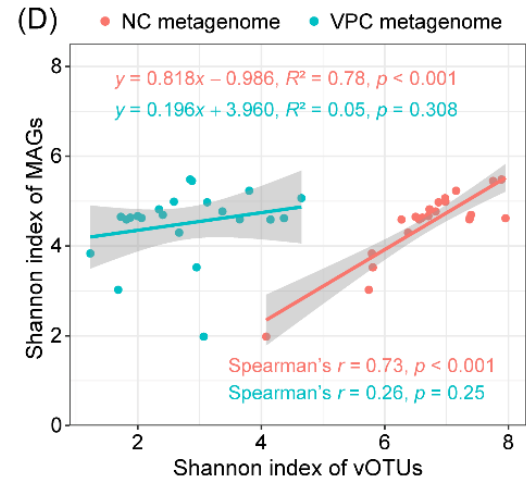
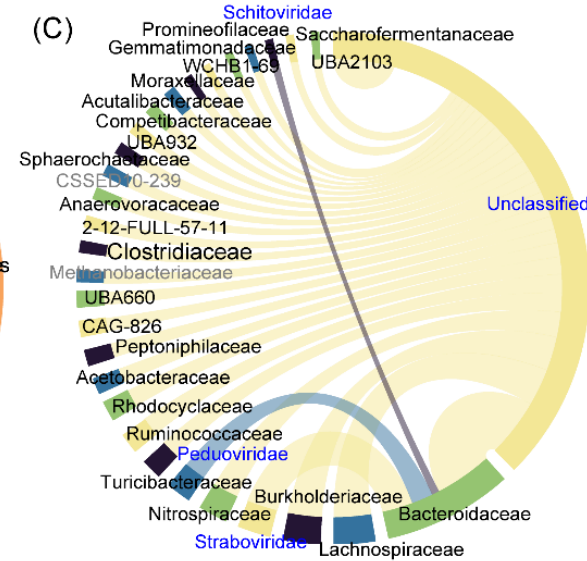
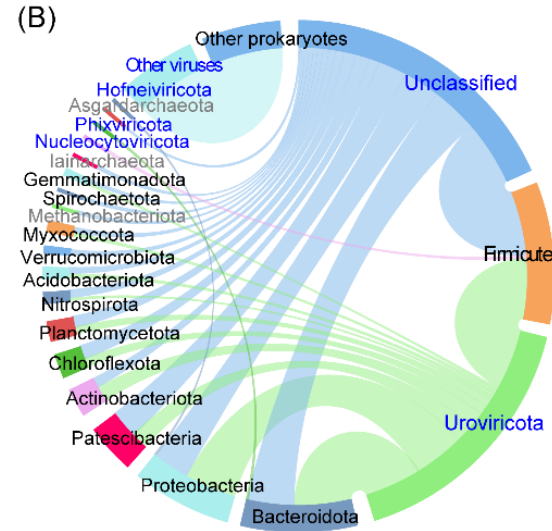
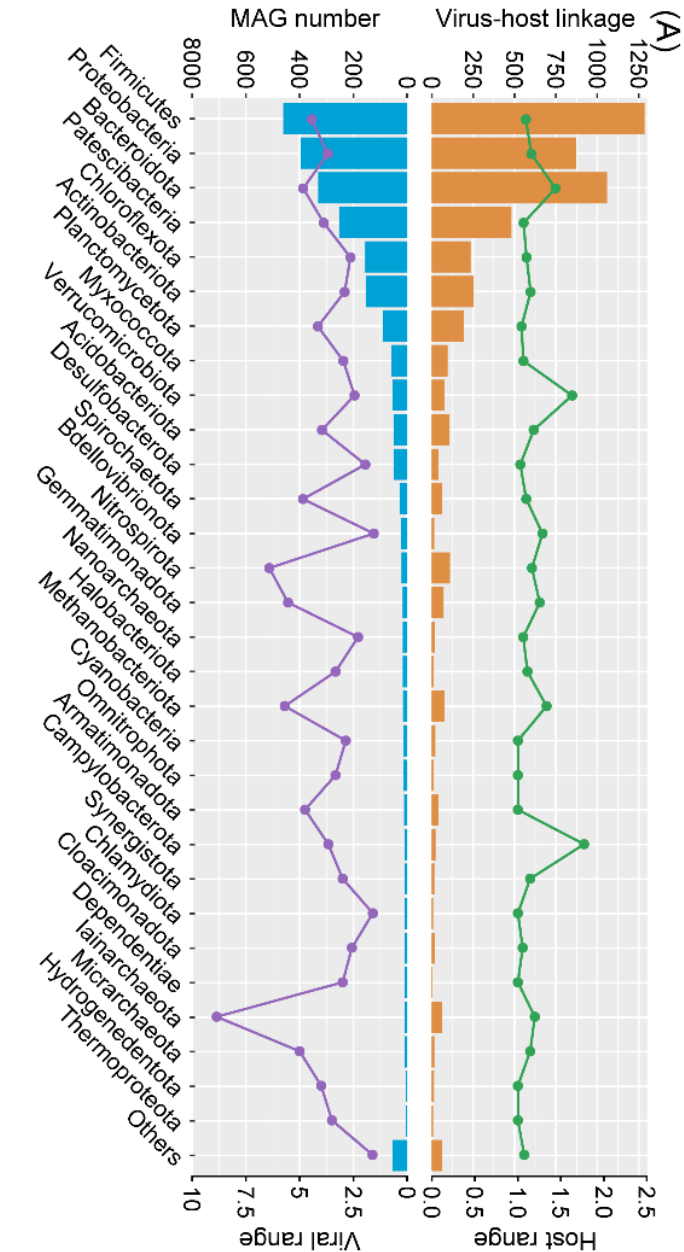




Results and discussion

Virus-prokaryote associations in wastewater treatment systems

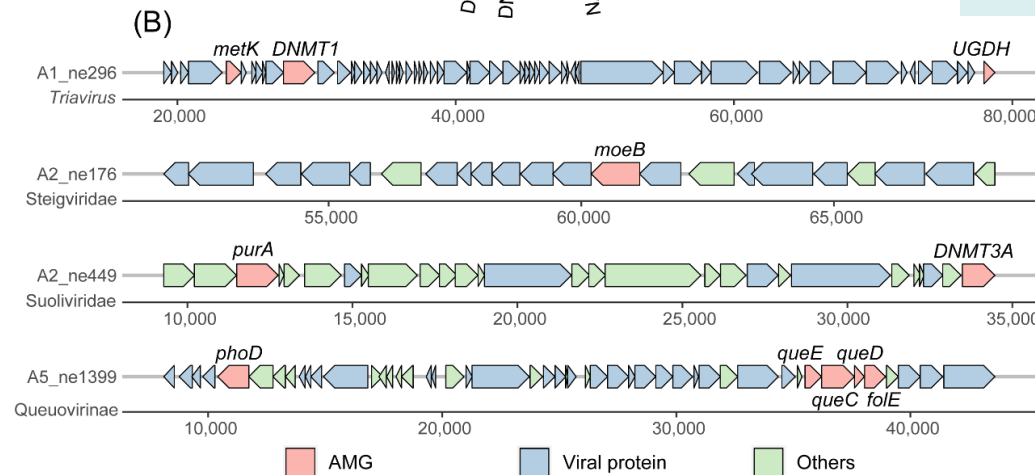
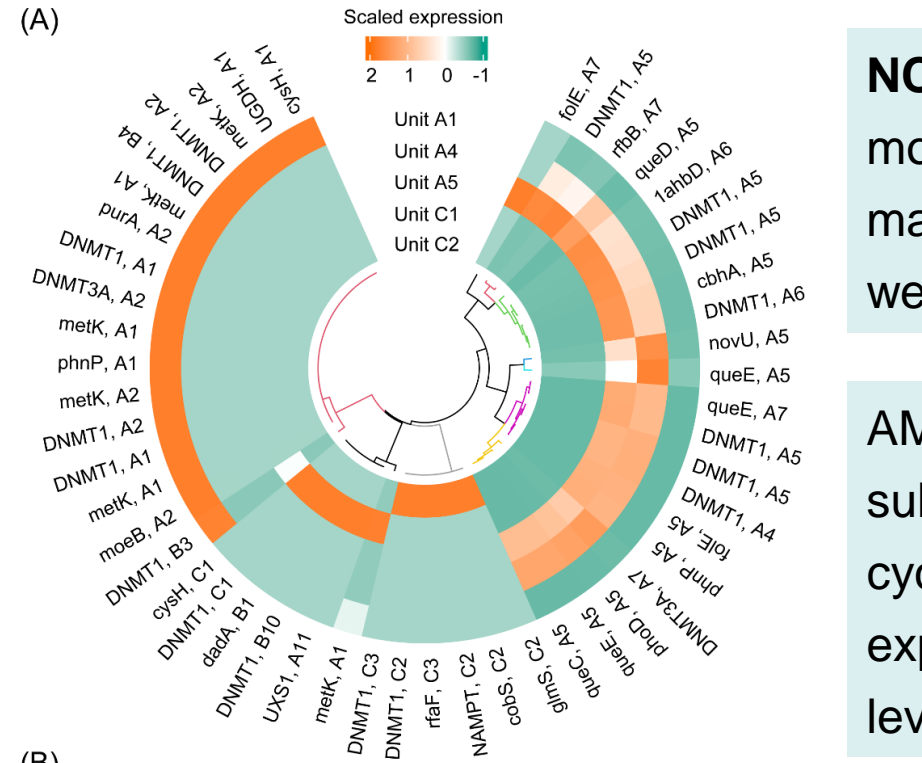
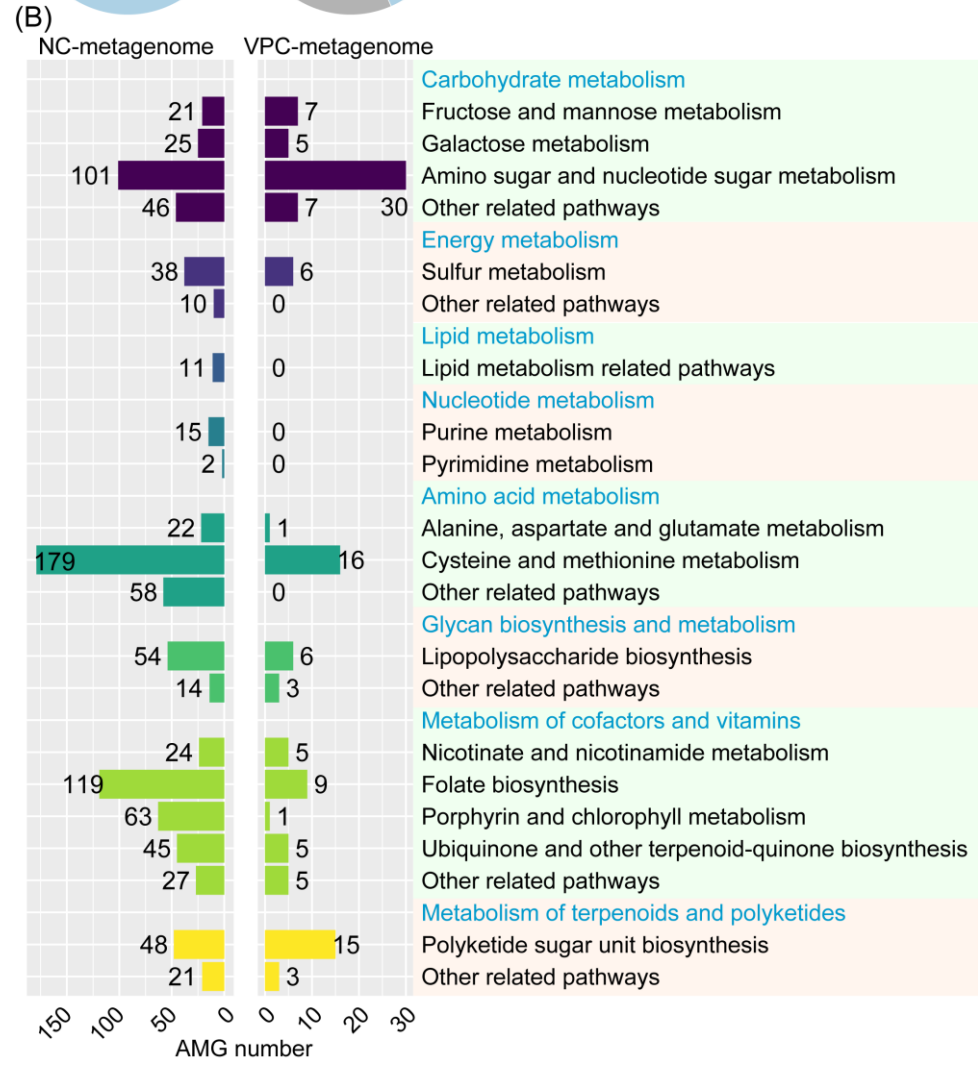
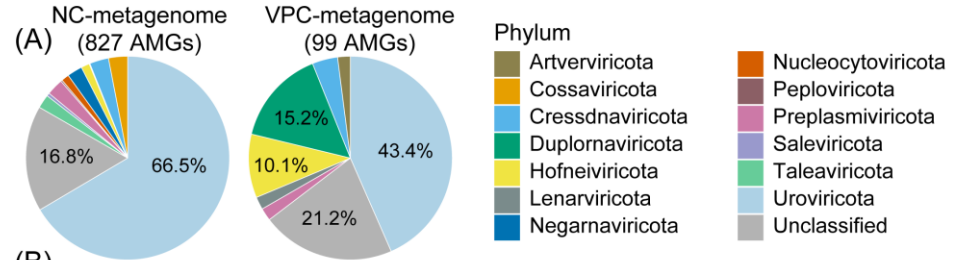
- A virus often has only one type of host, and a prokaryote can be infected by multiple viruses.
- Correlation between viral and bacterial diversity: $VPC < NC$.





Results and discussion

Auxiliary metabolic gene (AMG) composition and activity



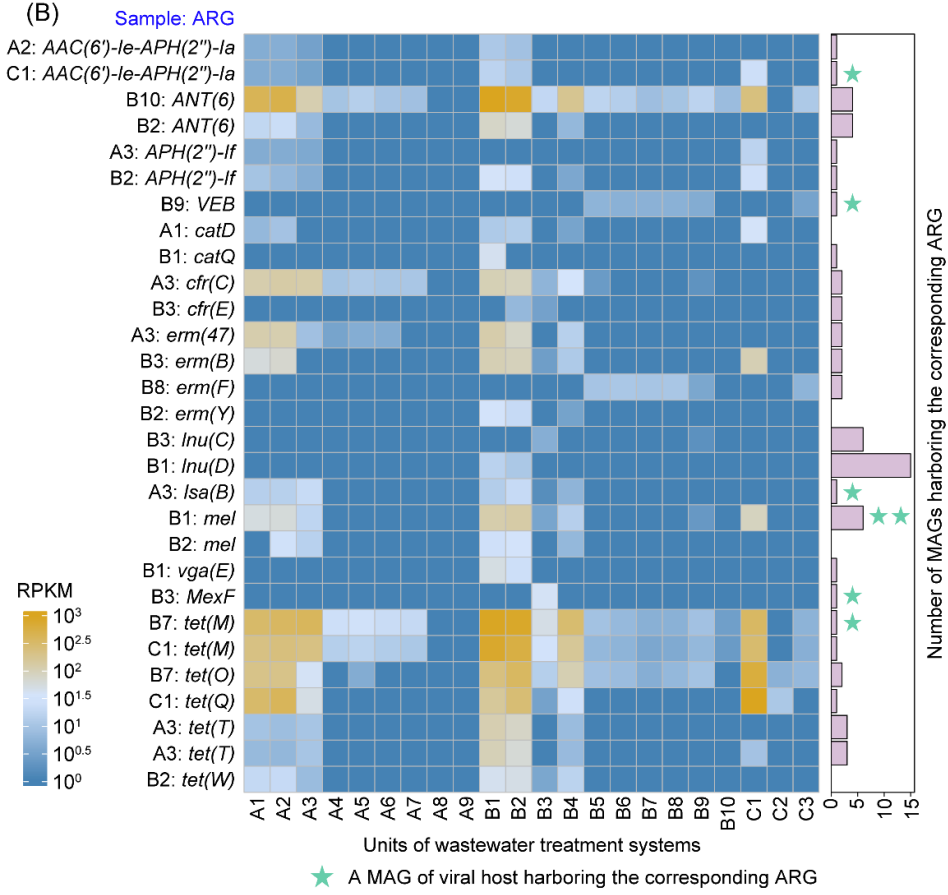
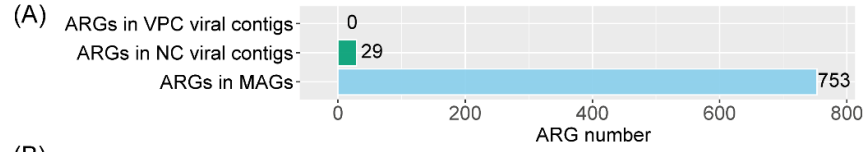
NC method identified more AMGs, and the majority carrying AMGs were bacteriophages.

AMGs in the carbon, sulfur, and phosphorus cycles were actively expressed at the RNA level.

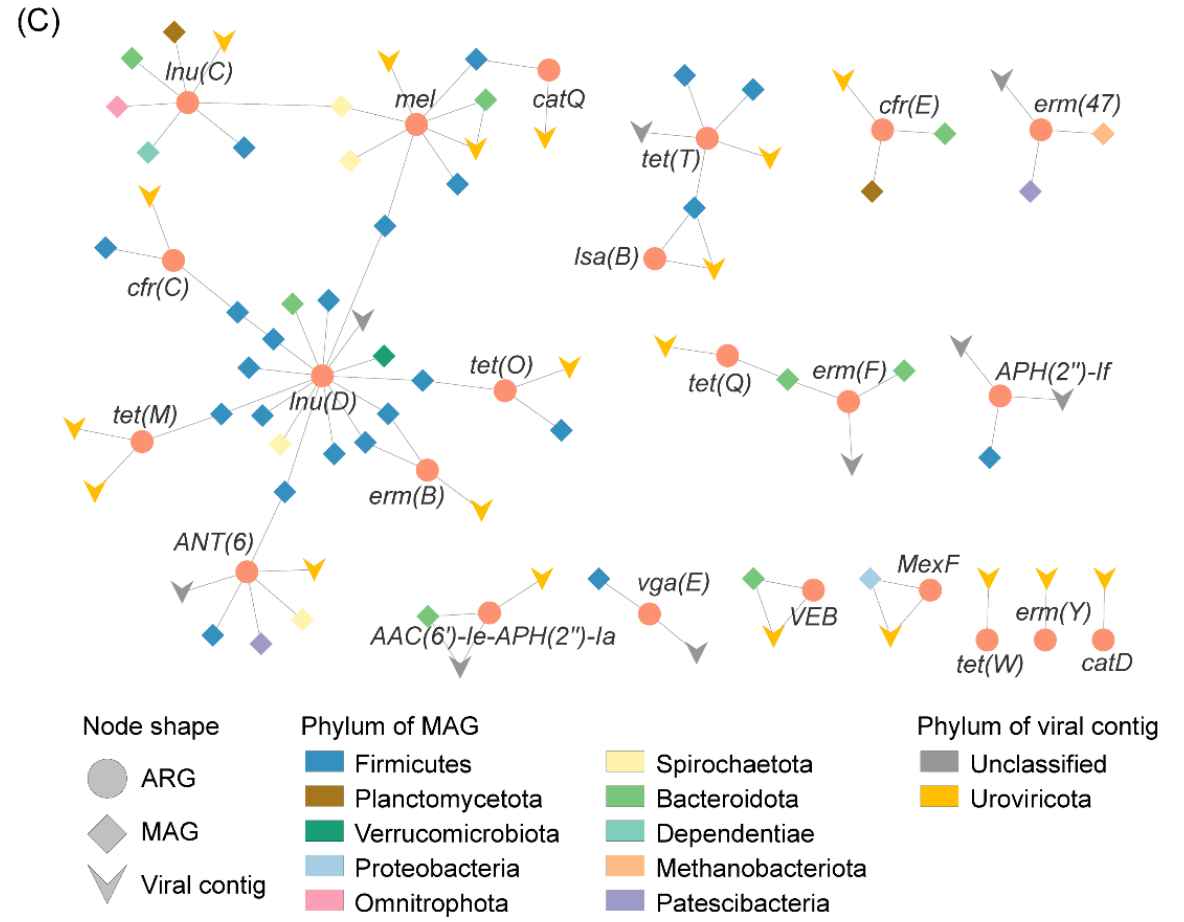


Results and discussion

Abundance of ARGs carried by viruses



ARGs sharing networks between viruses and bacteria

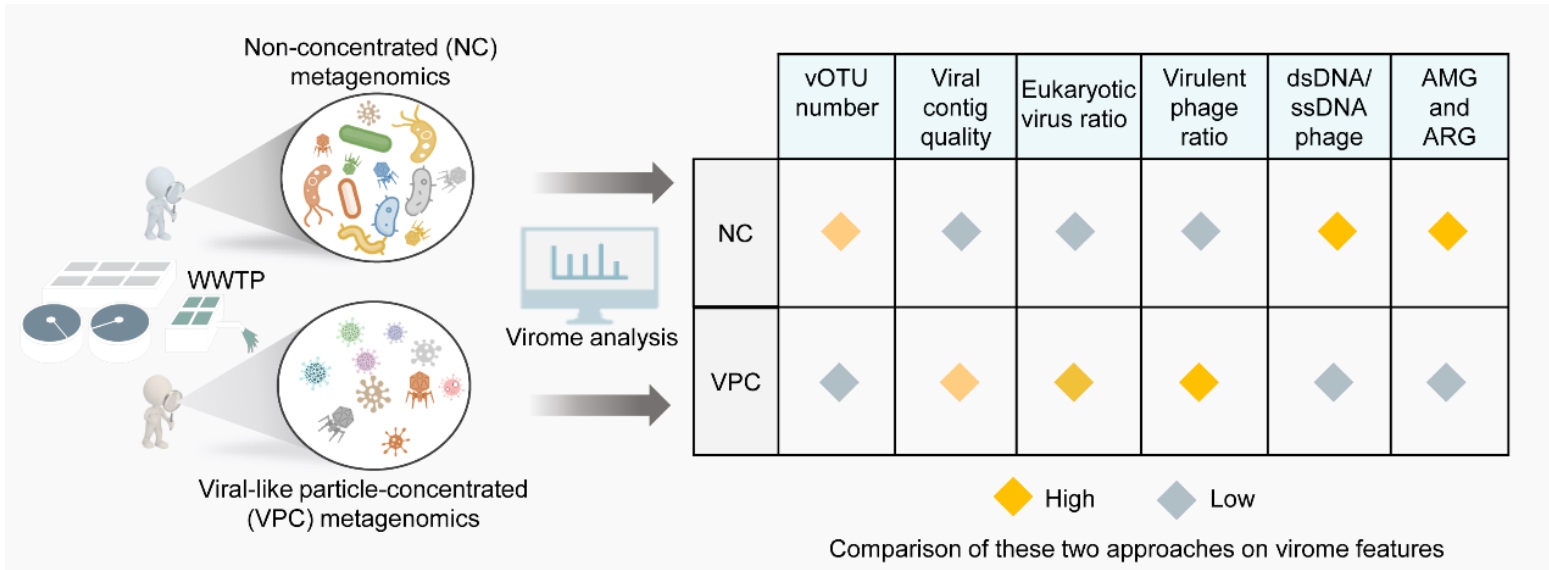


- **NC method** unveiled 29 ARGs carried by bacteriophages, including MLS, tetracycline, and aminoglycoside resistance types.
- Uroviricota phages were the primary ARG carriers. *Inu(D)* was shared by multiple bacteria and viruses.

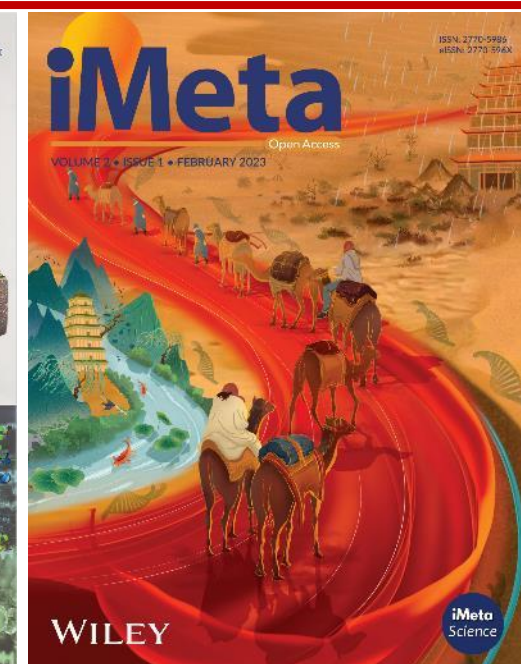


Summary

- ❑ A comprehensive comparison was made between VPC and NC metagenomic approaches in virome surveys, revealing distinct advantages of each method in analyzing viral communities and functions in wastewater treatment plants.
- ❑ Different sample processing methods resulted in different outcomes: eukaryotic viruses belonging to Cressdnaviricota were most abundant in VPC metagenomes, while bacteriophages belonging to Uroviricota were most abundant in NC metagenomes.
- ❑ A rich diversity of AMGs was discovered in the virome of wastewater treatment plants, with numerous AMGs involved in the biogeochemical cycles of carbon, sulfur, phosphorus, etc., showing transcriptional activity in wastewater treatment systems.
- ❑ Only a very small proportion (less than 0.08%) of viruses carrying ARGs was found in NC metagenomic data, with the types of ARGs carried by bacteriophages, mainly including macrolide-lincosamide-streptogramin (MLS), tetracycline, and aminoglycoside resistance.



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